Study title: ICI 182,780: One Month Intramuscular Toxicity Study in Rats.

Key study findings:

- Females exhibit histological changes in the ovaries (absent or reduced corpora lutea, multiple follicular cysts, luteal cysts, hemorrhagic Graafian follicles and hemorrhagic corpora lutea), uterus (atrophy), cervix (atrophy) and vagina (atrophy).
- There are no differences in the incidence or severity of the histological changes observed in rats administered ICI 182,780 alone or in combination with sulphone. These changes appear to be related to the anti-estrogenic activity of the compound.

Study no: TAR/2972
Volume #, and page #: N_000\2001-03-28\pharmtox\tox\Dose\ TAR/2972

Conducting laboratory and location: AstraZeneca UK Limited Safety Assessment Alderley Alderley Park Macclesfield Cheshire SK10 4TG England
Date of study initiation: April 21, 1999
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: Batch # P/1465/28, P/1465/26, P/1465/29, 99.9% w/w
Formulation/vehicle:

Ingredients	Strength placebo	Strength 2 % w/v	Strength 1.96 % w/v
	% w/v	% w/v	% w/v
ICI 182780	-	2.0	1.96
ICI 182780 sulphone	-	-	0.04
Poloxamer 407 USNF	1.0	1.0	1.0
Ethanol 96 % BP	10.0	10.0	10.0
Water for Injection Ph Eur	8.0	8.0	8.0
Propylene glycol	to 100 %	to 100 %	to 100 %
Formulation batch reference	P/1465/28	P/1465/26	P/1465/29

Methods: This study compares the toxicity of ICI 182,780 2% SA (short acting) formulation and a ICI 182,780 1.96% (with 0.04% sulphone) SA formulation administered intramuscularly to the rat once daily for 28 days. Note: The sulphone derivative is a degradation product of ICI 182,780 formulations and is a minor metabolite in the rat after IM administration. The results of this study are intended to support the final specification for sulphone content in ICI 182,780 parenteral formulations. The dose level selected for the study of 5 mg/kg/day (30 mg/m^2) x 28 days = 140 mg/kg (840 mg/m^2) is ~ 30x-(5x) the proposed human dose of 250 mg/30 days (4.17 mg/kg or 154.3 mg/m^2).

Dosing:

Species/strain:	Alpk: APfSD -Wistar derived rats
#/sex/group or time point (main study):	10/sex/group
Satellite groups used for toxicokinetics:	3/sex/group
Age:	37-41 days old
Weight:	127-217 g
Doses in administered units:	0, 5 mg/kg/d, and 5 mg/kg/d ICI 18270 + 0.4 mg/ml sulphone. Daily x 28 days

Route and volume:	IM; 0. 025 ml/100 g

Observations and times:

Clinical signs:	Twice daily
Body weights:	Day -7, pre- study, first day of dosing and then weekly for the remainder of the study.
Food consumption:	Daily
Ophthalmoscopy:	Pre-study and week 4
Hematology:	At scheduled necropsy. Blood samples for coagulation analyses were taken from designated 5/animals/group at scheduled necropsy (day 29)
Clinical chemistry:	At scheduled necropsy (day 29)
Urinalysis:	At scheduled necropsy (day 29)
Gross pathology:	Day 29
Organs weighed:	Adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, testes (including epididymides), thymus and uterus.
Histopathology:	Day 29
Toxicokinetics:	Pre-dose and 1, 3, 6, 12, and 24 hours on day 28. The AUC_{0-24} of ICI 182,780 and the AUC_{0-12} of the circulating metabolites (ZM208,917 sulphone and ZM366,472 17- ketone) for the male and female rats were compared within and between each dose group.

Results:

Mortality:	One ♂ animal dosed 5 mg/ kg ICI 182,780 with Sulphone, from the pharmacokinetic sub- group, died (day 5) as the result of an accident. The Sponsor did not indicate the nature of the accident. Necropsy findings included minimal focal acute myositis and minimal multifocal adjacent tissue acute inflammatory cell infiltration at the injection site. Also, mild unilateral hydronephrosis and minimal focal cortical tubular basophilia
Clinical signs:	Clinical observations included swelling of the hind limbs with associated transient limping in several animals in all groups including controls from day 3 to 24. The Sponsor considers this effect a consequence of exposure to the excipients and not ICI 182,780.
Body weights:	Males from both groups dosed with ICI 182,780 showed an approximate 10% body weight gain reduction when compared to control group. All females gained weight at a similar rate throughout the dosing period.
Food consumption:	Unremarkable (UR)
Ophthalmoscopy:	UR
Hematology:	Values represent percent change from control.

	Males			Females		
	Control	II[A]	III[A]	Control	II[A]	III[A]
Hemoglobin (g/dl)	15.3		↓4*	14.6	↑7*	
RBC ($x10^{12}$/l)	8.2		↓4*	7.6	↑8*	↑7*
Reticulocytes (%)	3.05		↑5*	3.29	↓11	↓8
Hematocrit (l/l)	0.48		↓4*	0.45	↑7	↑4*
MCV (fl)	59.6			59.3		↓4*
MCH (pg)	19			19.2		↓5*
Platelets (10^9/l)	974	↑11*	↑9*	938	↑9	
WBC (10^9/l)	8.6		↑14*	7.4		↑11
Neutrophil (%)	1.5	↑19	↑35*	1.53	↑10	↑56

[A] Group II-drug + sulphone; Group III-drug alone
*P<0.05

Clinical chemistry: Values represent percent change from control.

	Males			Females		
	Control	II[A]	III[A]	Control	II[A]	III[A]
Glucose (mmol.l)	14.6	↓16	↓18*	12.3		
Urea (mmol/l)	6.3			6.6	↓9	↓11*
Albumin (g/l)	30		↓7*	32	↓6*	↓9*
A/G Ratio	1		↓12*	1.1	↓16*	↓15*
T. calcium (mmol/l)	3.28		↓7*	3.14		1
ALP (IU/l)	344		↓8*	182	↑60*	↑41*
Triglycerides (mmol/l)	1.05		↓23*	0.68	↑19	↑35*

[A] Group II-drug + sulphone; Group III-drug alone
*P<0.05

Urinalysis: UR

Organ weights: At necropsy, there was a decrease in median body weight of male rats in Group II (11%) and in Group III (16%) given ICI 182,780. Thus, some absolute organ weights for male rats in these groups showed significant differences from the control values. The Sponsor considers these changes to be of no toxicological importance.
Median relative uterine weight was decreased by 79% for females in Group II and III given ICI 182,780.

Gross pathology:

Gross pathology findings	Group II[A]	Group III[A]
ovaries showed pale discoloration with red foci	5/10	8/10
cervix were thin/ small	4/10	3/10
uterus were thin/ small	10/10	9/10

[A] Group II-drug + sulphone; Group III-drug alone

Histopathology:

Females	**Control**	**II[A]**	**III[A]**
Cervix: Severe Atrophy		10/10	10/10
Ovaries			
No corporea lutea		5/10	7/9
Reduced corporea lutea		5/10	1/9
Follicular Cysts		9/10	8/9
Luteal Cysts			1/9
Hemorrhagic Corporea Lutea			1/9
Hemorrhagic Graafian follicles		2/10	9/9
Uterus: Severe Atrophy		10/10	10/10
Vagina: Epithelial Severe Atrophy	0/10	10/10	9/9
Estrus	5/10	0/10	0/9

Injection site-acute myositis Adjacent tissue acute inflammatory cell infiltration	5/10 0/10	5/10 4/10	5/10 7/10
Sciatic nerve-adjacent acue inflammatory cell infiltration	0/10	5/10	7/10
Males	**Control**	**II[A]**	**III[A]**
Epididymides-Chronic epididymitis	4/10	0/10	3/10
Injection site-acute myositis Adjacent tissue acute inflammatory cell infiltration	3/10 1/10	4/10 4/10	5/10 5/10
Sciatic nerve-adjacent acue inflammatory cell infiltration	0/10	0/10	2/10

[A] Group II-drug + sulphone; Group III-drug alone

Toxicokinetics:

	ICI 182,780		ZM 208,917[A]				ZM 366,472[B]			
	Group II*	Group III	Group II*	Group II*	Group III	Group III	Group II*	Group II*	Group III	Group III
	Combined	Combined	♂	♀	♂	♀	♂	♀	♂	♀
AUC $_{(0\text{-}12,24)}$** (ng*h/ml)	3300	3270	205	333	122	194	NC	104	NC	92.0
C_{min} (ng/ml)	48.2	43.4	3.76	4.52	<2.00	<2.00	NC	7.43	NC	5.07
C_{max} (ng/ml)	337	251	25.4	38.0	12.4	24.9	NC	15.6	NC	13.2
T_{max} (h)	1.00	3.00	1.00	1.00	6.00	3.00	NC	3.00	NC	3.00

*with 0.04% sulphone; ** ICI 182.780 was measured 0-24 and ZM 208,917 and ZM 366,472 were measured 0-12

[A] ZM208,917 is a sulphone metabolite

[B] ZM366,472 is a 17-ketone metabolite only demonstrated in females.

- There was no difference in exposure to ICI 182,780 between the sexes or between the groups given ICI 182,780 alone or in combination with sulphone.
- Absorption appeared faster in animals given ICI 182,780 and sulphone compared to administration of the drug alone. That is, on day 28, C_{max} for ICI 182,780 occurred at 1 h post-dose (t_{max}) for Group II (drug + sulphone) compared to 3 h post-dose for Group III (drug alone).
- Exposure to ZM208,917 (sulphone metabolite) was slightly greater in females compared to males (Group II and III : 62% and 59%, respectively).
- Exposure to ZM208,917 (sulphone metabolite) was approximately 70% greater in animals given ICI 182,780 in combination with sulphone than in animals given ICI 182,780 alone.
- There was a significant difference between the sexes in the $AUC_{0\text{-}12}$ of ZM366,472 (ketone metabolite) with male rats showing no systemic exposure. However, there was no difference between the $AUC_{0\text{-}12}$ of ZM366,472 of Groups II and III females.

Summary of individual study findings:

Study TAR/2972 compares the toxicity of the ICI 182,780 formulation (2% ICI 182,780-SA) and a ICI 182,780 formulation (SA) spiked with sulphone (1.96% ICI 182,780 with 0.04% sulphone), when administered intramuscularly to the rat once daily for 28 days. The sulphone derivative is the main degradation product of ICI 182,780 formulations and is a minor metabolite in the rat.

Pharmacokinetic monitoring demonstrated no difference in exposure to ICI 182,780 between the sexes or between the groups given ICI 182,780 alone or in combination with sulphone. Exposure to ZM208,917 (sulphone metabolite) was greater in females compared to males and ~ 70%

greater in animals given ICI 182,780 in combination with sulphone than in animals given ICI 182,780 alone (the increase in exposure probably due to administered ZM208,917). Exposure to ZM366,472 (ketone matebolite) was observed only in females.

Changes seen in animals given ICI 182,780 (alone or in combination with sulphone) were related to the anti-estrogenic activity of the compound. The changes seen included a 10% reduction in body weight gain in males and histological changes in the ovaries (absent or reduced corpora lutea, multiple follicular cysts, luteal cysts, hemorrhagic Graafian follicles and hemorrhagic corpora lutea), uterus (atrophy), cervix (atrophy) and vagina (atrophy). There were no differences in the incidence or severity of histological changes between the two groups.

Study title: ICI 182,780 : SIX MONTH INTRAMUSCULAR TOXICITY STUDY IN RATS.

Key study findings:

- Atrophy of the female reproductive tract; a specific constellation of ovarian alterations with increased late stage and cystic Graafian follicles, loss of mature corpora lutea and reduced vacuolation of the interstitial cells.
- A loss of spermatozoa from the seminiferous tubules with an accompanying dilatation, seminiferous tubular atrophy with some associated degenerative changes in the epididymides.
- Conversion of specific tissues in females to the morphology normally seen in males including mammary gland structure, splenic hemosiderosis, pituitary gonadotroph vacuolation and reduced hair loss and an earlier appearance of adrenal cortical congestion with hemocyst formation.

Study no: TPR/2042.
Volume #, and page #: N_000\2001-03-28\pharmtox\tox\Dose\ TAR/2972\TPR/2042.
Conducting laboratory and location: ICI Pharmaceuticals Safety of Medicines Department Alderley Park Macclesfield Cheshire England
Date of study initiation: April 2, 1992
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: ICI 182,780; ADM 44010/89; 98.8%
Formulation/vehicle: Sustained release LA formulation

Ingredients	Strength w/v%	Placebo w/v%
ICI 182,780	5.0	0
Ethanol 96% v/v	10.0	10.0
Benzyl Alcohol	10.0	10.0
Benzyl Benzoate	15.0	15.0
Castor Oil	To 100	To 100
Batch number	PH/6731/41	PH/6731/40
Analytical Number	ADM 48027/90	ADM 48026/90

Methods:
Dosing:

Species/strain: CR1:(WI)BR Wistar rats

#/sex/group or time point (main study):	20/sex/group
Satellite groups used for recovery:	10/sex/vehicle control or 10 mg/rat/30 days ICI 182,780 retained for a 19/20 week withdrawal period after the 6th dose.
Satellite groups used for PK:	5/sex/time point
Age:	36-43 days old
Weight:	110-199 g
Doses:	0 (saline and vehicle), 15 mg/kg/30 days, 10 mg/rat/30 days or 10 mg/rat/15 days.

- All main test animals received 7 doses at 30 day intervals of ICI 182,780 or control formulation, except Group V which received 13 doses at 15 day intervals. Animals were observed for at least 7 days prior to necropsy.
- Animals in withdrawal groups received 6 doses of ICI 182,780 or control formulation, at 30 day intervals and were observed for 19 or 20 weeks after cessation of dosing/before necropsy.
- On day 61, 5 male rats from Group III were erroneously dosed with the vehicle control. These animals were allowed to recover and then correctly dosed on day 64, giving inter-dose periods of 33 days before and 27 days after this dose. This error is not considered to have compromised the study.

Route, form, volume, and infusion rate: IM using a plastic syringe and a sterile needle 25G x 16 mm; 0.2 ml/rat for Groups I, II, IV and V and 0.03 ml/100 g body weight for Group III.

Group	Dose Levels ICI 182,780	Main test	Withdrawal
I	0 mg/rat/30 days vehicle control	20 M, 20 F	0 M, 0 F
II	0 mg/rat/30 days saline control	30 M, 30 F	10 M, 10 F
III	15 mg/kg/30 days	20 M, 20 F	0 M, 0 F
IV	10 mg/rat/30 days	30 M, 30 F	10 M, 10 F
V	10 mg/rat/15 days	20 M, 20 F	0 M, 0 F

Note: The dose of 15 mg/kg/30 d was chosen as a dose which could be administered once a month for 6 months within the constraint of the maximum permitted dose volume (0.1 ml/site in 2 sites). The 10 mg/rat dose was set to maximize exposure of the rats with doses at the limit volume every 30 or 15 days.

Observations and times:

Clinical signs:	Twice daily
Body weights:	Day -7 and -2 pre-study, on the first day of dosing, then weekly to week 13 and then monthly for the remainder of the study.
Food consumption:	Recorded 7 days pre-study, then weekly to week 13, and monthly for the remainder of the study.

Ophthalmoscopy:	Pre-study and all group I, II, and V animals in the main study were also examined during weeks 14 and 26 of the study
Hematology:	Weeks 13 and 24 of dosing and from the withdrawal animals on week 40.
Clinical chemistry:	Weeks 13 and 24 of dosing and from the withdrawal animals on week 40.
Urinalysis:	Weeks 13 and 24 of dosing and from the withdrawal animals on week 40.
Gross pathology:	Main group: week 27-29; withdrawal: week 46-47
Organs weighed:	adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, testes (including epididymides) and uterus.
Histopathology:	Main group: week 27-29; withdrawal: week 46-47
Toxicokinetics:	Samples were taken 3, 7 and 24 h after the first dose and on days 4, 7, 11, 16, 21, 25 and 30. In addition, samples were taken 3 and 24 hours after the second dose from rats in Group V. Samples were also taken after the sixth (Groups III and IV) and eleventh/twelfth (Group V) dose at the same time intervals given for the first and second dose above.

Results:

Mortality:	There were two premature sacrifices during the study, M 400826 (vehicle control) and F 400775 (10 mg/rat/30 days) due to broken teeth and body weight loss.
Clinical signs:	Bruising around the injection site was observed in one ♂ (vehicle control).

Body weights:

Cumulative Body Wt. Gains (g)

	Females				Males			
Week	**Vehicle**	**15 mg/kg/ days**	**10 mg/rat/ 30 days**	**10 mg/rat/ 15 days**	**Vehicle**	**15 mg/kg/ days**	**10 mg/rat/ 30 days**	**10 mg/rat/ 15 days**
1	25	20**	18***	21*	53	51	48***	46***
2	48	41*	33***	36***	110	102*	97***	94***
3	64	55*	46***	52**	157	152	144**	138***
4	76	71	60***	63**	194	188	182	176*
5	88	79**	74**	76*	223	215	211	206
6	99	85***	80***	85**	-	-	-	-
7	109	89***	85***	93**	-	-	-	-
8	116	98**	93***	98**	-	-	-	-
9	123	107**	101***	105**	-	-	-	-
10	128	109**	108***	113*	-	-	-	-
11	135	112***	112***	121*	-	-	-	-
12	138	116***	117***	124*	-	-	-	-
13	142	117***	120***	128*	-	-	-	-
17	160	133***	137***	144*	-	-	-	-
21	174	146***	156**	159*	-	-	-	-
25	184	149***	160***	169*	435	424	417	412

* $p<0.05$, **$p<0.01$,***$p<0.001$

- Female rats given 15 mg/kg/30 days showed impaired growth throughout the study with a cumulative gain over the 25 week measurement period of 81% of control value. Females given 10 mg/rat, at 30 or 15 day intervals, showed impaired growth over the study (cumulative gains of 87 and 92% of controls).
- Male rats given ICI 182,780 showed a slight impairment of growth up to 4 weeks after the first dose compared to controls.
- During the withdrawal phase male rats previously given 10 mg/rat/30 days gained less weight than the vehicle controls (gain between weeks 25-41 ~ 85% of control) while females from this group showed similar growth to vehicle controls.

Food consumption:

- Male rats given ICI 182,780 ate similar amounts of food to controls during the dosing period. On weeks 2 and 3, male rats given 10 mg/rat (at both 15 and 30 day intervals) had significantly lower (4-6%) group mean food consumption values compared to controls.
- Female rats given ICI 182,780 ate less food (9-15%) than controls during the dosing period. For rats given 15 mg/kg/30 days and 10 mg/rat/30 days, this effect began in week one and continued throughout the dosing period. Females given 10 mg/rat/15 days also had lower (9%) group mean food consumption from week 2 to week 8, after which time it was comparable to the control animals.
- During the withdrawal phase the food consumption of animals previously given 10 mg/rat/30 days (males and females) was lower than that of the control animals.

Ophthalmoscopy: UR

Hematology:

Treatment	Week	Vehicle		15 mg/kg/30 d		10 mg/rat/30 d		10 mg/rat/15 d	
		M	F	M	F	M	F	M	F
Hemoglobin (g/dl)	13	15.46	14.25	14.91*	15.06***	14.55***	15.24***	14.73**	15.27***
	24	15.13	14.24	14.09***	14.97**	14.25***	15.82***	14.58*	15**
RBC (x10 ^{12}/l)	13	8.94	7.82	8.57*	8.40***	8.48**	8.48***	8.63*	8.41***
	24	9.14	8.04	8.58**	8.179	8.51***	8.64***	8.63**	8.16
Hematocrit (l/l)	13	0.4480	0.411	0.4296**	0.4291**	0.4150***	0.4320**	0.4270**	0.4349***
	24	0.4695	0.429	0.4360***	0.4307	0.4160***	0.4534**	0.4220***	0.4300

Recovery	Week	Vehicle		10 mg/rat/30 d	
		M	F	M	F
Hemoglobin (g/dl)	13	15.472	14.102	14.55***	15.240***
	24	15.300	14.383	14.25***	15.269
RBC (x10 ^{12}/l)	13	8.902	7.83	8.48**	8.48**
	24	9.146	8.229	8.51***	8.486
Hematocrit (l/l)	13	.4494	.4070	0.4150***	.4320**
	24	.4629	.4343	0.4160***	.4629

* $p<0.05$, ** $p<0.01$, *** $p<0.001$

- Males given ICI 182,780 showed lower hemoglobin (4-7%), hematocrit (4-11%), and RBC (4-7%) values compared to vehicle controls. Decreases in RBC counts may be suggestive of hemolysis unrelated to vehicle.
- Female rats given ICI 182,780 showed higher hemoglobin (5-11%) concentrations compared to controls at both time-points in all dose groups. At week 13, this was associated with an increase (~8%) in red blood cell count however, at week 24, this increase was only observed in rats given 10 mg/rat/30 days (↑7%).

- The same pattern of changes was observed in male and female rats in the recovery group. However, it is noteworthy that the values for ♂s in the main test (10 mg/rat/30 days) and withdrawal group (10 mg/rat/30 days) are identical.

Clinical chemistry:

	Week	Vehicle		15 mg/kg/30 d		10 mg/rat/30 d		10 mg/rat/15 d	
		M	F	M	F	M	F	M	F
Total protein (g/l)	13	72	70.10	69.60	63.60***	68.5*	65.30***	71	65.6**
	24	76.34	75.90	73.50*	69***	73.80	69.80***	74.7	68.4***&
Albumin (g/l)	13	33.90	35.8	32.6*	32***	32.8	33.1***	33.7	32.7***
	24	35.24	38.1	34.1	33.90***	34.2	34.6***	35	33.5***&
Alkaline phosphatase (IU/l)	13	245.9	194.6	236.4	234.8*	285.7*	247.4**	250.2	266.9***
	24	248.6	166.6	233.6	210.9*	265.6	200.3	231.2	230.3**&
Calcium (mmol/l)	13	2.644	2.669	2.676	2.548**	2.643	2.584*	2.558*	2.508***
	24	2.773	2.775	2.727	2.624***	2.725	2.596***	2.767	2.60***&
Cholesterol (mmol/l)	13	2.34	1.97	2.07	1.95	1.80**	1.91	1.66***	1.97
	24	2.908	2.43	2.38*	2.03	2.13**	1.92*	2.140**	2.05
Triglycerides (mmol/l)	13	1.624	1.218	1.537	0.986	1.262	0.837*	1.476	0.813*
	24	1.651	1.480	1.355	0.905**	1.542	0.990*	1.510	0.819**&

p<0.05, **p<0.01,***p<0.001, &=continues into the withdrawal period

- Female rats given ICI 182,780 had lower plasma total protein (↓6-10%) and albumin concentrations (↓4-12%) and higher alkaline phosphatase activities (↑21-38%) compared to controls. These differences were still apparent at the end of the withdrawal period suggesting that full recovery had not occurred. According to the Sponsor, the lower plasma total protein and albumin concentrations may be attributed to the anti-estrogenic effect of the drug in the liver since protein synthesis is partially regulated by estrogens. The higher alkaline phosphatase activities may be a result of the removal of estrogen suppression allowing elevation to levels associated with males.
- Lower (5-7%) total calcium values than controls were observed in all females given ICI 182,780 and these small differences are probably related to the reduction in plasma albumin/total protein, which serve as carrier proteins for the calcium.
- Males given ICI 182,780 exhibited lower (18-29%) cholesterol concentrations than controls. At week 24, the values were similar to those observed in control females.
- Females given ICI 182,780 also showed lower (19-45%) triglyceride values than controls in weeks 13 and 24 and at the end of the withdrawal period this difference was still apparent between control and rats previously given 10 mg/rat/30 days.

Urinalysis:

	Week	Vehicle		15 mg/kg/30 d		10 mg/rat/30 d		10 mg/rat/15 d	
		M	F	M	F	M	F	M	F
Na mmol/l	13	30.2	37.5	43.2 (↑48%)	93.6* (↑3x)	70.3 (↑2x)	102.4* (↑3x)	43.2 (↑43%)	77.4* (↑2x)
	24	48.2	15.3	36.2	94.9** (↑6x)	60 (↑4x)	38.4 (↑3x)	32	52.2* (↑3x)

Organ weights: Absolute organ weights (g)

	Treatment								Recovey			
	vehicle		15 mg/kg/d		10 mg/rat/30 d		10 mg/rat/15d		Vehicle		10 mg/rat/30 d	
	M	F	M	F	M	F	M	F	M	F	M	F
Ovary (↑)		0.105		0.084*		0.114		0.135*		0.089		.108*

				**		&		**				
Spleen (↓)	1.08	0.69	0.95**	0.56** *	0.963*	0.629*	0.976*	0.625*	1.068	.745	1.028	.685
Liver (↓)	22.82	12.39	21.63	10.04* **	22.16	10.69* **	21.21	10.83* **	23.80	12.39	20.48	12.59
Uterus (↓)		0.539		0.120* **		0.086* **&		0.091* **		0.644		0.148* **
Adrenals	.005	.087	.053	.087	.054	.095	.052	.097	.0721	.0782	.0620	.1121*
Pituitary (↓)	0.013 6	0.015	0.0113 ***	0.0112 ***	0.012 3*	0.0106 ***	0.0112 ***	0.0103 ***	.0136	.0174	.0129	.0148

*p<0.05, **p<0.01,***p<0.001, &= trend continues into the recovery period

Gross pathology: Compared to controls, changes included a reduction in size of the female reproductive tract, changes in the size, constituency or color of the ovaries and testes, and a lower incidence of hair loss in the females that approximated to that observed in the males. The majority of these changes were also observed at termination of the withdrawal study.

	Males (n=20)				Females (n=20)			
Treatment	vehicle	15 mg/kg/ 30 min	10 mg/rat/ 30 day	10 mg/rat/ 15 day	vehicle	15 mg/kg/ 30 min	10 mg/rat/ 30 day	10 mg/rat/ 15 day
Small/thin cervix					0	8	3	7
Large ovaries					0	0	0	5
Small ovaries					0	2	0	0
Discolored ovaries					0	15	12	13
Thin uterus					0	20	20	20
Hair loss	0	1	3	2	8	4	2	5
Large testes	0	3	2	1				
Soft testes	0	2	4	4				
Small testes	0	3	7	3				
Discolored testes	0	2	4	7				

	Males (n=10)		Female (n=10)	
Recovery	vehicle	10 mg/rat/ 30d	vehicle	10 mg/rat/ 30d
Small thin cervix			0	3
Discolored ovaries			2	6
Change in ovary composition			0	3
Thin uterus			0	10
Inject site sac	5	8	7	7
Hair loss	1	3	0	7
Large testes	0	2		
Soft testes	0	3		
Small testes	1	4		
Discolored testes	1	5		

Histopathology: Blank values spaces indicate the tissue was not examined.

	vehicle		15 mg/kg		10 mg/rat/30 d		10 mg/rat/15 d	
Treatment	M	F	M	F	M	F	M	F
adrenal cortical congestion/hemocysts	0/20	1/20		1/20		2/20		5/20
cervical atrophy		0/20		20/20 mod		20/20 mod		18/20 mod

epididymides ductal epith. microcystic	2/20		6/20		11/20		6/20	
reduced/ loss of spermatozoa	0/20		4/20		9/20		4/20	
male like mammary glands	0/14	0/20		0/20		1/20	0/11	4/20
ovary reduced interstitial cell vacuolation		0/20		20/20 mild		20/20 mild		20/20 mild
ovary inc. later stage follicles		0/20		12/20		18/20		18/20
reduced /loss of mature corpora lutea		0/20		17/20		18/20		17/20
pituitary pars distalis vacuolation/foaminess of cells	15/20	0/19		1/20		4/20	13/20	5/20
testes seminiferous tubular atrophy	0/20		5/20 (3 severe, 2 min-mild)		9/20 (8 severe, 1 mod)		5/20 (4 severe, 1 mod)	
loss of spermatozoa with tubular dilation	0/20		6/20 (2 mod, 4 min-mild)		6/20 (3 mod, 3 min-mild)		10/20 (5 mod, 5 min-mild)	
uterus atrophy		0/20		20/20 mod		20/20 mod		20/20 mod
vagina atrophy		0/20		20/20 mod		20/20 mod		20/20 mod
injection site cysts	5/20 mild 11/20 mod	3/20 mild 17/20 mod	9/20 mild 6/20 mod	11/20mild 5/20 mod	2/20 min 5/20 mild 10/20 mod	6/20 mild 14/20 mod	2/20 mild 17/20 mod	19/20 mod
myocyte necrosis inflammation	3/20 min 14/20 mild	17/20 mild 2/20 mod	16/20 mild	3/20 min 13/20 mild 1/20 mod	2/20 min 14/20 mild 2/20 mod	15/20 mild 3/20 mod	17/20 mild 2/20 mod	18/20 mild 1/20 mod

Histopathology in Withdrawal Group

	vehicle		10 mg/rat/30 d	
Recovery	M	F	M	F
adrenal cortical congestion/hemocysts		6/10	0/10	7/10
reduction/ loss of spermatozoa	1/10		3/10	
cervix atrophy			0/10	6/10 mild
mammary gland gladular vauolation		3/9		6/10
ovary reduced interstitial cell vacuolation		0/10		9/10
ovary reduced/loss of mature corpora lutea		0/10		5/10
inc. later stage follicles		0/10		3/10
pituitary pars distalis vacuolation		0/10		2/10
seminiferous tubular mineralization	1/9		3/10	
seminiferous tubular atrophy	2/9		5/10 (4 severe, 1 mild)	
loss of spermatozoa	0/9		2/10	

with some dilation of seminiferous tubules				
utuerus atrophy		0/10		10/10 mild
vaginal atrophy		0/10		8/10 mild
mild inflam injection site	3/10	2/10	6/10	8/10
cysts injection site	4/10 mild 1/10 mod	5/10 mild 2/20 mod	6/10 mild 2/10 mod	9/10 mild

- In animals of all groups, changes at the injection sites included moderate cyst formation around the deposited material with mild inflammation. Rats given 10 mg/rat/15 days, by subjective assessment, appeared to show a greater number of identical cysts as a result of more frequent intramuscular administration. Following the withdrawal period, cysts were still present at the injection sites but the cysts appeared slightly more fibrous with evidence of resolution of the inflammation.
- In all females given ICI 182,780, there was a diffuse atrophy of the uterus, cervix, and vagina with a loss of normal cyclical estrous activity. In the ovaries, there was an increase in the number of later stage Graafian follicles (some cystic and others showed an apparent anovulatory leuteinization). A reduction in the number, or loss of mature corpora lutea and a loss of the normal ovarian interstitial cell vacuolation also occurred. These changes persisted to termination of the withdrawal study, although there was a decrease in the number of animals with increased numbers of later stage/cystic follicles.
- A proportion of males from all groups given ICI 182,780 showed a unilateral or bilateral reduction in numbers of mature spermatozoa within the seminiferous tubules with an accompanying dilatation. Also, a significant increase in incidence of multifocal or diffuse atrophy of the seminiferous tubules occurred. Microcystic degeneration of the epithelium of epididymal ducts was common in these rats. Changes in the testes and epididymides also persisted to termination of the withdrawal study.
- Conversion of specific tissues in females to the morphology seen in males (loss of sexual dimorphism) including mammary gland structure, splenic hemosiderosis, pituitary gonadotroph vacuolation and reduced hair loss, and an earlier appearance of adrenal cortical congestion with hemocyst formation was observed in animals receiving ICI 182,780.
- At the end of the withdrawal period, drug-treated females showed a higher incidence of microvacuolation of the mammary glandular epithelium even though general mammary gland morphology had returned to the normal state. Also, the increased incidence of pituitary gonadotroph vacuolation persisted in the withdrawal animals. Although there were no histological differences between the control and dosed withdrawal group adrenal glands, the increased mean adrenal weight for the high dose group may indicate a persistent increased severity of adrenal cortical congesion with hemocyst. The lowering incidence of splenic haemosiderin deposition (approximating males) did not appear to persist to termination of the withdrawal study.

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Toxicokinetics:

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

	Male				Female			
	BW (g)	Actual dose	BW (g)	Actual dose	BW (g)	Actual dose	BW (g)	Actual dose
Week	10 mg/rat/ 30 days	mg/kg/ 30 days	10 mg/rat/ 15 days	mg/kg/ 15 days	10 mg/rat/ 30 days	mg/kg/ 30 days	10 mg/rat/ 15 days	mg/kg/ 15 days
0	147	--	146	--	131	--	132	--
1	212	47	208	96	160	63	162	123
2	261	38	256	78	175	57	177	113
4	346	29	339	59	202	50	204	98
6	399	25	394	51	222	45	226	88
8	435	23	433	46	235	43	239	84
10	463	22	460	43	250	40	254	79
12	484	21	483	41	259	39	265	75
21	557	18	556	36	298	34	299	67
25	581	17	574	35	303	33	309	65

- It is noteworthy that as body weight increases (~2-3 fold) with time, the actual dose administered decreases in both male and female rats.
- Since females have lower (~30%) body weights compared to male rats, the actual dose administered to female rats is thus greater than males.

Serum Concentration (ng/ml)

Dose	15 mg/kg/30 days		10 mg/rat/30 days	
	Male	Female	Male	Female
1	3.34±0.56	7.74±1.19	15.50±1.52	42.4±1.55
2	6.19±1.35	12.6±0.72	23.6±2.20	39.5±3.59
3	12±1	10.3±1.92	16.4±2.69	30.9±4.14
4	12.3±1.35	16±1.83	14.1±2.67	38.6±5.14
5	12.3±0.67	15.8±1.29	13.8±2.49	31.5±6.15
6	13.8±0.86	23.7±4.62	6.46±0.82	13.4±2.37

Dose	10 mg/rat/15 days	
	Male	Female
1	49.9±5.13	51.5±9.28
2	42.7±4.3	95.5±15.7
4	34±2.89	64.6±6.11
6	61.7±13.5	136±13.7
8	52.9±4.72	109±11.4
10	42.7±2.68	111±18.6
11	48.6±4.63	124±28.2
12	58.9±13.6	95.3±15

Group	III (15 mg/kg/30 d)				IV (10 mg/rat/30 d)				V (10 mg/rat/15 d)							
Dose	1		6		1		6		1		2		11		12	
~Actual dose (mg/kg)	15	15	15	15	29	50	17	33	59	98	46	84	36	67	35	65
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
C_{max} (ng/ml)	55.3	62.4	56.3	60.2	94.5	125	82.3	194	70.2	129	94.8	132	105	372	88.3	208
T_{max} (h)#	1.29	1.13	2	4	2	11	1.30	1.13	2	7	1.13	1.13	4	1.13	2	1.13
AUC* (ng*d/ml)	326	483	719	998	1207	2083	638	1235	705	1352	763	1317	972	1931	963	1684
CL (ml/min)	8	5.4	3.62	2.61	5.75	3.33	10.9	5.63	9.86	5.14	9.11	5.27	7.15	3.6	7.21	4.12

1.13 = 3 h after dosing; 1.30 = 7 h after dosing

*AUC in the dosing interval: 0-30 d for groups III and IV; 0-16 d for group V.

- There was a significant difference between the sexes in serum ICI 182,780 concentrations, C_{max}, and AUC. The Sponsor attributes these differences to the lower body weight of female rats compared to male rats, given a fixed dose (10 mg/rat).

When body weight was taken into account, however, there was still a trend towards higher exposure in female rats.

- T_{max} showed considerable variability occurring between 3 h (day 1.13) and 11 days after dosing. Following the peak, the serum ICI 182,780 concentrations declined slowly following the first and sixth doses for rats given 15 mg/kg/30 days and 10 mg/rat/30 days and the first and twelfth doses for rats given 10 mg/rat/15 days. The decline was slower following the higher dose and more frequent dosing interval (Groups IV and V respectively)
- The group mean AUC in the dosing interval 0 – 30 days showed a proportional increase with dose.
- Following the 6th (or 12th) dose, AUC values were higher in both males and females compared to the first dose for Groups III and V. This trend was, however, reversed in Group IV, where AUC values following the sixth dose were lower than those following the first dose. The reason for this difference is unclear.

Summary of individual study findings:
Impairment of growth in females at all doses accompanied by lower food intake. Slightly higher red blood cell counts and mean cell hemoglobin concentrations in females at all doses (reversible). Lower plasma total protein, albumin, triglycerides and calcium, and higher alkaline phosphatase activity in females at all doses.

Histologically in rats at all doses: changes at the injection sites included moderate cyst formation around the deposited material with mild inflammation still present by the end of the recovery. Also, atrophy of the female reproductive tract; a constellation of ovarian alterations with increased late stage and cystic Graafian follicles, loss of mature corpora lutea and reduced vacuolation of the interstitial cells. Conversion of specific tissues in females to the morphology normally seen in males including mammary gland structure, splenic hemosiderosis, pituitary gonadotroph vacuolation and reduced hair loss and an earlier appearance of adrenal cortical congestion with hemocyst formation. A loss of spermatozoa from the seminiferous tubules with an accompanying dilatation, seminiferous tubular atrophy with some associated degenerative changes in the epididymides. Decreased weights of spleens, pituitary glands and, in females only, livers, which were unaccompanied by overt toxicological changes. Atrophy of the cervix, uterus, vagina, as well as, ovarian changes showed evidence of reversibility but not complete recovery at the end of the withdrawal period. Similarly, reduction/loss of spermatozoa and seminiferous tubular mineralization and atrophy were still present in male rats.

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Study title: ICI 182,780 : ONE MONTH TOXICITY STUDY IN DOGS.

Key study findings:

- Repeated daily dosing over 4 weeks resulted in non-proportional increases in systemic exposure and an ~3-fold accumulation in AUC_{0-24}. The majority of the accumulation took place during the first two weeks of dosing.
- Drug-induced histopathological changes comprised testicular Leydig cell hyperplasia and multiple ovarian Graafian follicles. These effects are likely a result of the antiestrogenic action of ICI 182,780.
- Chronic myositis was observed at injection sites.

Study no: TAD/583
Volume #, and page #: N_000\2001-03-28\pharmtox\tox\Dose\ TAR/2972\TAD/583
Conducting laboratory and location: ICI Pharmaceuticals Safety of Medicines Department Alderley Park Macclesfield Cheshire England
Date of study initiation: August 22, 1989
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: ICI 182,780 analytical reference number ADM 44026/88; 99.5%
Formulation/vehicle:

Ingredients	Placebo mg % w/v	ICI 182,780 mg 2 %w/v
ICI 182,780	-	2.0
Pluronic F127	1.0	1.0
Ethanol 96% BP	10.0	10.0
Water for injection	8.0	8.0
Propylene Glycol	to 100%	to 100%
Bulk-drug analytical reference number		ADM 44026/88
Formulation Batch Reference Number	PH 6124/144	PH 6124/145
Formulation Analytical Reference Number	ADM 49028/89	ADM 49033/89

Methods:
Dosing:

Species/strain:	Alderley Park Beagle dogs
#/sex/group or time point (main study):	3/sex group
Satellite groups used for recovery:	3/sex/group for 0 and 2.5 mg/kg/d
Age:	49-61 weeks old
Weight:	11.5-21.2 kg
Doses:	0, 1, 2.5, and 4 mg/kg/d; daily x 4 weeks
Route, form, volume:	IM and SC once daily

		Treatment		Recovery				
Group	Dose (mg/kg/d)	♂	♀	♂	♀	IM volume (ml/kg/d)	SC volume (ml/kg/d)	Total volume (ml/kg/d)
I	0 (saline)	3	3			0.025	0.1 (2 sites)	0.125
II	0 (vehicle)	3	3	3	3	0.025	0.1 (2 sites)	0.125
III	1	3	3			0.0125	0.0375	0.05
IV	2.5	3	3	3	3	0.025	0.1 (2 sites)	0.125
V	4	3	3			0.05	0.15 (2 sites	0.2

The main test animals received 28 - 31 daily doses. Withdrawal animals received 28 doses and were then left undosed for 42 - 43 days prior to necropsy.

Observations and times:

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Pre-study period and on day 28 of dosing. Withdrawal animals were also examined during the last week of the withdrawal period (day 64).

EKG: ECG, direct arterial blood pressure, and rectal temperature were recorded for all dogs in Groups II-V (main test and withdrawal) twice pre-study and during weeks 1 and 4 of dosing. No measurements were made during the withdrawal period.

Hematology: Twice pre-study, on day 25 of dosing, and day 67 for withdrawal animals.

Clinical chemistry: Pre-study and on day 25 of the dosing period

Urinalysis: Pre-study and on day 25 of the dosing period

Gross pathology: Main test: Day 28-31; Withdrawal: Day 42-43

Organs weighed: Adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, testes, thyroid glands, prostate and uterus.

Histopathology: Main test: Day 28-31; Withdrawal: Day 42-43

Toxicokinetics: Pre dose on Days 1, 14 and at 24 and 1, 2, 4, 8 and 24 hours after dosing.

Other:

Results:

Mortality: No mortality was reported.

Clinical signs: Changes included swelling around the intramuscular and subcutaneous dosing sites, discomfort immediately after dosing and scabs at the subcutaneous dosing sites. These changes

	appeared to increase in severity with increasing dose and were also present in the vehicle controls.
Body weights:	UR
Food consumption:	UR
Ophthalmoscopy:	UR
Electrocardiography:	UR
Hematology:	On day 25, platelet count was increased in Group IV (2.5 mg/kg/day; ↑25%) and Group V (4 mg/kg/day; ↑23%) compared to both control groups (I and II) main test and withdrawal animals. At the end of the withdrawal period results for Group II (vehicle control) and Group IV (2.5 mg/kg/day) were similar.
Clinical chemistry:	On day 25, albumin concentration was decreased in Group V (4 mg/kg/day; ↓6%) and Group IV, withdrawal, (2.5 mg/kg/day; ↓4%) compared to control groups. At the end of the withdrawal period results for Group II (vehicle control and Group IV (2.5 mg/kg/day) were similar. On day 25, sodium concentrations were reduced by 3% in the 4 mg/kg/day group. On day 25, triglycerides were reduced by 31% in the 2.5 mg/kg/day group, but were similar to control following withdrawal period.
Urinalysis:	UR
Organ weights:	Minor statistical differences in group mean organ weights of brain, epididymides and ovary expressed in absolute terms and as a percentage of body weight were seen between the groups dosed with ICI 182,780 and controls. The apparent decrease in ovary weight in saline controls (Group I) probably reflects the state of the estrous cycle. Decreased brain and epididymidis weights in the absence of any histopathological changes in the organs were considered by the Sponsor to not have toxicological importance.
Gross pathology:	Discoloration of muscle and skin at the intramuscular and subcutaneous injection sites respectively was observed in the vehicle control and all groups given ICI 182,780. At the end of the withdrawal period discoloration still persisted in the muscle tissue but no changes were observed at the subcutaneous injection sites.

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Histopathology:

End of treatment

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC) MALES I 0 mg/kg /day	II 0 mg/kg /day	III 1 mg/kg /day	IV 2.5 mg/kg /day	V 4 mg/kg /day	FEMALES I 0 mg/kg /day	II 0 mg/kg /day	III 1 mg/kg /day	IV 2.5 mg/kg /day	V 4 mg/kg /day
MUSCLE - SKELETAL:		(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Cellulitis			3	3	3	3		3	3	3	3
Myocyte necrosis			3	3	3	3		3	2	2	3
Fibrosis			1	3	3	3		3	3	3	3
Giant cells				2	3	3			2	2	2
Haemorrhage		1	2	2	3	2		3	1	2	3
Chronic myositis		2	3	3	3	3	1	3	3	3	3
Inflammatory cell infiltration					1		1				
OVARIES:							(3)	(3)	(3)	(3)	(3)
Multiple Graafian follicles									3	3	3
TESTES:		(3)	(3)	(3)	(3)	(3)					
Leydig cell hyperplasia					2	3					
SKIN - NON-PROTOCOLLED:			(2)	(2)	(3)	(2)		(3)	(2)	(2)	(3)
Necrosis			1	2	3	1		2	2	2	3
Cellulitis			2	2	3	2		3	2	2	3
Giant cells					2	2				2	1
Haemorrhage			1	2	3	2		1	2	2	3
Granulation tissue			1	1	3	2		2	2	2	2
Focal scab formation					2			1			
Inflammatory cell infiltration								1			

End of recovery

LESIONS	GROUP	INCIDENCE OF LESIONS (NUMERIC) MALES II 0 mg/kg /day	IV 2.5 mg/kg /day	FEMALES II 0 mg/kg /day	IV 2.5 mg/kg /day
MUSCLE - SKELETAL:		(3)	(3)	(3)	(3)
Fibrosis		3	2	3	1
Haemosiderosis		3	2	3	1
Chronic myositis		3		1	
Giant cells			1		
OVARIES:				(3)	(3)
Multiple Graafian follicles				1	1

Figures in brackets represent the number of animals

Toxicokinetics:

Dose mg/kg/day	Day	C_{max} (ng/ml)	C_{max}/ dose	↑ in C_{max} with dose	T_{max} (h) (Range)	AUC_{0-24} (ng.h/ml)	AUC/ dose	Fold accumulation
1	1	35.1±12.5	35		4 (1-8)	309±9.15	309	
	24	66±4.9	66		2 (1-24)	1016±64	1016	3.3
2.5	1	87.6±12.8	35	2.5-fold	4 (1-8)	1277±122	511	
	24	209±29.2	84	3.2-fold	4 (1-24)	3351±493	1340	2.6
4	1	150±21	38	1.7-fold	1 (1-4)	1650±166	413	
	24	407±88	102	1.9-fold	4 (1-24)	6878±1651	1720	4.2

- There were no discernible sex differences in serum concentrations, thus the mean pharmacokinetic parameters are for the sexes combined.
- On day 1, C_{max} increased proportionally with increasing dose. However, after multiple dosing, the increase in C_{max} was greater than proportional [3.2-fold increase in C_{max} for a 2.5-fold increase in dose (↑27%) or 1.9 fold increase in C_{max} for a 1.6-fold increase in dose (↑22%)].
- On day 14 of the study, the serum concentrations were higher in all animals. Mean C_{max} values had increased by between 1.8 and 2.3-fold. Slightly larger increases were observed in mean AUC_{0-24}, (2.5-3.2-fold) and C_{24} (2.6-3.7-fold). Between day 14 and day 24, the serum concentrations rose further but in general by only about 20% suggesting that the majority of the accumulation took place during the first two weeks of dosing.
- Accumulation between days 1 and 24 appears to be ~3 fold (range from 2.6-4.2).
- Inter-animal variability remained substantial throughout the study. t_{max} values ranged from 1-24 hours in many groups; coefficients of variation for C_{max} on day 24 ranged from 25 to 53%.

Summary of individual study findings:

Daily administration of ICI 182,780 to dogs by a combination of intramuscular and subcutaneous routes resulted in a dose-related exposure to the compound. On day 1, systemic exposure of dogs to ICI 182,780 increased in a dose-related manner. However, repeated dosing over 4 weeks resulted in non-proportional increases in systemic exposure and an ~3-fold accumulation in AUC_{0-24}. The majority of the accumulation took place during the first two weeks of dosing.

Histopathological changes associated with administration of ICI 182,780 comprised testicular Leydig cell hyperplasia and multiple ovarian Graafian follicles. These effects are likely a result of the antiestrogenic action of ICI 182,780. Chronic myositis was also observed at injection sites.

Study title: ICI 182,780 : SIX MONTH INTRAMUSCULAR TOXICITY STUDY IN DOGS.

Key study findings:

- Histological findings after IM administration of a sustained release (depot) formulation of ICI 182,780 at doses of up to 30 mg/kg/28 days included changes at the injection sites and increased medium sized Graafian follicles in one animal at the highest dose.
- At the end of the 6 month withdrawal period, the appearance of the ovaries was consistent with normal activity.
- Necrosis, myositis, fibrosis and granulomata (indicating presence of injected material) were seen at intramuscular injection sites. Myositis, fibrosis, and granulomata were still present at the end of the withdrawal period.

Study no: TPD/628
Volume #, and page #: N_000\2001-03-28\pharmtox\tox\Dose\ TAR/2972\TPD/628
Conducting laboratory and location: ICI Pharmaceuticals Safety of Medicines Department Alderley Park Maclesfield Cheshire England
Date of study initiation: December 11, 1990
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: ADM 44010/89; 98.8%
Formulation/vehicle:

Ingredients	Placebo mg % w/v	ICI 182,780 mg 2 %w/v
ICI 182,780	-	5.0
Ethanol 96% v/v BP	10.0	10.0
Benzyl alcohol BP	10.0	10.0
Benzyl benzoate BP	15.0	15.0
Castor oil Ph Eur	To 100	To 100
Bulk-drug analytical reference number		ADM 44010/89
Formulation Batch Reference Number	PH 6731/40	PH 6731/41
Formulation Analytical Reference Number	ADM 48026/90	ADM 48027/90

Methods:
Dosing:

Species/strain:	Alderley Park beagle dogs
#/sex/group or time point (main study):	4 animals/sex/group
Satellite groups used for recovery:	3 dogs/sex (Groups II and V)
Age:	50-56 weeks
Weight:	12.3-19.7 kg
Doses:	0, 10, 20 30 mg/kg/28d; once every 4 weeks for 6 months (7 doses)
Route, form, volume, and infusion rate:	IM

Group	Number of animals	Dose (mg/kg/28 days)	Dose volume (ml/kg)	
			Hind leg (one site)	Back (two sites)
I	4 ♂ 4 ♀	0 (saline control)	0.2 × 1	0.2 × 2
II	7 ♂ 7 ♀	0 (vehicle control)	0.2 × 1	0.2 × 2
III	4 ♂ 4 ♀	10	0.067 × 1	0.067 × 2
IV	4 ♂ 4 ♀	20	0.133 × 1	0.133 × 2
V	7 ♂ 7 ♀	30	0.2 × 1	0.2 × 2

Four animals/sex/group were sacrificed at the end of the six month dosing period (two weeks after the seventh dose) and three dogs/sex in Groups II and V were sacrificed after an additional 18 week withdrawal period (20 weeks following the final dose).

Observations and times:

Clinical signs: Twice daily

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: Pre-study and once during weeks 5, 13 and 26 of dosing.

EKG: Pre-study, week 14, and week 26

Hematology: Pre-study, week 14, and week 26

Clinical chemistry: Pre-study, week 14, and week 26

Urinalysis: Pre-study, week 15, and week 23

Gross pathology: Four animals/sex/group were killed at the end of the 6 month dosing period (2 weeks after the 7th dose) and 3 dogs/sex in Groups II and V were killed after an additional 18 week withdrawal period (20 weeks following the final dose).

Organs weighed: Adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, testes, thyroid glands, prostate gland and uterus.

Histopathology: Main Group: at the end of the 6 month dosing period (2 weeks after the 7th dose). Withdrawal: 20 weeks following the final dose.

Toxicokinetics: Pre-dose, 3 hours (day 1 and 141), and 24 hours (day 2 and 142) and then in the morning on days 4 (and 144), 7 (and 147), 10 (and 150), 14 (and 154), 17 (and 157), 21 (and 161) and 28 (and 168) associated with the first and sixth doses and on day 4 (days 32, 60, 88 and 116) following the second, third, fourth and fifth doses from four dogs/sex/group. In addition, from three dogs/sex in Groups II and V, pre-dose, 3 hours (day 169) and 24 hours (day 170) and days 4 (day 172), 7 (day 175), 10 (day 178), 14 (day 182), 17 (day 185), 21 (day 189), 28 (day 196), 35 (day 203), 42 (day 210), 49 (day 217), 56 (day 224), 63 (day 231) and 70 (day 238) associated with the seventh dose.

Other:

Results:

Mortality: All dogs survived until their scheduled necropsy.

Clinical signs: Swelling was seen frequently around the intramuscular injection sites in the hind leg and occasionally on the back of animals given ICI 182,780 or the vehicle control but rarely in dogs given the saline control. One dog on one occasion showed signs of pain on palpation of this swelling..

Body weights: UR

Food consumption: UR

Ophthalmoscopy: UR

Electrocardiography: UR

Hematology: UR

Clinical chemistry: Group mean creatinine concentration was marginally decreased at week 14 in Groups IV and V male dogs. The Sponsor reports that this decrease was considered to be caused by inter-animal variation.

	Vehicle	Saline	10 mg/kg	20 mg/kg	30 mg/kg
Creatinine µmol/l week 14 ♂ week 26 ♂	 101.1 104.4	 99.2 101.5	 96.8 99.8	 90.5* (↓10%) 94.8* (↓9%)	 90.7** (↓10%) 96.1* (↓8%)
Alkaline phosphatase (IU/l) week 26, ♀	113.7	118	121	154.2	152.3* (↑34%)
Albumin (g/l) (IU/l) week 14 ♂ Week 26 ♂	 29.68 31.22	 28.50 30.50	 29.75 31.50	 26.75* (↓10%) 29.00 * (↓9%)	 29.54 30.94

Urinalysis: UR

Organ weights: In Group V (30 mg/kg) females, a statistically significant increase (24%) in group mean adrenal gland weight was noted ($p<0.05$).

At the end of the withdrawal period, a reduction (↓35%) in prostate gland weight (significant for $p<0.05$) was present for Group V (30 mg/kg). Neither finding was associated with histopathological findings.

Occasional changes in group mean organ weight were seen for epididymides, heart and prostate gland. These changes showed no dose-relationship and were not associated with histopathological changes.

Abs organ weight (g)	Vehicle	Saline	10 mg/kg	20 mg/kg	30 mg/kg
Heart ♂	.766	.785	.781	.865* (↑13%)	.833
Epididymides	5.43	4.69	4.55	4.13* (↓24%)	4.94
Prostate gland	13.07	9.36	8.26* (↓37%)	9.67	10.36

Gross pathology: Included in histopathology table.

Histopathology:

Treatment

N=4	Males					Feamels				
mg/kg/28 d	0 Vehicle	0 (saline)	10	20	30	0 Vehicle	0 (saline)	10	20	30
Ovaries: Increased medium sized Graafian follicles										1
Implantation or injection site: Necrosis		3	2	4	2		3	3	2	3
Myositis		2 (mild)	1 (min)		1 (severe)			1 (mild)		1 (min) 1 mild)
Fibrosis		4 (severe)	1 (mild); 1(mod); 2 (severe)	4 (severe)	1 (mod) 3 (severe)		4 (severe)	1 (mild); 1(mod); 2 (severe)	1 (mod) 3 (severe)	4 (severe)
Granulomata		4	4	4	4		4	4	4	4
Degeneratopm										1

Recovery

N=3	Males		Females	
mg/kg/28 d	0	30	0	30
Myositis (minimal)	2	2	1	1
Fibrosis	1 mild 2 mod	2 mild 1 mod	2 mild	1 mild 2 mod
Granulomata	3	1	3	2
Ovaries: cysts			2	

- Necrosis, myositis, and fibrosis were common at the end of the dosing period. After the withdrawal period, necrosis was not seen, and severity of myositis and fibrosis was reduced. Granulomata indicated the presence of injected material.

Toxicokinetics:

	mg/kg	Cmax (ng/ml)		Cmax/dose		AUC (ng d/ml)		AUC/dose	
		1 Mo	6 Mo	1 Mo	6 Mo	1 Mo	6 Mo	1 Mo	6 Mo
III	10	27.8	23.1	2.8	0.8	270	390	11.7	1.4
IV	20	42.3	51.6	2.1	1.2	469	845	9.1	1.8
V	30	95.4	95	3.2	1.0	948	2341	10.0	2.5

	mg/kg	Cl (ml/min/kg	
		1 Mo	6 Mo
III	10	26.4	19.8
IV	20	31.9	18.5
V	30	25.5	10.9

- There was no statistically significant sex difference in serum ICI 182,780 concentrations (although in general females showed slightly higher levels than males within the same dose group). Thus, the mean pharmacokinetic parameters are presented for the sexes combined.
- Following the first and sixth monthly doses, all dogs in Groups III, IV and V showed systemic exposure to the compound. These data show a proportional increase in C_{max} with increasing dose.
- T_{max} occurred between 1.125 and 10 days. For most animals t_{max} occurred between days 2 and 7 showing slow absorption from the injection site.

- The group mean AUC in the dosing interval (0-28 days) showed a proportional increase with dose.
- Comparing AUC and CL values from doses one and six within the same dose group, it is clear that the AUC values increase 1.4 to 2.5 fold and CL values decrease following dose six in all dose groups. This accumulation is not reflected in increased C_{max} concentrations. However, positive pre-dose concentrations were found for Groups III, IV and U prior to dose six, (6.98, 11.03 and 23.96 ng/ml respectively) reflecting the slow decline in serum ICI 182,780 concentrations following the peak.

Summary of individual study findings:

Systemic exposure to ICI 182,780 was demonstrated in all drug-treated groups. There was no significant sex difference in serum concentrations of ICI 182,780. Positive pre-dose concentrations were found for all groups prior to dose six (and dose seven in Group V) reflecting the slow decline in serum ICI 182,780 concentrations and demonstrating that ICI 182,780 was continuously released from the site of administration throughout the dosing interval.

All dogs survived until their scheduled necropsy and the only clinical sign observed was swelling around the intramuscular injections sites. No drug-related differences were seen in the organ weight measurements. Histological findings were restricted to changes at the injection sites and increased medium sized Graafian follicles in one animal at the high dose. By the end of the 6 month withdrawal period, the appearance of the ovaries was consistent with normal activity. Necrosis, myositis, fibrosis and granulomata were seen at intramuscular injection sites. Myositis, fibrosis and granulomata were still present at the end of the withdrawal period.

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Study title: ICI 182,780 : Twelve Month Intramuscular Toxicity Study in Dogs.

Key study findings:

- ICI 182,780, when administered IM to dogs every 28 days for 12 months at doses of 10, 20 and 40 mg/kg/28 days produced reversible changes in the reproductive system consistent with anti-estrogenic activity of the compound.
- There were reversible multifocal foreign body type granulomatous responses at the injection sites of the majority of animals given the vehicle or the ICI 182,780 formulation.
- There was no sex difference in exposure to ICI 182,780. The plasma concentration and AUC_{0-28} of ICI 182,780 increased during the first 9 months of the dosing period suggesting accumulation. Exposure, both in terms of plasma concentration and AUC_{0-28d}, showed a dose proportional increase after the first and ninth dose.

Study no: TFD/913
Volume #, and page #: N_000\2001-03-28\pharmtox\tox\Dose\ TAR/2972\TFD/913
Conducting laboratory and location: AstraZeneca UK Limited Alderley Park Macclesfield Cheshire England
Date of study initiation: March 9, 1998
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, radiolabel, and % purity: ADM00192D98 (99.9%)and ADM00193A98 (99.4%)
Formulation/vehicle:

Ingredients	Vehicle mg % w/v	ICI 182,780 mg 2 %w/v
ICI 182,780	-	5.0
Ethanol 96% v/v BP	10.0	10.0
Benzyl alcohol BP	10.0	10.0
Benzyl benzoate BP	15.0	15.0
Castor oil Ph Eur	To 100	To 100
Bulk-drug analytical reference number		
Formulation Batch Reference Number	P/1359/45	P/1359/26, P/1359/46
Formulation Analytical Reference Number		

Methods: Measurements of clinical pathology indices included sex hormones.
urine.
Dosing:

Species/strain:	Alderley Park Beagle dogs
#/sex/group or time point (main study):	4 dogs/sex/group
Satellite groups used for recovery:	3 dogs/sex/group for Groups I, II, and V
Age:	18-32 months
Weight:	7.8-12.9 kg
Doses:	0, 10, 20, and 40 mg/kg/28 d

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Route, form, and volume: IM. once every 28 d; ≤0.2 ml/kg

Group	Dose (CI 182,780 (mg/kg 28 days)	Number of animals	
		Males	Females
I	0 (saline)	7*	7*
II	0 (vehicle)	7*	7*
III	10	4	4
IV	20	4	4
V	40	7*	7*

* 3 male and 3 females from Groups I, II and V were allocated for a 14 week withdrawal phase

Observations and tim

Clinical signs:

Body weights: f the study

Food consump f the study

Ophthalmosco id 54 and during

EKG: Pre-study and during weeks 14 and 27, and week 54 (main test) or weeks 50 and 67 (withdrawal period).

Hematology: Pre-study and during weeks 26 and 55 (main test) or 51 (withdrawal animals)

Clinical chemistry: Pre-study and during weeks 26 and 55 (main test) or 51 (withdrawal animals)

Urinalysis: Pre-study and during weeks 26 and 55 (main test) or 51 (withdrawal animals)

Gross pathology: Week 56 (day 386) and week 67 (day 467)

Organs weighed: Adrenal glands, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary gland, prostate gland, spleen, testes, thyroid glands and uterus.

Histopathology: Week 56 (day 386) and week 67 (day 467)

Toxicokinetics: Pre-dose and at 1, 3, 6, 15, 20, 24 and 28 days after the first dose and after the ninth dose in Groups III, IV and V and pre-dose and at 3, 6, 15, 20, 24 and 28 days after the thirteenth dose in Groups III, IV and V and 126 days after the thirteenth dose in Group V. Additionally, pre-dose samples were taken before doses 4, 5, 6, 7, 8, 11, 12 and 14 in Groups III, IV and V.

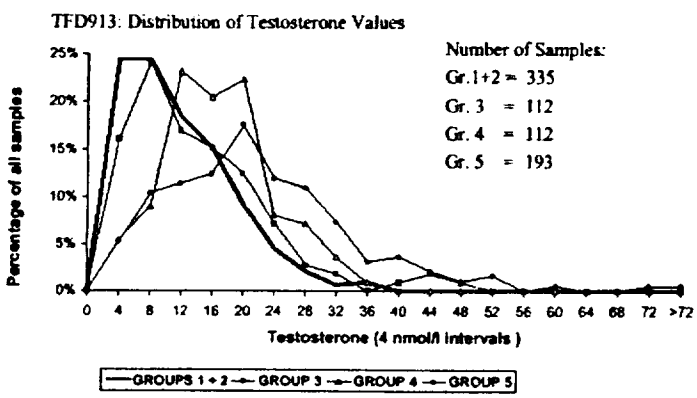
Other:

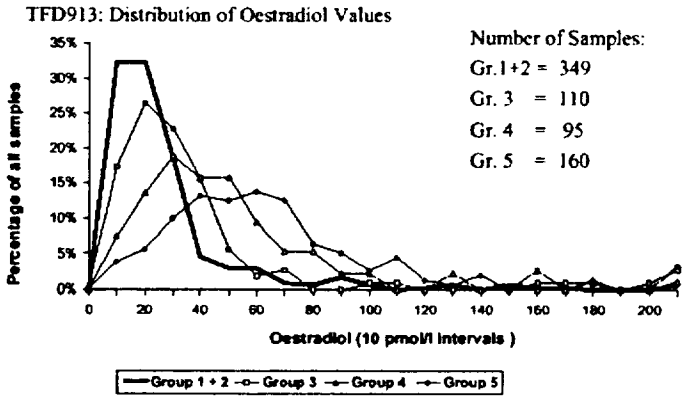
Results:

Mortality: Five animals were sacrificed during the study period.
On day 9, 3 control (2♂ and 1♀) were necropsied due to damage to the skin and eyes resulting from accidental contamination with undiluted HIBICET™ during routine cage cleaning.
One ♀ (40 mg/kg/28d) was sacrificed on day 59 after appearing disorientated, with unsteady gait and trembling and/or shaking salivation, piloerection, and vocalization.
One ♀ (20 mg/kg/28d) was sacrificed on day 141 after developing, within 1 hour of dose administration hind limb paresis, loss of placing reflex, reduced withdrawal reflex, and urinary retention.

Clinical signs: Four animals showed clinical signs. One ♀ (Group V: 40 mg/kg/28days) was subdued and had a reduced appetite between days 24 and 30 of the study. The animal was healthy for the remainder of the study. Three animals, (1 ♂ Group II, 1

	♀ placebo control, and 1 ♀Group V (40 mg/kg/28days), developed transient swellings at one of the injection sites but made a full recovery. Indications of estrous, characterized by a red discharge from the vagina, was recorded at least once and twice in the majority of animals in survivors from the two control groups. In animals given ICI 182,780 the incidence of animals showing estrous activity was reduced relative to the controls. Estrous activity was recorded in 3/4, 2/4 and 0/7 animals dosed 10, 20 and 40 mg/kg/28 days.
Body weights:	UR
Food consumption:	UR
Ophthalmoscopy:	Ocular inflammation, with corneal opacity in two cases, was noted for 392M (saline control), 349M and 258F (both vehicle control) from day 5 until their euthanasia on day 9.This is consistent with ocular irritation due to the accidental topical administration of undiluted HIBICET™ hospital concentrate during floor cleaning. One ♀ (231F (20 mg/kg/28 days) developed short linear opacities of the posterior lenticular sutures in both eyes during the dosing period; some of these linear opacities further developed into small oval opacities. This is an occasional finding in the Alderley Park beagle and is not considered to result from administration of ICI 182,780.
Electrocardiography:	UR
Hematology:	UR
Clinical chemistry:	Overall, the group median results for testosterone (♂) and estradiol (♀) show a dose-related increase in animals given ICI 182,780. Although there is marked individual variation for each of these hormones at each time point, the distribution graphs confirm increased levels. There was no effect on progesterone levels in females, with the expected cyclical pattern being maintained. During the withdrawal phase, there is good evidence, particularly for testosterone, of a return to normal levels in animals previously dosed 40 mg/kg/28 days.





Urinalysis:	UR
Organ weights:	In high dose dogs (40 mg/kg/28 d), absolute uterine weight was reduced (43 and 48%) compared to the saline and vehicle control groups respectively.
Gross pathology:	**Main test animals**. Findings were seen in the ovaries and at the injection sites. Enlarged ovaries of two ♀s (Group III and Group IV) and thickening or fluid at 1/4 injection sites of 2 high dose dogs (1/sex). **Decedent animals.** Eye discoloration was observed in the 3 control dogs killed on day 9. Meningeal vascular dilatation and free blood was noted around the brain, together with fluid in the pericardium of 1 ♀ dog (Group V-40 mg/kg/28 d) killed at 9 weeks. Discoloration, consistency changes, and masses were noted at 2/ 4 and 4/ 4 injection sites of 1 ♀ (Group V) and 1 ♀ (Group IV), respectively. There were no other macroscopic abnormalities in ♀ dog (Group IV-20 mg/kg/28d) killed at 21 weeks.
Histopathology:	**Main test animals** Drug-related histopathological changes were found in the ovaries and female reproductive tract and at the injection sites. Atrophy of the uterus, cervix and vagina was noted in 3/3 high dose (40 mg/kg 28d) females, together with a reduction in the number of active or regressing corpora lutea, although antral follicles were present in all animals and in increased numbers in one animal. Histologically the mid and low dose animals were in anoestrous or dioestrous. Multifocal foreign body type granulomatous responses were present within

the muscle component of the injection sites in all but one of the main test animals dosed with either the vehicle or test substance.

Multifocal foreign body responses were seen at the injection sites of 1 ♀each of the mid and high dose.
Other noteworthy histological findings included the presence of multifocal hepatic granulomata in high dose ♀(40 mg/kg/28d) and arteritis involving one site of a range of different tissues (heart, epididymis, spinal cord and urinary bladder) across the treatment groups.
Withdrawal animals. The high dose female animals were in anoestrous or dioestrous.
Granulomatous foreign body type responses were still noted at the injection site in vehicle and dosed animals but less sites were involved and in some instances the lesions were focal.
Decedent animals.

- Loss of epithelium and inflammation was present in the cornea and conjunctiva of control dogs.
- One ♀ (Group V 40 mg/kg/28days), sacrificed on day 59, showed meningeal vascular congestion, and widespread lymphocytolysis in the thymus, lymph nodes, spleen, Peyers patches and mucosa of the gut and gall bladder. No histological changes were note in the heart of this animal. Electron microscopy of the thymus and Peyers patch of this animal confirmed lymphocytolysis to be present but demonstrated no additional abnormality. Multifocal foreign body responses were seen at the injection sites.
- One ♀ (Group IV 20 mg/kg/28 days) showed no unexpected histological, and particularly no changes in the spinal cord, sciatic nerve or muscle. Multifocal foreign body responses were seen at the injection.

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Treatment	Males					Feamels				
mg/kg/28 d	0 Vehicle	0 (saline)	10	20	40	0 Vehicle	0 (saline)	10	20	40
N=	4	4	4	4	4	4	4	4	3	3
Cervix: Atrophy										3
Epididymides: Unilateral arteritis				1						
Injection site: Fat necrosis and foreign body granulomatous response (slide 35)										1
Multifocal inter and intrafasicular granulomatous foreign body response										
(4 injection sites)			3	1	2			1	1	3
(3 injection sites)		2		1	2		2	2	2	
(2 injection sites)		1	1	1			2	1		
LIVER Multifocal granuloma										1
MAMMARY GLANDS: Atrophy										1
Ovaries: Stage of cycle - dioestrus							1	2		
-late dioestrous						1				
-anoestrus						3	2	1		
Antral follicles present							1	1		1
Enlarged antral follicles									1	
Occasional antral follicles								1		
Increased antral follicles										1
Large corpora lutea									1	
Active corpora lutea								1		
Large inactive corpora lutea									1	
Regressing corpora lutea						3	1		1	
Occasional corpus luteum remnants						1	1	2	1	3
UTERUS: Atrophy										3
Vagina: Atrophy										3

Withdrawal	Males					Feamels				
mg/kg/28 d	0 Vehicle	0 (saline)	10	20	40	0 Vehicle	0 (saline)	10	20	40
N=	3	2			3	3	2			3
Multifocal inter and intrafasicular granulomatous foreign body response										
(4 injection sites)					1					1
(3 injection sites)		1			1					
(2 injection sites)										2

Ovaries: Stage of cycle							1			
- proestrus										
-dioestrus							1			2
-anoestrus						1				1
Antral follicles present						1				
Active corpora lutea										1
Regressing corpora lutea						1	1			1
Corpus luteum remnants						2				

Toxicokinetics:

	Dose 1			Dose 9			Dose 13		
Group	III	IV	V	III	IV	V	III	IV	V
mg/kg/28d	10	20	40	10	20	40	10	20	40
$AUC_{0\text{-}28\ d}$ (ng.d/ml)	154	369	683	353	685	1500	NC	NC	NC
AUC/dose	15	18	17	35	34	38	-	-	--
AUC Accumulation ratio				2.3	1.9	2.2	-	-	-
C_{28} (ng/ml)									
C_{28} Accumulation ratio							-	-	-
C_{max} (ng/ml)	13.3	36.0	55.5	20.9	35	87.9	NC	NC	NC
C_{max}/dose	1	2	1	2	2	2	-	-	-
t_{max} (d)	3	3	3	1	3	3	NC	NC	NC

C_{28} is the plasma concentration observed 28 days after administration of the dose.
NC not calculated

- There was no statistically significant difference between the sexes in the plasma concentrations of ICI 182,780. Thus, values presented are for the sexes combined.
- After doses 1 and 9, C_{max} increased proportionally with dose. For the thirteenth dose, C_{max}, t_{max}, and $AUC_{0\text{-}28}$ could not be determined because the blood samples scheduled for collection in the first 24 hours after dosing were not taken.
- Both AUC and C_{28} accumulation ratios show there is drug accumulation during the study. The monitoring of pre-dose plasma concentrations in all three dose groups throughout the dosing period showed a steady increase until the ninth dose, after which the concentrations leveled off.
- The group mean $AUC_{0\text{-}28}$ increases proportionally with increasing dose. For a 2-fold increase in dose there was a 2-fold increase in the $AUC_{0\text{-}28}$.
- The plasma concentrations of the withdrawal group were not used to derive any pharmacokinetic parameters because the protocoled samples at 4, 8 and 12 weeks were not taken.
- No plasma concentrations were determined for the 17-ketone or sulphone metabolites, however, the raw data from the analyses indicated that there were no significant levels of either metabolite in any of the representative samples.

Summary of individual study findings:

Pharmacokinetic monitoring demonstrated no sex difference in exposure to ICI 182,780. The plasma concentration and $AUC_{0-28\,d}$ of ICI 182,780 increased during the first 9 months of the dosing period suggesting accumulation. The plasma concentration after the thirteenth dose indicated no further accumulation after the ninth dose so that steady state was achieved after the ninth dose. Exposure both in terms of plasma concentration and $AUC_{0-28\,d}$ showed a dose proportional increase after the first and ninth dose. The $AUC_{0-28\,d}$ values after doses of 10, 20 and 40 mg/kg/28 days after 9 months were 353, 685 and 1500 ng.d/ml respectively.

Changes attributed to the anti-estrogenic actions of ICI 182,780 included atrophy of the uterus, cervix, and vagina, a reduction in the number of active or regressing corpora lutea, and an absence of clinical signs of estrous activity in females dosed at 40 mg/kg/28 days. In females dosed at 10 and 20 mg/kg/28 days there were similar but less pronounced changes. Histologically the animals were in anestrous or diestrous and there was little evidence of clinical signs of estrous activity. These changes showed reversibility following the withdrawal period with 2/3 animals previously given 40 mg/kg/28 days having histological evidence of resumption of the estrous cycle. Reversible elevations in plasma testosterone in males and estradiol in females given ICI 182,780 are considered to reflect hormonal pertubations as a result of the pharmacological actions of the compound. The effect on testosterone in males was not accompanied by any histological changes in the testes.

Systemic exposure to ICI 182,780 was demonstrated in all dogs at all dose levels. There was no evidence of significant levels of circulating 17-ketones or sulphone metabolites in the samples analyzed. The plasma concentration and $AUC_{0-28\,d}$ of ICI 182,780 showed an increase during the nine months dosing period suggesting accumulation. The plasma concentrations after the thirteenth dose indicated no further accumulation after the ninth dose. Exposure to ICI 182,780, both in terms of plasma concentration and $AUC_{0-28\,d}$ showed a proportional increase after the first and ninth dose.

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Histopathology Inventory for NDA

Study	TKR/ 1917	TAR/ 2972	TPR/ 2042	TAD/ 583	TPD/ 628	TFD/ 913
Species	Rat	Rat	Rat	Dog	Dog	Dog
Adrenals	X	x	X	X	X	X
Aorta		x	X	X	X	X
Bone Marrow smear		x	X		X	X
Bone (femur)	x	x	X	X	X	X
Brain		x	X	X	X	X
Cecum		x	X	X	X	X
Cervix	x	x	X	X	X	X
Colon		x	X	X	X	X
Duodenum		x	X	X	X	X
Epididymis	x	x	X	X	X	X
Esophagus	x	x	X	X	X	X
Eye		x	X	X	X	X
Fallopian tube						
Gall bladder					X	X
Gross lesions						
Harderian gland		x	X			
Heart	x	x	X	X	X	X
Ileum		x	X	X		X
Injection site	x	x	X	X	X	X
Jejunum		x	X	X	X	X
Kidneys	x	x	X	X	X	X
Lachrymal gland						X
Larynx						
Liver	x	x	X	X	X	X
Lungs	x	x	X	X	X	X
Lymph nodes, cervical			X	X	X	X
Lymph nodes mandibular		x	X	X	X	X
Lymph nodes, mesenteric		x		X	X	X
Mammary Gland	x	x	X	X	X	X
Nasal cavity						X
Optic nerves						
Ovaries	x	x	X	X	X	X
Pancreas		x	X	X	X	X
Parathyroid		x	X	X	X	X
Peripheral nerve				X		X
Pharynx						
Pituitary	x	x	X	X	X	X
Prostate	x	x	X	X	X	X
Rectum						
Salivary gland		x	X	X	X	X
Sciatic nerve		x	X	X		X
Seminal vesicles	x	x	X			
Skeletal muscle		x	X	X		X
Skin		x	X	X		
Spinal cord		x	X	X	X	X
Spleen	x	x	X	X	X	X
Sternum						
Stomach		x	X	X	X	X
Testes	x	x	X	X	X	X
Thymus	x	x	X	X	X	X
Thyroid	x	x	X	X	X	X
Tongue		x	X	X	X	X
Trachea	x	x	X	X	X	X
Urinary bladder		x	X	X	X	X
Uterus	x	x	X	X	X	X
Vagina	x	x	X	X	X	X
Zymbal gland						
Standard List						

X, histopathology performed
*, organ weight obtained

V. GENETIC TOXICOLOGY:

Study title: **ICI 182,780: Ames test: Bacterial Mutagenicity Study Using Selected Strains of Salmonella Typhimurium: Standard Method**

Key findings: Under the conditions of this assay, ICI 182,780 has shown no evidence of mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in both the presence and absence of metabolic activation.

Study no: TMV/399
Electronic NDA: N_000\2001-03-28\pharmtox\tox\muta\tmv399.pdf
Conducting laboratory/location: [redacted]
Date of study initiation: April 12, 1989
GLP/QA compliance: Yes
Drug, lot #, and % purity: ICI-182,780 (99.5%); lot # was not provided
Formulation/vehicle: The test article was dissolved in DMSO

Methods:
Strains: *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA1538)
Dose selection criteria:
Basis of dose selection: Dose levels were selected based on the results of the preliminary toxicity assay. In the first test the compound precipitated at 2500 µg/plate, partially obscuring the revertant colonies. Thus, the top dose level was reduced to 2000 µg/plate in the second test.
Range finding studies: TA100 treated with ICI 182,780 (100, 250, 500, 1000, 2500, and 5000 µg/plate)
Test agent stability: No analyses of stability, homogeneity, or achieved concentrations were carried out on the preparations of the test or positive control substances either prior to or after addition to the bacteria.
Metabolic activation system: Presence and absence of S-9 mix (Aroclor 1254-induced rat liver)
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: N-Methyl-N'-nitro-N-nitroguanidine, 9-aminoacridine, 2-nitrofluorene, daunomycin, methyl methanesulphonate, 2-aminoanthracene.
Exposure conditions:
Incubation and sampling times: Incubation for 2 days at 37°C in the dark
Doses used in definitive study: Test 1: 100, 250, 500, 1000, and 2500 µg/plate
Test 2: 100, 250, 500, 1000, and 2000 µg/plate
Study design: Plate incorporation assay
Analysis:

No. of replicates: 6 plates for each solvent control; triplicate plates for each treatment group.

Counting method: Automatic colony counter

Criteria for positive results: Reproducible statistically significant dose related increase in the number of revertant colonies of at least twice the concurrent solvent control.

Summary of individual study findings:

Study validity:

- Tester strain integrity data was not submitted (i.e., confirmation of the presence of the *rfa* wall mutation by demonstrating sensitivity to crystal violet; confirmation of the presence of the *uvr*A an *uvr*B mutation by demonstrating sensitivity to ultraviolet light; the presence of the pKM101 plasmid confirmed by demonstrating resistance to penicillin).
- Spontaneous revertant background frequencies were within historical control data.
- Tester strain titers were adequate (i.e., all tester strain culture titers must be equal or greater than 3×10^8 cell/ml)
- Positive control values-each mean positive control value exhibited a least a 3-fold increase over the respective mean vehicle control value for each tester strain.
- Toxicity-A minimum of 3 non-toxic dose levels were provided to evaluate assay data.

Study outcome:

- There were no significant increases in revertant colony numbers in any of the 5 tester strains following treatment with ICI 182,780 in the presence or absence of S-9 mix.
- ICI 182,780 was insoluble at doses greater or equal to 2500 µg/plate.
- The positive controls induced satisfactory mutagenic responses.
- The mean control revertant colony counts for TA 1535, TA 1537, and TA1538 and TA98 fell within the literature and historical ranges of the laboratory.
- The mean control revertant colony numbers for TA100 were lower than current historical data but within the range of previous historical data.

Thus, under the conditions of this assay, ICI 182,780 has shown no evidence of mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in both the presence and absence of metabolic activation.

APPEARS THIS WAY
ON ORIGINAL

Study title: **ICI 182,780: An Evaluation of the Mutagenic Potential Using *S. Typhimurium* and *E.coli.***

Key findings: Under the conditions of this assay, ICI 182,780 has shown no evidence of mutagenicity in *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and *Escherichia Coli* (WP2P [WP2 (pKM101)] and WP2P *uvr*A [WP2 *uvr*A (pKM101)] strains in the presence and absence of metabolic activation.

Study no: tmv/633
Electronic NDA: N_000\2001-03-28\pharmtox\tox\muta\tmv633
Conducting laboratory/location:
Date of study initiation: November 6, 1995
GLP/QA compliance: Yes
Drug, lot #, and % purity: ICI-182,780 (97.1%); Batch # ADM 37175E95
Formulation/vehicle: The test article was dissolved in DMSO

Methods:
Strains: *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and *Escherichia Coli* (WP2P [WP2 (pKM101)] and WP2P *uvr*A [WP2 *uvr*A (pKM101)].
Dose selection criteria:
Basis of dose selection: ICI 182,780 was initially assayed using the standard plate incorporation protocol over a dose range of 5000 to 5.0 µg/plate, both in the presence and absence of S9-mix prepared from phenobarbital-naphthaflavone-induced Sprague-Dawley rats using strain TA100 only.
In view of the lack of toxicity observed in this first experiment, ICI 182,780 was subsequently tested in the other five strains over the narrower dose range of 5000 to 100 µg per plate (both with and without S9-mix).
The test substance was subsequently re-tested in all six strains over the dose range of 5000 to 100 µg per plate; the +S9-mix phase of this repeat assay was conducted using a Pre-Incubation protocol.
Range finding studies: TA100 treated with ICI 182,780 (5, 10, 20, 50, 100, 200, 500, 1000, 2500, and 5000 µg/plate)
Test agent stability: No analyses of stability, homogeneity, or achieved concentrations were carried out on the preparations of the test or positive control substances either prior to or after addition to the bacteria.
Metabolic activation system: Presence and absence of S-9 mix (induced with a combined phenobarbital and β-Naphthaflavone corn oil solution)

Controls:
Vehicle: DMSO
Negative controls: DMSO

Positive controls: Acridine Mutagen ICR191, 2-Aminoanthracene (2A'A), Daunomycin HC1 (DR), N-Ethyl-N'-nitro-N- nitrosoguanidine (ENNG), Mitomycin C (MMC), and Sodium Azide (NaZ)

Comments:

Exposure conditions: Plate incorporation assay. The test substance was subsequently re-tested in all six strains over the dose range of 5000 to 100 µg per plate; the +S9-mix phase of this repeat assay was conducted using a Pre-Incubation protocol.

Incubation and sampling times: The incubation period for each experiment was 3 days at 37°C.

Doses used in definitive study: Test 1: 100, 200, 500, 1000, 2500, and 5000 µg/plate (+/- S9)
Test 2: 100, 200, 500, 1000, 2500, and 5000 µg/plate (+/- S9)
Test 3: 100, 200, 500, 1000, 2500, and 5000 µg/plate (+ S9)

Study design: Plate incorporation assay/Pre-incubation assay

Analysis:

No. of replicates: 5 plates for each solvent control; triplicate plates for each treatment group; 2 plates/ positive controls

Counting method: Automatic colony counter

Criteria for positive results: If a reproducible statistically significant dose related increase in the number of revertant colonies of at least twice the concurrent solvent control is obtained
A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:
a) a statistically significant dose-related increase in the mean number of revertant colonies is obtained;
b) a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) which is statistically significant, is observed at one or more concentrations.

Summary of individual study findings:

Study validity:

- The overnight culture from each new frozen culture was screened for the deep-rough characters, DNA repair deficiency, and Ampicillin resistance. The presence of the *uvr*B deletion (*Salmonella*) and the *uvr*A mutation (*E.coli*) was confirmed by testing the sensitivity of each culture to mitomycin C in the same manner as sensitivity to crystal violet was tested.
- The concurrent solvent control data are acceptable
- The positive control data show unequivocal positive responses.

Study outcome:

- In two separate assays with each strain, ICI 182,780 did not induce any significant, reproducible increases in the observed number of revertant colonies in any of the tester strains used, either in the presence or absence of S9-mix.

- The positive controls for each experiment induced the expected responses indicating the strains were responding satisfactorily in each case.

Under the conditions of this assay, ICI 182,780 was non- mutagenic in *S.typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strains WP2P and WP2P *uvr*A in the presence and absence of S9-mix.

Study title: **ICI 182,780: L5178Y TK$^{+/-}$ Mouse Lymphoma Mutation Assay.**

Key findings: Under the conditions of this assay, ICI 182,780 was not mutagenic or clastogenic in L5178Y TK$^{+/-}$ cells treated *in vitro* in either the presence or absence of S9-mix.

Study no: TMV/635
Electronic NDA: N_000\2001-03-28\pharmtox\tox\muta\TMV635
Conducting laboratory and location:
Date of study initiation: May 14, 1997
GLP/QA compliance: Yes
Drug, lot #, and % purity: Batch #: C166/1, ADM 36212F96; 98.8%
Formulation/vehicle: DMSO

Methods:
Cell line: L5178Y cells
Dose selection criteria:
Basis of dose selection: ICI 182,780 was tested over a range of concentrations limited by the solubility of the test substance in the treatment medium.
Range finding studies: In the first experiment, the maximum concentration of ICI 182,780 considered appropriate for testing in the mutation experiments was determined as 125µg/ml in both the presence and absence of S9-mix. A higher concentration (250µg/ml) was tested but produced excessive levels of precipitation.
Test agent stability: No analyses of stability, homogeneity, or achieved concentrations were carried out on the preparations of the test or positive control substances either prior to or after addition to the cell cultures.
Metabolic activation system: rat liver induced by combined phenobarbital and βnaphthoflavone corn oil preparation.
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Ethyl methanesulphonate (EMS) and benzo-α-pyrene (BP)

Comments:
Exposure conditions:
Incubation and sampling times: Treatment for 4 hours in the presence or absence of S9-mix. At the end of the 48-hour expression time, samples were grown in

both selective and non-selective medium, and the results obtained used to determine the mutant frequency per viable cell.

Doses used in definitive study: 16, 31, 63, and 15μg/ml

Analysis:

No. of replicates:	Duplicates
Counting method:	Cell growth was assessed after 10-13 days using a x10 dissecting microscope.

Criteria for positive results:
A statistically significant dose-related increase in mutant frequency is required at concentrations eliciting excessive toxicity. An associated absolute increase in mutant number above the solvent control values is a further requirement. Such a response must be reproducible in an independent experiment for the test substance to be described as positive in this assay.

Summary of individual study findings:
Study validity:

- Cell growth and maintenance. Cell growth and maintenance was demonstrated throughout the course of an experiment by post-expression cloning efficiencies >50% for the solvent control viability plates. The maximum concentration tested (125μg/ml) resulted in survival, relative to the controls, of 115% and 74% in the first experiment and 78% and 113% in the second experiment, in the presence and absence of S9-mix respectively.
- Spontaneous control data. The spontaneous mutant frequency in this study ranges from 1.2 - 2.9 x 10^4. Based on the historical data for the assay in this laboratory, the spontaneous mutant frequency using TFT as the selective agent, both in the presence and absence of S9-mix, should be with the range of 0.8-6.0 x 10^4 mutants per survivor.
- Positive control data. Ethylmethanesulphonate and benzo-a-pyrene produced the expected increase in mutant frequency in the absence and presence of S9-mix, respectively.

Study outcome:

- ICI 182,780 was tested up to a maximum concentration of 125μg/ml in both the presence and absence of S9-mix, a concentration limited by the solubility of the test substance in the treatment medium. This concentration resulted in survival, relative to the controls, of 115% and 74% in the first experiment and 78% and 113% in the second experiment, in the presence and absence of S9-mix respectively.
- No significant increases in mutant frequency, compared to the solvent control cultures, were observed in cultures treated with ICI 182,780 at any concentration tested in either the presence or absence of S9-mix.
- There were no significant differences in the distribution of either large or small colonies at any of the doses tested, compared to the solvent control, in either the presence or absence of S9-mix.
- The positive controls, EMS and BP, induced appropriate increases in mutant frequency in all mutation experiments.

Study title: **ICI 182,780 (ZD9238, Faslodex): in vitro mouse lymphoma TK assay**

Key findings: Under the conditions of this assay, ICI 182,780 was not mutagenic or clastogenic in L5178Y $TK^{+/-}$ cells treated *in vitro* in the absence of S9-mix.

Study no: TMV981
IND: [redacted] (N-176) IT (11-20-01)
Conducting laboratory and location: Astra Zeneca R&D Charnwood
Date of study initiation: April 6, 2001
GLP/QA compliance: Yes
Drug, lot #, and % purity: Batch #: ADM 65190D99; Certificate of analysis was not submitted.
Formulation/vehicle: DMSO

Note: This study was conducted using 24-hour exposure in the absence of metabolic activation. A previous lymphoma assay was performed with a 4-hour exposure in the presence and absence of rat liver S9 metabolic activation. The Sponsor notes that this current study ensures that mouse lymphoma assay data are fully compliant with the requirements of ICH Topic S2B (July 1997).

Methods:
Cell line: L5178Y cells
Dose selection criteria:
Basis of dose selection: ICI 182,780 was tested over a range of concentrations limited by the solubility of the test substance in the treatment medium.

Range finding studies: 7.6, 15.1, 30.3, 60.5, 121. And 242 µg/ml
Test agent stability: No analyses of stability, homogeneity, or achieved concentrations were carried out on the preparations of the test or positive control substances either prior to or after addition to the cell cultures.
Metabolic activation system: n/a
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: 4-nitroquinoline-1-oxide (4-NQO)

Comments:
Exposure conditions: Incubation and sampling times: Treatment for 24 hours in the absence of S9-mix. At the end of the 48-hour expression time, samples were grown in both selective and non-selective medium, and the results obtained used to determine the mutant frequency per viable cell.
Doses used in definitive study: 3.8, 7.6, 15.1, 30.3, 60.5, and121 µg/ml

Study design: Microtitration technique with an expression period of 48 hours after the end of the treatment and before selection for trifluorothymidine-resistant mutants.

Analysis:

No. of replicates: Duplicates

Counting method: Cell growth was assessed using a low-powdered stereio microscope.

Criteria for positive results:

For the solvent control, an individual test was considered acceptable if the mean solvent control mutant frequency value was within 99% confidence limits of the historical mean (mean ± 2.58 x standard deviation).

For the positive control, an individual test was considered acceptable if a clear response (minimum mean increase of 4 times the concurrent solvent control) was seen in the positive control cultures.

For the test article, an individual test was positive if

a) the mutant frequency at one or more concentrations was significantly greater than the negative control and;
b) there was significant dose-relationship as indicated by linear trend analysis and;
c) any positive results obtained at leass than 10% relative total growth were concluded.

Summary of individual study findings:

Study validity:

- Cell growth and maintenance. Cell growth and maintenance was demonstrated throughout the course of an experiment by post-expression cloning efficiencies >79% for the solvent control viability plates. The maximum concentration tested (121 µg/ml) resulted in survival, relative to the controls, of 37% and 40% in the in the absence of S9-mix respectively.
- Spontaneous control data. The spontaneous mutant frequency in this study ranges from 74-91 x 10^{-6}. Based on the historical data for the assay in this laboratory, the spontaneous mutant frequency is 91 ± 19.8 x 10^{-6} (n=36; 99% confidende limits 40-142 x 10^{-6})
- Positive control data. NQO increased (10-fold) the mutant frequency in the absence of S9-mix.

Study outcome:

- ICI 182,780 was tested up to a maximum concentration of 121 µg/ml in the absence of S9-mix, a concentration limited by the solubility of the test substance in the treatment medium. This concentration resulted in survival, relative to the controls, of 37% and 40% in the in the absence of S9-mix. Precipitate was also observed at 60.6 µg/ml, with survival of 37-41% compared to solvent control.
- No significant increases in mutant frequency, compared to the solvent control cultures, were observed in cultures treated with ICI 182,780 at any concentration tested in either the presence or absence of S9-mix.
- There were no significant differences in the distribution of small or large colonies at any of the doses tested, compared to the solvent control. However, compared to the solvent control, at the highest dose tested (121 µg/ml), there was an increase (1.6 fold) in the percent of small colonies.
- The positive control induced appropriate increases in mutant frequency.

Study title: **ICI 182,780: Micronucleus Test in the Rat: Oral Administration.**

Key findings: Oral administration of up to 2,000 mg/kg ICI 182,780 body weight (limit dose) did not increase the incidence of micronucleated polychromatic erythrocytes in the bone marrow of female Alderley Park rats.

Study no: TQR/2474
Electronic NDA: N_000\2001-03-28\pharmtox\tox\muta\TQR2474
Conducting laboratory and location: ZENECA Pharmaceuticals Safety of Medicines Department Cheshire, England
Date of study initiation: October 9, 1995
GLP/QA compliance: Yes
Drug and lot #: A solution containing 100 mg/ml of ICI 182,780 was supplied in accordance with current GMP regulations.
Batch reference number P0039/40 (placebo) and P0039/38 (ICI 182,780).

Formulation/vehicle:

Ingredients	Strength Placebo %/w/v	Strength 100 mg/ml
ICI 182,780	-	10.0
Ethanol 96%	13.3	12.0
BP Imwitor 988	43.1	38.8
Cremophor RH40	29.6	26.6
Fractionated Coconut Oil	14.0	12.6
Batch Reference Number	P0039/40	P0039/38

Methods:

Species: ♀ Alderley Park rats (Wistar derived) (Alpk:APySD)
#/sex/group: 14 ♀ rats/group (ICI 182,780 and placebo); 3 ♀ rats (positive control)
Age: 5 to 6 weeks old
Weight: 102.0 g and 138.0 g

Dose selection criteria:
Basis of dose selection: No range finding study was performed/submitted.
Controls:
Vehicle: Placebo (described in table above)

Negative control: Placebo
Positive control: Cyclophosphamide ([redacted] batch number 72H0088)
Comments: The controls utilized in the study are appropriate.

Exposure conditions:

Incubation and sampling times: Bone marrow sampling took place at 24 and 48 h post-dosing except for the positive control group (24 h only).

Doses: 0, 200, 700, 2,000 mg/kg ICI 182,780, oral gavage (see table below).

	Dose Level (mg/kg)	Volume (ml/100 g body weight)
Placebo	0	2.0
ICI 182,780	200	0.2
ICI 182,780	700	0.7
ICI 182,780	2,000	2.0
Cyclophosphamide	20	2.0

Study design: Single administration of ICI 182,780 to mice.

Analysis:

No. of replicates: Not applicable

Counting method: The bone marrow from the femur was flushed and smears were taken on slides. Two thousand polychromatic erythrocytes of a blue/gray hue were scored per slide (4 smears per slide) and the number of micronucleated polychromatic erythrocytes was recorded.

Animal observations: All animals were observed at least twice daily throughout the acclimatization and study periods. Any atypical signs or mortality were noted.

Criteria for positive results: A test for dose-related trend in the number of micronucleated polychromatic erythrocytes of the vehicle control and dosed groups, excluding the positive control, for 24 hours and 48 hours, was done using the Cochran-Armitage trend test.

Summary of individual study findings:

Study validity:

The study was deemed valid for the following reasons:

1) Previous pharmacokinetic assessments demonstrated systemic exposure.
2) The positive control, cyclophosphamide, exhibited appropriate responses. The incidence of micronucleated polychromatic erythrocytes for the positive control compared to the 24 hour vehicle control (using a one-sided Fisher's exact test) was statistically significant ($p<0.001$).
3) The proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

Study outcome:

Clinical signs:

Dose (mg/kg)	Clinical signs
0	• 14/14 animals salivated post-dose. • One animal appeared pale, showed subdued behavior, hunched posture, urine staining and abnormal feces within approximately 1 hour post-dose. This animal's condition improved by day 2.
200	• 4/14 animals had slight salivation up to 2 hours post-dose.
700	• 4/14 animals had slight salivation up to 2 hours post-dose. • One animal had an abnormal left eye it was slightly closed and had red exudate around it.
2000	• 10/14 animals salivated post-dose, • Other observations included urine staining, staining around muzzle, noisy breathing, hunched posture, and subdued behavior.

- There were no significant increases in the incidence of micronucleated polychromatic erythrocytes above vehicle control values for ICI 182,780 treated rats 24 or 48 hours post-dose.
- The incidence of micronucleated polychromatic erythrocytes in the cyclophosphamide (positive control) treatment group was significantly increased ($p<0.001$) above the vehicle control value at the 24 hour sampling time.
- The ratio of polychromatic to normochromatic erythrocytes in 1,000 cells (presented as mean % polychromatic erythrocytes), an assessment of cytotoxicity, was decreased at 48 h at the highest dose tested (55.2 in ICI 182,780 versus 63.6 in placebo) suggesting direct bone marrow toxicity of ICI 182,780.
- Finally, a test for dose-related trend in the number of micronucleated polychromatic erythrocytes of the vehicle control and dosed groups using the Cochran-Armitage trend showed no significant differences between the groups.
- It is noteworthy that the route of administration in this assay (oral) differs from the proposed clinical route (IM). The sponsor argues that the oral route produces higher systemic drug exposure making the inclusion of a study with IM dosing unnecessary.

Study title: **ICI 182,780: *In Vitro* Cytogenetic Study using Cultured Human Lymphocytes.**

Key findings: Under the conditions of the test, ICI 182,78O was not clastogenic in cultured human peripheral blood lymphocytes.

Study no: TYX/35
Electronic NDA: N_000\2001-03-28\pharmtox\tox\muta\ TYX35
Conducting laboratory and location: ICI Pharmaceuticals Safety of Medicines Department Cheshire, England
Date of study initiation: June 12, 1989
GLP/QA compliance: Yes
Drug, lot #, and % purity: ICI 182,780 (99.5%); Analytical reference ADM 44026/88
Formulation/vehicle: The dosing solutions of ICI 182,780 were formulated in DMSO. The final concentration of DMSO in the culture medium was 1% (v/v).

Methods:
Cell line: Human lymphocytes
Dose selection criteria: No range finding study was conducted.

Basis of dose selection: ICI 182,780 (125 µg/ml) was the highest dose tested based on the limit of solubility in culture medium.

Test agent stability: ICI 182,780 is predicted to be stable in DMSO for at least 7 days when stored at room temperature and protected from light

Metabolic activation system: Aroclor 1254-induced rat liver S-9 mix.

Controls:

Vehicle control: DMSO

Positive controls: Cyclophosphamide ([redacted] batch # 86F-0101) in DMSO to a final concentration in the culture medium of 20 µg/ml
Mitomycin C ([redacted] batch # 58F-04351) in distilled water to a final concentration in the culture medium of 0.5 µg/ml.

Comments:

Exposure conditions: Human lymphocytes were obtained from whole blood from 2 healthy donors (donor A = male, donor B = female) on the day of culture.

Incubation and sampling times: Thirty six human lymphocyte cultures were prepared and incubated at 37°C for 72 h.
The cultures were incubated in the presence of the test compound, positive control, or vehicle control (DMSO) for 2 hours at 37°C.
The cultures were returned to the incubator and maintained at 37°C for a further 26 hours.

Doses used in definitive study: 9, 12.5, 45, 62.5, 90 and 125 µg/ml

Study design:

Analysis:

No. of replicates: Two slides per culture-100 metaphase cells/culture

Counting method: "Blind evaluation" microscopic examination

Criteria for positive results: Statistically significant increase in the percentage of cells with aberrations for cultures treated with ICI 182,780 compared to the appropriate vehicle control group.

		(+S9)		(-S9)	
Treatment	Donor	Polyploids	Frequency	Polyploids	Frequency
DMSO	A	0	0.5	3	2
	A	1		1	
	B	1	0.5	2	2.5
	B	0		3	
12.5	A	1	1	1	0.5
	B	1		0	
62.5	A	2	2	2	2.5
	B	2		3	
125	A	4	3.5	2	2
	B	3		2	

Summary of individual study findings:

Study validity:

- The MI in the control cultures was within the accepted range for this laboratory.
- A marked reduction in MI was also observed in the positive controls, cyclophosphamide and mitomycin C-treated cultures.

Study outcome:

- At 125 µg/ml (limit of solubility in culture medium), a reduction in MI of ~30X was observed in the absence of S-9; however in the presence of S-9 no reduction in MI was observed.
- The incidence of cells with aberrations was not significantly different between the two donors within the dose groups. Thus, results from both donors were combined.
- The percentage incidence of cells with aberrations (including or excluding gaps) was similar to the levels of abnormal cells observed in the corresponding vehicle control cultures, both in absence and presence of metabolic activation.
- Both cyclophosphamide and mitomycin C induced statistically significant increases in the incidence of cells with aberrations.
- A dose related increase in polyploid cells was observed with ICI 182,180 in the presence of S-9. However, in this study, polyploid cells were observed in all dose and negative control groups. Also, the mean level of polyploid cells at 125 µg/ml was within historical control levels for this laboratory. Thus, it appears that this increase in polyploid cells has no biological significance.

Genetic toxicology summary:

The mutagenic and clastogenic potential of ICI 182,780 has been studied in Ames tests (TMV/399 and TMV/633), an *in vitro* cytogenetics assay (TYX/35), mouse lymphoma mutation assay (TMV/635 and TMV/981) and an *in vivo* rat micronucleus test (TQR/2474). ICI 182,780 has shown no evidence of genotoxic potential in this battery of tests.

The mutagenic potential of ICI 182,780 was assessed in bacterial mutagenicity studies using selected strains of S. *typhimurium* (TMV/399 and TMV/633) and E. *coli* (TMV/633). In both studies, ICI 182,780 did not induce any significant increases in the observed numbers of revertant colonies in the tester strains used, either in the presence or absence of S-9 mix. The potential for clastogenicity was also assessed *in vitro* using the L5178Y TK $^{+/-}$ mouse lymphoma mutation assay (TMV/635 and TMV/981). No significant increases in mutations or chromosomal aberrations were observed in cultures treated with ICI 182,780 in either the presence or absence of S- 9 in the independent experiments. Thus, under the conditions of these assays, ICI 182,780 showed no evidence of bacterial mutagenic or mutagenic/clastogenic activity in the L5178Y TK $^{+/-}$ assay.

An *in vitro* cytogenetic study using cultured human lymphocytes (TYX/ 35) was also used to detect the clastogenic potential of ICI 182,780. The percentage incidence of cells with aberrations (including or excluding gaps) was similar to the levels of abnormal cells observed in the corresponding vehicle control cultures, both in absence and presence of metabolic activation. A

dose related increase in polyploid cells was observed with ICI 182,180 in the presence of S-9. However, this finding was concluded to have no biological significance when examined in light of the historical and negative controls. Thus, under the conditions of this assay, ICI 182,780 was not clastogenic in cultured human peripheral blood lymphocytes.

ICI 182,780 was also evaluated *in vivo* using the micronucleus test in female rats. Single oral administration of ICI 182,780 (200, 700 and 2000 mg/kg) did not increase the incidence of micronucleated polychromatic erythrocytes in the bone marrow. Thus, under the conditions of this assay, ICI 182,780 tested negative in the *in vivo* micronucleus assay indicating that the substance does not induce micronuclei resulting from chromosomal damage or damage to the mitotic apparatus in the erythroblasts. It is noteworthy that the route of administration in this assay (oral) differs from the proposed clinical route (IM). The sponsor argues that the oral route produces higher systemic drug exposure making the inclusion of a study with IM dosing unnecessary.

Genetic toxicology conclusions:

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.

Labeling recommendations:
Proposed Labeling:

Mutagenesis

Draft

FDA recommendations:

Draft

APPEARS THIS WAY
ON ORIGINAL