Oral Rat Carcinogenicity Bioassay

2. **Report of a 104 week oral carcinogenicity study of 311C90 in the Wistar rat,** (study no. BDRE/93/0129), Glaxo Wellcome R&D, in-life phase by 311C90 batch no. Q6, September 17, 1996, GLP.

Study Description

Animals: Wistar rats (50/sex/group with an additional 10/sex/group in each treated group and one control group included for blood sampling); supplier was IFFA CREDO, France; age at study initiation 5 weeks; body wts. 100-156 g males, 97-136 g females; housed 5 cage of same sex.

Drug: 311C90 (batch no. Q6) supplied by the Wellcome Foundation

Treatment: The study protocol is shown in the following sponsor's table:

4.3.1. EXPERIMENTAL DESIGN

MAIN GROUP

Group number	Group designation	Dose level (mg/kg/day)	Dose volume (ml/kg/day)	Dose concentration (mg/ml)	Number of animals	
					Males	Females
1	Control I	0	10	0	60	60
2	Low dose	5	10	0.5	60	60
3	Intermediate dose I	25	10	2.5	60	60
4	Intermediate dose II	100	10	10	60	60
5	High dose	400	10	40	60	60
6	Control II	0	10	0	50	50

Group 1 and 6 animals (control) received the control article (sterile water for injection).
The last 10 males and females in groups 1 to 5 were additional animals for clinical pathology investigations. The intention was to use these animals as possible replacements for animals whose death was known to have been caused by intubation error (up to week 26). In fact, no animals were replaced.

SATELLITE GROUP (test article blood concentration)

Group number	Group designation	Dose level (mg/kg/day)	Dose volume (ml/kg/day)	Dose concentration (mg/ml)	Number of animals	
					Males	Females
2	Low dose	5	10	0.5	5	5
3	Intermediate dose I	25	10	2.5	5	5
4	Intermediate dose II	100	10	10	5	5
5	High dose	400	10	40	5	5

As a result of higher than expected mortality in group 5 animals, females were necropsied

Animals received daily oral gavage dosing with 311C90. Dose levels were selected based on results of previous studies with the compound. The oral route was used because it mimicks the proposed human therapeutic route.

Animals in all groups except group 5 (high dose) were treated for a total of 104 (males) or 105 (females) weeks. Due to excessive mortality in the high dose group and to ensure at least 20 animals per group at study termination, these animals were killed at 101 weeks (males) or 86 weeks (females).

Observations:

Morbidity/mortality, clinical observations, body weight, food consumption, clinical pathology (hematology, blood chemistry), macropathology, histopathology, organ wieghts, bone marrow smears.

Tissues sampled for histopathology included adrenals, aorta, bone (femur), bone marrow smears, bronchi, brain, caecum, colon, duodenum, Harderian gland, eyes, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph node (submaxillary), lymph node (mesenteric), lymph node (adjacent to any subcutaneous mass), mammary gland, esophagus, optic nerves, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary gland (submaxillary), sciatic nerves, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes (with epididymides), thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina, Zymbal's glands, all gross lesions. Bone marrow smears were prepared at necropsy for all animals and were fixed and stne to the study sponsor. All tissues were sent for histological processing and examination to the Study Sponsor, and then on to

Results

Mortality

Mortality data are shown on the following sponsor's table 9.1.

9.1. MORTALITY

(Table 1 - Figures 1 and 2)

A total of 137 males and 130 females were found dead or killed moribund during the study. The distribution of the survivors across the treatment groups at the end of the study (weeks 87 and 102 for group 5 females and males, respectively) was as follows :

Group no.	Dose level (mg/kg/day)	Surviving animals	
		Males	Females
1	0	42 (34)	42 (36)
2	5	44 (37)	40 (33)
3	25	31 (26)	37 (31)
4	100	38 (33)	37 (31)
5	400	25 (21)	29 (23)
6	0	33	35

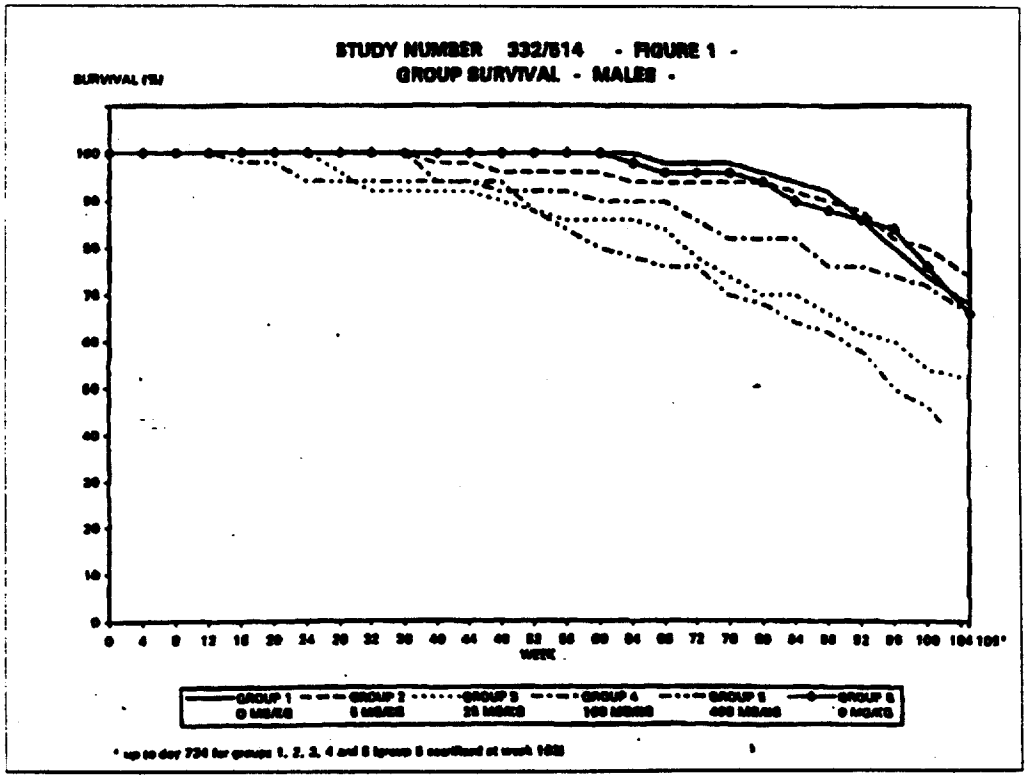
The figure in parentheses is the number of survivors excluding clinical pathology animals.

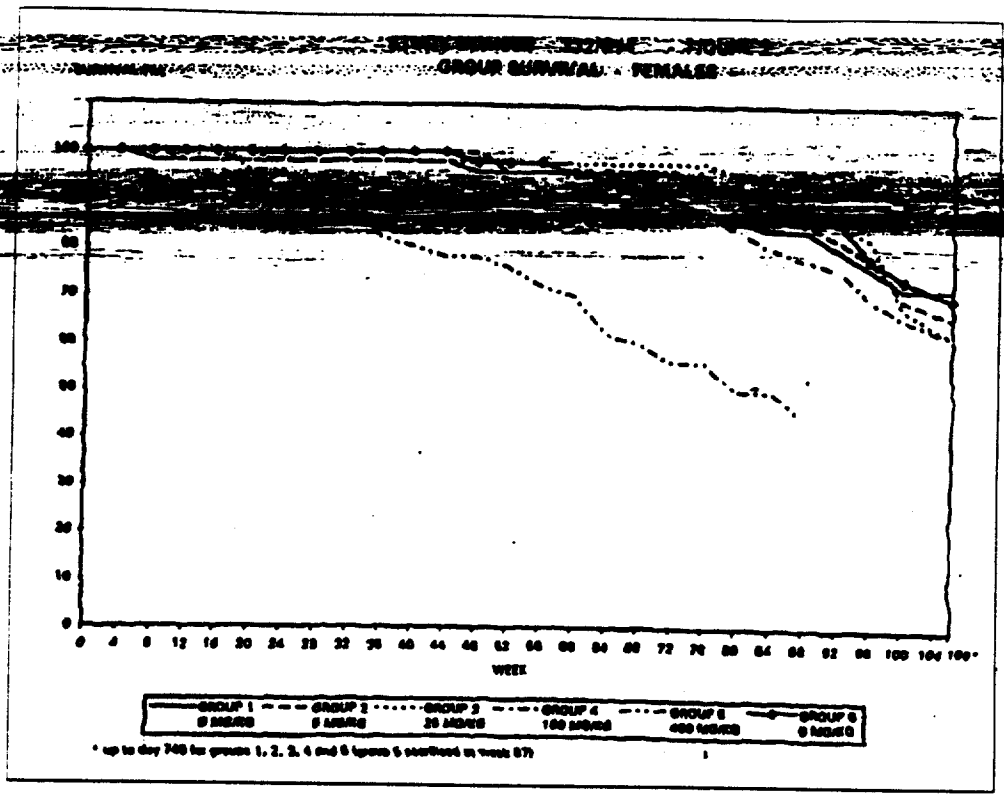
Mortality data expressed as per cent are shown in the following table:

% Mortality

Group #	Dose level (mg/kg/day)	# Deaths (% Mortality) Males	Females
1	0	18 (30%)	18 (30%)
2	5	16 (27%)	20 (33%)
3	25	29 (48.3%)	23 (38.3%)
4	100	22 (36.7%)	23 (38.3%)
5	400	35 (58.3%)	31 (51.6%)
6	0	17 (34%)	25 (30%)

The data show that mortality in the high dose (400 mg/kg/day) animals was highest, while the other treatment groups had similar incidence of mortality to controls. Based on these results, it is clear that the 400 mg/kg/day dose is above the MTD based on excessive mortality. This is also demonstrated in the following survival curves:





The death rate of animals in the high dose group was greater than in other groups, and these animals were terminated at 86 (females) and 101 (males) weeks. The decision to terminate was based on the numbers of surviving animals not subjected to bleeding in an attempt to maintain sufficient live animals for appropriate statistical analysis.

Clinical Signs

High incidence of stained fur and hairloss: high dose animals (400 mg/kg/day)
Salivation: Group 3, 4 and 5 animals
Pedalling movements soon after dosing: Group 4 and 5 animals
Muscular tonus (arched back, erect tail, body raised off of cage floor) within 30 minutes of dosing from week 25: Group 4 and 5 animals
Other miscellaneous symptoms:
head tilt, subdued behaviour, piloerection, swollen abdomen, ptosis, noisy respiration, tremors, cold to touch, tail lesions.

Subcutaneous masses

The following sponsor's table shows the number of animals presenting with at least one sub-cutaneous mass clinically and the mean time (in weeks) to first mass:

The number of animals bearing at least one sub-cutaneous mass clinically which was confirmed at necropsy was as follows :

Group no.	Dose level (mg/kg/day)	No. of animals	
		Males	Females
1	0	14[a]/60	18/60
2	5	19/60	23/60
3	25	19/60	28/60
4	100	21/60	15/60
5	400	12/60	2/60
6	0	17/50	15/50

(a) excludes two animals having masses clinically one of which was found to be distension of the bladder and the other a sore at necropsy.

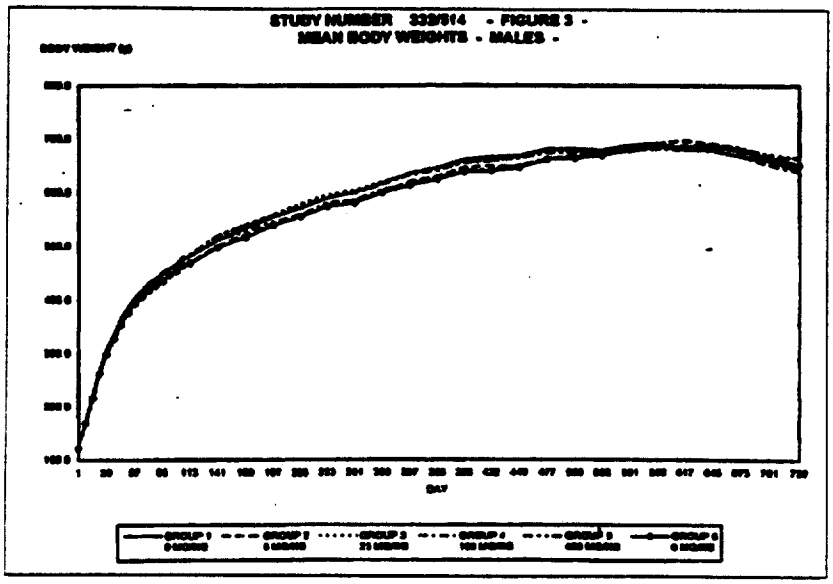
The mean time (in weeks) to first mass (masses confirmed at necropsy only) was as follows :

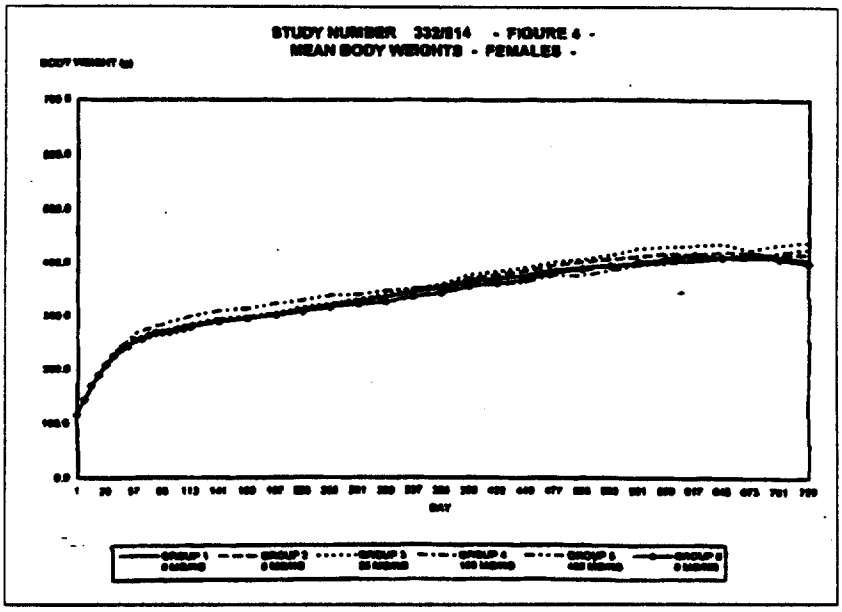
Group no.	Dose level (mg/kg/day)	Week	
		Males	Females
1	0	85	84
2	5	92	85
3	25	82	88
4	100	77	91
5	400	76	79
6	0	86	91

The number of sub-cutaneous masses appeared to be slightly increased in the Group 3 animals. Even though there was a higher mortality rate in the Group 5 (high dose) animals, the number of animals with at least one sub-cutaneous mass was slightly decreased in this treatment group. Drug administration also appeared to have no effect on "time to first mass".

Body weights

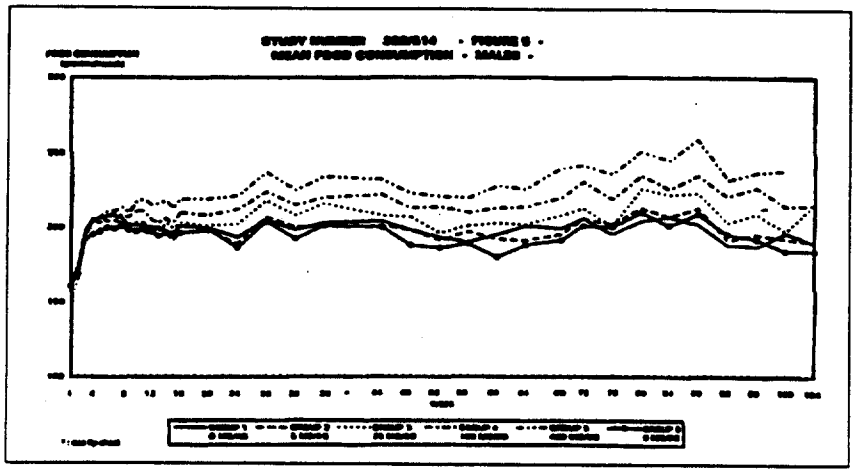
No effects (see sponsor Figures 3 and 4 below).

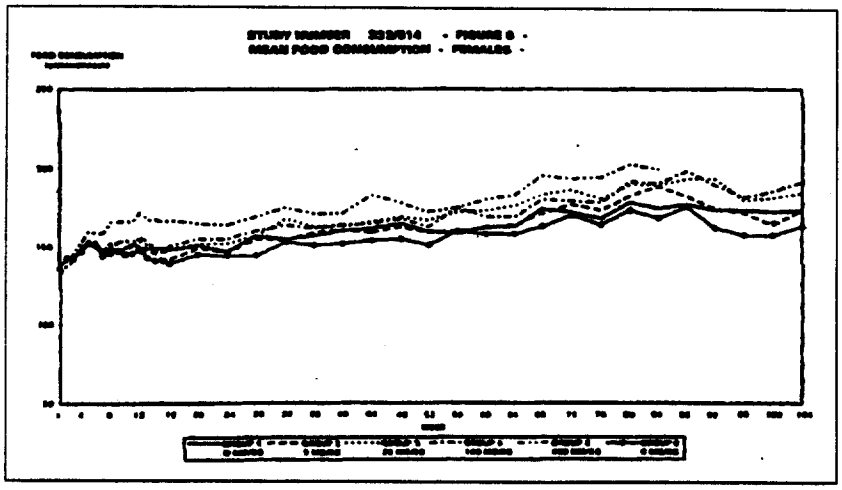




Food consumption

Food consumption data are shown in sponsor Figures 5 and 6 below. High dose animals ate more food than controls in the first 16 weeks, and then all treatment group except the low dose group ate more food than controls for the remainder of the study.





Hematology

No treatment-related effects.

Clinical Chemistry

The only notable clinical chemistry effect was a higher (26%) glucose concentration in Group 5 (high dose) females than controls at weeks 26, 52 and 78. Group 4 females had a similarly high glucose concentration in week 26 only.

Mean AST and ALP levels in group 5 females were 358% and 103%, respectively, higher than controls in weeek 26. The increased AST levels were mainly due to a single animal, but the increase in ALP was representation of the whole treatment group. ALP was also increased 39% in Group 5 (high dose) males on Week

Macropathology

All animals

pale and enlarged kidneys: group 4 males

enlargement and nodules on uteri: group 5 females: with no histopathological sequelae

skin sores : group 3 and 5 males

disended stomachs: group 5 males

dark areas in stomach: group 3 and 4 males

Terminally sacrificed animals

opacities in eyes of group 2 and 4 males

dark areas on livers of group 2 females

Histopathology

Non-neoplastic lesions: increased incidence of thyroid follicular hyperplasia, increased incidence of edema in the lungs, and increased incidence of acute thymic hemorrhage, all in high dose animals (400 mg/kg/day), as shown in the sponsor's tables below:

THYROID

Dosage level(mg/kg/day) Sex (males only)	0	5	25	100	400	0
Follicular cell hyperplasia	5	5	2	6	10	3
No. thyroids examined	60	60	60	60	60	50

LUNGS

Dosage level (mg/kg/day)	0		5		25		100		400		0	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Edema	1	0	1	1	5	0	5	3	24	12	1	2
No. examined	60	60	60	60	60	60	60	60	60	60	50	50

THYMUS

Dosage level (mg/kg/day)	0		5		25		100		400		0	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Hemorrhage	0	0	3	1	5	1	3	0	9	7	0	0

Neoplastic lesions: significant increase in the incidence of thyroid follicular cell neoplasma in 400 mg/kg males, as shown below. Statistical treatment involved combining the adenoma and adenocarcinomas, although the incidence of carcinomas was actually about the same in treated and control animals.

Incidence of rat thyroid follicular cell neoplasms

Group	1		2		3		4		5		6	
Dose			5 mg/kg		25 mg/kg		100 mg/kg		400 mg/kg			
	M	F	M	F	M	F	M	F	M	F	M	F
No. Examined	60	60	60	60	60	60	60	60	60	60	50	50
Follicular Hyperplasia	5	2	5	0	2	2	6	6	10	3	3	1
Adenoma	1	0	0	1	2	0	2	3	7	0	1	1
Carcinoma	2	0	1	0	1	0	1	0	2	0	1	0
Lesions Combined	8	2	6	1	5	2	9	9	19	3	5	2
Mean Maximum Plasma Level ng/ml	-	-	250	1750	1900	1900	7450	9700	29000	26000	-	-
Mean Total Exposure ng/ml.h	-	-	3300	9600	18000	13000	64800	82300	305300	258700	-	-

The sponsor stated that the mechanism for this increase in neoplasms may be due to an increased rate of clearance of thyroid hormones causing trophic feedback. As evidence they cite an increase in thyroid weight at 1000 mg/kg/day 311C90 in a 1-month oral rat study and a slight increase in the incidence of thyroid follicular hyperplasia together with an increase in thyroid and liver weights at 400 mg/kg/day in a 6-month rat study. The sponsor also states that the absence of a clear effect on TSH in their opinion does not rule out the possibility of mild stimulation of the thyroid as a possible mechanism.

Dose-comparison with humans

The sponsor concluded that the NOEL was 100 mg/kg/day with respect to the neoplasms and decreased survival, with a mean AUC of about 65-82,000 ng.h/ml, or about 406-500-fold greater than at the mrdd in patients. The AUC at the high dose is in the 300,000 ng.h/ml range, which is about 1875-fold greater than the AUC (160 ng.h/ml) for patients at the mrdd.

Histopath effects associated with mortality

Animal deaths at the high dose (400 mg/kg/day) were associated with an increased incidence of pulmonary edema and acute thymic hemorrhage, as shown in the summary tables of histopathology effects in decedent animals shown below:

Study ID: 893129 — AD9402/ 3
Compound: 511C90 — V3.0
Species: Rat
Pathologist: Dr. A. Mecklin

Histopathology Group Incidence Summary
Found Dead

Group	Group 1		Group 2		Group 3		Group 4		Group 5		Group 6	
Dose(Mg/Kg/Day)	0		5		25		100		400		0	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
Animals On Study	60	60	60	60	60	60	60	60	60	60	50	50
Animals Lapped	5	3	5	4	22	3	15	10	31	29	4	7
Lung/Bronchi(Continued)	5	3	5	4	22	3	15	10	31	29	4	7
Congestion	1	0	1	1	5	1	5	5	25	20	1	2
Edema	1	0	1	1	5	0	5	3	24	12	1	2
Emphysema	0	0	0	0	0	0	0	0	0	1	0	0
Fibrous Histiocytoma	0	0	0	0	0	0	0	0	0	0	0	1
Foamy Alveolar Macrophages	0	0	0	0	0	0	1	1	0	0	0	0
Hemorrhage	1	1	0	1	1	0	0	0	1	1	0	1
Inflammation	0	0	0	0	1	0	0	0	0	0	0	1
Leukemia, Lymphocytic	0	0	0	0	0	0	1	0	0	0	0	0
Lymphoma	0	0	0	0	1	0	0	0	0	0	0	0
Mineralization	0	0	0	0	1	0	0	0	0	0	0	0
Mononuclear Cells, Aggregates Of	0	0	0	0	0	1	0	0	0	0	0	0
Pneumonia, Foreign Body	0	1	0	0	0	0	0	0	1	0	0	0
Pneumonitis, Interstitial	0	0	0	0	1	0	0	0	0	0	0	0
Within Normal Limits	0	0	1	0	0	0	0	0	1	0	0	0
Thymus	5	3	5	4	22	3	15	10	31	29	4	7
Missing	1	0	0	0	0	1	1	0	3	4	0	0
Not Remarkable	4	1	3	1	13	2	8	8	18	16	4	5
Remarkable Observations	0	2	2	3	9	0	6	2	10	9	0	2
Atrophy	0	2	2	1	2	0	3	2	1	2	0	1
Congestion	0	0	0	0	0	0	1	0	0	0	0	0
Edema	0	0	0	0	1	0	0	0	0	0	0	0
Fibrous Histiocytoma	0	0	0	0	0	0	0	0	0	0	0	1
Hemorrhage	0	0	1	1	4	0	3	0	9	7	0	0
Lymphoma	0	0	0	0	2	0	0	0	0	0	0	0
Thymoma	0	0	0	1	0	0	0	0	0	0	0	0

Toxicokinetics

311C90

A summary of AUC_{0-T} (ng.h/ml) values for the rat carcinogenicity study are shown in the following sponsor's table:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5 mg/kg	not estimated	1390	8544	2036	1190
	25 mg/kg	not estimated	10562	37772	12740	10728
	100 mg/kg	12882	49295	110097	64059	87904
	400 mg/kg	66162	216440	380141	418342	445719 (Wk 102)
Females	5 mg/kg	523	2202	41908	1892	1458
	25 mg/kg	1171	12099	25251	16584	10433
	100 mg/kg	20318	69418	199287	59142	63637
	400 mg/kg	89194	215050	431252	256366	301811 (Wk 87)

The average Cmax (ng/ml) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	49	194	652	217	141
	25mg/kg	264	1696	3817	2362	1397
	100mg/kg	1770	7364	11134	7260	9778
	400mg/kg	7729	22245	31501	43548	39917 (Wk 102)
Females	5mg/kg	75	379	7949	193	204
	25mg/kg	229	2139	2712	3028	1503
	100mg/kg	3413	9745	16228	8636	10593
	400mg/kg	14301	26433	35231	25047	28764 (Wk 87)

These data show that AUCs for 311C90 parent drug increased with dose, in a supra-proportional manner. They also increased fairly daramatically (5-5.7-fold in males and females, respectively) with time up to week 52 of the study, after which they decreased at week 78 and then remained fairly constant on week 104. Plasma C_{max} showed a similar pattern.

183C91

Exposure to this metabolite was also dose-dependent, although levels did not increase to the same dose- or time-dependent extent as with 311C90 parent. Therefore, at higher exposures to 311C90, the 311C90:183C91 ratio actually increased. The exposure to 183C91 also decreased after week 52 and remained constant, same as 311C90 (see following sponsor tables).

The average $AUC_{0-\tau}$ (ng/ml x h) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	255	not estimated	not estimated	not estimated	not estimated
	25mg/kg	not estimated	408	1483	not estimated	not estimated
	100mg/kg	930	1924	3803	1712	2094
	400mg/kg	4168	6299	9150	6439	9731 (Wk 102)
Females	5mg/kg	not estimated	not estimated	4271	not estimated	not estimated
	25mg/kg	not estimated	426	955	265	not estimated
	100mg/kg	1182	1890	4182	878	905
	400mg/kg	2837	4409	9728	4124	4754 (Wk 87)

The average Cmax (ng/ml) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	22	11	13	<10	<10
	25mg/kg	35	51	181	55	33
	100mg/kg	86	225	391	143	155
	400mg/kg	312	520	742	507	659 (Wk 102)
Females	5mg/kg	<10	13	198	<10	<10
	25mg/kg	15	44	82	43	27
	100mg/kg	103	204	381	84	95
	400mg/kg	265	372	703	299	342 (Wk 87)

1652W92

In rat, exposure to 1652W92 was somewhat greater than 183C91 (2-3-fold greater at 400 mg/kg/day dose). Exposure tended to be greater in males than females. Exposures were dose-related but did not increase as dramatically with respect to dose or time as did 311C90 parent drug. Therefore, at higher exposure, the AUC ratio of 311C90:1652W92 was greater than at lower doses. Similar to 311C90, exposures decreased after week 52 and remained constant thereafter (see sponsor's tables below).

The average $AUC_{0-\tau}$ (ng/ml x h) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	not estimated	not estimated	877	306	not estimated
	25mg/kg	not estimated	1273	6674	1257	not estimated
	100mg/kg	2252	5211	13589	5619	6009
	400mg/kg	8057	15974	27017	17914	21616 (Wk 102)
Females	5mg/kg	not estimated	not estimated	8522	not estimated	not estimated
	25mg/kg	not estimated	435	1730	not estimated	not estimated
	100mg/kg	1362	2941	7369	1759	1942
	400mg/kg	3677	7788	18355	7663	9534 (Wk 87)

The average Cmax (ng/ml) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	<10	40	52	23	11
	25mg/kg	53	198	1042	204	86
	100mg/kg	294	764	1563	552	503
	400mg/kg	792	1454	2437	1508	1662
Females	5mg/kg	<10	23	728	<10	<10
	25mg/kg	21	62	142	92	51
	100mg/kg	191	391	730	212	283
	400mg/kg	503	776	1406	664	877

2161W92

Exposure levels of 2161W92 were in the same range as 1652W92, which means they were also about 2-3-fold greater than levels of 183C91 and about 16-fold lower than 311C90. Similar to parent drug, exposures of 2161W92 increased up to week 52, decreased after week 52 and tended to remain constant thereafter (see sponsor tables below).

The average $AUC_{0-\tau}$ (ng/ml x h) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	not estimated	not estimated	710	546	not estimated
	25mg/kg	not estimated	487	not estimated	784	671
	100mg/kg	1024	3430	9532	4124	5924
	400mg/kg	4938	11339	20588	16782	23860 (Wk 102)
Females	5mg/kg	not estimated	not estimated	4661	not estimated	not estimated
	25mg/kg	not estimated	759	2209	1005	558
	100mg/kg	1491	5332	14256	9195	2644
	400mg/kg	4797	13787	30516	12437	14699 (Wk 87)

The average Cmax (ng/ml) were as follows:

		Week 1	Week 26	Week 52	Week 78	Week 104
Males	5mg/kg	<11	15	39	22	16
	25mg/kg	20	87	583	125	72
	100mg/kg	89	519	1110	369	484
	400mg/kg	398	927	1986	1305	1919 (Wk 102)
Females	5mg/kg	<11	29	533	21	17
	25mg/kg	22	115	244	158	89
	100mg/kg	164	688	1211	327	309
	400mg/kg	516	1364	2366	1013	1178 (Wk 87)

Summary and Conclusions Regarding Toxicokinetics

Concentrations of 311C90 and metabolites in plasma increased during the first 52 weeks, decreased at 52 weeks and remained fairly constant thereafter. The $T_{1/2}$ was originally calculated to be about 14 hours in rats in this dose range, and thus steady state should have been reached in the first week. However, the AUC for 311C90 obviously increased with time up to 52 weeks. These data are consistent with a saturation of clearance, although the reason for this effect is unknown. The sponsor pointed out that this effect was specific to the rat, and did not occur in mouse or dog.

The sponsor stated that the decrease in exposure after 52 weeks could be due

accounts for about 32% of the excreted drug). However, this increased clearance was inconsistent in that it greater at the higher doses.

The AUC ratio of 311C90:metabolite (183C91 and 1652W92) increased with time over the dose range, indicating that these metabolic pathways might be saturable in the rat. Since this ratio increased with time, even after the 52-week timepoint at which exposure levels decreased, data are also consistent with a decrease in the efficiency of these metabolic pathways in the older rats.

Determination of an MTD

As with the mouse carcinogenicity study, the only parameter on which one could base an MTD for purposes of determining appropriate dose selection for this study was excessive mortality at the high dose. It was apparent that mortality was fairly consistent with controls (30-34% males and females) in the dose range from 6-100 mg/kg/day (27-48% mortality in males; 33-38% in females). However, mortality was higher in male (58.3%) and female (51.6%) rats at the high dose (400 mg/kg/day), indicating that the dose-limiting level is somewhere in the range of 400 mg/kg/day. Therefore, the 400 mg/kg/day dose was probably somewhat greater than the MTD based on excessive mortality and was an appropriate dose for the high dose in this rat CAR study.

With respect to the low dose, 5 mg/kg/day resulted in exposure levels (AUC) of 1200 (week 104) to 8500 (week 52) ng.h/ml. Using the premise that the low dose should provide some information relevant to the mrdd (15 mg/day; 160 ng.h/ml), this low dose was somewhat (about 10-fold) higher than would have been desirable.

Summary and Evaluation of Rat Carcinogenicity Study

As with the mouse study, the rat carcinogenicity study results also demonstrated excessive mortality at the high dose (400 mg/kg/day). There was some increase in liver enzymes (AST and ALP) in the high dose animals, but no effect of TSH (thyroid stimulating hormone). Unlike the mouse study, there were notable histopathological findings in the rat. Non-neoplastic lesions were found in high dose animals in thyroid (follicular cell hyperplasia), lungs (edema) and thymus (acute thymic hemorrhage). There was also an increase in neoplastic lesions, that being rat thyroid follicular cell adenoma and carcinoma. The NOEL with respect to both neoplasms and decreased survival for this study was 100 mg/kg/day, which resulted in an AUC of about 65-80,000 ng.h/ml, which is on the order of 406-500-fold greater than the AUC at the mrdd. The AUC at the high dose (400 mg/kg/day) was about 300,000 ng.h/ml, which is about 1875-fold greater than the AUC at the mrdd in patients. The sponsor concluded, based on this information, that these tumors were not relevant to the clinic. However, these findings should be included in the drug labelling.

The data for excessive mortality are consistent with an MTD in the range of 400 mg/kg/day, and therefore support the use of this as the high dose in this study. The low dose of 5 mg/kg/day was probably about 10-fold higher than desirable in order to show relevance to the AUCs at the mrdd.

rat, exposure levels of 311C90 and metabolites increased dramatically with time up to 52 weeks, at which time levels decreased and then stabilized. Data are consistent with a saturation of the clearance pathway for parent 311C90, followed by an increased clearance after 52 weeks, possibly due to increased age of the animals. Furthermore, data are consistent with a saturation of the metabolic pathways for 183C91 and 1652W92 metabolites.

Reproductive Toxicology

The following table summarizes the reproductive toxicology studies.

2.6 **Reproductive toxicity studies**

Species Type of Study	No./Group (M/F)	Doses (mg/kg/day)	Route	Duration	Ref No
CD Rat Fertility Reproduction	25/25	0, 25, 100,400	Oral	See experimental plan	54
CD Rat Teratology	0/30	0, 100, 400, 1200	Oral	10 days	55
NZW Rabbit Teratology	0/20	0, 10, 30, 100	Oral	13 days	56
NZW Rabbit Teratology	0/20	0, 3, 10, 30	Oral	13 days	57
CD Rat Pre- and Post Natal	0/25	0, 15, 100, 400	Oral	14 days	58

1. **311C90: Reproduction/fertility in rats given 311C90 by gavage (report TTEP/96/0018), Study No. TOX 677, Glaxo-Wellcome R&D, May 29, 1996, GLP.**

Animals: Crl:CD®BR VAF/Plus® rats; Charles River; 25/sex/group; F0 generation males 8 weeks old, females 12 weeks old; individually housed after acclimation period.

Drug: 311C90 in 0.5% methylcellulose; lot 311C90WQ (reference No. 92/0015-134-4.

Study description:

Three groups of 25 animals/sex/group received 311C90 by daily oral gavage at doses of 25, 200, or 400 mg/kg/day as per the above experimental schedule. A similar group received vehicle alone as control.

The experimental plan was as follows:

F0 males dosed for 73 days prior to mating
F0 females dosed for 14 days prior to mating
Dose males and females during mating period

Dose females to Day 7 of pregnancy
Dose males until termination

/ \

Sacrifice females Day 13 of pregnancy
Caesarian Examination
Necropsy Examination

Sacrifice males after female C. Section
Necropsy examination
Retain and weigh reproductive organs
Seminology
Histopathology on reproductive organs

Observations:

Clinical signs and mortality
Body weights (F0 males and females weekly; bred dams Day 0, 7, 8, 12 and 13 of gestation.
Food consumption (F0 dams; bred dams gestation days 0, 6, and 12)
Estrus cycle (daily evaluation of vaginal smears until evidence of insemination)
F0 generation cesarean section dams and fetal data (females scheduled for C-section sacrificed gestation day 13; gravid uterus weight; numbers of corpora ltea, implantation sites, live and ddead embryos, and early and late resorptions recorded.
Necropsy exams (F0 males sacrideced study day 103 or 104; first 10 males per dose group-weights of testes, epididymides and prostate gland recorded; right epididymis removed for sperm count and motility; microscopic examof reproductive tissues performed on control and high dose rats only).

Pharmacologist's comment: This study design is consistent with the ICH guidelines for "Detection of toxicity to reproduction of medicinal products" for a reproduction/fertility study.

Results:

Important findings from this study are summarized in the following two sponsor's tables: Table 1.

No. of rats/sex/group	Dosages mg/kg/day	Females with sperm	Pregnant females	Evaluated pregnant females[a]
25	0 (control)	23	19	18
25	25	24	23	23
25	100	24	24	23
25	400	24	23	22

Generation of parental animals: F0	Sperm in vaginal smear = Day 0 of gestation Day of Caesarian section: 13
Study in compliance with GLP: Yes	Batch No.: 311C90WQ Reference No. 92/0015-134-4

Parent	Litters (arithmetic mean per litter)			
Dosage. mg/kg/day	Corpora lutea	Implantations	Live foetuses	Resorptions[b]
0 (control)	17.7	16.8	15.9	0.9
25	17.7	15.1	14.4	0.7
100	18.3	15.3	14.3	1.0
400	18.5	16.7	15.6	1.0

Key:

a = Excludes females in which evidence of mating was not detected in vaginal smear

b = Early intra-uterine deaths

Table 2. Important reproduction/fertility findings

Dosage mg/kg/day	(control)	25	100	400
F0 Males Clinical Signs	None	post-dose salivation	post-dose salivation	post-dose salivation
Deaths	0	0	3 (dosing accidents)	1 (dosing accident)
Body weight gain dose period, grams	423.4	423.6	427.1	400.2
Mean Testes weight, grams	3.965	3.964	3.662	3.861
Mean Epididymides weight, grams	1.730	1.716	1.650	1.654
Mean Prostate weight, grams	0.667	0.703	0.747	0.745
Sperm Count[a]	1257.9 ± 270.0	1195.4 ± 359.7	1240.3 ± 232.9	1255.9 ± 456.4
Sperm, Percent Motility	97.0	97.3	95.3	95.0
F0 Females Clinical Signs	None	post-dose salivation	post-dose salivation	post-dose salivation
Deaths	0	0	0	0
Pregnancy Rate[b]	76.0 (19/25)	92.0 (23/25)	96.0 (24/25)	92.0 (23/25)
Fertility Index[c]	78.3 (18/23)	95.8 (23/24)	95.8 (23/24)	91.7 (22/24)
Mean pre-coital interval, days[d]	3.3	4.1	2.8	3.2

[a] Sperm count X 10^6, per gram caudal tissue, Mean S.D

[b] Pregnancy rate = % of paired females that were pregnant.

[c] Fertility Index = % of sperm positive females that were pregnant.

[d] Number of days from pairing to sperm positive.

Mortality and clinical signs

F0 males:

Mortality: 4 died during study; 3@100 mg/kg/day, 1@400 mg/kg/day; death due to gavage accident.

Clinical signs: post-dose salivation 5-10 minutes after dosing (100 and 400 mg/kg).

F0 females:

Mortality: no deaths.

Clinical signs: post dose salivation 5-10.min after dosing.

Body weights

F0 males: high dose males (400 mg/kg/day) gained significantly ($p<0.05$) less weight than control males during days 1-8 (66.7 g vs. 58.5 g; 12.3% less) and 85-92 (14.4 g vs. 5.2 g; 64% less). Also high dose males had less body weight gain (6%) over whole treatment period (400.2 grams; see table above) than controls (423.4 g).

Terminal body weights of males in high dose (400 mg/kg; 608.3 g) lower (7.5%) than controls (657.65 g).

F0 females:

Pregestation: no effect.

During gestation: 100 and 400 mg/kg/day rats gained more weight than controls.

Food Consumption

No dose-related effect.

Reproductive Performance (see table previous page)

No effect on fertility index (% of sperm positive females that were pregnant).

No effect on pre-coital interval.

Pregnancy and litter data

No effect on numbers of implantations per dam, viable fetuses, corpora lutea or resorptions in F0 females.

Necropsy (macropath and micropath)

Single high dose male had right cauda epididymis enlarged and yellow in color. No other gross or histopathologic lesions in testes, epididymides, prostate or seminal vesicles from 10 high dose and 10 control male rats observed in this study.

F0 male organ weights

No effect on weight or organ:body weight ratio for organs examined.

Sperm counts and motility

No effects on sperm counts or % motility.

F0 females

No gross lesions related to drug administration.

Toxicokinetics

No Toxicokinetics data were submitted for this study. The ICH guidelines allow for this, in that animals are dosed before pregnancy and toxicokinetics should be very similar to those in subchronic and chronic rat toxicology studies. In a 28-day oral gavage study in Wistar rat (see "Subchronic Toxicology" section of this review), rats were administered 100, 400 or 1000 mg/kg/day of 311C90. At the 400 mg/kg/day dose there were a number of animal deaths (3/15 animals), and the AUC for parent 311C90 drug was in the range of 200,000 to 400,000 ng h/ml (males and females

respectively), which is on the order of 1250- to 2500-fold larger than the AUC in humans at the mrdd (160 ng.h/ml). The NOEL in that study by their determination was 100 mg/kg, with AUCs of 47,000 (males) to 53,000 (females) ng.h/ml, which is 294-331-fold larger than the human mrdd. These AUC values were taken on Day 26 in that study. It is known that the exposure to 311C90 increases significantly in a time-dependent manner.

The 25 mg/kg dose, in a previous 26-week daily oral gavage study in Wistar rats, gave AUCs on the order of 3500-5000 ng.h/ml on Day 1 of the study, which is still about 22- to 31-fold greater than at the mrdd. However, on Day 169 of that same study, the AUCs at this dose had increased to 39,000 (males) to 56,000 (females), which is 243-350-fold greater than at the mrdd. Therefore, the low dose was on the order of 10-100-fold too high to give relevance to the human dose.

Summary and Conclusion

The study design complies with the ICH guideline ("Detection of toxicity to reproduction for medicinal products") for a reproduction/fertility study and an adequate number of animals were utilized.

With respect to choice of dose, the sponsor used the same doses as they used in the Wistar rat subchronic toxicology studies, and in which they saw some mortality (3/15 animals) at the high dose (400 mg/kg/day). Furthermore, while there was no effect of body weight on the females in this repro study, male body weight gain at the high dose decreased 12-64% compared to controls at various time intervals during this study. The overall decrease in body weight gain for the males was about 6% at the high dose. These data could be interpreted to support an MTD, which further supports the use of 400 mg/kg as the high dose in this reproductive toxicology study. Although no PK data were submitted for this study, from data reported in a 28-day and 26-week oral gavage studies in Wistar rats, one might predict that these animals in the reproduction/fertility study experienced exposure levels of 311C90 on the order of 1250- to 2500-fold higher at the high dose (400 mg/kg/day) than humans at the mrdd, and about 20-30-fold greater (at a minimum) at the low dose (25 mg/kg/day) than humans at the mrdd.

There were virtually no effects of 311C90 on reproductive performance or fertility when administered at daily oral gavage doses up to 400 mg/kg/day. There was no mortality associated with drug treatment, and the only clinical sign was excessive salivation, which the sponsor attributes to effects of taste aversion.

2. 311C90: Teratology Study in CD Rats Given 311C90 by Gavage, Study Number TTEP/93/0001-1, Protocol Tox 582, Burroughs Wellcome Co., Research Triangle Park, NC, January 1993, GLP.

Animals: CD rats (Rattus norvegicus): Strain CRL:CD/BR VAF/Plus (Charles River Breeding Labs, Inc, Raleigh, NC.
initial body wt: approximately 300 g
initial age: 14-15 weeks
housing: 1/cage
30/dose for teratology, 15/dose for PK

Drug: 311C90 Batch W1 (free base); ref no. 91/0017-108-2.
vehicle: 0.5% methylcellulose
route: oral gavage
dosing: 0, 100, 400 or 1200 mg/kg
duration: **single dose per day for days 6-15 of gestation (total 10 doses)**
dose volume: 10 ml/kg
concentration: 0, 10, 40, or 120 mg/ml

Study design: The study design is outlined in the sponsor's table shown below:

No. Animals/ Dose Group:	30
Dose Group (mg/kg/day):	0 (0.5% methylcellulose), 100, 400, 1200
Dose Volume (mL/kg):	10
Dosing Schedule:	Gestation days 6 to 15
Observations:	Maternal body weight and food consumption Maternal pharmacokinetic evaluation Maternal and fetal examinations at day 20 laparotomy

Observations:

Clinical signs and mortality: determined daily

Maternal body weights and food consumption: body weights recorded on gestation days 0, 6, 9, 12, 15, 16 prior to sacrifice on day 20. Food consumption recorded over a 24-hour period from individual animals on gestation days 0, 6, 12, 15, and 19.

PK data: Samples from 3 dams were collected at the following times:
Gestation Day 6: Group 9 (Control)-pre-dose and 2 hours post-dose, Group 10-12 (100, 400 and 1200 mg/kg/day)-pre-dose, 2, 4, 6, 8 and 24 hours post-dose.
Gestation Day 15: Group 5 (Control)-Pre-dose and 2 hours post-dose, Groups 6-8 (100, 400 and 1200 mg/kg/day)-pre-dose, 2, 4, 6, 8 and 24 hours post-dose.

Maternal and Fetal Examinations at Day 20 Laparotomy: On gestation Day 20 all dams were sacrificed, gravid uterus weighed, number of corpora lutea, implantation sites, live fetuses, dead fetuses and early and late resorptions were determined. Live fetuses were weighed, measured, sexed and examined for external malformations. Half of the fetuses in each litter were dissected and examined for visceral anomalies and heads examined for brain, eye and other abnormalities. All fetuses were cleared and examined for skeletal defects.

Pharmacologist's comment: The study design and observations are consistent with the ICH guidelines for a teratology study.

Results:

Results of this rat teratology study are given in the sponsor's table shown below:

311C90 (mg/kg/day)

Parameter	Vehicle Control	100	400	1200
MATERNAL				
Total number of dams	30	30	30	30
Death total:	2	0	0	1
Death due to Dosing Accident:	0	0	0	0
Clinical Signs: (postdose salivation):	No	Yes	Yes	Yes
Body Weight and Weight Gain:	Normal	Normal	Normal	Dec. ($p<0.05$)
Food Consumption:	Normal	Normal	Normal	Normal
Pregnancy Rate:	Normal	Normal	Normal	Normal
Pharmacokinetic Data:*				
FETAL				
Number Viable:	Normal	Normal	Normal	Normal
Increased Dead/Resorbed:	No	No	No	Yes. ($p<0.05$)Slight
Body Weight/Length:	Normal	Normal	Normal	Normal
Increased Malformations:	No	No	No	No
Increased Variations:	No	No	No	No

Dec. = Decreased

* = Pharmacokinetic data will be included in an addendum.

Clinical signs and mortality

3 deaths:

Control: found dead from pyelonephritis on gestation day 5
Control: found dead on gestation day 17; cause of death undetermined
High dose (1200 mg/kg/day): found dead on gestation day 14; cause of death undetermined.

Clinical signs: post dose salivation, lasting 5-10 minutes at all doses.

Maternal body weights and food consumption

Body weights significantly decreased (6% decrease versus controls; $p<0.05$) in high dose (1200 mg/kg) animals (see sponsor's table below).

Decreased mean body weight gain days 9-12 (30%) and days 6-15 (21%) significantly ($p<0.05$) different from controls (See table below).

TABLE 2
A TERATOLOGY STUDY IN RATS GIVEN 311C90 BY GAVAGE
MEAN BODY WEIGHTS (GRAMS) DURING GESTATION

STUDY NO.: TOX-582 PAGE 1
COMPOUND: 311C90

DOSE GROUP:	0 MG/KG/DAY	100 MG/KG/DAY	400 MG/KG/DAY	1200 MG/KG/DAY
DAY 0	306.8	302.1	307.8	297.1
DAY 6	338.0	338.0	338.3	329.6
DAY 9	345.6	341.2	340.9	337.1
DAY 12	365.0	357.3	359.6	350.7
DAY 15	382.1	375.8	377.7	364.4
DAY 16	391.3	384.9	386.4	374.0
DAY 20	443.4	438.0	432.7	435.8*

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST

TABLE 2
A TERATOLOGY STUDY IN RATS GIVEN 311C90 BY GAVAGE
MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION

STUDY NO.: TOX-582 PAGE 1
COMPOUND: 311C90

DOSE GROUP:	0 MG/KG/DAY	100 MG/KG/DAY	400 MG/KG/DAY	1200 MG/KG/DAY
DAY 0- 6	30.8	35.9*	30.5	32.5
DAY 6- 9	7.6	3.2	2.9	7.5
DAY 9- 12	19.4	16.1	18.7	13.6*
DAY 12- 15	17.1	18.5	18.2	13.7
DAY 15- 16	9.2	9.0	8.7	9.6
DAY 16- 20	48.7	45.1	44.3	61.8
DAY 6- 15	44.1	37.8	39.4	34.9*

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

There was no effect of 311C90 administration of food consumption during gestation.

Toxicokinetics data

The following table shows AUC and Cmax of 311C90 parent drug and metabolites 1652W92, 183C91 and 2161W92 in pregnant animals on days 6 and 15 of gestation following oral administration of 311C90:

Pharmacokinetics

Profiles were taken on Days 6 and 15 for both parent 311C90 and its metabolites (1652W92, 183C91 and 2161W92).

	311C90 (mg/kg/day)			
	Control	100	400	1200
311C90 Day 6				
$AUC_{0-\infty}$	-	29.2	114.4	255.4[a]
Cmax	-	4.47	8.17	15.59
Day 15				
AUC_{0-24}	-	33.3	133.8	604.9
Cmax	-	4.24	16.79	50.60

1652W92 Day 6				
$AUC_{0-\infty}$	-	1.9	5.6	11.9
Cmax	-	0.217	0.373	0.571
Day 15				
AUC_{0-24}	-	1.3	4.0	21.6
Cmax	-	0.134	0.473	1.948

183C91 Day 6				
$AUC_{0-\infty}$	-	1.1	3.1	6.9
Cmax	-	0.134	0.219	0.259
Day 15				
AUC_{0-24}	-	1.7	4.8	18.2
Cmax	-	0.156	0.397	1.342

2161W92 Day 6				
$AUC_{0-\infty}$	-	3.2	9.4	18.6
Cmax	-	0.352	0.614	1.038
Day 15				
AUC_{0-24}	-	4.0	9.3	24.9
Cmax	-	0.353	0.826	1.991

AUC (µg/ml x h) — area under the plasma concentration–time curve

[a] AUC_{0-24} (µg/ml x h) — area under the plasma concentration–time curve for the daily dose

Cmax (µg/ml) — maximum observed plasma concentration

311C90

Day 6: dose-proportional increase in AUC and Cmax
Day 15: dose-proportional increase between 100 and 400 mg/kg, supra-proportional increase at 1200 mg/kg/day
Time-dependent increase in AUC, possibly due to saturation of clearance.

1652W92

Day 6: dose-proportional increase AUC and Cmax
Day 15: dose-proportional increase between 100 ang 400 mg/kg, supra-proportional increase at 1200 mg/kg
Concentration of this metabolite was about 19-fold (Day 6) to 29-fold (Day 15) lower than parent 311C90 drug.

183C91

Day 6 and Day 15: exposure levels increased less than dose-proportionally at all doses.
Day 15: exposure levels increased supra-proportionally between 400 and 1200 mg/kg/day.
Concentrations of this metabolite were about 30-fold lower than parent 311C90 on both days 6 and 15.

2161W92

Day 6: exposure levels increased in a less than dose-proportional manner at all doses.
Day 15: Exposure increased less than dose-proportionally between 100 and 400 mg/kg, and in a dose-proportional manner between 400 and 1200 mg/kg/day.
Concentrations of metabolite were 9-14-fold lower than 311C90 parent of Day 6 and 8-14-fold lower on Day 15.

Overall, there appears to be a dose-dependent increase in AUC on Day 6 at all doses. However, there is apparently a time-dependent increase in 311C90 parent drug as well as in at least two of the metabolites, 1652W92 and 183C91. The AUC comparison from highest to lowest exposure level is as follows:

311C90>2161W92>1652W92>183C91

Maternal and Fetal Examinations at Day 20 Laparotomy

No effect on number of pregnant females per group:

Control:	22/29*	75.9%
100 mg/kg:	27/30	90%
400 mg/kg:	26/30	86.7%
1200 mg/kg:	24/30	80%

*pregnancy status of one animal was not determined.

Slightly increased incidence early resorptions and post-implantation loss at high dose as seen in sponsor Table 5 below:

TABLE 5
A TERATOLOGY STUDY IN RATS GIVEN 311C90 BY GAVAGE
SUMMARY OF MEAN FETAL DATA AT TIME OF LAPAROTOMY

STUDY NO.: TOX-882
COMPOUND:311C90

GRP.		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	EARLY RESORPTIONS	LATE RESORPTIONS	POST IMPL. LOSS	IMPLANTATION SITES	CORPORA LUTEA	FETAL WEIGHTS	FETAL LENGTHS
1	TOTAL	166	172	338	1	16	0	17	355	419	NA	NA
	MEAN	7.9	8.2	16.1	0.0	0.8	0.0	0.8	16.9	20.0	3.6	3.8
	S.D.	2.8	2.5	1.8	0.2	0.9	0.0	0.9	1.8	2.6	0.4	0.2
2	TOTAL	225	204	429	3	26	1	30	459	537	NA	NA
	MEAN	8.3	7.6	15.9	0.1	1.0	0.0	1.1	17.0	19.9	3.4	3.6
	S.D.	2.1	2.0	2.6	0.4	1.2	0.2	1.3	2.0	2.6	0.4	0.3
3	TOTAL	198	187	385	0	30	0	30	415	513	NA	NA
	MEAN	7.4	7.2	14.8	0.0	1.2	0.0	1.2	16.0	19.7	3.6	3.7
	S.D.	3.0	2.5	3.8	0.0	1.3	0.0	1.3	3.6	3.0	0.5	0.3
4	TOTAL	177	168	343	0	36	1	37	382	468	NA	NA
	MEAN	7.4	7.0	14.4	0.0	1.5*	0.0	1.5*	15.9	19.5	3.5	3.7
	S.D.	2.4	2.4	2.8	0.0	1.3	0.2	1.3	2.9	2.6	0.4	0.2

* = SIGNIFICANTLY DIFFERENT FROM CONTROL AT 0.05 LEVEL
NA = NOT APPLICABLE

SEX RATIO COMPARED USING CHI SQUARE TEST.
MEAN NUMBER OF VIABLE FETUSES COMPARED USING DUNNETT'S TEST.
TOTAL NUMBER OF DEAD FETUSES COMPARED USING MANN-WHITNEY TEST.
TOTAL NUMBER OF EARLY RESORPTIONS COMPARED USING MANN-WHITNEY TEST.
TOTAL NUMBER OF LATE RESORPTIONS COMPARED USING MANN-WHITNEY TEST.
TOTAL POST IMPLANTATION LOSS COMPARED USING MANN-WHITNEY TEST.
MEAN NUMBER OF IMPLANTATION SITES COMPARED USING DUNNETT'S TEST.
MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST.
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
FETAL LENGTHS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG/DAY 2- 100 MG/KG/DAY 3- 400 MG/KG/DAY 4- 1200 MG/KG/DAY

Pharmacologist's comment: The sponsor states that the 1.5 post-implantation losses per litter at the high dose is within historical control values for CD-1 rats at Charles River, but the study controls are the appropriate values for this study. Therefore, there was some increase in early resorptions and thus post-implantation losses. However, since maternal body weights did decrease somewhat at this high dose, this increase in early resorptions may very well be due to maternal toxicity.

No effect on litter size, fetal body measurements or incidences of fetal malformations. A single dam (100 mg/kg group) presented with abscessed lungs, which the sponsor concluded was due to a dosing accident. This dam had three dead fetuses, with the 14 surviving fetuses undersized and showing delayed skeletal ossification.

Summary and Conclusions for Oral Teratology Study in Rats

The study design was consistent with the ICH guidelines for teratology studies. Maternal body weights were decreased (6% versus controls overall) at the high dose, indicating the possibility of maternal toxicity at this dose. Also, there was a decrease in mean body weight gain on days 9-12 (30%) and days 6-15 (21%) that were statistically significant ($p<0.05$). Toxicokinetics data indicated a time-dependent increase in 311C90 parent drug as well as at least two of the metabolites (1652W92 and 183C91).

The only finding of note was an increase in early fetal resorptions (0.8 per litter in controls to 1.5 per litter at 1200 mg/kg/day). This was a statistically significant increase ($p<0.05$), and resulted in the same increase in post-implantation losses. However, the decrease in maternal body weights at the high dose suggests that this effect may be due to maternal toxicity.

2. **An Oral Teratology Study in New Zealand White Rabbits (8)** (Protocol Tox TTEP/93/0005 (Tox 583), Burroughs Wellcome Co., Research Triangle Park, NC, March 22, 1993, GLP)

Animals: Female New Zealand White rabbits

initial body wt: approximately 4000-4400 g
initial age: 19 months
housing: 1/cage
20/dose for teratology, 8/dose for PK

Drug: 311C990 Batch W1 (free base) (Reference No. 91/0017-108-2
vehicle: 0.5% methylcellulose
route: oral gavage
dosing: 0, 10, 30 and 100 mg/kg/day
duration: single dose per day for days 6-18 of gestation (Day of insemination was Day 0)
dose volume: 5 ml/kg
concentration: 0, 2, 6 and 20 mg/ml

Observations: Maternal body weights (gestation days 0, 6, 9, 12, 15, 18 and 19 and prior to sacrifice Day 29) and food consumption (gestation days 0-6, 6-12, 12-19, 19-24 and 24-29), PK data (maternal blood collected on gestation days 6 and 18 at predose and 2h for Controls and predose, 1, 2, 4, 8 and 24 h for Treated animals), and maternal and fetal exams at Day 29 laparotomy were recorded.

Pharmacologist's comment: The study design is consistent with the ICH guidelines for a teratology study.

Results: There was a poor reproductive performance (decreased litter size and number of implantation sites) in controls as well as treated animals, probably due to their advanced age (19 months). There was also excessive toxicity (12/20 died) in the HD (100 mg/kg) group (See sponsor's Table below for summary of results). Therefore, the sponsor concluded that these data were invalid, and a second teratology study was completed in New Zealand White rabbits.

The results are summarized as follows:

	311C90 (mg/kg/day)			
PARAMETER MATERNAL	Vehicle Control	10	30	100
Total Number of Does[a]	20	20	20	20
Total Number of Dead or Sacrificed	3	3	6	12
Death due to Dosing Accident:	0	2	1	3
Aborted and Sacrificed:	2	1	3	7
Death of Undetermined Cause:	1	0	2	2
Clinical Signs:	None	None	None	None
Body Weight and Weight Gain:	Normal	Dec, NS	Dec.	Dec

* = Pharmacokinetic data will be issued as an addendum.
** = Insufficient for study interpretation at mid and high dose; study has been repeated.
a = Rabbits used for pharmacokinetic evaluations are not included.
b = Litter size was lower than typically observed in this species for all groups, including controls.
Dec = Decreased
NS = Not statistically significant

	311C90 (mg/kg/day)			
PARAMETER MATERNAL	Vehicle Control	10	30	100
Food Consumption:	Normal	Normal	Normal	Dec
Pregnancy Rate:	Normal	Normal	Normal	Normal
Pharmacokinetic Data*				
FETAL -				
Number of Litters with Live Fetuses**	11	11	4	2
Number Viable Fetuses/Litter	b, Dec	b, Dec, NS	b, Dec	b, Dec, NS
Increased Post Implantation Loss/Litter	No	Yes, NS	Yes, NS	Yes
Increased Resorbed:	No	Yes, NS	Yes, NS	Yes
Body Weight/Length	Normal	Normal	Normal	Normal
Increased Malformations:	No	No	No	No
Increased Variations:	No	No	No	No

* = Pharmacokinetic data will be issued as an addendum.
** = Insufficient for study interpretation at mid and high dose; study has been repeated.
a = Rabbits used for pharmacokinetic evaluations are not included.
b = Litter size was lower than typically observed in this species for all groups, including controls.
Dec = Decreased
NS = Not statistically significant

Toxicokinetics: (Protocol BPAT 93/0018, The Wellcome Foundation, GLP) The animals used for the PK studies were younger (10 months), and no adverse effects on reproductive capacity were reported in these animals. Therefore, the sponsor believes that these PK data are valid. They incorporated these results into the table for the second rabbit teratology study, but for accuracy I am including them here instead (See sponsor's table below).

Toxicokinetics of the parent 311C90

	10 mg/kg	30 mg/kg	100 mg/kg
Day 6			
Cmax	251 ng/ml	611 ng/ml	2886 ng/ml
AUC	1.2 µg/ml-hr	3.4	16.8
Day 18			
Cmax	389 ng/ml	1164	8960
AUC	1.3	4.9	76.9

Plasma levels were linear with dose between 10 and 30 mg/kg at both Day 6 and Day 18, but not between 30 and 100 mg/kg, suggesting the possibility of saturation of clearance. Also, plasma and exposure levels with dose were higher at Day 18 at both 30 mg/kg (C_{max} 1164; AUC 4.9) and 100 mg/kg (C_{max} 8960; AUC 3.4) than at Day 6 at 30 mg/kg (C_{max} 611; AUC 3.4) and 100 mg/kg (C_{max} 2886; AUC 16.8), indicating that exposure rates were time-dependent. This is a problem with respect to toxicity, as under these circumstances there can be no clear predictability between dose and toxicity. The half-life was variable, ranging from 1.4 to 9.1 hours, but there was no obvious correlation with time or dose. The T_{max} was from 1-2 hours.

Toxicokinetics of the active metabolite 183C91

	10 mg/kg	30 mg/kg	100 mg/kg
Day 6			
Cmax	7.8 ng/ml	29 ng/ml	149 ng/ml
AUC	Not estimated	0.08 µg/ml-h	0.6 µg/ml-h
Day 18			
Cmax	11 ng/ml	67 ng/ml	1154 ng/ml
AUC	Not estimated	0.16 µg/ml-h	10.1 µg/ml-h

The toxicokinetics of the active metabolite 183C91 followed the same pattern as the parent drug, with the lack of linearity between the 30 and 100 mg/kg dose suggesting saturation of clearance and the time-dependence of exposure resulting in much higher exposure rates on Day 18 than Day 6 of gestation. The mean half-life for this metabolite was 5.6 hours. The T_{max} was 1-2 hours.

Drug exposure compared to the mrdd in humans

The toxicokinetics data for this study were collected in younger animals, and are therefore most likely valid. Animals at the high dose (100 mg/kg) received AUCs of 16,800 ng.h/ml, which is about 105-fold greater than the AUC (160 ng.h/ml) at the mrdd in humans. Animals at the low dose (10 mg/kg/day) attained AUCs of about 1,200 ng.h/ml, which is still about 7.5-fold greater than at the human mrdd.

Summary and Conclusions for First Rabbit Oral Teratology Study

There were excessive maternal deaths (12/20) at the high dose (100 mg/kg/day), in addition of a dramatic decrease in the number of litters with live fetuses (2 versus 11 in controls). There was also a decrease in maternal body weight gain during gestation days 6-18 as shown below:

Mean maternal body weight changes (grams) during gestation

Day	Dose (mg/kg/day)			
	0	10	30	100
6-18	-33.3	-173.3	-271.4*	-415.4**

*statistically significantly different from control ($p<0.05$)
**statistically significantly different from control ($p<0.01$)

There were also a number of aborted fetuses at all doses as well as in control animals. The sponsor stated that indicated that these effects were most likely due to the advanced age of the animals.

The sponsor also concluded, and I concur, that based on these data 100 mg/kg/day is probably a limiting dose in the rabbit, as 12/20 animals died prematurely at this dose. 6 of 20 animals also died prematurely at the 30 mg/kg dose, indicating that this may still be above the MTD. Therefore, the sponsor's choice of 30 mg/kg as the high dose in the repeat rabbit teratology study is probably appropriate.

3. **An Oral Teratology Study in New Zealand White Rabbits Given 311C90 by Gavage (9)** (Protocol Tox TTEP/93/0008, (Tox 598) Burroughs Wellcome Co., Research Triangle Park, NC, May 10, 1993,GLP)

Animals: Female New Zealand White rabbits

initial body wt: approximately 3500 g
initial age: 9 months
housing: 1/cage
20/dose for teratology, 8/dose for PK (*PK reported is actually from previous study)

Drug: 311C990 WI (free base) (Reference No. 91/0017-108-2)
vehicle: 0.5% methylcellulose
route: oral gavage
dosing: 0, 10, and 30 mg/kg/day
duration: single dose per day for days 6-18 of gestation (Day of insemination was Day 0)
dose volume: 5 ml/kg
concentration: 0, 2, or 6 mg/ml

Observations: Maternal body weights (gestation days 0, 6, 9, 12, 15, 18 and 19 and prior to sacrifice Day 29) and food consumption (gestation days 0-6, 6-12, 12-19, 19-24 and 24-29), PK data (maternal blood collected 2 h post-dose on gestation day 18), and maternal and fetal exams at Day 29 laparotomy were recorded.

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ON ORIGINAL

Results:

Overall summary of results are shown in the following sponsor's table:

The results are summarized as follows:

	311C90 (mg/kg/day)			
PARAMETER	Vehicle Control	3	10	30
MATERNAL				
Total Number of Does [a]	20	20	20	20
Found Dead/Died	0	2	2	2
Death due to Dosing Accident:	0	1	2	2
Aborted and Sacrificed:	1	0	0	0
Death of Undetermined Cause:	0	1	0	0
Clinical Signs:	None	None	None	None
Body Weight Gain (dose period):	Normal	Normal	Dec	Dec
Body Weight Gain (postdose):	Normal	Normal	Inc	Inc
Food Consumption:	Normal	Normal	Normal	Dec
Pregnancy Rate:	Normal	Normal	Normal	Normal
Maternal Drug Plasma Data Plasma Concentrations (ng/ml.)[b]				
311C90	NA	30	91	510
1652W92	NA	14	39	146
183C91	NA	12	13	45
2161W92	NA	36	98	371
FETAL				
Number of Litters with Live Fetuses:	14	16	14	15
Number of Viable Fetuses/Litter:	Normal	Normal	Normal	Normal
Increased Dead/Resorbed:	No	No	No	No
Body Weight/Length:	Normal	Normal	Normal	Normal
Increased Malformations:	No	No	No	Yes, NS[c]
Increased Variations:	No	No	No	No

a Plasma animals are not included.

b Mean plasma concentrations of 311C90 and metabolites at 2 hr postdose on gestation day 18.

c Six fetuses (four litters) with sternebral or rib anomalies (within historical control range).

Inc Increased; Dec Decreased NA Not Applicable

NS Not statistically significant, not out of range for same sternebral and rib malformations in controls in other studies in this laboratory.

Clinical signs and mortality

Premature deaths: 2 of 20 animals in each dosing group (10 animals per sex per dosing group) (all dosing accidents except for one low dose animals, whose cause of death was undetermined)

Clinical signs: ataxia (one high dose animal); labored breathing (1 mid dose animals; 2 high dose animals)

Maternal body weights and food consumption

Mean body weight change was decreased in pregnant females over the course of the treatment period day 6-18 of gestation. Mean body weight gain was decreased 24.7%, 44.3% and 91% at 3, 10 and 30 mg/kg/day doses, respectively. Classically, an MTD is defined as the dose at which a 10% decrease in body weight change occurs, which occurred in this study at the low dose. However, in this study there was a fairly large variability in body weight changes within a given dosing group such that only the 91% decrease in body weight change was statistically significant at $p<0.05$ (there was also statistical significance to the decreased body weights at 10mg/kg/day, but only on gestation days 6-9). Therefore, the 10 mg/kg/day dose, or somewhere between the 10 and 30 mg/kg doses, is probably the correct choice for the MTD, and the 30 mg/kg dose may have been slightly higher than necessary for the high dose animals.

Food consumption was decreased significantly ($p<0.05$) in the high dose group during days 12-19 only.

TABLE 2

STUDY NO.: TOX-598
COMPOUND:1311C90

A TERATOLOGY STUDY IN RABBITS GIVEN 1311C90 BY GAVAGE
MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION

DOSE GROUP:	0 MG/KG/DAY	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
DAY 0- 6	252.9	233.3	250.0	235.3
DAY 6- 9	41.2	-6.3	-33.3*	-6.3
DAY 9- 12	5.9	37.5	28.6	-13.3
DAY 12- 15	70.6	56.3	28.6	40.0
DAY 15- 18	23.5	18.8	50.0	-6.7
DAY 18- 19	-11.8	6.3	7.1	0.0
DAY 19- 29	125.0	143.8	214.3*	220.0*
DAY 6- 18	141.2	106.3	78.6	13.3*

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

Maternal and fetal examinations at Day 29

No effect on number or percentage of female rabbits per group that were pregnant:

	Dose (mg/kg/day)			
	0	3	10	30
Number of pregnant females	17/19	16/18	15/18	17/18
% of live females that pregnant	85	88.9	78.9	85

No effect on the number of viable fetuses, late resorptions, implantation sites, corpora lutea, fetal weights or fetal lengths (see sponsor Table 5 below).

The number of early resorptions (and thus number of implantation losses) increased at the mid (10 mg/kg) and high (30 mg/kg) doses. However, in the mid-dose, 9 of the 12 early resorptions occurred a single dam and at the high dose (30 mg/kg/day) 8 of 11 of the early resorptions occurred in a single dam. Therefore, the sponsor stated that these were not statistically significant effects (by the Mann-Whitney Test).

TABLE 5

STUDY NO.: TOX-598
COMPOUND:1311C90

A TERATOLOGY STUDY IN RABBITS GIVEN 1311C90 BY GAVAGE
SUMMARY OF MEAN FETAL DATA AT TIME OF LAPAROTOMY

PAGE 1

GRP.	SEX	N	F	VIABLE FETUSES	DEAD FETUSES	EARLY RESORPTIONS	LATE RESORPTIONS	POST IMPL. LOSS	IMPLANTATION SITES	CORPORA LUTEA	FETAL WEIGHTS	FETAL LENGTHS
1	TOTAL	48	39	87	0	4	0	4	91	134	NA	NA
	MEAN	3.0	2.4	5.4	0.0	0.3	0.0	0.3	5.7	8.4	48.7	7.4
	S.D.	1.9	2.2	3.3	0.0	0.8	0.0	0.8	3.8	3.3	3.9	0.5
2	TOTAL	31	46	77	0	1	0	1	98	136	NA	NA
	MEAN	3.2	2.9	4.1	0.0	0.1	0.0	0.1	6.1	8.5	46.4	7.5
	S.D.	1.8	1.7	2.5	0.0	0.3	0.0	0.3	2.6	2.6	4.5	0.5
3	TOTAL	37	48	77	0	12 a	1	13	90	134	NA	NA
	MEAN	2.6	2.9	5.5	0.0	0.9	0.1	0.9	6.4	9.7	50.4	7.6
	S.D.	1.4	1.7	3.2	0.0	2.4	0.3	2.4	2.4	3.0	3.1	0.6
4	TOTAL	32	34	65	0	11 b	0	11	96	137	NA	NA
	MEAN	3.4	2.3	5.7	0.0	0.7	0.0	0.7	6.4	9.1	49.4	7.3
	S.D.	2.6	2.0	3.1	0.0	2.1	0.0	2.1	3.2	2.9	7.0	0.6

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL AT 0.05 LEVEL
NA = NOT APPLICABLE

SEX RATIO COMPARED USING CHI SQUARE TEST.
MEAN NUMBER OF VIABLE FETUSES COMPARED USING DUNNETT'S TEST.
TOTAL NUMBER OF DEAD FETUSES COMPARED USING MANN-WHITNEY TEST.
TOTAL NUMBER OF EARLY RESORPTIONS COMPARED USING MANN-WHITNEY TEST.
TOTAL NUMBER OF LATE RESORPTIONS COMPARED USING MANN-WHITNEY TEST.
TOTAL POST IMPLANTATION LOSS COMPARED USING MANN-WHITNEY TEST.
MEAN NUMBER OF IMPLANTATION SITES COMPARED USING DUNNETT'S TEST.
MEAN NUMBER OF CORPORA LUTEA COMPARED USING DUNNETT'S TEST.
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
FETAL LENGTHS COMPARED USING DUNNETT'S TEST

a = A single rabbit (92-3879) had 9 early resorpt
b = A single rabbit (92-3901) had 8 early resorpt

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 10 MG/KG/DAY 4- 30 MG/KG/DAY

Pharmacologist's comment:

The sponsor did not offer any explanation as to why these two animals (one mid dose and one high dose) should present with such a high percentage of early resorptions. This could be explained by maternal toxicity in the high dose animal (#92-3901), as her body weight at week 29 was 3900 g, considerably less than the body weight of control animals (4406.3) or high dose animals (4406.7) on day 29. However, the mid dose animal (#92-3879) body weight (4800 g) on day 29 was greater than mean control weights (4406.3 g) or mid dose animal weights (4521.4 g) on day 29, suggesting that maternal toxicity may not have been an issue with this animal. No other explanation for the large number of early resorptions was offered by the sponsor for this animal.

Malformations (see sponsor's table 6 below)

External: single low dose fetus with spina bifida

Visceral: single fetus with intervertricular septal defect and common truncus arteriosus

Skeletal: a number of skeletal abnormalities occurred in fetuses from high dose does, including sternebrae fused (20%), rib anomaly (13.3%) and rib anomaly with or without vertebral anomaly (6.7%).

Pharmacologist's comments: The sponsor states that three of these fetuses with rib or sternebral anomalies were from a single doe that showed weight loss (lost 2.2% of body weight over 29 days) during gestation, indicative of maternal toxicity. I concur with this observation. The sponsor also makes the point that such rib anomalies, especially fused ribs, have been observed among control new Zealand White rabbits in a control range of 0-16.7%. 6 of 85 fetuses (7.1%) and 4 of 15 litters (26.7%) demonstrated these rib anomalies. The anomalies only occurred at the high dose (30 mg/kg/day).

TABLE 6

STUDY NO.: TOX-598 — A TERATOLOGY STUDY IN RABBITS GIVEN 311C90 BY GAVAGE — PAGE

COMPOUND:311C90 — NUMBER OF FETUSES AND LITTERS WITH MALFORMATIONS — DAY

DOSE GROUP:	FETUSES 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED EXTERNALLY	87	97	77	85	14	16	14	15
SPINA BIFIDA	0	1	0	0	0	1	0	0
NUMBER EXAMINED VISCERALLY	87	97	77	85	14	16	14	15
INTERVENTRICULAR SEPTAL DEFECT	0	1	0	0	0	1	0	0
COMMON TRUNCUS ARTERIOSUS	0	1	0	0	0	1	0	0
NUMBER EXAMINED SKELETALLY	87	97	77	85	14	16	14	15
STERNEBRAE FUSED	0	0	0	4	0	0	0	3
RIB ANOMALY	0	0	0	3	0	0	0	2
RIB ANOMALY WITH OR WITHOUT VERTEBRAL ANOMALY	0	0	0	1	0	0	0	1
TOTAL MALFORMATIONS								
NUMBER WITH EXTERNAL MALFORMATIONS:	0	1	0	0	0	1	0	0
NUMBER WITH SOFT TISSUE MALFORMATIONS:	0	1	0	0	0	1	0	0
NUMBER WITH SKELETAL MALFORMATIONS:	0	0	0	6	0	0	0	4
TOTAL NUMBER WITH MALFORMATIONS:	0	2	0	6	0	2	0	4

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 10 MG/KG/DAY 4- 30 MG/KG/DAY

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL AT 0.05 LEVEL USING FISHER'S EXACT TEST

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TABLE 6

STUDY NO.: TOX-598
COMPOUND: 311C90

A TERATOLOGY STUDY IN RABBITS GIVEN 311C90 BY GAVAGE
PERCENT OF FETUSES AND LITTERS WITH MALFORMATIONS

PAGE

DAY

DOSE GROUP:	FETUSES 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED EXTERNALLY	87	97	77	85	14	16	14	15
SPINA BIFIDA	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0
NUMBER EXAMINED VISCERALLY	87	97	77	85	14	16	14	15
INTERVENTRICULAR SEPTAL DEFECT	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0
COMMON TRUNCUS ARTERIOSUS	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0
NUMBER EXAMINED SKELETALLY	87	97	77	85	14	16	14	15
STERNEBRAE FUSED	0.0	0.0	0.0	4.7	0.0	0.0	0.0	20.0
RIB ANOMALY	0.0	0.0	0.0	3.5	0.0	0.0	0.0	13.3
RIB ANOMALY WITH OR WITHOUT VERTEBRAL ANOMALY	0.0	0.0	0.0	1.2	0.0	0.0	0.0	6.7
TOTAL MALFORMATIONS								
PERCENT WITH EXTERNAL MALFORMATIONS:	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0
PERCENT WITH SOFT TISSUE MALFORMATIONS:	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0
PERCENT WITH SKELETAL MALFORMATIONS:	0.0	0.0	0.0	7.1	0.0	0.0	0.0	26.7
TOTAL PERCENT WITH MALFORMATIONS:	0.0	2.1	0.0	7.1	0.0	12.5	0.0	26.7

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 10 MG/KG/DAY 4- 30 MG/KG/DAY

Variations (see sponsor table 7 below)

While the sponsor states that there were no statistically significant ($p<0.05$) variations in the fetuses, the data intable 7 indicate that there was certainly trend toward in an increase in major vessel variation (3.4% control fetuses, 20.0% high dose fetuses) and irregular ossification pattern of rib(s) (9.0% control fetuses, 15.3% high dose fetuses).

TABLE 7

STUDY NO.: TOX-598
COMPOUND: 311C90

A TERATOLOGY STUDY IN RABBITS GIVEN 311C90 BY GAVAGE
PERCENT OF FETUSES AND LITTERS WITH VARIATIONS

PAGE

DAY

DOSE GROUP:	FETUSES 1	2	3	4	LITTERS 1	2	3	4
NUMBER EXAMINED EXTERNALLY	87	97	77	85	14	16	14	15
NO FINDINGS								
NUMBER EXAMINED VISCERALLY	87	97	77	85	14	16	14	15
MAJOR BLOOD VESSEL VARIATION	3.4	2.1	9.1	20.0	21.4	12.5	28.6	33.3
INCOMPLETE SEPARATION OF LIVER LOBES	0.0	0.0	0.0	1.2	0.0	0.0	0.0	6.7
NUMBER EXAMINED SKELETALLY	87	97	77	85	14	16	14	15
13TH FULL RIB(S)	43.7	57.7	44.2	43.5	92.9	75.0	85.7	73.3
13TH RUDIMENTARY RIB(S)	31.0	18.6	28.6	28.2	92.9	75.0	71.4	73.3
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	5.7	7.2	7.8	5.9	28.6	31.3	21.4	26.7
IRREGULAR OSSIFICATION PATTERN OF RIB(S)	8.0	4.1	6.5	15.3	28.6	25.0	28.6	66.7
HYOID UNOSSIFIED	1.1	0.0	1.3	0.0	7.1	0.0	7.1	0.0
25 PRESACRAL VERTEBRAE	0.0	0.0	0.0	1.2	0.0	0.0	0.0	6.7
14TH RUDIMENTARY RIB(S)	0.0	0.0	0.0	1.2	0.0	0.0	0.0	6.7
HYOID ARCH(ES) BENT	0.0	1.0	0.0	0.0	0.0	6.3	0.0	0.0

1- 0 MG/KG/DAY 2- 3 MG/KG/DAY 3- 10 MG/KG/DAY 4- 30 MG/KG/DAY

Toxicokinetics data

Toxicokinetics data for 311C90 parent drug and it's metabolites are shown below in sponsor tables from Appendix 3:

311C90 - 3mg/kg/day (BPAT/93/0031 - TOX 608)		
	AUC_{0-t} (µg/ml x h)	Cmax (ng/ml)
311C90	0.10	40
1652W92	0.03 (AUC_{0-2})	19
183C91	NE	9 (n=1)
2161W92	0.18	74

311C90 - 10mg/kg/day (BPAD/93/0018 - TOX 583)		
	$AUC_{0-\tau}$ (µg/ml x h)	Cmax (ng/ml)
311C90	1.3	389
1652W92	0.2	61
183C91	NE	11
2161W92	0.6	214

NE - not estimated as concentrations were low.

311C90 - 30mg/kg/day (BPAT/93/0018 - TOX 583)		
	$AUC_{0-\tau}$ (µg/ml x h)	Cmax (ng/ml)
311C90	4.9	1164
1652W92	0.6	226
183C91	0.16	67
2161W92	4.3	1102

Blood samples were collected at 2 hours post dose on gestation day 18 and plasma levels of 311C90 and metabolites determined (see previous results summary table under "Results" in this section of my review). In order to compare AUCs for the pregnant New Zealand rabbits to those at the human mrdd (15 mg; 160 ng.h/ml), the sponsor actually used AUCs from two other teratology studies, Tox 583 and another study, Tox 608, both also in New Zealand White rabbits. Those data are shown in the above three sponsor tables from Appendix 3.

These data show that at the high dose, animals receiving 30 mg/kg/day drug received AUCs of 4,900 ng.h/ml, which is about 31-fold greater than at the mrdd in humans. At the low dose, 3 mg/kg/day, pregnant rabbits presented with AUCs in the 100 ng.h/ml range, which is in the range of the mrdd and therefore should provide relevance to the proposed maximum clinical dose.

Data also show that AUCs and Cmax for 311C90 parent drug and two of the metabolites

(1652W92 and 183C91) increase in an approximately dose-proportional manner when the dose is increased from 10 to 30 mg/kg/day. However, the AUC for metabolite 2161W92 increases in a supra-proportional manner (3-fold increased in dose resulting in 7-fold increase in AUC).

Dose comparison to human mrdd

The human mrdd is 15 mg/day, with an AUC of about 160 ng.h/ml. In this rabbit teratology study, there was a statistically significant decrease in pregnant female body weights (90%; 0<0.05) on treatment days 6-18 of gestation, and there was also a decrease in food consumption in the high dose animals. There was also about a 44% decrease in body weight gain at the 10 mg/kg/day dose. These data are consistent with an MTD for the pregnant rabbits on the order of 10 mg/kg/day, which gave an AUC in the range of 1,300 ng.h/ml, about 8-fold greater than the mrdd for humans. At the low dose of 2 mg/kg/day exposures in pregnant females were on the order of 100 ng.h/ml, which is in the range of the human at the mrdd (160 ng.h/ml). At the high dose, which by these data is above the MTD, the animals were exposed to AUCs on the order of 4,900 ng.h/ml, which is about 31-fold greater than at the mrdd in humans.

A strict NOEL for this study, based on the early resorptions, would be 3 mg/kg/day. This gives an AUC in the range of the human at the mrdd. However, as the sponsor stated, the majority of those early resorptions at both doses (9 of 12 at 10 mg/kg/day; 8 or 11 at 30 mg/kg/day) occurred in a single female animal. Based on other effects, such as skeletal abnormalities, the NOEL would be 10 mg/kg/day, which provided an AUC about 8-fold greater than the mrdd for humans.

Summary and conclusion for 2nd rabbit teratology study

The study design was consistent with the ICH guidelines for a teratology study. Clinical signs included ataxia and labored breathing, mainly in high dose animals. Mean body weight change was significantly less in the mid- and high dose pregnant females during various time intervals, supporting an MTD of 10 mg/kg/day, or at least an MTD somewhere between 10 and 30 mg/kg/day (only the decrease in body weight gain at the high dose was statistically significantly different than controls ($p<0.05$) due to the large variability in body weight changes). These data indicated that the high dose of 30 mg/kg/day may have actually been a little too high, and 10 mg/kg/day may have been sufficiently high for rabbits with respect to an MTD. 10 mg/kg/day gave AUCs about 8-fold higher than in humans at the mrdd, and 30 mg/kg/day gave AUCs about 31-fold greater.

There were no effects on the number or percentage of pregnant female rabbits, number of viable fetuses, late resorptions, implantation sites, corpora lutea, fetal weights or fetal lengths. The number or early resorptions (and thus implantation losses) increased at the mid and high doses, but the majority of these early resorptions 9/12 mid dose; 8/11 high dose) occurred in a single pregnant female. This effect could be explained by maternal toxicity in the high dose female, as her body weight was considerably less than mean weight of either control or other high dose animals. However, this was not the case with the mid-dose animal. The NOEL for early resorptions was 3 mg/kg/day, which gives an AUC of about 100 ng.h/ml for 311C90 parent drug. This is in the same range of exposure as in humans at the mrdd (160 ng.h/ml).

There were also a number of fetal skeletal abnormalities, but only at the high dose (30 mg/kg/day). The sponsor pointed out that 3 of these skeletal abnormalities were from a single high dose doe, and that there was a considerable (90%) and statistically significant decrease in body weight gain in the pregnant females in the high dose group, suggesting that this effect could be due to maternal toxicity. I concur with this, especially in light of the overall body weight data that suggest that the MTD is actually at 10 mg/kg/day, and the 30 mg/kg dose may be well above the MTD.

While the sponsor concluded that there were no statistically significant variations in the fetuses, data indicated that there was certainly a trend toward increase in major vessel variation and irregular ossification pattern of rib(s), but again this was only at the high dose (see Table 7). The NOEL for skeletal abnormalities, major vessel variations and irregular ossification pattern of ribs is 10 mg/kg/day, which gave an AUC for 311C90 parent drug of 1300 ng.h/ml, which is about 8-fold greater than in humans at the mrdd.

With respect to toxicokinetics, AUCs for parent drug and two metabolites (1652W92 and 183C91) appear to increase proportionally with dose on Day 18 of gestation. However, metabolite 2161W92 appears to increase in a supra-proportional manner when the dose increases from 10 to 30 mg/kg/day (note: toxicokinetics data for the 3 mg/kg/day dose is from a different study than the 10 and 30 mg/kg/day doses, so I am not very confident in comparing to the higher doses).

The only effect of concern in this study was the early fetal resorptions, with a NOEL of 3 mg/kg/day, and with the exception of a single mid dose animal, these could be explained by maternal toxicity (decreased body weights in pregnant females).

4. Pre- and Postnatal development study in rats given 311C90 by gavage (study number TTEP/96/0019), Glaxo Wellcome R&D, (Tox 687), July 3, 1996, GLP.

Animals: Sprague-Dawley rats (male and female); females approx 12 weeks old;

Drug: 311C90 Batch #311C90WR in 0.5% methylcellulose (vehicle=0.5% methylcellulose.

Study design:

Three groups of 25 bred female CD rats received 311C90 by daily oral gavage (0, 25, 100 or 400 mg/kg/day) from Day 6 of pregnancy (day of mating=day 0 of pregnancy) until lactation day 20 (day of littering=lactation day 0). A similar group received vehicle alone.

Animals allowed to deliver young spontaneously and (following culling on lactation day 4) to rear them to weaning. All littering females euthanized on day 21 of lactation. Necropsy was performed and implantation scars counted. Development of F1 offspring was assessed until completion of all developmental markers. Upon completion of these observations, minimum of 1 male and 1 female were selected from each F0 litter, all remaining pups and dams euthanized, and post mortem exams performed. Selected F1 offspring were allowed tor each sexual maturity and mated within dosage groups. F1 females were euthanized on gestation day 20 and F2 fetuses examined externally.

Observations and measurements

F0 (parental): clinical signs, body weights, food consumption, littering, litter retrieval.

F1 Offspring: survival, growth, developmental milestones (pinna detachment, surface righting, eye opening, startle response, vaginal opening or cleavage of the balanopreputial gland), behavior (open field, swimming M-maze), opthalmology, reproductive capacity.

F2 Fetuses: external examinations.

Pharmacologist's Comment: This study design is consistent with the ICH guidelines for a pre- and postnatal reproductive toxicology study.

Results:

A summary of study results is shown in the following two sponsor tables:

No. F0 Females/group	Dosages mg/kg/day	F0 Females with sperm	F0 Females with delivery	Mean duration of gestation (days)
25	0 (control)	25	20	21.7
25	25	25	22	21.9
25	100	25	22	21.8
25	400	25	23	21.7
Generation of parental animals: F0 Study in compliance with GLP: Yes			Sperm in vaginal smear = Day 0 of gestation Day of birth = Day 0 of lactation	
Parameters Evaluated: F0 (parental) - Clinical signs, body weight, food consumption, littering, litter retrieval F1 Offspring - Survival, growth, developmental milestones (pinna detachment, surface righting, eye opening, startle response, vaginal opening or cleavage of the balanopreputial gland), behaviour (open field, swimming M-maze), ophthalmology, reproductive capacity F2 Fetuses - External examination			Batch No.: zolmitriptan WR Reference No. 93/0019-121-D	

Parent (F0) Dosage mg/kg/day	No. of F1 litters evaluated	F1 Litters (arithmetic mean per litter)						
		Live births	Survivors on Day 4 pp before culling	Survivors on Day 4 pp after culling	Survivors at weaning (Day 21 pp)	Weight on Day 1 pp Males/ Females(g)	Weight at weaning Males/ Females(g)	Sex ratio of live newborns (M:F)
0 (control)	20	15.1	14.7	8.0	7.6	6.9/6.6	62.8/60.2	1.3
25	22	14.5	14.4	8.0	7.7	6.8/6.6	63.4/61.3	1.5
100	22	13.4	13.1	7.5	7.0	6.8/6.7	61.0/58.4	1.2
400	23	14.3	13.9	7.9	7.7	7.1/6.6	62.0/59.3	1.3

Dosage mg/kg/day	0 (control)	25	100	400
F0 Females Body weight change, gestation days 6-9 (g) arithmetic mean	11.1	9.0	6.0[a]	6.2[a]
Food consumption, gestation days 6-7 (g) arithmetic mean	26.8	25.4	24.2	23.6[a]
F1 Offspring Total No. Stillborn	5	1	0	3
Total No. Dead, lactation (postpartum 1-21)	3	4	5	4
Total No. Dead, post-weaning	0	0	0	2[b]

a $p < 0.05$.

b One F1 male died on postpartum day 44 during the M-maze trials (possibly due to aspiration of water); and one high dose F1 female selected for reproductive performance died on calculated gestation day 20 (postpartum day 112).

Clinical signs and mortality

F0 dams during gestation and lactation

Post-dose salivation sporadically in mid-dose (100mg/kg) and high dose (400 mg/kd) animals.
3 control dams died (probably dosing accident) during gestation.

F1 pups during postpartum

Three pups (2M, 1F) from same pregnant dam (10599) in mid dose group (100 mg/kg) presented with splayed hindlimbs, abnormal gate, dragging hindlimbs-so severe animals were sacrificed moribund postpartum day 20-21. Sponsor states that since from only single dam and at mid dose only, probably not treatment related.

Single female pup (high dose; 400 mg/kg)-macrophthalmia right eye), severe opacity and protrusion of right eye. Eye ulcerated on postpartum day 49 and was sacrificed early.

F1 dam selected to breed to F2

Single pregnamt F1 dam died day 20 of gestation (gravid with 16 implants); cause of death undetermined.

Body weights

F0 generation dams during gestation

Table 3. Mean Body Weight Changes (Grams) During Gestation: F0 Generation Dams

TABLE 3
STUDY NO.: TOX-687 — PRE-AND POSTNATAL DEVELOPMENT STUDY IN RATS GIVEN 311C90
COMPOUND:311C90 — MEAN BODY WEIGHT CHANGES (GRAMS) DURING GESTATION
SPONSOR:DOW — F0 Generation Dams

DOSE GROUP:	0 MG/KG/DAY	25 MG/KG/DAY	100 MG/KG/DAY	400 MG/KG/DAY
DAY 0- 6	28.5	33.3	34.7	31.5
DAY 6- 9	11.1	9.0	6.0*	6.2*
DAY 9- 12	16.3	15.8	14.7	14.1
DAY 12- 15	18.9	16.8	17.8	19.2
DAY 15- 18	36.7	33.2	36.0	36.2
DAY 18- 20	24.5	21.5	21.7	23.2
DAY 6- 20	107.1	96.3	98.2	98.9
DAY 0- 20	137.3	129.7	132.9	130.4

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST
MEAN DIFFERENCES CALCULATED FROM INDIVIDUAL DIFFERENCES

Pregnant rats at 100 or 400 mg/kg/day drug gained significantly ($p<0.05$) less weight (46%) than controls on gestation days 6-9, the first three days of dosing. There were no significant difference in mean body weight changes over the entire dosing period (days 6-20 of gestation).

F0 generation dams during lactation

Low dose (25 mg/kg) dams gained significantly more weight than controls on lactation days 14 and 21, while high dose lactating dams ans gained significantly ($p<0.05$) more weight on postpartum days 7-14.

F1 generation pups during postpartum

No effect on body weights.

Pharmacologist's comment: In Table 6 (vol 48, pg 101), the number of F1 female litters declines dramatically on about day 98, or at least they are no longer being included in the body weight data. Presumably this is because pregnant female animal body weights are no longer relevant to this data set, as the concern is for the effect of drug treatment, and not pregnancy, on F1 female body weights.

However, my concern is that the number of F1 female LITTERS declines beginning on day 91 and continuing on day 98 and thereafter. The protocol states that the sponsor will take 1 male and 1 female from each F1 litter and use these to examine the reproductive capability of the F1 generation. Therefore, while the number of animals per litter should decline, the total number of litters should theoretically remain the same. These data raise the question in my mind of whether or not the sponsor actually used a single male and female animal from each litter (up to a total of 25 pairs) to examine F1 generation reproductive capacity.

F1 generation dams selected to breed F2 generation

No effect on body weights or body weight gain.

Food consumption

F0 generation dams during gestation

High dose (400 mg/kg/day) animals consumed less (12%; $p<0.05$) food than control dams (gestation days 6-7 only).

F0 generation dams during lactation

Mid dose (100 mg/kd/day) dams consumed less (11%; $p<0.05$) than controls on lactation days 4-5.

F0 Generation littering dams and F1 generation pups-observations during lactation

Pregnancy and litter data

No effect on duration of gestation, number of live pups, sex ratio or viability index. There was a slight increase in the post-implant loss (control, 7.3%; 25 mg/kg, 7.7%; 100 mg/kg, 9.2% and 400 mg/kg, 11.8%). However, due to fairly large within-group variability, this was not a statistically significant ($p<0.05$) difference. The lactation index (% pups surviving to weaning on postpartem day 21) was actually significantly ($p<0.05$) higher at the high dose (400 mg/kg/day) than in control animals.

F1 generation dead pups external, visceral and skeletal findings

Results are outlined in the following sponsor's table (vol. 48, pg. 86):

Fate	0 (Control) mg/kg/day	25 mg/kg/day	100 mg/kg/day	400 mg/kg/day
Stillborn	5	1	0	3
Dead	3	4	5	4[a]
Sacrificed	0	0	4	1
Missing	6	5	7	5

[a] An additional high dose F1 male died on postpartum day 44 during the M-maze trials (possibly due to aspiration of water); and one high dose F1 female selected for reproductive performance died on calculated gestation day 20 (postpartum day 112).

Sacrificed moribund

100 mg/kg/day: 4 pups sacrificed moribund; 3 from same litter with splaying of hindlimbs and abnormal gait with dragging of hind limbs; oen male pup with laceration on its head.

Found dead

No external, visceral or skeletal findings on animals found dead during lactation period of study. Cause of death was undetermined for animals that did die. A number of the dead animals were cannibalized and gross necropsy was not possible. Also, some of the animals were too autolyzed to receive visceral examination.

Litter retrieval test

No effect on litter retrieval.

Pinna detachment

No effect.

Surface righting response

No effect.

Eye opening

No effect.

Startle response

No effect.

Necropsy exams

single F0 generation dam at 100 mg/kg/day had firm subcutaneous mass in right axillary area; not examined histologically.

F1 Generation pups, postweaning observations and behavioral tests

No effect on auditory function test, ophthalmology exam, open field test, swimming M-maze, vaginal opening, or cleavage of balanopreputial gland (males).

Necropsy:

Animals sacrificed or found dead postpartum day 70

Controls (10 F): no hydronephrosis
25 mg/kg (9 F): no hydronephrosis
100 mg/kg (9F, 1M): 1 (M) hydronephrosis (moderate, left)
400 mg/kg (7M, 11F): 3 (M) hydronephrosis (bilateral)
4 (M) hydronephrosis (right)
3 (F) hydronephrosis (right)
1 (F) hydronephrosis (bilateral)

Animals sacrificed or found dead postpartum day 140

1 Control with hydronephrosis, left
1 @100mg/kg/day with hydronephrosis, right
1 @400 mg/kg/day with hydronephrosis, bilateral

Pharmacologist's comment: a number of F1 generation pups presented with hydronephrosis, dilation of the pelvis and calices of one or both kidneys (indicative of obstruction to the flow of urine flow), on day 70 postpartum. This actually appeared to occur in a dose-related manner, in both male and female animals. The sponsor overlooked these results in the "results" section of their report, and no explanation was offered for this finding.

It is of some concern that hydronephrosis was found in 7 high dose male pups sacrificed on postpartum day 70 and in 4 female high dose animals sacrificed at this time. No explanation was offered, and no histopathological data were included to determine whether or not there were histopathological effects that might explain this. These 11 animals presenting with hydronephrosis were from a total of about 140 animals (20 litters, about 7 pups per litter) at the high dose, although it is unclear to me from the protocol as to how many animals per dose group were actually sacrificed at day 70 postpartum. I believe that all but a single pair (one male, one female) of animals per litter were sacrificed at this time.

The sponsor did mention the day 140 results in which hydronephrosis was found at low levels, and in one control animal as well.

The sponsor's table summarizing these data 70 days postpartem is shown below:

Table 25. Individual Necropsy Findings of Dead and Sacrificed Animals – F1 Generation Pups Sacrificed on Postpartum Day 70/F1 Generation Male Pups Sacrificed on Postpartum Day 140

Pre- and Postnatal Development Study in Rats Given 311C90 by Gavage

Individual Necropsy Findings of Dead and Sacrificed Animals

A. F1 Generation Pups Sacrificed on Postpartum Day 70

Group	Dose (mg/kg/day)	Animal No. (Prefix 94)	Pup No.	Sex	Finding
1	0	10531	6	F	Hydrometra, Moderate, Bilateral
1	0	10539	5	F	Hydrometra, Moderate, Bilateral
1	0	10541	6	F	Enlarged Kidneys, Bilateral
1	0	10545	4	F	Hydrometra, Moderate, Bilateral
1	0	10545	8	F	Hydrometra, Moderate, Bilateral
1	0	10547	8	F	Hydrometra, Moderate, Bilateral
1	0	10547	5	F	Hydrometra, Moderate, Bilateral
1	0	10549	6	F	Hydronephrosis, Bilateral
1	0	10550	5	F	Hydrometra, Moderate, Bilateral
1	0	10550	6	F	Hydrometra, Moderate, Bilateral
2	25	10555	6	F	Hydrometra, Moderate, Bilateral
2	25	10556	7	F	Hydrometra, Moderate, Bilateral
2	25	10558	6	F	Hydrometra, Moderate, Bilateral
2	25	10567	7	F	Hydrometra, Slight, Bilateral
2	25	10571	5	F	Hydrometra, Moderate, Bilateral
2	25	10573	5	F	Hydrometra, Moderate, Bilateral
2	25	10573	6	F	Hydrometra, Moderate, Bilateral
2	25	10573	8	F	Hydrometra, Moderate, Bilateral
2	25	10575	7	F	Hydrometra, Moderate, Bilateral
3	100	10578	1	M	Hydronephrosis, Moderate, Left
3	100	10578	3	F	Hydrometra, Moderate, Bilateral
3	100	10578	4	F	Hydrometra, Moderate, Bilateral
3	100	10578	6	F	Hydrometra, Moderate, Bilateral
3	100	10579	1	F	Hydrometra, Moderate, Bilateral
3	100	10580	6	F	Hydrometra, Moderate, Bilateral
3	100	10594	8	F	Hydronephrosis, Right
3	100	10595	8	F	Hydrometra, Moderate, Bilateral
3	100	10596	4	F	Hydrometra, Moderate, Bilateral
3	100	10597	6	F	Hydrometra, Moderate, Bilateral
4	400	10608	2	M	Hydronephrosis, Bilateral
4	400	10608	4	M	Hydronephrosis, Bilateral
4	400	10612	1	M	Hydronephrosis, Bilateral
4	400	10612	4	M	Hydronephrosis, Right
4	400	10614	2	M	Hydronephrosis, Right
4	400	10616	4	M	Hydronephrosis, Right
4	400	10623	4	M	Hydronephrosis, Right
4	400	10606	8	F	Hydrometra, Moderate, Bilateral
4	400	10608	6	F	Hydronephrosis, Right
4	400	10608	8	F	Hydronephrosis, Right
4	400	10609	8	F	Hydrometra, Moderate, Bilateral
4	400	10612	5	F	Hydronephrosis, Bilateral
4	400	10612	6	F	Hydronephrosis, Right
4	400	10615	8	F	Hydrometra, Moderate, Bilateral
4	400	10616	6	F	Hydrometra, Moderate, Bilateral
4	400	10620	5	F	Hydrometra, Moderate, Bilateral
4	400	10620	6	F	Hydrometra, Moderate, Bilateral
4	400	10625	8	F	Hydrometra, Moderate, Bilateral

Footnote: Litter No. 94-10544 (Group 1) – Female pups No. 5, 6 and 8 were found to be pregnant at Lactation Day 70 necropsy. Male pup No. 1 was found in the female cage (shoe box) 8 days earlier. Otherwise their necropsy findings were nonremarkable.

F1 generation pups-reproductive capacity observations

F1 generation reproductive performance

As shown in sponsor's table 26 below (vol. 48, pg. 131), there were no effects on F1 mating or pregnancy rate of F1 generation animals.

Summary of F1 Generation Reproductive Performance

Group/Dose Level (mg/kg/day)	1/0	2/25	3/100	4/400
No. of Females Paired	25	25	25	25
No. Females Which Showed Evidence of Mating	24	23	24	25
No. Pregnant	23	19	18	21*
No. Females Which Did Not Show Evidence of Mating	1	2	1	0
No. Pregnant	0	1	0	-
Total No. Pregnant Females	23	20	18	21
Fertility Index	92%	80%	72%	84%
Mean Pre-coital Interval (Days)	3.2	3.5	4.9	3.2
± S.D.	2.53	4.21	4.77	1.29
N	24	23	24	25

$$\text{Fertility Index (\%)} = \frac{\text{Total No. Pregnant Females}}{\text{No. of Females Paired With Males}} \times 100$$

*No. 94-10623 Pup 7 was found dead on gestation day 20, this female was gravid.

F1 generation dams selected to breed F2 generation

At the 100 mg/kg/day dose, the ratio of males to females was significantly ($p<0.05$) altered in favor of males, although this did not appear at the high dose.

No effect on viable fetuses per dam, post-implantation loss, number of implantations or corpora lutea per dam or fetal weights and lengths were seen in F2 pups.

At the mid dose group, 7 fetuses from the same litter presented with fetal anasarca (generalized infiltration of edema fluid into subcutaneous connective tissue). A single fetus at the high dose showed this effect.

F1 generation dams sacrificed on gestation day 20

3 mid dose (100 mg/kg) and 3 high dose (400 mg/kg) F1 generation dams presented with hydronephrosis when sacrificed on gestation day 20.

Toxicokinetics results

No toxicokinetics data were included with this study. However, based on results of the rat oral teratology study (CD-1 rats), in which animals were administered similar doses of drug (100 and 400 mg/kg/day) by daily oral gavage during days 6 through 15 of gestation, rats at 100 mg/kg/day experienced AUCs on the order of 20,000 ng.h/ml of parent 3111C90 drug, which is about 125-fold greater than humans at the mrdd. At 400 mg/kg/day, the rats most likely experienced AUCs on the order of 114,000 ng.h/ml, which is about 712-fold greater than at the mrdd in humans.

Dose comparison with mrdd in humans

With respect to an MTD, pregnant F0 rats at the mid and high doses gained about 46% less weight than controls on gestation days 6-9 (statistically significant $p<0.05$). High dose animals also consumed less food over this same time period. Based on these transient effects on body weight gain, an MTD of 100 mg/kg/day could be established. However, overall Days 0-20 or on treatment days 6-20 of gestation there was no difference in body weight gain between the various treatment groups and controls. At a dose of 100 mg/kg/day, animals were exposed to AUCs of 311C90 parent of about 29,000 ng.h/ml (based on findings in the oral rat teratology study), which is about 181-fold greater than in humans at the mrdd. At the high dose of 400 mg/kg/day, animals were probably exposed to levels of about 114,000 mg/kg/day, which is about 712-fold greater than humans at the mrdd.

The NOEL, based on the report of F1 generation pups with splayed hindlimbs and findings of hydronephrosis in F1 generation pups is 25 mg/kg/day. One would predict, based on toxicokinetics data from the oral teratology study in rats, an AUC of about 7,500 ng.h/ml for animals receiving this dose. This is about 47-fold higher than the AUC at mrdd (160 ng.h/ml). There is some question as to the validity of this NOEL, because the splayed hindlimbs findings only occurred at the mid dose (not the high dose), and mainly in three animals from the same litter. Also the hydronephrosis most likely occurred at a low incidence, although it is unclear how many animals were actually sacrificed in the groups where this effect was reported.

Summary and conclusions regarding pre- and postnatal rat reprotoxicology study

This study design was consistent with the recommendation in the ICH guidelines for studies of effects on pre- and postnatal development. Clinical signs in F0 dams included only post-dose salivation. Animal weights were transiently decreased (46%) in mid and high dose animals on gestation days 6-9, which could support an MTD of 100 mg/kg/day (with an AUC of about 29,000 ng.h/ml, about 181-fold higher than in humans at the mrdd; predicted from the oral rat teratology study). Three F1 pups (postpartum) from the same pregnant mid dose (100 mg/kg) dam presented with splayed hindlimbs, abnormal gate, and dragging hindlimbs so severe they were sacrificed moribund. However, this was probably not a significant toxicological finding because all three animals were from the same dam and because no such findings were reported in the high dose animal group. The only other finding of concern was the report of hydronephrosis in F1 generation pups, mainly at the high dose. It is unclear how many animals were included in these sacrifice groups, but the incidence of hydronephrosis was probably fairly low (11 animals in a total of about 140 at the high dose). Considering these two possible drug effects, a NOEL of 25 mg/kg/day would be

appropriate, which would give a predicted (from oral rat teratology study toxicokinetics) AUC on the order of 7,500 ng.h/ml, about 47-fold higher than in humans at the mrdd. However, the toxicological significance of the splayed hindlimb effects is questionable because it happened mainly in 3 pups from a single litter and only at the mid dose. The toxicological significance of the hydronephrosis is of more concern, since it was clearly a dose-dependent effect. However, this occurred at a fairly low incidence, and is know to occur in certain rat strains in absence of treatment.

Genetic Toxicology

The following diagram shows a summary of the genetox studies for this NDA:

Genetic toxicology studies

Study	Species	No./Group (M/F)	Doses	Duration	Ref No
Ames Assay	*Salmonella typhimurium*	NA	Up to 3160µg/Plate	NA	59
Clastogenicity in Human lymphocytes	*in vitro*	NA	Up to 2000µg/ml	NA	60
Chinese Hamster Ovary Cells	*in vitro*	NA	Up to 3600µg/ml	NA	61
Unscheduled DNA synthesis	Rat *in vivo* *in vitro*	5 males	500, 750, 1000 mg/kg	Single dose	62
Micronucleus Assay	Mouse	5/5	0, 100, 300, 500 mg/kg/orally	3 days	63

NA = Not Applicable

1. 311C90: Evaluation for mutagenicity using Ames Salmonella/microsome incorporation test and the Yahagi modification, study #BPAT/91/0048, The Wellcome Foundation Ltd, Beckenham, Kent, UK, Sept. 4, 1992, GLP.

Test strains: Salmonella typhimurium TA1535, TA1537, TA1538, TA98, TA100

Pharmacologist's comment: The use of S. typhimurium strains TA1535, TA1537, TA98 and TA100 are consistent with the 1994 OECD guidelines. However, those guidelines also recommend the use of E. Coli WP2 uvrA, E. Coli WP2 uvrA pKM101, or S. typhimurium TA 102 to detect certain oxidizing mutagens, cross-linking agents and hydrazines that are not detected by the other S. typhimurium strains.

Toxicity screen: The protocol states that the sponsor would carry out a toxicity screen to determine the appropriate dose-range to test based on decreased bacterial cell viability. In the study report, they state that "at a concentration of 10000 µg/plate, toxicity towards the tester strains precluded further evaluation of results." Therefore, apparently a concentration of 10000 µg/plate was the limiting toxicity. However, no data were submitted to support this statement. It is recommended that this toxicity screen be carried out in cultures both +S9 and -S9.

Study procedures: Other study procedures were consistent with the OECD guidelines for genetic toxicology testing (1994).

Results:

Strain TA1535

Negative results in repeat studies; appropriate positive controls (sodium azide and 2-aminoanthracene) used, resulting in appropriate responses.

Strain TA1537

Negative results in repeat studies; appropriate positive controls (2-aminoanthracene and 9-aminoacridine) used, resulting in appropriate responses.

Strain TA1538

Study #1 2/3/92 Ames Test and Yahaghi modification (preincubation with drug)
Results are shown below:

Table 3

Laboratory Ref: 1438
Test Type: Ames Test
S9Batch: 66 Rebens
Test Date: 28.2.92
Direct Control : Hycanthone (HYC)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 1538
Reading Date: 02/03/92
Reading Time: 09:36

Compound Batch Number: WDCP 91/0017/104/1 01R
Compound Source: Mr.S. Salmon
DSE
Beckenham.
Compound Name: 311C90
Comment: No comment
0

Proc	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	121	6	P	131(M)	14	P
		122	18	P	132(M)	22	P
		123	13	P	133(M)	24	P
		MEAN	12		MEAN	21	
.	100	124	10	P	134(M)	11	P
		125	21	P	135(M)	30	P
		126	10	P	136(M)	82	P
		MEAN	14		MEAN	48	
.	316	127	14	P	137(M)	48	P
		128	18	P	138(M)	31	P
		129	23	P	139(M)	11	P
		MEAN	17		MEAN	30	
.	1000	130	18	P	140(M)	17	P
		131	21	P	141(M)	91	P
		132	10	P	142	76	P
		MEAN	16		MEAN	61	
.	3160	133	12	P	143	718	P
		134	3	P	144	687	P
		135	8	P	145	627	P
		MEAN	8		MEAN	677	
.	10000	136	4	R	146	88	R
		137	7	R	147	45	R
		138	0	R	148	88	R
		MEAN	4		MEAN	34	
	HYC	139	191	P	149	160	P
		140	138	P	150	149	P
		141	178	P	151	118	P
		MEAN	169		MEAN	142	
	2AA	142	11	P	152	1412	P
		143	11	P	153	1411	P
		144	22	P	154(M)	1344	P
		MEAN	16		MEAN	1389	
CONTAMINATION		145(M)	0		155(M)	0	
		146(M)	0		156(M)	0	
		147(M)	0		157(M)	0	
HISTIDINE		148(M)	-		158(M)	+	
		149(M)	+		159(M)	+	
		150(M)	+		160(M)	+	

KEY for BACKGROUND GROWTH

P - present
R - reduced

Signed: GTGrime
Witnessed:
Date: 3/2/92

Table 10

Laboratory Ref: 1438
Test Type: Yahaghi Modification
S9Batch: 66 Rebens
Test Date: 28.2.92.
Direct Control : Hycanthone (HYC)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 1538
Reading Date: 02/03/92
Reading Time: 11:33

Compound Batch Number: WDCP 91/0017/104/1
Compound Source: Mr.S.Salmon
DSE
Beckenham.
Compound Name: 311C90
Comment: No comment
-

Proc	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	121	16	P	131	21	P
		122	18	P	132	16	P
		123	12	P	133	21	P
		MEAN	15		MEAN	19	
.	100	124	13	P	134	20	P
		125	11	P	135	24	P
		126	13	P	136	18	P
		MEAN	12		MEAN	21	
.	316	127	17	P	137	21	P
		128	10	P	138(M)	19	P
		129	18	P	139(M)	18	P
		MEAN	18		MEAN	19	
.	1000	130	21	P	140	25	P
		131	23	P	141	12	P
		132	17	P	142(M)	22	P
		MEAN	20		MEAN	20	
.	3160	133	12	P	143	36	P
		134(M)	11	P	144	48	P
		135	22	P	145	30	P
		MEAN	15		MEAN	36	
.	10000	136	7	R	146	172	
		137	9	R	147	120	
		138	3	R	148	358	R
		MEAN	6		MEAN	217	
	HYC	139	244	P	149	279	P
		140	261	P	150	299	P
		141	278	P	151	333	P
		MEAN	261		MEAN	311	
	2AA	142	83	P	152	1918	P
		143	10	P	153	2134	P
		144	16	P	154	2321	P
		MEAN	16		MEAN	2187	
CONTAMINATION		145(M)	0		155(M)	0	
		146(M)	0		156(M)	0	
		147(M)	0		157(M)	0	
HISTIDINE		148(M)	-		158(M)	-	
		149(M)	-		159(M)	-	
		150(M)	-		160(M)	+	

KEY for BACKGROUND GROWTH

P - present

Signed: GTGrime
Witnessed:

In the first Ames test (sponsor Table 3; vol. 49, pg 30; sponor Table 10; vol. 49, pg 25), a very strong positive response was seen at 1000 µg/plate (+S9; 2.9-fold increase over controls; controls 21, treated 61) and at 3160 µg/plate (+S9; 32-fold increase over controls; control 21; treated 677). This constitutes a very substantial dose-related increase. This increase is not found at the 10000 µg concentration, which may be due to toxicity (Table 15 indicates that the background lawn was reduced at this dose). It is the sponsor's contention that 10000 µg is the concentration at which there is limiting cytotoxicity. The sponsor also contends that for a response to be considered positive, a 2-fold increase is required at 3 doses, but this is not consistent with the OECD guidelines (1994) for Genetic Toxicology Testing.

In the first Yahaghi modification study, an 11-fold increase (217 in treated versus 19 in controls) in the number of colonies is seen at the 10000 µg dose +S9, and a 1.9-fold increase (36 in treated versus 19 in controls) is seen at the 3160 µg concentration. While the 1.9-fold increase does not quite meet the 2-fold increase requirement for a positive, it is very close, and somewhat troublesome due to the much greater increase at 10000 µg. The sponsor states that 10000 µg is the concentration at which limiting toxicity occurs, and Table 19 shows that the background lawn is reduced at this concentration. In both of these initial studies the positive controls gave an appropriate response.

Therefore, in the first set of experiments using Strain TA 1538, a strong positive, dose-related response was seen in the standard Ames test. The Yahagi test yielded a weak positive response at the 3160 µg concentration, and a strong positive response at the highest concentration (10000 µg), the concentration at which the bacterial lawn was reduced suggesting cytotoxicity.