CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20768

PHARMACOLOGY REVIEW(S)

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

NDA Original Review

NDA #: 20,768

Review Date: September 11, 1997

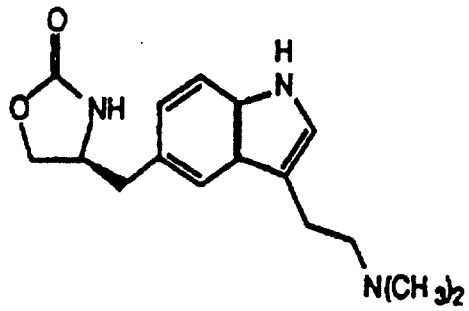
Date of Submission: November 29, 1997

Safety Review Date: November 28, 1998

Sponsor: Zeneca Pharmaceuticals

Drug: ZOMIG™ (Zolmitriptan) 2.5/5 mg. tablets (formerly 311C90 tablets when owned by Burroughs Wellcome)

Structure:



Chemical Name: (S)-4-[3-(2-Dimethylaminoethyl)-1H-indole-5-yl) methyl-2-oxazolidinone

Molecular Formula: MF: $C_{16}H_{21}N_3O_2$

Molecular Weight: 287.36

Chemical Class: Serotonin Analogue

Pharmacological Category: Serotonin 5-HT_1 Receptor Agonist

Indication: Treatment of Acute Migraine Headaches

Related INDs/NDAs: IND

Table of Contents

Subject *Page Number*

Pharmacology....4

Zolmitriptan

Mechanism of action....4

Receptor studies....4

Cranial hemodynamic/extravasation studies....6

Safety pharmacology....7

Cardiovascular....7

CNS....9

Autonomic....9

Respiratory....9

Renal and G.I....10

183C91 Active Metabolite

Receptor studies....11

Cardiovascular safety studies....11

ADME....12

Absorption....13

Mouse....13

Rat....15

Rabbit....16

Dog....17

Human volunteers....18

Distribution....19

Metabolism....21

In Vivo

Human....23

Mouse....23

Rat....23

Rabbit....24

Dog....24

In Vitro....24

Excretion....25

Toxicology Studies....27

Acute....27

Subchronic....28

Chronic....29

26-week rat....29

28-week dog....39

1-year dog....54

Carcinogenicity.....67
Oral mouse study.....67
Oral rat study.....89
Reproductive Toxicology.....104
Reproduction/fertility rat study.....104
Teratology study; rat.....110
Teratology study; rabbit.....116
Teratology study #2; rabbit.....120
Pre/Postnatal development study; rat.....127
Genetic Toxicology.....136
Ames Test.....136
Human lymphocyte assay (clastogenicity).....143
CHO/HGPRT forward mutation assay.....147
Mouse micronucleus test.....150
Unscheduled DNA synthesis test.....153
Miscellaneous Toxicology Studies.....154
Rat study; 311C90 with 0.2% degradation product (420C90).....154
Rat study; intranasal formulation/accelerated degradation.....157
Rat study; assessment thyroid function with 311C90.....158
Rat study; telemetry and respiration with 311C90.....160
Summary and Evaluation.....163
Pharmacology.....163
ADME.....164
Toxicology.....167
Carcinogenicity.....171
Reproductive Toxicology.....174
Genetic Toxicology.....180
Miscellaneous Toxicology Studies.....181
Toxicokinetics.....184
Conclusions.....186
Recommendations.....189
Labeling.....191

Date of Previous Pharmacology Reviews (Amendments):
IND . (reviewed by John J. Jessop, Ph.D., M.P.H., June 10, 1994; Sponsor was Burroughs Wellcome Co.; Zeneca later purchased ZOMIG™ as part of SEC requirement for merger between Glaxo and Burroughs Wellcome)

Proposed Clinical Use:

The recommended dose to treat a migraine attack is 2.5 mg. (oral tablets). If symptoms persist, a second 2.5 mg. tablet can be taken 2 hours after the initial dose, for a total of 5 mg in a 24 hour period. If a patient does not achieve satisfactory relief with 2.5 mg doses, subsequent attacks can be treated with 5 mg doses.

The maximum recommended daily dose of ZOMIG™ should not exceed 15 mg in a 24 our period.

Note: 15 mg in a 60 kg person is 0.25 mg/kg (or 9.25 mg/m^2). According to the sponsor, this dose results in an exposure in humans of about 160 ng.h/ml, which is seen following a dose of 15 mg in man either as a single or divided dose in a 24 hour period.

PHARMACOLOGY

ZOLMITRIPTAN

Mechanism of Action

The pathophysiology of migraine is unclear, but based upon the effects of certain drugs effective in acute migraine treatment it is thought that at least two factors are involved. It is believed that both cranial vasoconstriction and inhibition of trigeminal neuronal activity are involved in successful treatment of migraine. It is now apparent that inhibition of trigeminal neuronal activity is regulated independently from vessel constriction, and that "5-HT_{1D}-like" receptors are involved in both activities. Recent evidence suggests that cranial vasoconstriction if regulated by 5-$HT_{1D\beta}$ receptors, while neuronal inhibitory effects involve 5-$HT_{1D\alpha}$ receptors. Zolmitriptan is a 5-HT_{1D} receptor agonist, and it is based on this activity that it was initially proposed to treat migraine.

Receptor Pharmacology Studies

Fourteen human 5-HT receptor types have been cloned and sequenced. These are divided into seven classes (5-HT_1-5-HT_7) based on structural and functional characteristics. Within the 5-HT_1 subclass there are five subtypes: 5-HT_{1A}, 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E} and 5-HT_{1F}. Furthermore, a "5-HT_{1D}-like" receptor has been identified in certain blood vessels where it mediated vasoconstriction and inhibition of transmitter release from certain perivascular sympathetic or sensory (C-fiber) nerve terminals. It is unknown whether or not this receptor corresponds to the 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ receptor.

The following sponsor Table (vol. 11, pg 12) gives a summary of the receptor profile for zolmitriptan *in vitro*, including both radioligand binding and functional assays:

Receptor profile of zolmitriptan *in vitro*

Receptor	Functional assay	Radioligand binding assay
	p[A_{50}] / max or pK_B [a]	pKi (pIC_{50})
"5-HT_{1D}-like"	6.8/0.77	N/A
5-HT_{1A}	6.4 / 1.00	7.0 (6.5)
5-HT_{1B}	6.7 / 1.00	8.7
5-$HT_{1D\alpha}$	9.9 / 1.00	(9.2)
5-$HT_{1D\beta}$	9.5 / 1.00	(8.2)
5-HT_{2A}	<4.5/-	N/A
5-HT_{2C}	N/A	4.1
5-HT_3	<4.5/-	N/A
5-HT_4	<4.5/-	N/A
5-HT (atypical, endothelial receptor)	<4.5/-	N/A
5-HT (smooth muscle relaxation, 5-ht_7)	5.3/-	N/A
α_1-adrenoceptor	<4.5/-	<4.0
α_2-adrenoceptor	N/A	4.1
β_1-adrenoceptor	4.8/-	N/A
Histamine H_1	5.5/-	N/A
Histamine H_2	<4.5/-	N/A
Muscarinic M_2	5.0/-	N/A
Muscarinic M_3	<4.5/-	N/A
Dopamine D_1	N/A	<4.0
Dopamine D_2	N/A	<4.0

[a] - where zolmitriptan behaved as an agonist, the result is expressed as p[A_{50}] / maximum c.f. 5-HT (=1). When the compound failed to display agonism (indicated by -), it was evaluated as an antagonist and the result expressed as a pK_B value (6, 7).

Functional assays: *in vitro*

rabbit saphenous vein contraction: zolmitriptan affinity p[A_{50}]=6.8>sumatriptan affinity p[A_{50}]=6.5.

dog basilar artery rings: zolmitriptan potency (p[A_{50}]=6.9 similar to effect in rabbit saphenous vein; attained 32% of 5-HT maximum response.

human coronary artery rings: zolmitriptan higher potency p[A_{50}]=7.3 but low maximum response (37% of 5-HT maximum response).

Receptor binding

High affinity for human recombinant 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors in membranes from transfected CHO cells, pIC_{50} 9.2 and 8.2 respectively. Also good specificity expect for 5-HT_{1A} receptors (pIC 6.5).

Cranial hemodynamic and extravasation studies

1. Preclinical studies related to the proposed mechanism for migraine induction
A. Vascular theory: Carotid arterial and intracranial AVA blood flow, vasodilation causes migraine (vasoconstriction to treat).
-dose-related and sustained reduction in anesthetized dog carotid arterial blood flow **(311C90 2-3-fold more potent than sumatriptan)**

Animal	Drug	ED_{50}	maximum reduction
Dog	311C90	2.9±0.8µg/kg	67%
	sumatriptan	6.0µg/kg	70%
Cat	311C90	1.13±0.22µg/kg	39%
	sumatriptan	3.97µg/kg	46%

-dose-related sustained reduction intra-cranial arteriovenous anastomosis blood flow, cat **(ED_{60} 11µg/kg vs sumatriptan, 56µg/kg; 5-fold).**
-since total cerebral blood flow unchanged, sponsor stated that decrease in carotid arterial conductance mainly due to decreased AVA shunting.

B. Neurogenic theory: trigeminal nerve stim--calcitonin gene related peptide (CGRP) and substance P release--increased vascular permeability/vasodilation, plasma protein extravasation, inflammation, pain.
-inhibits neurally-evoked plasma protein extravasation into the dura mater (anesthetized guinea pigs)**(311C90 10 µg/kg reduced S/U ratio 30%; sumatriptan 10µg/kg reduced 38%)**

-inhibits neurally-evoked (electrical stimulation of trigeminal ganglion) increase in blood flow in ipsilateral superficial cortex (index of cerebral blood flow) and CGRP and VIP increased in jugular vein in anesthetized cats.

Pharmacologist's comment: Based on these data, there is a valid scientific rationale for use of 311C90 for treatment of acute migraine.

Safety Pharmacology

Cardiovascular Pharmacology

Conscious animals

1. Conscious rabbit-311C90 (3-30 mg/kg, i.v.) produced significant and sustained increase in b.p. (38 mmHg) and h.r. (60 bpm). B.P. returned to normal in 25 min.
2. Conscious rat-serial bolus injections 311C90 (0.1-10,000 µg/kg, i.v.)- transient, dose-related decrease in b.p. at doses ≥100 µg/kg; maximum fall in diastolic pressure of 21 mmHG at the high dose; reflex baroreceptor-related increase in heart rate.
3. Conscious non-human primate-311C90 (3000 µg/kg-3 mg/kg) produced transient decrease in b.p. followed by more sustained increase in b.p. Effects on h.r. were inconsistent.
4. Conscious dog: see Table below

Table 4. Changes in Blood Pressure and Heart Rate Following 311C90 and Sumatriptan in the Conscious Dog

Dose	Mean Maximal change in b.p. systolic/diastolic ±S.E.M. (mmHg)	Mean maximal change in H.R. (bpm)
311C90		
Oral		
2mg/kg (n=1)	+75/59	+118
I.V.		
0.5 mg/kg (n=3)	+49/37 (±11/8)	+68 (±15)
1.0 mg/kg (n=1)	+85/52	+58
1.0 mg/kg (n=3)	+52/23 (±8/4)	+38±10
Sumatriptan		
Oral		
2 mg/kg (n=2)	+32/21	+34
I.V.		
1 mg/kg (n=2)	+34/27	+80

Interpretation-311C90 vs Sumatriptan Cardiovascular Effects

1. 311C90 2-3-fold more potent than Sumatriptan in rabbit saphenous vein and carotid artery blood flow assays.
2. 2 mg/kg **oral**- (see above table)
   3311C90 2-3-fold greater effect at increasing blood pressure and heart rate than sumatriptan.
   *sponsor also stated that time to recovery was 2-3 hours for 311C90, making cardiovascular effects more persistent than with sumatriptan.

Relationship to human dose:
311C90-dog (oral)- 2 mg/kg C_{max} 0.5-1 µg/ml

human maximum proposed dose-15 mg C_{max} 20 ng/ml

dose in dog 25-50-fold higher than in man based on plasma C_{max}.

Pharmacologist's Comment: It appears, based on these minimal data, that the 311C90 is about 2-3-fold more potent with respect to cardiovascular side-effects than sumatriptan.

Anesthetized animals

1. Anesthetized dog- 311C90 (and sumatriptan)-no effects on coronary vasculature, but in anesthetized dogs, a significant dose-related decrease in renal blood flow and conductance was seen (ED_{50} 0.14-1µg/kg; max reduction 56-87%)-unique to the dog. Human coronary artery in vitro showed dose-related contraction by 311C90 (37% of the maximum caused by serotonin).

2. Anesthetized rat-(100-10,000 µg/kg)-dose-related reduction in blood pressure (max. Reduction 51 mmHg (ED_{50} 161 µg/kg)-effects on heart rate inconsistent.

3. Anesthetized cat-low doses zolmitriptan (dose-related, persistent decrease in carotid arterial conductance; ED_{50} 1.1-1.90 µg/kg, i.v.); maximum reductions 36-39%. At doses up to 100 µg/kg were no changes in femoral arterial blood flow or pulmonary vascular conductance. 100 µg/kg, i.v. resulted in reduced systemic blood pressure up to 83 mmHg (ED_{50} 515 µg/kg)-inconsistent effect on heart rate Regional blood flow studies (radiolabelled microspheres)-up to 1,000 µg/kg, i.v.-no consistent or significant change in vascular conductance to sub-regions of myocardium or brain. Ocular, splanchnic and stomach vascular conductance reduced about 55%; renal, adrenal and hepatic conductance increased 55, 150 and 220%, respectively.

Overall cardiovascular effects

311C90 in conscious animals appeared to increase blood pressure and heart rate with potency about 2-3-fold greater than sumatriptan on dose per dose basis. In anesthetized animals, there appeared to be a no effect on conductance to myocardium, coronary vasculature, subregions of the brain or pulmonary vascular conductance. However, there did appear to be a decrease in renal blood flow and conductance as well as ocular, splanchnic and stomach vascular conductance. *In vitro* studies on human coronary artery did, however, show dose-related contraction by 311C90 (37% of the maximum caused by serotonin).

Summary of Other Pharmacology Effects

1. CNS effects: probably through central 5-HT_{1A} receptor sites (similar effects for sumatriptan and 311C90-
   conscious dog: distress, restlessness, agitation, ataxia-due to these receptor sites affecting mood control and sympathetic outflow.
   conscious mouse: up to 300 mg/kg oral dose-no CNS effects observed.
   -also no pro- or anticonvulsant effect or analgesic effects.
   conscious rat: 10-2000 mg/kg oral-peripheral vasodilatation, slight hypothermia, respiratory depression, decreased locomotor activity (dose-related effects)-lethal oral dose was 2000 mg/kg (2-7 hr post-dose) and lethal i.v. dose was 100 mg/kg.
   conscious rabbit: 1-30 mg/kg-no CNS effects.
   conscious non-human primates (cyno monkeys): repeat i.v. injections 1-10,000 µg/kg-≤100 µg/kg gave restlessness, agitation, emesis-higher doses included initial agitation with ataxia and depression by study end.

2. Autonomic pharmacology
   SNS (sympathetic nervous system) studies in anesthetized dogs: zolmitriptan (1-100 µg/kg) prevented stimulation-evoked ∝-adrenoreceptor mediated falls in carotid arterial blood flow (dose-dependent)-prejunctional effect on sympathetic innervation to these blood vessels
   SNS studies in anesthetized cats: 900 µg/kg zolmitriptan augmented 1Hz nictitating membrane contractions (90 and 9000 µg/kg had no effect).
   PSNS (parasympathetic nervous system) studies in anesthetized cats: 9000 µg/kg zolmitriptan attenuated frequency-dependent bradycardia induced by electrical stimulation of the right vagus nerve.
   In vitro autonomic receptor pharmacology studies: no binding affinity(pK_B/pK_i<4.5) for monoamine receptors, including ∝- and ß- adrenergic receptors, dopamine receptors, and muscarinic cholinergic receptors. Was low potency antagonism (pK_B=5.5) at H_1 histamine receptors.

3. Respiratory pharmacology:
   Conscious rat: 200-2000 mg/kg oral zolmitriptan-respiration slower and deeper.
   Conscious rabbit: up to 30 mg/kg i.v.-no effect on respiration
   Conscious dog: 1-2 mg/kg oral-no effect on respiration.
   Conscious non-human primate: 1-10,000 µg/kg i.v.-no discernible effects on respiration.
   Anesthetized dog: 1-10 mg/kg, i.v. produced small, dose-related decreases in respiratory tidal volume, accompanied by increases in respiratory rate (respiratory minute volume unchanged).

Anesthetized cat: uniquely sensitive-dose-related inhibition of respiration (0.09 mg/kg- No effect; 0.9 mg/kg-reduction in tidal volume, increased respiratory rate; 9 mg/kg-apnea, hypercapnea, hypoxia, death in 2/6 animals)-not due to effects of airway smooth muscle, probable central effect.

In vitro guinea pig isolated trachea: 30 µg/kg did not modify the intrinsic tone in isolated strips of guinea pig trachea.

4. Renal and G.I. pharmacology-no significant effects
5. Interactions with metabolizing enzymes-311C90 does not affect duration of barbiturate-induced sleep in conscious rats.
6. Barbiturate sleep time interactions: No changes in barbiturate sleep time in conscious mouse (1-30 mg/kg, oral) or conscious rat (10-100 mg/kg oral; 0.1-10 mg/kg i.v.)

183C91 METABOLITE

Pharmacological profile of 183C91, the N-demethylated metabolite of zolmitriptan

The following information, taken from my review of IND (the original IND for 311C90 for migraine, April 28, 1994), shows the metabolic profile by species for 311C90 (zolmitriptan) and some previously studies effects of the active metabolite 183C91:

Metabolism of 311C90 by oral route of administration

- Four metabolites: 183C91 (active metabolite: N-demethylation of 311C90), 614C91 (dog) and 2161W92 (man)(indole acetic acids), and 1652W92 (N-oxide analogue).
- Plasma metabolite profile by species, in descending order of occurrence:
  Human: 2161W92>183C91>1652W92.
  Mouse: 183C91>1652W92>2161W92.
  Rat: 2161W92>1652W92>183C91.
  Rabbit: 1652W92>2161W92>183C91.
  Dog: 1652W92>2161W92>183C91.

- 183C91 (Active metabolite) vs 311C90 parent-activity
  -6-fold more potent at reducing carotid artery blood flow and conductance (ED_{50} 0.52µg/kg vs 311C90, 3.3µg/kg)
  -2-fold more potent at transient reduction in renal blood flow in the anesthetized dog (ED_{50} 0.07µg/kg vs 311C90, 0.14µg/kg)
  -2-fold more potent in the rabbit saphenous vein assay (EC_{50} $9x10^{-8}$ vs $1.6x10^{-7}$ M for 311C90)
  -partial agonist with respect to 5-HT

In the NDA, the sponsor states that 2161W92 and 1652W92 would be expected to have no pharmacological activity at the 5-HT receptor based on their knowledge of the inactivation of 5-HT. For this reason, they examined mainly the pharmacological profile of the 183C91 metabolite.

Receptor pharmacology

The following sponsor's Table (volume 11, pg. 24) summarizes the *in vitro* receptor profile for 183C91:

Receptor profile of 183C91 *in vitro*

	p[A_{50}] / max or pK_B [a]	pIC_{50}
"5-HT_{1D}-like"	7.1 / 0.80	N/A
5-HT_{1A}	6.8 / 1.00	6.8
5-HT_{1B}	8.0 / 1.00	N/A
5-$HT_{1D\alpha}$	10.8 / 1.00	9.3
5-$HT_{1D\beta}$	10.3 / 1.00	8.8
5-HT_{2A}	<4.5 / -	N/A
5-HT_4	<4.5 / -	N/A
5-HT (atypical, endothelial receptor)	<5.0 / -	N/A
5-HT (smooth muscle relaxation, 5-ht_2)	<4.5 / -	N/A
		N/A
α_1-adrenoceptor	<4.5 / -	N/A
β_1-adrenoceptor	<4.5 / -	N/A
β_2-adrenoceptor	<5.0 / -	N/A
Histamine H_1	<5.0 / -	N/A
Histamine H_2	<4.5 / -	N/A
Muscarinic M_2	<5.0 / -	N/A

[a] - where 183C91 behaved as an agonist, the result is expressed as p[A_{50}] / maximum *c.f.* 5-HT (=1). When the compound failed to display agonism (indicated by -), it was evaluated as an antagonist and the result expressed as a pK_B value (6)

High affinity at 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors (p[A_{50}] 10.8 and 10.3, respectively. Also fairly high affinity for other 5-HT_1 receptors (see above table). Little or no affinity for 5-HT_2 or 5-HT_4, ∝- or ß-adrenoreceptors, histamine H_2 or muscarinic M_2 receptors. Some low affinity for histamine H_1 (p[A_{50}] 5.0).

Cardiovascular safety pharmacology

Conscious dog studies

Single dose of 183C91 (0.5 mg/kg, i.v. or 1 mg/kg, p.o.)-sympathomimetic response (hypertension, tachycardia, mydriasis, behavioral stress) similar to those obtained with about 2-fold higher doses of zolmitriptan.

Cumulative administration 183C91 (0.1-100 µg/kg, i.v.)-rapid, brief increases in blood pressure and heart rate, dose-dependent over the range 0.1-10 µg/kg, diminishing at 20-100 µg/kg. Higher doses (100-500 µg/kg, i.v.) caused more marked increases in blood pressure and heart rate that increased with dose. No ECG changes

Anesthetized dogs

Open-chested anesthetized dogs-(0.03-30 µg/kg i.v. 183C91)-dose-related, long lasting decrease in carotid arterial conductance similar to zolmitriptan, but six-times more potent than zolmitriptan (ED_{50} 0.52 µg/kg 183C91; 3.3 µg/kg zolmitriptan). Like zolmitriptan, 183C91 had no effect on coronary arterial blood flow, but produced transient decreases in renal arterial flow (twice the potency of 311C90; 183C91 ED_{50} 0.07 µg/kg; zolmitriptan ED_{50} 0.14 µg/kg). 300-1000 µg/ml zolmitriptan caused significant reduction in blood pressure and dose-related reductions in heart rate to a maximum of 32%, similar to zolmitriptan.

ADME (Absorption, distribution, metabolism, excretion)

Absorption

For 311C90, there are a number of metabolites that are important in addition to the parent drug. These metabolites include 2161W92, 183C91 and 1652W92. In human plasma from patients receiving oral 311C90, metabolite 2161W92 is the major metabolite, found at plasma levels similar to parent zolmitriptan. 183C91 is the metabolite in humans that is known to be an active metabolite, with affinity for 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ and potency in a number of *in vitro* assays similar to zolmitriptan. Both 183C91 and 1652W92 are were reported to occur in humans at about 50% of the concentration of parent drug. While these same metabolites are also found in animals (rats, dogs), plasma levels compared to parent drug vary with species. Since these metabolites are so predominant in humans receiving zolmitriptan by the oral route, it was important that the sponsor monitor them in the nonclinical toxicology studies, including the carcinogenicity and reproductive toxicology studies.

For a summary of the pharmacokinetics data for the animal and human studies, see sponsor's Table 4 (volume 11, pg. 97) below:

Table 4 Plasma pharmacokinetics of zolmitriptan in laboratory animals and man

Species/ Strain	Dose route	Sex (n)	Dose (mg/kg)	T½ (h)	Cmax (ng/ml)	Tmax (h)	Cl/F (ml/min/kg)	Vd/F (l/kg)	AUC (ng/ml.h)	Bioavailability (%)	Reference
Mouse/CD-1	po	m (33)	9.8	1.3	326	0.25	NC	NC	586[d]		BDRR/93/0071
	po	f (33)	9.8	1.1	274	0.25	NC	NC	848[d]		(74)
	iv	m (39)	3.5	0.9	1300	0.08	NC	NC	501[d]		
	iv	f (39)	3.5	1.0[c]	811	0.08	NC	NC	529[d]		
Rat/Wistar	po	m (30)	9.3	1.3	336.7	4.0	NC	NC	1465.0		BPAT/91/0113
	po	f (30)	9.3	1.3	633.6	2.0	NC	NC	1786.4		(75)
	iv	m (36)	2.5	1.0	1694.8	0.08	39.37	NC	1058.3		
	iv	f (36)	2.5	1.2	1438.3	0.08	38.56	NC	1080.6		
Rabbit/New Zealand White	po	f (4)	9.84	2.0	272	0.75	205.0	33.5	826		BDRE/93/0028
	iv	f (4)	2.86	1.0	1416	0.08	53.3	3.6[c]	827		(79)
Dog/Beagle											BPAT/91/0177

Table 4 **Plasma pharmacokinetics of zolmitriptan in laboratory animals and man (continued)**

Species/ Strain	Dose route	Sex (n)	Dose (mg/kg)	T½ (h)	Cmax (ng/ml)	Tmax (h)	Cl/F (ml/min/kg)	Vd/F (l/kg)	AUC (ng/ml.h)	Bioavailability (%)	Reference
Human	po	m (10) f (10)	2.5[b]	2.50	3.79	2.5	-	-	20.7		BLVS/96/0017 (105)
	po	m (10) f (10	5[b]	2.77	7.81	3	-	-	46.4		
	po	m (9)	6[b]	2.50	10.8	2.0	24.90	5.22	60.7	-	BLVS/93/0023 (81)
	po	m (9)	12[b]	2.78	12.9	3.0	29.09	6.70	100.5	-	
	po	m (11)	25[b]	2.67	34.8	3.0	26.99	6.07	229.2	-	
	po	m (7)	50[b]	3.01	68.2	4.0	29.07	6.75	515.4	-	

Cmax: Maximum plasma concentration observed (at time Tmax). Cl/F: Apparent plasma clearance (divided by the dose fraction).

Vd/F: Apparent volume of distribution (divided by the dose fraction). AUC: Area under the plasma concentration-time curve (0 - ∞ unless otherwise stated)

T½: Apparent plasma elimination half-life. NC: Not calculated

Bioavailability: Apparent fraction of the dose of the dose available to the systemic circulation.

[a] Vss: Volume of distribution at steady state

[b] Total dose (the mean values for Cl/F and Vd/F were divided by the mean group body weights to give figures for comparison with the animal data).

[c] Half-life of 6.1h may be true elimination half-life but obscured by limit of quantitation of the assay.

[d] AUC from 0 to the last measured data point (curve not extrapolated).

Mouse Pharmacokinetics

Zolmitriptan was absorbed rapidly by the oral route in the mouse, with a Tmax of 0.25 h and a bioavailability of about 50% (compared by AUC to single i.v. dose). The $T_{1/2}$ was about 1.2 h (see Sponsor's Table 4 on previous page). Females had a somewhat greater exposure level than males at the 10 mg/kg dose.

For a summary of mouse PK data for both 311C90 and metabolite 183C91 following single oral dose 10 mg/kg or i.v. dose 3.5 mg/kg, see the following sponsor's table (vol 11, pg. 13).

		Radioactivity	311C90	183C91
PO	AUC_{0-t} (ng/mL x h)	1340	717	96[a]
	Cmax (ng/mL)	503	300	25
IV	AUC_{0-t} (ng/mL x h)	1147	515	62
	Cmax (ng/mL)	2269	1056	60

n = 2, [a] n = 1

AUC_{0-t} = area under the plasma concentration–time curve from zero to the last detectable timepoint (ng/mL x h).

Cmax = observed maximum plasma concentration (ng/mL).

These data show that exposure to the metabolite 183C91 in the mouse was about 13% of the parent 311C90 exposure for a single oral dose of 10 mg/kg.

For a summary of mouse exposure to 311C90 during the course of the mouse carcinogenicity study, see the following sponsor's table 5 (vol. 11, pg. 107):

Table 5 Summary of plasma zolmitriptan multiple dose pharmacokinetic data from oral carcinogenicity studies in the mouse

Sample Time	Sex	Parameter	Dose (mg/kg/day)				Reference
			6	60	400	600*	
Day 1	m	Cmax	124	1455		17146	BDRE/94/0010 (52)
		AUC	NE	NE		145440	
	f	Cmax	124	3818		27740	
		AUC	NE	NE		149338	
Week 6	m	Cmax			19199		
		AUC			69309		
	f	Cmax			13418		
		AUC			62910		
Week 26	m	Cmax	241	2286	15259		
		AUC	NE	7804	71773		
	f	Cmax	93	1814	16997		
		AUC	NE	NE	78688		
Week 52	m	Cmax	115	2965	11474		
		AUC	NE	9617	49166		
	f	Cmax	121	2313	19198		
		AUC	NE	8149	106166		
Week 74	m	Cmax	89	1821	14615		
		AUC	NE	6497	74670		
	f	Cmax	139	2841	22207		
		AUC	NE	10724	104716		

*Dosing was started with 600mg/kg/day as the highest dose but due to high mortality this was reduced to 400mg at 10 days and samples collected in week 6.

Cmax - Maximum plasma concentration (ng/ml).

AUC - Area under the plasma concentration-time curve (ng/ml.h).

NE - Not estimated.

PK data from the mouse carcinogenicity study show that exposure (AUC) to 311C90 remained fairly constant with time in male animals, while AUCs in females (40 mg/kg) increased from about 62,000 ng.h/ml on Week 6 to about 105,000 ng.h/ml by Week 74.

Rat Pharmacokinetics

Following is a sponsor's summary Table 3 (volume 50, pg. 45) for PK for zolmitriptan in the rat:

TABLE 3: PHARMACOKINETIC PARAMETERS OF PLASMA 311C90 IN THE RAT FOLLOWING ORAL AND I.V. ADMINISTRATION OF ^{14}C-311C90

PARAMETER	ORAL		INTRAVENOUS	
	Male	Female	Male	Female
Dose ($mg.kg^{-1}$)	9.3	9.3	2.5	2.5
Cmax ($ng.ml^{-1}$)	336.7	633.6	-	-
Tmax (h)	4.0	2.0	-	-
$t_{\frac{1}{2}}$ (h)	1.3	1.3	1.0	1.2
AUC ($ng.h.ml^{-1}$)	1465.0	1786.4	1058.3	1080.6
Clp ($ml.min^{-1}.kg^{-1}$)	NC	NC	39.37	38.56
Vc ($l.kg^{-1}$)	NC	NC	0.85	0.64
Bioavailability (%)	37.2	44.4	100.0	100.0

Cmax = observed maximum plasma concentration.

Tmax = time to observed maximum plasma concentration.

$t_{\frac{1}{2}}$ = half-life.

AUC = area under the concentration-time curve from 0 to infinity.

Clp = plasma clearance.

Vc = volume of central compartment.

Bioavailability = systemic bioavailability using i.v. as a reference dose.

NC = not calculated.

The drug was rapidly absorbed by the oral route (Tmax 2-4 hours) with a bioavailability of about 37-44%. The oral route actually resulted in an initial peak concentration at about 0.5 h after dosing, followed by a second peak 3 h after dosing, in both males and females for unchanged drug and total radioactivity in plasma. This may indicate a secondary absorption lower down the gastro-intestinal tract. The $T_{1/2}$ was about 1.3 h following oral and 1.1 h following i.v. administration. No difference in exposure levels between males and females appeared to occur.

Levels of radioactivity in plasma were considerably higher than those of the unchanged drug, indicating presence of circulating metabolites.

Following is a summary of 311C90 exposure (AUC_{0-T}, ng.h/ml) for the rat carcinogenicity study, demonstrating multiple dose exposure level information:

		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)

These data show that exposure levels (AUC) in the rats were generally linear with dose, but increased with time up to 52 weeks of chronic dosing. Exposure levels subsequently dropped again on weeks 78 and 104. The sponsor suggested that this may be due to varying age effects on the different routes of elimination (absorption, metabolism, renal excretion).

Rabbit Pharmacokinetics

Following is sponsor's Table 14 (volume 50, pg. 90) summarizing rabbit PK:

Table 14. Pharmacokinetics parameters of plasma 311C90 in the female rabbit following a single oral dose of [^{14}C]-311C90 at 10mg.kg^{-1}.

	Subject 938003	Subject 938004	Subject 938005	Subject 938006	Mean ± SD
$AUC_{0-\infty}$	973	731	859	740	826 ± 114
$C_{max_{fit}}$	135	297	340	316	272 ± 93
$T_{max_{fit}}$	0.8	0.9	0.3	0.4	0.6 ± 0.3
$C_{max_{obs}}$	140	377	361	336	304 ± 110
$T_{max_{obs}}$	1.0	0.75	0.75	0.75	0.75 & 1.0
$t^1/_2{}_{obs}$	0.15	0.43	0.06	0.10	0.19 ± 0.17
$t^1/_2{}_{elim}$	4.4	0.9	1.5	1.3	2.0 ± 1.6
Cl/F	10.3	13.7	11.7	13.5	12.3 ± 1.6
V/F	65.6	16.8	25.9	25.5	33.5 ± 21.8
F					0.25

$AUC_{0-\infty}$ ($ng.ml^{-1} \times h$) - Area under the plasma concentration-time curve
$C_{max_{fit}}$ ($ng.ml^{-1}$) - Modelled maximum plasma concentration
$T_{max_{fit}}$ (h) - Time at which Cmax was modelled
$C_{max_{obs}}$ ($ng.ml^{-1}$) - Observed maximum plasma concentration
$T_{max_{obs}}$ (h) - Time at which Cmax was observed

Zolmitriptan was rapidly absorbed by the oral route in the female rabbit (Tmax 0.75h). $T_{1/2}$ of elimination was about 2 hours. Bioavailability by the oral route was about 25%. Apparently levels of total radioactivity in plasma were significantly higher than those of zolmitriptan and the relative exposure ratios of zolmitriptan and radioactivity were about 1:17 after oral dosing, suggesting the presence of circulating metabolites.

Dog Pharmacokinetics

Following is sponsor's Table 13 (volume 50, pg. 140) summarizing zolmitriptan PK information in the dog:

TABLE 13: PHARMACOKINETIC PARAMETERS OF PLASMA 311C90 IN THE DOG FOLLOWING ORAL AND INTRAVENOUS ADMINISTRATION OF ^{14}C-311C90

PARAMETER	ORAL		INTRAVENOUS	
	MALE	FEMALE	MALE	FEMALE
Dose (mg.kg^{-1})	1.919	1.919	0.969	0.980
Cmax (ng.ml^{-1})	993.5	583.6	NC	NC
Tmax (h)	0.25	0.51	NC	NC
$t_{\frac{1}{2}\alpha}$ (h)	0.07	0.28	0.06	0.11
$t_{\frac{1}{2}\beta}$ (h)	2.17	1.88	1.59	1.78
AUC (ng.h.ml^{-1})	1686.5	1417.1	1171.3	862.0
Clp (ml.min^{-1}.kg^{-1})	18.96*	22.57*	13.79	18.95
Vc (l.kg^{-1})	0.18*	1.35*	0.33	0.59
Bioavailability (%)	73	84	NC	NC

Cmax - Observed maximum plasma concentration.
Tmax - Time to observed maximum plasma concentration.
$t_{\frac{1}{2}}$ - Half-life.
AUC - Area under the concentration-time curve from 0 to infinity.
Clp - Plasma clearance.
Vc - Volume of central compartment.
Bioavailability - Systemic bioavailability using iv as a reference dose.
NC - Not calculated.
* - Apparent values only.

Drug was rapidly absorbed by the oral route (Tmax 0.25-0.5 h). $T_{1/2}$ of elimination was about 1.9-2.2 h. There were no significant sex differences with respect to exposure levels. Oral bioavailability was about 73-84%.

Plasma exposure to zolmitriptan was about half that of total radioactivity, indicating the presence of circulating metabolites.

The sponsor's Table summarizing plasma exposure levels for 311C90 in the one year toxicology study in Beagle dogs is shown below:

The average exposures to 311C90 were as follows:

	5mg/kg/day		25mg/kg/day		100mg/kg/day	
	Day 23	Day 366	Day 23	Day 366	Day 23	Day 366
AUC_{0-t} (ng/ml x h)	3069	1362	22183	27180	106836	120150
Cmax (ng/ml)	627	398	4395	7069	19490	29153

These data show that, during multi-dosing studies in dogs, there was little change in plasma exposure levels (AUC) on Day 366 versus Day 23 of the study. Therefore, in the dog there did not appear to be any accumulation of drug with time.

Human Volunteer Pharmacokinetics

In healthy human male volunteers: oral doses of 6 to 50 mg drug (about 0.08 to 0.66 mg/kg); PK was linear across this dose range; peal plasma levels of 10.8 to 68.2 ng/ml; Tmax 2-6 h after dosing; $T_{1/2}$ elimination of 2.5-3 h; plasma clearance 24-31 mg/min/kg.

Protein Binding

In vitro plasma protein binding for animals and humans is summarized in the following sponsor's Table 8 (volume 11, pg. 110):

Table 8 Plasma protein binding of zolmitriptan *in vitro*

Species	Initial plasma zolmitriptan concentration (ng/ml)				Reference
	10	200	500	1000	
Mouse	18.46	11.70	14.68	13.67	BPAT/92/0001 (82)
Rat	26.97	27.13	26.41	27.41	BPAT/92/0001 (82)
Rabbit	11.49	11.76	19.80	12.97	BPAT/92/0001 (82)
Dog	17.97	20.10	19.30	20.25	BPAT/92/0001 (82)
Human	26.85	21.38	18.81	17.61	BDDM/92/0004 (83)

Binding is expressed as the % of initial concentration bound to plasma protein.

Zolmitriptan was bound only at low levels (ranging from) in all species. Although the actual level of binding was dependent on the species, binding within each species appeared to be independent of drug concentration for the most part.

Pharmacologist's comment: Plasma protein binding does not appear to be a concern for zolmitriptan, certainly not at the plasma levels attained in humans at the mrdd.

Tissue Distribution

Tissue Distribution:

Qualitative tissue distribution (whole body autoradiography)- in rats- The animals received a single oral dose of [^{14}C]-311C90 at 10 mg/kg (25 µCi) and then were sacrificed at 2, 6 or 24 h post-dose. One animal was examined at each time point.

Results:

2 h-highest levels in stomach, intestine and bladder, lower levels in liver, and some radioactivity found in kidney cortex. None detected in brain or spinal cord.

4 h-highest levels in intestine, with lower levels in kidney medulla, liver and salivary glands.

24 h-low levels in stomach only

In a second study, using male albino and pigmented rats, data showed that at 2 h brain levels were slightly above background, indicating a slight amount of blood/brain barrier crossing by 311C90 and/or its metabolites. These data were not quantified. By 6 hours, radioactivity was below the level of detection in most organs. Also, at up to 24 h, higher levels of radioactivity were seen in the eyes of pigmented animals, indicating that 311C90 and/or its metabolites probably bind to melanin.

In a third study, in pregnant female rats following oral administration of [^{14}C]-311C90 at 100 mg/kg (50 µCi) on the 18th day of gestation, radioactivity was detected in the fetal tissue, most notably fetal liver, and in the wall of the uterus by 6 h after administration. Also, high levels were seen in the kidney 2 and 6 h after dosing, indicating rapid elimination from this tissue.

Quantitative Tissue Distribution: See sponsor's Table 9 (vol. 11, pg. 111) below:

Fifteen pigmented Lister Hooded rats received a single oral dose of 10 mg/kg (13 µCi) of [^{14}C]-311C90 and tissue distribution of total radioactivity was assessed at 4, 12, 24, 72 and 168 h post-dose. Levels of total radioactivity were also determined in urine and feces for each animal up to sacrifice. The following organs, tissues, and

intestine, thyroid, fat, heart, plasma, brain, small intestine, lungs, muscle, bone marrow, eyes, spleen, kidneys, testes, liver and skin (pigmented vs. non-pigmented).

Table 9 Tissue distribution of radioactivity in the male pigmented rat (Lister Hooded) following oral administration of [^{14}C]-zolmitriptan [a]

(Results are expressed as µg equiv./g tissue or ml fluid)

Tissue	Time point (h) [b]				
	4	12	24	72	168
Adrenals	2.10	0.18	0.04	0.00	0.01
Blood	0.69	0.03	0.03	0.01	0.01
Bone	0.30	0.04	0.02	0.00	0.00
Bone marrow	1.33	0.01	0.00	0.00	0.00
Brain	0.04	0.00	0.00	0.00	0.00
Eyes	0.94	0.78	0.80	0.49	0.71
Fat	0.36	0.03	0.01	0.00	0.01
Heart	0.91	0.03	0.01	0.00	0.00
Kidneys	2.78	0.06	0.08	0.01	0.01
Liver	3.99	0.34	0.25	0.05	0.02
Lungs	1.00	0.03	0.02	0.01	0.00
Muscle	1.30	0.04	0.02	0.00	0.01
Skin (non-pigmented)	0.76	0.08	0.03	0.01	0.02
Skin (pigmented)	0.73	0.18	0.15	0.03	0.01
Plasma	0.53	0.02	0.01	0.00	0.00
Spleen	1.07	0.05	0.03	0.01	0.00
Testes	0.48	0.28	0.22	0.05	0.01
Thyroid	1.43	0.20	0.12	0.02	0.04
Remaining Carcass	0.30	0.04	0.02	0.00	0.00
Large Int. (+ contents)	160.96	10.02	5.27	0.03	0.00
Small Int. (+ contents)	42.49	0.45	0.24	0.01	0.00
Stomach (+ contents)	1.34	0.07	0.06	0.00	0.00

Reference BDRE/92/0053 (88)

[a] Dose was 10.35 mg/kg.

[b] Each time point was the mean of 3 animals.

Results: The main route of elimination was fecal, with 46-68% of the dose in animals surviving to 168 h (probable incomplete absorption and biliary elimination of the absorbed dose). High levels of radioactivity were also found in urine (29-48%), indicating significant absorption of the orally administered drug. Highest levels of radioactivity were found 4h post-dose, indicating rapid absorption. Most tissues contained levels of total radioactivity similar to or up to 2 times those observed in plasma. Levels of total radioactivity in the organs of metabolism and excretion i.e. liver (mean 3.99 µg.equiv.g^{-1}) and kidney (mean 2.78 µg.equiv.g^{-1}) were significantly greater than those of plasma. 168 h post-dose levels above background indicating retention were found in the eye (0.94 at 4 h post-

retention). Very small amounts (0.04 µg.equiv.g^{-1}, 4 h only) reached the brain. In addition to the eye, a high level of retention of radioactivity occurred in the skin up to 72 h, suggesting that the drug may be associated with melanin.

Secretion into milk in lactating rats

Lactating female Wistar rats dosed orally with [^{14}C]-zolmitriptan had milk levels of radioactivity equivalent to plasma at 1 h post-dose and 4X higher than plasma at 4h.

Pregnant rabbit tissue distribution

Qualitative whole body autoradiography study in pregnant female Dutch rabbits-oral administration of [14C]-zolmitriptan (10 mg/kg). Rabbits showed generalized distribution with highest levels in liver and minimal penetration to the CNS.

24 h-levels of radioactivity declined in most tissues to less than background levels-still detectable at low levels in liver, kidney, and uveal tact of maternal eye (melanin).

Placenta-levels of radioactivity similar to those in maternal cardiac blood-detectable at very low levels in fetuses at 2 and 6 h after dosing, evenly distributed throughout the fetal tissue. By 24 h low levels of radioactivity found in placenta and intestinal contents of one of the fetuses, but not in any other fetal tissue.

Metabolism

Metabolism of zolmitriptan was studied *in vivo* and *in vitro* using radiolabel and analysis by HPLC and LC-MS-MS. The major metabolites identified in man were all represented in the various animal species used for toxicity studies, although quantitative differences did occur from species to species. Plasma metabolite data were derived from toxicokinetic and clinical analyses. The structures of the known metabolites of zolmitriptan are shown in sponsor's Figure 2 (volume 11, pg. 76) shown below:

Figure 2 Structure of zolmitriptan and some of its major metabolites

In Vivo Studies

A summary of the plasma exposure to zolmitriptan and its metabolites in animals and man is shown in the following sponsor's Table 11 (vol. 11, pg. 113):

Table 11 Summary of the plasma kinetics of zolmitriptan and its metabolites in animals and man

Species	Parameter	zolmitriptan	183C91	2161W92	1652W92	Reference
Mouse	AUC	66110	3886	2144	3174	BDRE/94/0010
	Cmax	16309	580	214	522	(52)
Rat	AUC	167607	4719	14146	14125	BDRE/93/0082
	Cmax	24786	619	2092	2089	(49)
Dog	AUC	27180	1066	7271	11462	TTEP/96/0016
	Cmax	7069	192	1407	2733	(51)
Man[a]	AUC	160.4	75.6	162.0	53.1	BLVS/95/0029
	Cmax	23.3	10.5	21.0	6.8	

Animal doses are at NOEL (mouse 400 mg/kg/day; rat 100 mg/kg/day and dog 25 mg/kg/day). Human dose was 15 mg.

[a]data from young male (n=6) and female (n=6) subjects combined.

Human Metabolism

plasma: major metabolite 2161W92 with concentrations similar to zolmitriptan 183C91 and 1652W92 present in levels approximately 50% of parent.

urine: 0-48 h urine collection; parent drug 10% of total dose; 2161W92 major urinary metabolite (30% of total dose); 1652W92 (9% of total dose); 183C91 (4% of total dose); 4441W94 (4% of total dose).

feces: predominately unchanged drug; minor amounts of 2161W92 .

Therefore, metabolites for humans in descending order were 2161W92>183C91>1652W92.

Mouse Metabolism

plasma: All metabolites, 2161W92, 1652W92 and 183C91 were found in plasma at concentrations of 5-10% of the parent compound. The descending order was 183C91>1652W92>2161W92.

urine and feces: 183C91, 1652W92 and 2161W92.

Rat Metabolism

plasma: major metabolites were, in descending order, 2161W92>1652W92>183C91. The first two metabolites were present in levels of 5-10% of parent and 183C91 had levels of 2-5% of parent drug.

urine and feces: unchanged zolmitriptan was 50% of drug-derived material, with 2161W92, 183C91 and 1652W92. At least six other metabolites were detected, but could not be identified due to low concentrations present.

Rabbit Metabolism

plasma: contained much higher levels of metabolites than parent compound. Major metabolites were, in descending order, 1652W92>2161W92>183C91. 1652W92 was found at levels up to 3 times the parent level, 2161W92 at similar levels to parent drug, and 183C91 in low levels only.

urine and feces: 2161W92, 1652W92 and 183C91. Ten other minor metabolites were also detected in excreta, with three in urine tentatively identified as glucuronic acid conjugates.

Dog Metabolism

plasma: in descending order 1652W92 (30-50% of parent drug)>2161W92 (20% of parent)>183C91 (5% of parent).

urine and feces: 2161W92>1652W92>183C91.

In Vitro

rat and human liver enzymes: very low levels of metabolites produced in these systems; enzymes responsible for metabolism of zolmitriptan have not been characterized.

Rat microsomal fractions: 183C91 and 1652W92

Human microsomal fractions: 1652W92 only in trace amounts.

Human liver (elevated levels of most cytochrome p450 isoenzymes including 3A4 and 2D6): some conversion zolmitriptan to 183C91 in presence NADPH, but insufficient to determine cytochrome P450 that is responsible.

Major urinary metabolite of zolmitriptan is 2161W92, structurally similar to major metabolite of sumatriptan; sumatriptan metabolism thought to be mediated through monoamine oxygenase (MAO) system. Examined zolmitriptan interaction with MAO enzyme system, and no interactions were found between zolmitriptan and MAO-A or MAO-B over the range of 2.5-250 µM.

Summary of Metabolism in Animals Compared to Human

The plasma levels of metabolites after oral administration are presented below in descending order by species, including human:

Human: 2161W92>183C91>1652W92.
Mouse: 183C91>1652W92>2161W92.
Rat: 2161W92>1652W92>183C91.
Rabbit: 1652W92>2161W92>183C91.
Dog: 1652W92>2161W92>183C91.

With respect to human plasma levels of metabolites, the rat is the only animal species that also presented with 2161W92 as the major metabolite with the highest levels. However, 2161W92 in humans was found in plasma at a similar level to parent drug while in the rat this metabolite was only found at a level of about 10% that of parent drug.

Drug Interactions

Zolmitriptan not appear to induce liver enzymes; no indications of falling plasma levels of drug or liver weight changes in chronic toxicity tests.
Zolmitriptan-no effect on barbiturate sleep times in rats or mice.
Zolmitriptan is weak inhibitor of isozyme $2D_6$ and $1A_2$, but only at concentrations in excess (at least 1000 times) of that found in plasma in the clinic.

Excretion

In all species, the predominant route of excretion following i.v. dosing was urinary. Following oral dosing the major excretory route was fecal in mice and rats and urinary in rabbits and dogs.

Mice

oral dosing: 99% of dose recovered within 96h, 37% in urine and 54% in feces.

Rats

Wistar: i.v. dosing-73-74% of total dose in urine at 72h; 16-19% in feces; 8-9% exhaled CO_2. Study of excretion of radioactivity in bile following oral administration revealed only minimal secretion into feces via this route.
Lister-Hooded: oral dosing-27% of total dose in urine; 70% in feces.

Female Rabbits

oral dosing: 55% of total dose in urine, 33% in feces.
i.v. dosing: 67% of total dose in urine, 9% in feces.

Dogs
oral dosing: 56% in urine, 20% in feces.
i.v. dosing: 70% in urine, 12% in feces.

Humans
healthy human volunteers: oral administration (25 mg)-64% of total dose in urine, 27% in feces.

TOXICOLOGY

Acute Toxicology Studies

Following is a list of acute toxicology studies that were submitted with the NDA, and that were already reviewed in the original IND submission (IND , reviewed June 10 1994). For my detailed review of these acute toxicology studies, please see the attached review of IND (Attachment #1).

Acute Studies Reviewed in Original IND

1. Report of a acute oral toxicity study on 311C90 in the CD1 mouse (BPAT/91/0190) (8/1F/1020597).
2. Report of an acute intravenous toxicity study in 311C90 in the CD1 mouse (BPAT/91/0192) (8/IF/1020599).
3. Report of an acute oral toxicity study in the Wistar Rat (BPAT/91/0189) (8/1F/1020673).
4. Report of an acute intravenous toxicity study on 311C90 in the Wistar Rat (BPAT/91/0191) (8/IF/1020598).

Summary of Acute Toxicology Study Results

ACUTE STUDIES-Single oral dose

Note: maximum recommended daily dose proposed to be 15 mg=0.25 mg/kg (60 kg person)

Species	Route/dose (mg/kg)	LD50 (mg/kg)	Times human dose (mg/m^2 basis)	Major findings
CD-1 mouse	Oral/0, 500, 1000, 1500	1000	324-fold>human	vasodil, piloerect, dypsnea, muscle spasm, ataxia.
Wistar rat	Oral/0, 1000, 1500	1000-1500	650-973-fold>human	clin: vasodil, salivate, piloerect, HD convulse, muscle spasms, ataxia. Histo: kidney, bilat hydroneph.
CD-1 mouse	I.V./0, 25, 50, 100	50-100	16-32-fold>human	clin: convulse, collapse, tachyapnea, spasms -all doses
Wistar rat	I.V./0, 25, 50, 100	none determined LLD>50 mg/kg	32-fold>human	clin: vasodil, lethargy, piloerect, muscle spasm, ataxia

Animals died at an LD_{50} of about 1000 mg/kg, which for the mouse is about 324-fold greater than the maximum recommended human dose on a mg/m^2 basis and for rat is about 650-fold greater. The cause of death was undetermined, although the sponsor suggested that death was due to exaggerated pharmacological effects of the drug.

Subchronic Toxicology Studies

Following is a list of subchronic toxicology studies that were submitted with the NDA, and that were already reviewed in the original IND submission (IND reviewed June 10 1994). This included one-month oral toxicity studies in rat and dog. For my detailed review of these two oral toxicology studies, please see the attached review of IND . . . (Attachment #1).

Subchronic Studies Reviewed in Original IND

1. Report of a one-month oral toxicity study on 311C90 in the Wistar Rat (BPAT/91/0137) (8/IF/1021169).
2. Report of a one-month oral toxicity study of 311C90 in beagle dog (BPAT/91/0114) (8/1F/1021269).

SUBCHRONIC TOXICOLOGY STUDIES (28 day)
Note: maximum recommended human daily dose 15 mg= AUC 160 ng.h/ml

Species	Route/dose (mg/kg)	# Animals per group (M/F)	Plasma levels (µg/ml)	Major findings	NOEL (toxicity)
Wistar rat	Oral, gavage/0, 100, 400, 1600/1000	15/15 5/5 in recovery group 3/3 for PK	Day 1: 2, 6, 12.5 Day 26: 7, 24, 77 (means of M/F values)	Mortality: C 1/15, LD 2/15, MD 3/15, HD, 10/15 (8F) dilated renal pelvis/kidney histopath=pyelo-nephritis (HD)	100 mg/kg* based on animal death/kidney effects AUC=47,000ng.h/ml 293-fold>human AUC
Beagle dog	Oral, gavage/0, 5, 25, 100	3/3; 2/2 in recovery group	Week 3: 1, 7.5, 28 Week 6: 1, 7.5, 34 (mean of M/F values)	No deaths Clin: pupil dilation, trembling, aggressive behavior, unsteady gait 1 HD female-convulsed 4 separate times in recovery period HD urine Na ↑ (62%) K ↓ (40%) (Day 6)	25 mg/kg based on clinical signs AUC=19,000ng.m/ml 119-fold>human AUC

* Note: the two animals at 100 mg/kg were sacrificed moribund, and did not die directly from drug.

Summary of 28-day study results:

The major toxic effects of this drug involved high incidence of death in rats at the HD (1600 mg/kg), kidney pathology (rats; NOEL at 100 mg/kg; 294-fold>human AUC) and effects on levels of urinary Na and K (dogs; NOEL 119-fold> human AUC), and clinical effects in dogs (pupil dilation, aggressive behavior, trembling, 1 HD female with convulsions). All of these effects occurred at doses/plasma levels of drug that provided an appropriate safety index with respect to the proposed human dose (NOEL 119-293-

Chronic Toxicology Studies

1. Report of a 26-week oral toxicity study of 311C90 in the Wistar Rat, followed by a 4-week treatment-free period (study# BDRE/93/0082) (8/IF/1021267), The Wellcome Fouindation Ltd., Ken, UK, Dept. of Pharmacology/Toxicology, June 9, 1995, 311C90 Batch: lot Q5, GLP.

Study design: Wistar Rats (30/sex/group); 4 study groups (Control (de-ionized water), 25, 100 or 400 mg/kg/day 311C90); daily oral gavage (stomach tube).

EXPERIMENTAL DESIGN

Dose Groups

Group	Treatment	Dose (mg/kg)	Conc. (mg/ml)	Cage No.		Animals Numbers (93 prefix)	
				M	F	M	F
1	Control	0	0	1-6	28-33	2790-2819	2919-2948
2	311C90	25	2.5	7-12	34-39	2820-2849	2949-2978
3	311C90	100	10	13-18	40-45	2850-2879	2979-3008
4	311C90	400	40	19-24	46-51	2880-2909	3009-3038
Satellite (Pharmacokinetic) Groups:							
5	311C90	25	2.5	25	52	2910-2912	3039-3041
6	311C90	100	10	26	53	2913-2915	3042-3044
7	311C90	400	40	27	54	2916-2918	3045-3047

The last two cages in each main group for each sex were designated as the recovery animals.

The following were evaluated:

Mortality, clinical signs, body weights, food, water, clinical path (hematology, clinical chemistry), urinalysis, ophthalmoscopy, pharmacokinetic methods, necropsy (adrenals, bone marrow, brain, caecum, cervical lymph node, colon, duodenum, eyes/Harderian glands, femur and femoro-tibial joint, heart, ileum, jejunum, kidneys, larynx, liver, lungs and mainstem bronchi, mesenteric lymph node, oesophagus, ovaries, pancreas, pituitary, prostate, sciatic nerve, seminal vesicles, skeletal muscle (biceps, femoris), skin/mammary gland, spinal cord, spleen, sternum, stomach, submandibular salivary glands, tail, testes/epididymides, thoracic aorta, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, vagina/cervix), macro- and histopathology.

With respect to histopathology, microscopic examination was carried out on all

from animals that died before study end. Tissues with macroscopic findings were also examined microscopically. Finally, after initial examination, the thyroid was identified as a potential target organ and thyroid was examined from all animals in control and high dose groups (including recovery groups).

Results:

Mortality

1. Summary of unscheduled deaths

Group	Sex	Total deaths	Toxicity not determined	Procedural[1]/ Incidental
1	M	0	0	0
2	M	2	1	1
3	M	0	0	0
4	M	10	10	0
1	F	1	0	1
2	F	4	0	4
3	F	1	0	1
4	F	13	13	0

[1]Procedural i.e. killed for humane reasons because of swollen eye/red matter/scab

Note: Group 1 (saline control); Group 2 (25 mg/kg/day); Group 3 (100 mg/kg/day); Group 4 (400 mg/kg/day).

There was a clear effect at the high dose, with 10 of 30 male and 13 of 30 female unscheduled deaths. Most of the deaths occurred from on or after Week 12 of the study. All deaths occurred during the dosing phase, without any preemptory signs.

The sponsor stated that the cause of death for the majority of the animals was related to procedure (ocular lesions from orbital bleeding, necrotizing laryngitis due to gavage). The cause of death was not determined for one male (25 mg/kg) or any of the females at 400 mg/kg. The animals generally presented with pulmonary congestion (minimal to marked severity), usually accompanied by thymic congestion/hemorrhage. Congestion was apparently also noted sporadically in various other tissues in these

Clinical Signs

The only consistent clinical sign seen with 311C90 was *flushed extremities,* seen consistently from Day 10 onward in the majority of male (30 of 30, high dose, Week 12) and female (27 of 30, high dose, Week 12) animals at 100 and 400 mg/kg/day and in males at 25 mg/kg/day. This effect occurred only sporadically in females at the low dose. The cause for this flushing is unclear, but the effect completely disappeared in both high dose males and females by Week 27 of the study.

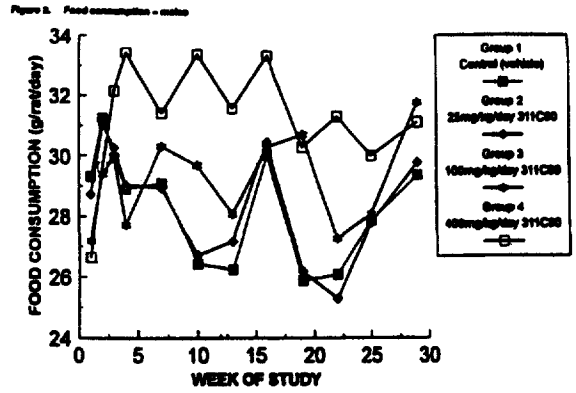
Body Weight

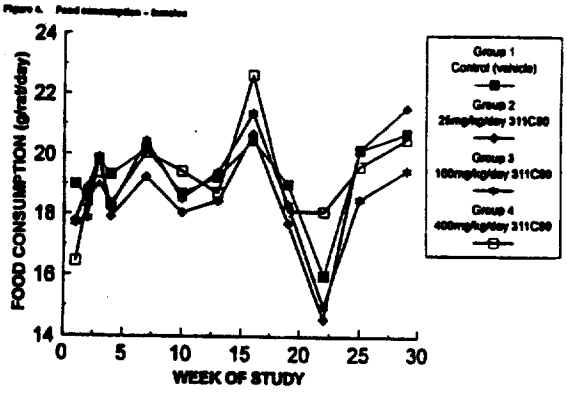
Between Weeks 8 and 9, body weights in male and female high dose (400 mg/kg/day) animals were about 5-6% greater than vehicle controls (saline). These differences were statistically significantly different ($p<0.05$).

Food Consumption

For approximately the first 20 weeks of the study, high dose (400 mg/kg/day) males had a higher food consumption than controls and both high dose males and females showed a greater water consumption than controls (see sponsor's Figures 3-6 below).

Food Consumption (figures 3 and 4)





Water Consumption (figures 5 and 6)

Hematology

There were no consistent, dose-dependent effects.

Clinical Chemistry

On observation day 183, glucose levels were increased in high dose males about 18.5% (stats. significant $p<0.05$) over saline controls.

On observation day 183, ALP (alkaline phosphatase) levels were increased in high dose females 55.6% (stats. significant $p<0.001$) over saline controls.

On observation day 88, plasma urea increased 10% in males at 100 mg/kg/day compared to saline controls, but they decreased on day 183 and remained unchanged on day 211.

On observation day 8, creatinine levels increased 16.2% in high dose males, while no effects was seen in female animals.

There were no clearly dose-dependent effects that occurred consistently in both genders.

Urinalysis

Urine volume increased in high dose males 37-44% during the last two observations periods (days 164 and 206) of the study. No such effect was seen in females.

Organ Weights

Organ weights relative to body weights: liver increased 13.6% in HDM (day 183) and 7.6% in HDF (day 183)

Absolute organ weights: Spleen weights increased 19% in HDF (day 183); liver weights increased 17% in HDM (day 183) and 11.5% in HDF (day 183); thyroid weights increased 33% in HDM (day 183) and 33% in HDF (day 183). All of these increases are relative to saline controls of the same gender.

Ophthalmoscopy

No effects.

Pathology

Macropath

There were no consistent, dose-dependent effects observed in the study animals that survived to the end of the study. While no cause was determined for the unscheduled deaths, animals demonstrated the following upon macropath examination:

Unscheduled deaths

Heart:

Firm heart: 3 females (400 mg/kg)

Kidneys:

Unilateral dilated pelvis: 2 males and 1 female (400 mg/kg)
Dark discoloration: 1 male (400 mg/kg)

Liver:

Dark discoloration: 2 males and 2 females (400 mg/kg)

Lungs:

Red focus/foci: 1 male (400 mg/kg)
Generalized red discoloration: 2 males and 1 female (400 mg/kg)
Pale discoloration: 1 male (400 mg/kg)
Dark discoloration: 2 females (25 mg/kg); 4 males and 7 females (400 mg/kg)

Thyroid:

Enlarged: 2 females (400 mg/kg)

Thymus:

Mottled: 1 male and 5 females (400 mg/kg)
Red discoloration: 3 males and 1 female (400 mg/kg)
Discoloration: 1 male and 1 female (400 mg/kg)
Dark discoloration: 1 male (400 mg/kg)

With respect to the thyroid, macropath effects were also in animals that were part of an interim sacrifice, as follows:

Interim sacrifice

Thyroid:

Enlarged: 1 of 13 males examined (400 mg/kg)

Histopathology

Histopathology results of note were as follows:

Interim Sacrifice

	Males				Females			
Dose (mg/kg)	**0**	**25**	**100**	**400**	**0**	**25**	**100**	**400**
Number in group	**20**	**18**	**20**	**13**	**19**	**17**	**19**	**13**
Thyroid								
Mononuclear Cell Foci	0	0	0	0	0	0	0	1
Hypertrophy (increased follicular-epithelial height)								
>minimal	2	3	1	9	1	0	2	6

Final Sacrifice

	Males				Females			
Dose (mg/kg)	**0**	**25**	**100**	**400**	**0**	**25**	**100**	**400**
Number in group	**10**	**10**	**10**	**7**	**10**	**9**	**10**	**4**
Lungs								
Alveolar histiocytosis								
>minimal	0	1	1	1	0	0	0	0
>slight	0	0	1	0	0	0	0	0
>moderate	0	0	1	0	0	0	0	0
Foci of hepatocellular necrosis								
>slight	0	1	0	0	0	0	0	0
Hepatocyte vacuolation								
>slight	0	0	0	1	0	0	0	0
Thyroid								
Hypertrophy (increased follicular-epitheliall height)								
>minimal	2	2	0	4	1	0	2	0

Unscheduled Deaths

		Males				Females		
Dose (mg/kg)	**0**	**25**	**100**	**400**	**0**	**25**	**100**	**400**
Number in group	**0**	**2**	**0**	**10**	**1**	**4**	**1**	**13**
Lungs								
Alveolar histiocytosis								
>minimal	0	0	0	1	0	0	0	1
Congestion								
>minimal	0	0	0	1	0	0	0	2
>slight	0	0	0	2	0	0	0	2
>moderate	0	0	0	3	0	1	0	3
>marked	0	0	0	1	0	0	0	3
Spleen								
Extramedullary hemato-poiesis								
>minimal	0	2	0	4	1	0	1	6
>slight	0	0	0	3	0	0	0	1
Thymus								
>minimal	0	0	0	1	0	0	0	2
>slight	0	1	0	0	0	0	0	2
>moderate	0	0	0	3	0	0	0	4
>marked	0	0	0	1	0	0	0	0
Liver								
Leucocyte foci								
>minimal	0	0	0	5	0	1	0	3
>slight	0	1	0	1	0	0	0	0
Foci of hepatocellular necrosis								
>minimal	0	0	0	1	0	0	0	0
>slight	0	1	0	2	0	0	0	0
Kidney								
Tubular basophilia								
>minimal	0	0	0	2	0	0	0	1
>slight	0	0	0	2	0	0	0	0
Mononuclear cell foci								
>minimal	0	0	0	1	0	0	0	1
Thyroids								
Hypertrophy (increased follicular-epithelial height)								
>minimal	0	1	0	1	0	0	0	5

Toxicokinetics

311C90 at 25 mg/kg/day: C_{max} and AUC_{0-T} for 311C90 and metabolites

DAY 1				
	AUC_{0-T} (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	3546	5299	352	746
183C91	425	not done	31	29
1652W92	332	not done	43	33
2161W92	not done	201	25	58
DAY 169				
zolmitriptan	38681	56504	5065	7976
183C91	1927	2474	250	333
1652W92	8296	3778	1117	528
2161W92	4973	7706	726	1090

311C90 at 100mg/kg/day: C_{max} and AUC_{0-T} for 311C90 and metabolites

DAY 1				
	AUC_{0-T} (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	24343	29714	2083	3135
183C91	2410	1422	87	99
1652W92	2961	1565	182	184
2161W92	2695	3141	136	332
DAY 169				
zolmitriptan	140742	194472	25320	24252
183C91	5021	4417	762	475
1652W92	16305	11944	2470	1707
2161W92	12718	15573	2208	1976

311C90 at 400mg/kg/day: C_{max} and AUC_{0-T} for 311C90 and metabolites

DAY 1				
	AUC_{0-T} (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	127999	180969	6639	7403
183C91	7951	5945	248	239
1652W92	13235	6892	585	255
2161W92	12359	14899	451	608
DAY 169				
zolmitriptan	653895	612173	91757	81840
183C91	18287	19200	2483	2593
1652W92	51111	32714	7411	4217
2161W92	56164	67510	8101	8405

311C90 (zolmitriptan)

On Day 1, exposure (AUC) increased proportionally with dose in both males and females. On Day 169, AUCs increased proportionally with dose in both males and females, but overall exposures had increased about 6-10-fold over the same dose on Day 1. Therefore, exposure increased about 6-10-fold with time (Day 1 to Day 169).

C_{max} was reported to occur at *2-6 hours* post-dose. $T_{1/2}$ was about *14 h* at the mid and high doses.

183C91

On Day 1 exposure levels (AUC) increased proportionally with dose in males and females. On Day 169, AUCs also increased proportionally with dose. However, on Day 169 AUCs were 2-3-fold greater than on Day 1 at the same doses, and the zolmitriptan:183C91 ratio had increased at each dose. On Day 1, the zolmitriptan:183C91 ratios were in the 10-20-fold range whereas on Day 169 this ratio is in the 20-40-fold range.

$T_{1/2}$ was at about 29 hours for the 183C91 metabolite in this study.

1652W92

On Day 1, exposure levels increased proportionally with dose. On Day 169, AUCs at each dose were increased about 2-8-fold over those on Day 1. Ratios of zolmitriptan:1652W92 increased with time. C_{max} was 2-6 h and $T_{1/2}$ was about 21h.

2161W92

Again, AUCs increased dose-proportionally at both Day 1 and Day 169, while AUC was about 4-6-fold on Day 169 as compared to same doses on Day 1. Therefore, ratio of AUC for zolmitriptan:2161W92 increased with time. Plasma C_{max} occurred at about 2-6 hours after dosing and the $T_{1/2}$ was about 23h.

Comparison to Human Exposure

Based on the fact that the majority of the deaths (they chose to ignore the single male death at 25 mg/kg/day with unexplained cause of death) as well as the increased incidence of minimal thyroid hypertrophy occurred occurred at the 400 mg/kg/day dose, the sponsor designated the NOEL effect level to be 100 mg/kg/day. This resulted in an AUC (Day 169) of 140742 (males) to 194472 (females) ng.h/ml, which is in the range of 880-1215-fold greater than the AUC (160 ng.h/ml) at the maximum proposed human dose.

However, upon careful review of the mortality and macro- and histopathology data, it is my opinion that designation of 100 mg/kg/day as the NOEL for this study is not as straightforward as the sponsor indicated. A single male animal whose cause of death was not determined to be due to dosing or bleeding procedures died at a dose of 25 mg/kg/day, the lowest dose administered in this study. Therefore, with respect to death, no true NOEL or LLD (lowest lethal dose) can be determined. At 25 mg/kg/day, AUCs (Day 169) for zolmitriptan were 38681 ng.h/ml for males and 56504 ng.h/ml for females. This is about 242-353-fold greater than the AUC reported in humans (160 ng.h/ml) receiving the maximum recommended daily dose of zolmitriptan (15 mg/day).

Furthermore, although I will agree that the majority of the macro- and histopathological effects were seen at the high dose (400 mg/kg/day), there were a few animals in the "interim sacrifice", "final sacrifice" and "unscheduled death" groups that demonstrated such effects as increased incidence of thyroid hypertrophy, alveolar histiocytosis in lungs, foci of hepatocellular necrosis, and moderate congestion of the lungs even at the lowest dose (25 mg/kg/day). Granted these effects occur at very low incidence compared to those at the high dose, but they did occur. However, at 25 mg/kg/day, there is still about a 242-353-fold margin of safety with respect to AUC between this dose and the maximum recommended daily dose in humans.

Study Summary and Conclusions

The main effect of note in this study was the large number of unscheduled deaths, especially at the high dose (10 of 30 males; 13 of 30 females). The sponsor stated that the cause of death for the majority of these animals was related to dosing or bleeding procedures, but in fact, no cause of death was determined for one male at 25 mg/kg/day or for any of the female animals receiving 400 mg/kg/day. Furthermore, these deaths occurred without any apparent preemptory signs.

The other effects include scattered weak evidence of effects on kidney, including increased creatinine levels (HDM), plasma urea (males, 100 mg/kg/day) and urine

increase in the incidence of thyroid hyperplasia, which occurred mainly at the high dose (400 mg/kg/day), and a low incidence (1 of 10 animals) of alveolar histiocytosis, hepatocellular necrosis, or moderate lung congestion at the low or intermediate doses in "interim sacrifice", "final sacrifice" or "unscheduled death" animals.

If one accepts the sponsor's NOEL of 100 mg/kg/day, there is an 880-1215-fold margin of safety with respect to AUC (160 ng.h/ml) in the human at the maximum proposed human dose. The lowest dose in the study (25 mg/kg/day) still provides a margin of safety of about 242-353-fold greater than the AUC reported at the maximum proposed human dose.

With respect to toxicokinetics, the exposure to 311C90 (zolmitriptan) increased 6-10-fold with time. Exposure (AUC) to 183C91, 1652W92, and 2161W92 metabolites also increased with time, but since exposure to zolmitriptan increased at a greater rate with time the overall effect was an increase in the zolmitriptan:metabolite ratio for all three metabolites over time. This result could be interpreted to indicate a saturation of the metabolic pathway(s) over time, resulting in increased exposure to parent and overall decreased exposure to the three known metabolites.

2. **Report of a 28-week oral toxicity study of 311C90 in beagle dogs (study# BDRE/92/0166), The Wellcome Foundation Ltd., Ken, UK, Dept. of Pharmacology/Toxicology, June 9, 1995, 311C90 Batch: lot Q5, GLP.**

Study Design: 311C90 (zolmitriptan) was administered orally to three groups of Beagles (6/sex/group), once daily at dose levels of 5, 25 or 100 mg/kg/day for 26 weeks. These dose levels were attained through an initial escalating dosing regimen for two weeks, as shown below. A fourth group of animals (6/sex/group) received vehicle (deionized water) and served as controls. A subset of animals from each group served as a recovery group (4 weeks recovery after the 28-week treatment period).

A series of solutions of the following concentrations were made by serial dilution from the high level solution to achieve the required dosage levels.

Escalating dose phase (Weeks 1 - 2)

Dosage/treatment (mg/kg/day)	Concentration (mg/ml)	Dose volume (ml/kg)
2	0.4	5
5	1.0	5
10	2.0	5
15	3.0	5
25	5.0	5
50	10.0	5
100	20.0	5

Fixed dose phase (Weeks 3 - 25)

Group/	Dosage/treatment	Concentration	Dose volume

Dosing of animals was accomplished as follows:

Day(s)	Highest dose level administered (mg/kg/day)	Comments
1 - 3	2	
4 - 6	5	Low dosage achieved Day 4
7 - 8	10	
9	15	
10 - 11	25	Intermediate dosage achieved Day 10
12 - 14	50	
15	100	High dosage achieved

The 26-week fixed dose phase commenced, for all groups, on Day 15 when the high dose group reached the target dose. Blood samples were taken on four occasions during the study to measure plasma levels of 311C90 and metabolites.

The following observations were made during the study:

Mortality, clinical signs, body weights, food consumption, ophthalmoscopy, electrocardiography, toxicokinetics, hematology (including differentials), serum chemistry, urinalysis, bone marrow smears and macro- and micropathology. Organ weights included adrenals, brain, heart, kidneys, liver, lung, ovaries, pancreas, pituitary, prostate, spleen, submandibular salivary glands, testes, thymus, thyroids (with parathyroids), and uterus. Tissues preserved for histopath were as follows: adrenals, alimentary tract, aorta, brain, cervix, eyes, femur, gall bladder, heart, kidneys, lachrymal glands, liver, lungs (with bronchi), lymph nodes, mammary gland, nictitating membrane, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum (with marrow), tatoo, testes (with epididymides), thymus, thyroids (with parathyroids), tongue, trachea, ureter, urinary bladder, uterus, vagina.

Microscopic analysis was carried out on all protocolled tissues from all animals. Any tissues for which a macroscopic finding was recorded was also examined microscopically.

Results

Mortality

Single male (#821; 25 mg/kg/day): sacrificed moribund on Week 26 (Day 4) due to a series of convulsions/collapse and self-mutilation. Sponsor stated that these effects were drug-related, due to exaggerated pharmacological effects. This animal presented with elevated plasma urea, creatinine, cholesterol and triglyceride values (see Appendix 1 below), but no notable findings in organ weights or macroscopic pathology.

APPENDIX 1

(Clinical histories and pathological findings - continued)

Biochemistry for Dog 821♂

Week	Glucose mg/dl	Protein g/dl							A/G	Urea	Creatinine	ALP	GPT	GOT	γGT	OCT	Bilirubin	Na	K	Ca	P	Cl	Chol	Triglyc
		Total	Alb	A1	A2	B	G	Glob																
-4	112	5.2	2.3	0.8	0.4	1.4	0.3	2.9	0.22	19	0.6	303	27	16	1	5.3	0.2	151	4.3	5.6	5.5	105	168	33
-1	114	5.6	2.6	1.0	0.4	1.3	0.3	3.0	0.15	24	0.7	300	31	11	3	2.6	0.1	151	4.7	5.6	4.9	106	181	38
6	96	5.8	2.6	0.9	0.5	1.5	0.3	3.2	0.79	24	0.8	256	23	20	<1	2.0	0.3	151	4.3	5.7	4.4	107	213	39
13	90	5.3	2.7	0.7	0.5	1.2	0.3	2.7	1.00	22	0.9	219	21	24	<1	3.5	0.3	146	4.8	5.4	3.2	108	176	22
26 (Day 1)	103	6.3	3.1	0.8	0.6	1.4	0.4	3.2	0.96	36	1.2	158	42	23	3	2.7	0.3	157	3.7	5.9	3.8	103	220	44
26 (Day 4)	99	6.1	3.0	0.9	0.6	1.3	0.4	3.1	0.96	51	1.5	144	36	30	2	13.2	0.3	150	3.8	5.4	2.8	103	300	43

Histopathological examination revealed the following:

Liver: *Leucocyte foci*, minimal multifocal; *granulomatous inflammation*, minimal, multifocal.

Lungs: *Alveolar hemorrhage*, minimal, multifocal; *inflammatory foci*, minimal focal; *granuloma*, slight, focal.

Tongue: *Chronic mucosal inflammation*, minimal, multifocal.

Urinary bladder: *Mucosal hemorrhage*, minimal, multifocal.

Spleen: *Congestion*, slight.

Thymus: *Involution*, slight.

Apparently the bone marrow smear was missing and they were unable to examine this tissue.

Single male (#833; 100 mg/kg/day) found dead on Day 56 of dosing. This animal apparently had surgery to repair a strangulated hernia, and died of a ruptured anastomosis repair. At necropsy, breakdown of the anastomosis site and other related gut lesions were seen. The pathologist's report stated "*...In summary, breakdown of the ileal anastomosis site was evident, marked serosal and mucosal congestion were noted on the ileum, the caecum was full of hard, compacted contents and the colon was distended by solid feces. There were multilple adhesions between the gut and omentum, and the abdominal cavity contained a large quantity of purulent fluid. These findings were considered related to the post-operative clinical condition of the animal, and were considered the principal cause of death.*"

Clinical Symptoms

Escalating and Fixed Dose Phases of the study:

pupillary dilation: all treated animals (onset immediately to several hours after dosing; duration several hours).

pupil constriction: in a few animals in all dose groups during escalating phase and then in all treated animals from Week 17.

vasodilation: (reddening of eyes, gums, ears, muzzle and/or abdomen); high incidence in all treated animals, especially during fixed dose phase; dose-related incidence; onset minutes to hours; duration hours to full day.

Abnormal posture/gait: moderate incidence during escalating and first 9 Weeks of fixed dose phase; effect disappeared after Week 9; onset variable (25 minutes to hours); duration hours.

Trembling: low incidence in all treated groups, mainly during Weeks 1 through 3, decreasing in incidence from Week 4 to 6 with very little occurrence by Week 8; onset 25 minutes to several hours; duration hours.

Aggressive behavior: related to treatment but not dose and occurring commonly during feeding; onset 1 to 2-1/2 hours after treatment; duration about 2 hours after onset.

Abdominal contractions: mainly during escalation phase, only in low incidence; onset about 2 to 3 hours after dosing.

Abnormal drinking/bloated appearance: moderate incidence in all treatment groups; treatment but not dose-related; not seen after Week 7; onset 1 to 4-1/2 hours after dosing; duration hours.

Difficulty awakening/staying awake: all treatment groups; dose-related; onset 2-1/2 to 6 hours after dose administration.

Subdued behavior: beginning Day 7; dose-related incidence by end of Week 3; no occurrence beyond Week 8.

Prominent nicitating membranes: about half the docs at high dose (100 mg/kg/day) during Week 4 mainly; onset 45 minutes to 2 hours; duration hours.

Salivation: slightly more frequent at 25 and 100 mg/kg/day; no occurrence beyond Week 3.

Recovery Phase

Pupil constriction: high incidence in all previously treated animals; persists for 1 to 2 weeks in some animals.

Photophobia: noted on 5 different days for one male animal at 25 mg/kg/day and one female animal during Week 1 of recovery, at this same dose.

Body Weights

During the first two weeks of treatment, bodyweight gain was impaired in all animals treated with 5 mg/kg/day, and animals receiving 25 or 100 mg/kg/day showed an actual loss in body weight.

During the recovery period, one dog of each sex previously receiving 5 mg/kg/day, all dogs receiving 25 mg/kg/day and two females previously receiving 100 mg/kg/day all showed bodyweight losses in excess of those seen in the control group.

Food Consumption

No effect.

Ophthalmoscopy
No effect.

Electrocardiography
Heart rate: Day 28 there was some slight increase in heart rate (140 bpm in control; 152 bpm at 100 mg/kg/day). During the recovery period (R4), the heart rates actually decreased in animals that had received the higher drug doses (see table below).

TABLE 5

Heart rates - group mean values (beats/min)

Fixed dosage mg/kg/day	Week no.				
	-4	-3	15	28	R4
Males					
Control (vehicle)	151	135	135	136	
5	148	139	130	140	
25	146	149	133	135	
100	129	142	145	153	
Females					
Control (vehicle)	136	130	135	144	
5	132	135	139	145	
25	139	144	140	146	
100	158	163	150	151	
Combined					
Control (vehicle)	143	132	135	140	146
5	140	137	135	143	113
25	142	147	137	141	79
100	143	152 +	148	152	97

+ P <0.05
R Recovery

No effects on the ECG were reported.

Hematology

As shown in sponsor's Table 6 below, group mean APTT values were reduced for dogs of both sexes receiving 100 mg/kg/day at Weeks 6, 15 and 28. Also lymphocyte numbers decreased at 25 and 100 mg/kg/day at Week 6 and Week 15.

TABLE 6

(Haematology - continued)

Males and females combined

Plasma dosage mg/kg/day	PCV %	Hb g/dl	RBC $\times 10^6$/mm^3	MCHC %	MCV fl	WBC - Diff $\times 10^3$/mm^3 Total	N	L	E	B	M	Plts $\times 10^3$/mm^3	PT s	APTT s
Week -4														
Control (vehicle)	48	13.8	6.1	29.1	78	11.8	7.08	4.25	0.16	0.00	0.32	400	6.5	14.7
5	46	13.3	5.8	29.2	79	10.9	6.56	4.04	0.11	0.00	0.17	379	6.8	13.9
25	44 *	12.8 **	5.6 **	28.8 *	80	10.8	6.30	4.09	0.17	0.00	0.20	386	6.5	14.7
100	45 *	13.3	5.8	29.5	78	11.1	6.81	3.88	0.16	0.00	0.28	381	6.5	14.2
Week -1														
Control (vehicle)	50	14.4	6.3	29.1	78	10.0	5.65	3.83	0.23	0.00	0.31	331	6.5	17.5
5	48	13.9	6.1	28.7	79	10.5	6.12	4.01	0.12	0.00	0.29	320	6.8	16.2
25	47	13.6	6.0	28.8	79	10.3	5.99	3.78	0.16	0.00	0.37	341	6.4	17.4
100	48	13.7	6.0	28.7	79	10.2	6.41	3.32	0.21	0.00	0.23	326	6.5	16.0
Week 6														
Control (vehicle)	51	14.0	6.4	27.2	81	8.8	5.09	3.35	0.12	0.00	0.27	305	6.7	18.4
5	52	13.9	6.2	26.9	83	8.5	5.19	3.01	0.09	0.00	0.24	282	6.9	18.2
25	52	14.1	6.4	27.1	82	8.4	4.97	2.79 *	0.35 *	0.00	0.26	303	6.4	17.5
100	50	13.4	6.1	27.1	81	8.8	5.93	2.41 **	0.24 *	0.00	0.24	292	6.5	13.5 **

+ P <0.05, ++ P <0.01 Student's 't' test
* P <0.05, ** P <0.01 Williams' test

TABLE 6

(Haematology - continued)

Males and females combined

Plasma dosage mg/kg/day	PCV %	Hb g/dl	RBC $\times 10^6$/mm^3	MCHC %	MCV fl	WBC - Diff $\times 10^3$/mm^3 Total	N	L	E	B	M	Plts $\times 10^3$/mm^3	PT s	APTT s
Week 15														
Control (vehicle)	51	15.4	6.0	30.6	86	11.6	7.03	4.27	0.21 *	0.00	0.09	313	6.7	17.1
5	49	14.6 *	5.6 **	30.1	86	11.6	7.18	4.04	0.22	0.00	0.21	303	6.9	17.8
25	47 **	14.3 **	5.5 **	30.4	85	10.4	6.04	3.74	0.41	0.00	0.19	317	6.5	17.2
100	48	14.4 **	5.7 **	30.1	84	10.8	6.80	3.53	0.38	0.00	0.13	312	6.7	12.7 **
Week 28														
Control (vehicle)	56	15.4	6.2	27.7	90	10.8	6.66	3.73	0.20	0.00	0.21	295	6.8	18.7
5	55	15.1	6.0	27.5	92	10.3	6.63	3.39	0.17	0.00	0.11	299	7.0	18.9
25	55	15.0	6.0	27.3 *	92	10.2	6.57	2.85	0.30	0.00	0.21	295	6.5	17.4
100	53	14.5 *	5.8 *	27.1 **	92	11.1	7.12	3.23	0.47 **	0.00	0.18	268	6.6	13.5 **
Week 54														

APTT (activated partial thromboplastin time) is used to screen for functioning of factors VIII, IX and XI blood clotting factors. Deficiencies in these factors or inhibition of their function can lead to excessive bleeding problems.

APTT values had recovered to control levels on recovery week 4 (R4). Therefore, this was a reversible process.

Biochemistry

Triglyceride levels were reduced in animals (100 mg/kg/day) on Weeks 6 and 15 (See sponsor's Table 7 below), and in females only receiving 25 mg/kg/day on Week 15 (data not shown). By Week 28 levels were still reduced, but the effect was not statistically significant ($p<0.05$), and by recovery week 4 levels were back to those of controls indicating that the effect was reversible.

TABLE 7

(Biochemistry - continued)

Males and females combined

Final dosage mg/kg/day	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl	Tri-glyc mg/dl
Week -4												
Control (vehicle)	24	18	<2	3.6	<0.2	150	4.8	5.6	5.0	107	134	38
5	24	21	<2	3.6	0.3	150	4.4	5.5	4.7	106	138	34
25	25	22	<2	3.5	0.2	150	4.5	5.5	5.0	107	125	31
100	25	20	<2	4.5	0.2	149	4.2	5.5	4.8	107	134	33
Week -1												
Control (vehicle)	26	18	<3	3.2	<0.2	150	4.5	5.4	4.8	108	133	34
5	25	18	3	3.5	0.1	149	4.2	5.4	4.6	108	131	31
25	28	17	<3	3.0	<0.2	149	4.3	5.3	4.5	109	126	30
100	26	17	<3	3.2	<0.2	150	4.3	5.4	4.5	109	133	30
Week 6												
Control (vehicle)	25	17	<2	3.3	0.3	153	4.4	5.5	3.9	112	118	32
5	92	19	<2	3.3	0.3	153	4.2	5.6	3.8	111	143	36
25	24	17	<2	2.7	0.3	152	4.2	5.6	3.7	110	138	26
100	24	16	<2	3.1	0.3	153	4.3	5.7	3.7	110	135	20

+ $P<0.05$ Student's 't' test
* $P<0.05$, ** $P<0.01$ Williams' test

TABLE 7

(Biochemistry - continued)

Males and females combined

Final dosage mg/kg/day	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl	Tri-glyc mg/dl
Week 15												
Control (vehicle)	30	20	<1	3.3	0.3	148	4.1	5.5	3.8	110	117	36
5	31	20	<1	3.0	0.2	147	4.1	5.5	3.4	108	143	36
25	25	19	<1	3.2	0.2	148	4.1	5.4	3.1	110	134	27
100	26	20	<1	3.2	0.2	148	4.1	5.5	3.3	110	120	22
Week 28												
Control (vehicle)	32	22	<2	3.2	0.2	149	4.0	5.4	2.7	108	108	36
5	33	22	<2	3.8	0.2	147	3.9	5.3	2.7	108	130	45
25	25	21	<2	3.4	0.2	148	4.1	5.3	2.5	108	124	37
100	32	24	<2	3.7	0.2	148	4.0	5.4	2.6	109	124	29
Week R4												
Control (vehicle)	29	18	2	3.6	0.2	150	4.1	5.3	2.5	109	120	25
5	36	20	<3	2.9	0.3	149	4.1	5.5	3.1	109	114	32
25	28	22	2	2.9	0.2	148	4.0	5.2	2.5	110	103	33
100	48	23	2	4.8	0.2	149	4.1	5.5	3.0	109	117	35

* $P<0.05$, ** $P<0.01$
R Recovery

On Week 6, animals receiving 5 mg/kg/day drug had elevated GPT, a liver enzyme. Apparently this increase was caused mainly by elevated GPT levels in 2 male and 2 female animals. The effect occurred only at the low dose and was therefore not dose-dependent. At Week 4 of the recovery period, the high dose animals (100 mg/kg/day) demonstrated a statistically significant ($p<0.05$) elevation in GPT as well. This increase appeared to be real, as two of the three recovery animals examined had GPT levels well above control as seen below (sponsor's Appendices 7 and 8; circled

APPENDIX 8

(Biochemistry - continued)

Recovery Week 4 (29 September 1993)

Fixed dosage mg/kg/day	Animal no.	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl	Tri-glyc mg/dl
Control (vehicle)	7954	35	16	2	4.2	0.1	149	4.0	5.3	2.5	108	120	17
	7974	37	19	3	3.3	0.2	150	4.2	5.2	2.4	111	88	21
	7949	25	18	1	4.2	0.2	150	4.0	5.4	2.9	107	143	32
	7969	25	17	3	2.7	0.2	151	4.0	5.4	2.3	110	127	31
	Mean	29	18	2	3.6	0.2	150	4.1	5.3	2.5	109	120	25
	SD	6.4	1.3	1.0	0.73	0.05	0.8	0.10	0.10	0.26	1.8	23.1	7.4
5	8074	30	21	4	3.9	0.2	146	4.3	5.0	2.8	111	119	29
	8094	32	25	3	3.1	0.3	149	4.2	5.7	3.6	108	131	32
	8089	39	18	<1	3.1	0.3	151	4.2	5.9	3.4	107	117	35
	8109	41	17	3	1.6	0.2	150	3.6	5.2	2.4	109	87	31
	Mean	36	20	<3	2.9	0.3	149	4.1	5.5	3.1	109	114	32
	SD	5.3	3.6		0.96	0.06	2.2	0.32	0.42	0.55	1.7	18.7	2.5

SD Standard deviation

APPENDIX 8

(Biochemistry - continued)

Recovery Week 4 (29 September 1993)

Fixed dosage mg/kg/day	Animal no.	GPT mU/ml	GOT mU/ml	γGT mU/ml	OCT mU/ml	Bili-rubin mg/dl	Na mEq/l	K mEq/l	Ca mEq/l	P mEq/l	Cl mEq/l	Chol mg/dl	Tri-glyc mg/dl
25	8194	27	25	3	4.0	0.3	149	4.3	5.3	2.8	112	74	28
	8209	28	21	1	2.7	0.2	148	4.2	5.0	2.4	112	103	22
	8229	29	21	3	2.0	0.2	148	3.6	5.4	2.4	107	132	49
	Mean	28	22	2	2.9	0.2	148	4.0	5.2	2.5	110	103	33
	SD	1.0	2.3	1.2	1.01	0.06	0.6	0.38	0.21	0.23	2.9	29.0	14.2
100	8314	49	23	2	2.4	0.2	150	4.5	5.6	3.0	109	120	25
	8329	63	26	3	8.0	0.2	149	4.1	5.3	3.3	110	92	52
	8349	31	20	1	4.0	0.3	149	3.7	5.5	2.6	109	139	28
	Mean	48	23	2	4.8	0.2	149	4.1	5.5	3.0	109	117	35
	SD	16.0	3.0	1.0	2.88	0.06	0.6	0.40	0.15	0.35	0.6	23.6	14.8

SD Standard deviation

These data are consistent with an effect of the drug on the liver in the dog. Effects on triglycerides can relate to protein metabolism.

Urinalysis

Total protein was reduce at Week 6 (combined; 100 mg/kg/day) and Week 28 (combined; 5, 25 and 100 mg/kg/day). Cl was decreased at Week 6 (combined; 5, 25 and 100 mg/kg/day) and Week 15 (combined; 100 mg/kg/day).

At recovery Week 4, protein levels had returned to normal levels, while Cl remained decreased at the high dose (100 mg/kg/day) (see sponsor's Table 8 below).

TABLE 8

(Urinalysis - continued)

Males and females combined

Fixed dosage mg/kg/day	Volume ml	pH	SG	Protein mg/dl	Na mEq/vol	K mEq/vol	Cl mEq/vol
Week -4							
Control (vehicle)	139	5.9	1043	34	21.65	33.97	39.72
5	147	5.8	1044	35	27.57	33.17	44.80
25	129	5.8	1045	37	23.39	32.55	40.14
100	108 ++	5.8	1044	40 +	17.15	27.88 +	33.56
Week -1							
Control (vehicle)	137	5.8	1042	34	26.68	30.14	43.30
5	123	5.8	1043	33	26.21	29.36	44.64
25	138	5.9	1045	35	29.49	33.68	46.75
100	120	5.9	1044	33	24.21	30.10	40.24
Week 6							
Control (vehicle)	138	6.3	1048	37	18.14	38.61	36.96
5	127	6.5	1047	34	16.03	38.00	26.70 *
25	133	6.3	1046	33	15.48	36.88	26.28 *
100	155	6.2	1046	32 *	18.35	41.89	31.44 *

+ P <0.05, ++ P <0.01 Student's 't' test
* P <0.05 Williams' test

TABLE 8

(Urinalysis - continued)

Males and females combined

Fixed dosage mg/kg/day	Volume ml	pH	SG	Protein mg/dl	Na mEq/vol	K mEq/vol	Cl mEq/vol
Week 15							
Control (vehicle)	197	6.6	1044	24	34.05	47.99	47.97
5	180	6.8	1044	26	30.19	49.68	40.53
25	175	6.8	1044	23	29.67	46.80	41.09
100	163	6.4	1044	25	20.24 **	44.65	35.36 *
Week 28							
Control (vehicle)	154	6.5	1050	30	21.19	43.35	40.11
5	133	6.6	1048	23 *	20.42	42.81	35.95
25	141	6.5	1048	21 **	18.32	44.90	32.95
100	143	6.4	1045	21 **	13.43	43.84	30.76
Week R4							
Control (vehicle)	139	5.9	1044	36	14.75	37.34	40.43
5	119	5.9	1055	48	19.97	33.36	37.41
25	115	6.1	1059	49	20.52	33.34	31.41
100	67 *	6.1	1043	36	7.70	16.32 *	16.01 *

* P <0.05, ** P <0.01
R Recovery

Organ Weights

Heart weights (means adjusted to body weights) were decreased at the high dose (100 mg/kg; combined genders) (see sponsor's Table 9 below). Female heart weights were statistically significantly decreased ($p<0.01$), while there was a trend toward a decrease in male animals (data not shown).

Salivary gland weights (means adjusted to body weights) were increased (5, 25 and 100 mg/kg/day; combined genders) (Table 9 below).

Heart and salivary gland weights were similar to controls in the recovery animals (data not shown).

TABLE 9

(Absolute organ weights - continued)

Terminal kill - males and females combined

Fixed dosage mg/kg/day	Body wt kg	Brain g	Pituitary mg	Heart g	Lungs g	Liver g	Spleen g	Pancreas g
Unadjusted means								
Control (vehicle)	14.1	86.1	81	112.3	114.6	423.4	121.1	32.5
5	13.6	82.7	80	108.1	110.8	423.7	116.6	31.9
25	13.1	84.4	80	100.0	107.5	398.5	112.4	26.4
100	13.7	85.7	83	97.7	107.6	396.4	110.0	29.3
Adjusted means								
Control (vehicle)		84.6	80	108.8	111.3	410.9		
5		82.9	80	108.4	111.1	424.6		
25		86.0	82	103.6	110.8	411.2		
100		85.6	83	97.3**	107.3	389.1		

** P <0.01

TABLE 9

(Absolute organ weights - continued)

Terminal kill - males and females combined

Fixed dosage mg/kg/day	Thymus g	Kidneys g	Thyroids g	Adrenals g	Salivary glands g
Unadjusted means					
Control (vehicle)	16.2	60.2	1.30	1.83	12.34
5	11.9	60.7	1.19	1.85	13.81
25	10.4	58.2	1.25	1.95	13.40
100	15.4	58.8	1.44	1.98	14.70
Adjusted means					
Control (vehicle)	15.3	58.9	1.22		11.93
5	12.0	60.8	1.20		13.84*
25	11.3	59.5	1.33		13.83*
100	15.3	58.7	1.43		14.66**

* P <0.05, ** P <0.01

Macropathology

According to the sponsor, the only macropath finding of note was the breakdown of the anastomosis repair contribuing to the death of the single 100 mg/kg/day male animal.

Micropathology

There only a minimal number of notable histopath findings.

Interim sacrifice:

Heart: valvular myxomatous change--1 control, 1@25 mg/kg/day (minimal); 1@5 mg/kg/day, 2@100 mg/kg/day (slight).

Kidneys: tubular basophilia--minimal=1@5, 2@25, 3@100 mg/kg/day.

Adrenals: vacuolation (zona glomerulosa cells)--minimal (1 control, 4@5, 6@25, 1@ 100 mg/kg/day; slight (1@5, 2@100 mg/kg/day); moderate (1@25 mg/kg/day).

Tongue: chronic submucosal inflammation--minimal (2 controls, 4@5, 4@25, 5@100); slight (1 control, 2@5, 1@10, 2@ 100 mg/kg/day).

Brain: mononuclear cell focus--minimal (1 control, 1@5, 4@25, 1@100

Final sacrifice:

Liver: ganulomatous inflammation--minimal (3@5, 2@25, 2@100 mg/kg/day); slight (1@25 mg/kg/day).

Kidneys: tubular basophilia--minimal (1@5, 1@100 mg/kg/day).

Lungs: inflammatory foci--minimal (2@5, 1@25, 1@100 mg/kg/day).

Toxicokinetics

The toxicokinetics for 311C90 parent and metabolites 183C91, 1652W92, and 2161W92 are as follows:

Toxicokinetics

311C90 at 5 mg/kg/day: C_{max} and AUC_{0-T} for 311C90 and metabolites

WEEK 3				
	AUC_{0-T} (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	2920	2422	1079	824
183C91	291	224	80	70
1652W92	913	779	348	272
2161W92	1543	1328	471	332
WEEK 28				
zolmitriptan	3424	2752	1496	996
183C91	435	240	94	65
1652W92	1283	911	496	347
2161W92	1738	1089	472	311

311C90 at 25 mg/kg/day: C_{max} and $AUC_{0-\tau}$ for 311C90 and metabolites

WEEK 3				
	$AUC_{0-\tau}$ (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	21239	20061	7145	5446
183C91	1964	1969	368	286
1652W92	6873	7270	2412	2093
2161W92	6893	7096	1778	1354
WEEK 28				
zolmitriptan	25351	24685	8841	7427
183C91	1706	1665	343	320
1652W92	9120	8315	3285	2999
2161W92	8546	7224	1897	1565

311C90 at 100 mg/kg/day: C_{max} and $AUC_{0-\tau}$ for 311C90 and metabolites

WEEK 3				
	$AUC_{0-\tau}$ (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	112724	120712	26480	33373
183C91	7530	7732	1088	1539
1652W92	40402	44727	8265	13477
2161W92	24620	27084	4145	5090
WEEK 28				
zolmitriptan	161568	149650	35237	42636
183C91	9676	7639	1321	1402
1652W92	60151	46416	11938	13091
2161W92	37002	33007	5216	5620

311C90

AUCs increased with dose in a supra-proportional manner (5- and 4-fold increases in dose resulted in about 7- and 6-fold increases in AUC, respectively). T_{max} occurred at 0.5-1h post-dose. The $T_{1/2}$ of elimination was about 3 hours. At the 100 mg/kg/day dose, AUCs increased about 1.4-fold with time (Week 3 versus Week 28), which the sponsor suggested may be due to saturation of absorption of the metabolites 183C91 and 2161W92. Data support this hypothesis in that AUCs for these two metabolites did not increase to the extent of the parent drug over this same time period.

183C91

In previous discussions with the sponsor, it was revealed that this metabolite occurs in humans at about 50% of the concentration of 311C90 parent drug. It is 2-3-fold more potent in the dog and cat (carotid artery and AVA models) than parent drug. Apparently, this active metabolite has about the same receptor specificity and slightly greater potency for the $5HT_{1D}$ receptor than 311C90 parent drug. Finally the sponsor has stated in the pre-NDA meeting that this metabolite has caused tachycardia and increased blood pressure in the conscious dog, and decreased carotid arterial and renal blood flow but no coronary arterial blood flow.

In this dog study, AUCs increased in a dose-dependent manner and approximately proportionally with dose, which also means that AUC levels increased at a lesser rate than those of parent 311C90 drug. C_{max} levels occurred at either 1 or 4 hours post-dose. Elimination was slower than for parent drug, with $T_{1/2}$ of elimination of about 5-8 hours. Average ratios of the AUC for 311C90:183C91 were about 10, 13 and 16-fold at 5, 25 and 100 mg/kg/day doses, respectively.

1652W92

AUCs increased proportionally to those of parent 311C90. T_{max} was 1 or 4 hours post dosing. The $T_{1/2}$ was similar to parent drug at about 3 hours. The ratios of AUC for 311C90:1652W92 remained at about 3-fold at all three doses (5, 25 and 100 mg/kg/day).

2161W92

AUCs increased sub-proportionally with respect to those for parent 311C90, with T_{max} at 0.5-1hour. $T_{1/2}$ for elimination was about 4-5 hours, slightly slower than parent 311C90 drug. Ratios of AUC for 311C90:2161W92 were about 2, 3, and 4-fold at 5, 25 and 100 mg/kg/day doses.

The following table lists the average (combined genders, average value over total 28 weeks) AUC and C_{max} values for 311C89 and metabolites and the average ratio of 311C90:metabolite:

Average C_{max} and $AUC_{0-\tau}$ for 311C90 and metabolites over entire study period

WEEK 3				
	Dose (mg/kg/day)	$AUC_{0-\tau}$ (ng.h/ml)	C_{max} (ng/ml)	Ratio 311C90:metabolite
zolmitriptan	5 25 100	3140 22609 132478	1221 7651 36022	
183C91	5 25 100	310 1755 8104	86 341 1343	10 13 16
1652W92	5 25 100	1008 7292 43402	394 2573 11021	3 3 3
2161W92	5 25 100	1682 7668 31080	470 1727 5338	2 3 4

NOEL and dose comparison to maximum recommended daily dose in humans

The maximum recommended daily dosing for ZOMIG™ is 15 mg (0.25 mg/kg) per day. This resulted in an AUC in humans of about 160 ng.h/ml.

The sponsor argues that the only effects caused by drug administration are exaggerated pharmacological effects, and therefore the NOEL for this study is 100 mg/kg/day. I would argue that there are, in fact, a number of toxicological effects that do not fall in the category of "exaggerated pharmacological effects". APTT values were reduced about 30% on Weeks 6, 15 and 28 for dogs of both sexes at 100 mg/kg/day (high dose). Triglyceride levels were reduced up to 39% at the high dose on week 15. GPT levels were increased about 39% at the high dose during Recovery Week 4. Finally, heart weights were decreased 11% at the high dose. Therefore, I would argue that the NOEL is approximately 25 mg/kg/day, which gave an average AUC of about 22609 ng.h/ml over the course of the 28 week study. This gives about a 141-fold safety margin with respect to AUC for 311C90 drug between the NOEL in animals and the maximum recommended daily dose in humans.

Summary and Conclusions for 28-week dog study

Animals demonstrated a number of clinical symptoms consistent with exaggerated pharmacological effects of the drug such as pupillary dilation, vasodilation, trembling, and difficulty awakening at all treatment groups, with a dose-dependent pattern. However, none of these symptoms were of a serious nature and the majority disappeared over time. Even at the low dose (5 mg/kg/day), the average AUC (3140 ng.h/ml) gives a 20-fold safety margin over that for the maximum recommended daily dose (AUC 160 ng.h/ml) in the clinic.

A single animal was killed moribund after a number of convulsions. However, this was only a single animal at 25 mg/kg/day, while none of the high dose animals were reported to convulse. At 25 mg/kg/day, the margin of safety (AUC) is 141-fold with respect to 311C90 parent drug.

Heart rates increased about 5-12% at the high dose on Day 28, while heart rates decreased at the 4 Week Recovery point. APTT values decreased about 30% at Weeks 6, 15 and 28 at the high dose. GPT levels increased at the high dose at the 4 Week Recovery timepoint. Heart weights decreased at the high dose. Finally, histopath evaluation revealed a number of sites of inflammation (tongue submucosa, liver, lungs), basophilia (kidneys) or mononuclear cell foci (brain) that occurred in a dose-dependent manner.

With respect to toxicokinetics, 311C90 parent exposure increased in a supra-proportional dose-dependent manner, especially at the 100 mg/kg/day dose. This may due, in part, to a saturation of metabolic pathways for 183C91 and 1652W92 metabolites. The active metabolite 183C91 occurred at AUC in the range of 6-10% of the parent 311C90 drug, depending on dose. In humans it has been reported that this metabolite occurs at about 50% of 311C90 parent drug levels.

The sponsor concluded that the NOEL was 100 mg/kg/day, based on their contention that all effects were the result of exaggerated pharmacological effects of the drug and therefore no toxicity was apparent. However, my conclusion based on effects on APTT level, triglyceride levels, heart weights and GPT levels is that the NOEL is in the range of 25 mg/kg/day, which still gives a margin of safety of about 141-fold by AUC compared to the maximum recommended daily dosing in humans.

3. A one year oral toxicity study in Beagle dogs given 311C90 (study# TTEP/96/0016), Glaxo Wellcome R&D, Research Triangle Park, N.C., June 3, 1996, 3111C90 Batch reference# 93/0019-121-D, GLP.

Study design

The study design is summarized in the following sponsor's table:

SUMMARY

Materials and Methods Summary:

Dose (mg/kg/day)	0, 5, 25, or 100. Final doses were attained via gradual dose escalation over a 15 day period. The drug concentration varied while the dose volume remained unchanged (see table below).
No of Dogs/Dose Control or 100 mg/kg/day 5 or 25 mg/kg/day	 6 4
Formulation	311C90 was dissolved in 1M HCl and titrated to a pH of 5.0 to 5.6 then QS to final concentration of 100 mg/mL with deionized water. Dilutions of this were prepared with deionized water to accommodate all doses.
Solution Concentrations (mg/mL) stock for dose esculation final concentration (Group 2) final concentration (Group 3) final concentration (Group 4)	 100 2, 5, 10, 15, 25, 50 and 100 The drug concentration varied while the dose volume remained unchanged (see table below) 5 25 100
Frequency of Dosing	Once daily
Dose Volume	1 mL/kg
Dose Route	Oral – liquid filled gelatin capsule
Vehicle	Deionized water in gelatin capsules
Toxicity Parameters	Clinical signs, body weights, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, organ weights, gross pathology, histopathology and ECG's
Toxicokinetic Parameters	Concentrations of 311C90 and metabolites 0.75, 4, 8, 12 and 24 hours after dosing on study days 23 and 366

The number of animals was actually 4/sex/group or 6/sex/group.

Also, 2 animals/sex/group from the Control and high dose (100 mg/kg/day) group were chosen as 30-day recovery animals.

The gradual dose escalation in the dogs (1st 15 days of study) is described in the sponsor's table below:

Dose Day(s)	Dose Administered (mg/kg/day)	Comments
1-3	2	
4-6	5	Low dose achieved on study day 4
7, 8	10	
9	15	
10, 11	25	Mid dose achieved on study day 10
12-14	50	
15	100	High dose achieved on study day 15

Following is the timing of the observations:

Observations and Measurements

Clinical Signs	Daily
Body Weights	Weekly
Food Consumption	Weekly
Clinical Pathology[a]	Study days -5, 87, 178, 268, 365 and +29 (also sacrificed animals 94-8226 Group 1 male and 94-8267 Group 3 female on study days 14 and 36, respectively)
Urinalysis[a]	Study days -6 (94-8237 recollected -1), 177, 364 and +28
Ophthalmic Examination[b]	Study days -15, 182, 364, +28
Electrocardiographic Examination	Study days -22 or -21, -13 or -12, 84 or 85, 176 or 177, 265 or 266, 359 and +27
Drug Plasma Concentration	0.75, 4, 8, 12 and 24 hours after dosing on study days 23 and 366

Scheduled Sacrifice	Dose days +1 and +30
Organ Weights	All dogs.
Gross Examination	On all animals which died were sacrificed for humane reasons or killed on scheduled sacrifice dates.

Results

Mortality

Two treated animals were sacrificed early due to aggressive behavior and one control was sacrificed early due to hemorrhaging in the submucosa of the penis. A single high dose animal (100 mg/kg/day) was found dead on study day 280 (see sponsor's table below):

L.1 ***Deaths***

Animal No./Sex (94–prefix)	Dose (mg/kg/day)	Study Day	Reason for death
8226 M	0	14	Sacrificed due to severe hemorrhaging. Grossly found hemorrhage in the submucosa of the penis from middle to end of penis and the left testicular vein and artery appeared larger than the right.
8246 M	5	367	Aggression on study day 366. Animal sacrificed on study day 367
8267 F	25	36	Aggression on study day 23. Animal was held undosed quarantine for 13 days and then sacrificed.
8241 M	100	280	Found dead. On study day 275 animal was observed having the following clinical signs: emesis, salivation, cyanosis, ataxia, body tremors, difficult to arouse and labored breathing/panting. The previous body weight on this dog was 19.52 kg.

Apparently the cause of death was undetermined. The sponsor states that histopathology results did not point to a particular cause of death for animal 8241M. In this study, only a single high dose animal (100 mg/kg/day) was found dead before the scheduled sacrifice.

Clinical symptoms

Clinical symptoms fell into two categories, 1) symptoms observed mainly during the dose escalation phase (Days 1-16) and 2) symptoms seen intermittently throughout the study in treated animals. Clinical symptoms are shown in the following sponsor's table:

L2 ***Clinical Observations***

Signs noted in animals were primarily limited to the beginning of dosing during the dose escalation phase (days 1–16). The clinical signs were noted and the number of dogs affected are shown below:

Group	Dose (mg/kg/day)	N=	Ataxia	Decreased Activity	Intermittent Howling	Lying unresponsive in cage – able to arouse with difficulty	Pupils Dilated	limited limb movement, weakness and/ or extention in the hindquarters
2	5	8	8	3	8	4	8	0
3	25	8	8	6	8	3	8	4
4	100	12	12	12	12	11	12	6

Signs noted intermittently during dosing in limited numbers of animals attributed to drug effects are tabulated below with the numbers of animals per dose exhibiting the sign.

	Dose (mg/kg/day)		
	5	25	100
Clinical Sign	N=8	N=8	N=12
Body tremors, twitching		2	1
Clonic convulsions			3
Aggressive	2	2	1
Growling			2
Excessive drinking		2	6
Cyanotic		1	
Prostration			3
Exophthalmia		1	
Excessive panting or slow breathing			1
Pale mucosa			1
Red gingiva or skin (ear and/or neck)		2	2
Ulcer gingiva			1
Eye movement back and forth			1[a]
Relaxed nictitan			1[a]

[a] These signs were noted during the same time as prostration.

The clinical symptoms, both those seen up to day 16 and those seen intermittently throughout the study, occurred in a dose-dependent manner. The symptoms including ataxia, aggressiveness, body tremors/twitching, may be due to increased sympathetic outflow, probably through $5HT_{1A}$ receptor sites. The most disconcerting effect was the occurrence of clonic convulsions in 3 of 12 high dose (100 mg/kg/day) dogs. These occurred in 2 males (before Day 63 of study) and 1 female (about Day 91 of the study). Convulsions were also reported in one dog (25 mg/kg/day) in the 28-week dog study and in one high dose (100 mg/kg/day) female in the 28-day subchronic study in dogs.

With respect to convulsions, these appear to occur mainly at the 100 mg/kg/day dose in dogs. Therefore, 25 mg/kg/day would constitute the NOEL for convulsions in this study. At the end of 1 year, the mean AUC in dogs receiving 25 mg/kg/day was 27180 ng.h/ml for 311C90. This provides a safety margin of about 170-fold compared to the maximum recommended daily dose for humans (AUC=160 ng.h/ml). With respect to all clinical symptoms in this study, the NOEL would be 5 mg/kg/day, which gave an AUC for 311C90 of about 1362 ng.h/ml at 1 year. This provides a safety margin of about 9-fold compared to the maximum recommended daily dose for humans.

Body weight and food consumption

Table: Mean change in body weight (kg) from study Day 1 to study Day 369

group and sex	Kg gain
1M	2.42
2M	0.62[a]
3M	0.59
4M	2.02
1F	1.87
2F	1.62
3F	4.89
4F	3.70

[a] Study day 363 used due to early sacrifice of 1 of 4 dogs.

While body weight change was quite variable, there was no dose-dependent decrease in body weight of the treated animals over the course of the study. There was also no change in food consumption (data not shown).

Hematology

The sponsor concluded that there were no effects of ZOMIG™ on hematology parameters. However, there was an apparent effect on neutrophils and monocytes. At the high dose (100 mg/kg/day) in males, absolute neutrophil counts decreased an

average of about 17% on Days 87 and 178 and then increased about 28% after 29 days of recovery. In females at the high dose, neutrophil numbers increased 111% after 29 days of recovery. Monocyte numbers decreased about 32% on Days 87 and 178 in males, and then monocyte numbers increased about 48% after 29 days of recovery. Monocyte numbers in females increased 61% after 29 days of recovery. Effects in monocytes also occurred mainly at the high dose.

These effects are consistent with presence of an inflammatory response involving activated neutrophils (and possibly monocytes), which would appear to decrease in peripheral blood as they migrated to the site of inflammation. In the recovery animals, the dramatic increase in peripheral blood neutrophils could be explained by the body's replenishment of these inflammatory cells with drug withdrawal. It is interesting to note that 5HT agonists are known to activate macrophages.

Clinical chemistry

The sponsor concluded that there were no effects of the drug on clinical chemistry parameters. However, as shown in the following excerpt from sponor's table, alkaline phosphatase (ALP) was increased up to 32% (Day 268) and 113% (Day 365) in treated male animals and 78% in high dose recovery animals (recovery day 29). ALP was also increased 27% (Day 268) and 38% (Day 365) in female treated animals, and 24% in female recovery animals (recovery Day 29). Finally, SGPT was increased about 39% in high dose female animals on Day 365.

Increases in these clinical chemistry parameters can be indicative of liver toxicity.

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ON ORIGINAL

Table: Alkaline phosphatase and SGPT levels in animals treated with ZOMIG™

SEX: MALE

		STUDY DAY						
OBSERVATION NAME	MG/KG/DAY	-5	14	87	178	268	365	+29
ALKALINE PHOSPHATASE IU/L	0.000	59.2	70.0	54.2	49.0	47.6	44.4	29.0
	5.000	51.5		62.3	64.3	74.5	69.3	
	25.000	67.3		85.0	82.5	98.5	113.5	
	100.000	55.5		53.7	49.7	62.7	72.8	51.5

GROUP MEAN CLINICAL CHEMISTRY

SEX: FEMALE

		STUDY DAY						
OBSERVATION NAME	MG/KG/DAY	-5	36	87	178	268	365	+29
ALKALINE PHOSPHATASE IU/L	0.000	71.0		75.8	77.5	74.8	66.3	43.0
	5.000	54.0		82.3	73.8	90.3	91.8	
	25.000	51.0	46.0	57.0	54.0	57.7	35.0	
	100.000	78.3		72.0	74.2	84.3	77.3	53.5
SGPT (ALT) IU/L	0.000	28.7		28.2	26.8	31.0	28.0	47.5
	5.000	28.3		30.0	29.8	32.8	37.0	
	25.000	31.0	29.0	24.0	34.7	25.0	27.0	
	100.000	32.3		27.5	32.3	28.5	39.2	32.0

Urinalysis
No effect.

Ophthalmic examination
No effects.

Electrocardiographic examination
The submission includes a "Summary Electrocardiographic Report" only, signed by Clarke E. Atkins, DVM, stating that "There were no clinically significant

included for heart rates, blood pressure, or ECG tracings.

According to the methods section, ECG exams were done of Study days -22 or -21, -13 or -12, 84 or 85, 176 or 177, 265 or 266, 359 and recovery day 27. However, it is unclear when the ECGs were taken with respect to drug administration on a given day.

Organ weights

Adrenal weights in females given 311C90 were higher than controls at the end of the dosing period, and this increase was dose-dependent (Sponsor's table below). This difference was not apparent at the recovery sacrifice.

Organ Weights

Adrenals weights in females given 311C90 were higher than controls at the end of dosing but not at the recovery sacrifice.

	Males			Females		
Dose mg/kg/day	Mean (g)	% of Control	Mean (g) Recovery	Mean (g)	% of Control	Mean (g) Recovery
0	1.663		1.360	1.215		1.545
5	1.493	-10		1.383	+14	
25	1.613	-3		1.577	+30	
100	1.783	+7	1.550	1.663	+37	1.435

There was also about a 33% decrease in thymus weights in male animals at the high dose at the end of the dosing period. This was not seen in female animals.

Macropathology

No effects.

Histopathology

The sponsor stated that there were no histopathological effects that could be attributed to drug administration. Furthermore, there were no histopathological findings in the single male animal (high dose) that was found dead.

However, it has been reported that serotonin (and 5HT agonists) can activate macrophages (Silverman et al., *Biochem., Biophys. Res. Commun.* 131:1160, 1985), possibly through binding to a muramyl peptide receptor. In reviewing the histopathology data, I noticed a number of "inflammatory foci" and sites of "mononuclear cell infiltration", and have summarized them below. I have some concern that administration of this drug may result in the presence of activated macrophages that invade various organs and tissues according to the tissue distribution of the drug.

Summary of histopathology data with respect to "inflammatory foci" and "mononuclear cell infiltration":

Main Study (4/group examined)

Sex	Organ	Effect	Dose (mg/kg/day) Control	5	25	100
M	Caecum	Focal submucosal acute Inflammation--slight	0	0	1	0
M	Liver	Perivascular mononuclear Cell infiltration--minimal	1	0	1	1
M	Pancreas	Focal mononuclear cell Infiltration--minimal	0	0	1	0
M	Prostate	Focal inflammation				
		--slight	0	1	0	0
		--moderate	0	0	1	0
M	Tongue	Perivascular mononuclear Cell infiltration				
		--minimal	1	1	0	0
		--slight	0	0	0	1
F	Brain	Perivascular inflammatory Infiltrate--minimal	0	1	0	0
F	Kidney	Focal inflammatory cell Infiltration--minimal	1	1	0	0
F	Lacrimal Gland	Focal inflammation				
		--minimal	0	1	0	0
		--slight	0	0	0	1
F	Lung	Perivascular mononuclear Cell infiltration				
		--minimal	1	2	1	2
		--slight	0	1	0	0
		Focus(i) of alveolar Macrophages--minimal	0	1	1	0
F	Mammary Gland	Focal inflammation				
		--minimal	0	1	0	0
		--slight	0	1	0	0
F	Oesophagus	Focal submucosal Inflammatory infiltration				
		--minimal	0	3	1	1
F	Salivary Gland	Periductular mononuclear cell infiltration				
		--minimal	0	2	0	0
		--slight	1	0	0	0
F	Tongue	Perivascular mononuclear Cell infiltration--minimal	0	0	1	1

Recovery Group (2/sex/group examined)

Sex	Organ	Effect	Dose (mg/kg/day) Control	5	25	100
M	Lung	Perivascular mononuclear Cell-minimal	0			1
M	Oesophagus	Focal submucosal inflammatory Infiltration--minimal	0			1
M	Tongue	Perivascular mononuclear Cell infiltration--minimal	0			1
F	Liver	Microfoci of inflammation				
		--minimal	1			2
		--slight	1			0

While it it true that at least 7 control animals (combined male and female) presented with "inflammatory foci" or "mononuclear cell infiltration" in various tissues or organs, a substantial larger number of treated animals also presented with these inflammatory lesions is a whole host of tissues and organs. While these data certainly do not support a firm conclusion that administration of 311C90 to dogs resulted in activation of macrophages and resultant formation of these inflammatory lesions, they do indicate a somewhat unusual occurrence of these inflammatory sites in test animals.

Toxicokinetics

The following table shows toxicokinetic data for 311C90 parent drug (zolmitriptan) and metabolites 183C91, 1652W92 and 2161W92 when drug was administered to dogs at 5, 25 or 125 mg/kg/day. Data are given for both Day 23 and Day 366 of the study.

311C90 at 5 mg/kg/day: Mean C_{max} and AUC_{0-T} for 311C90 and metabolites

DAY 23				
	AUC_{0-T} (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	3466	2672	966	687
183C91	NE	NE	32	37
1652W92	1038	1056	229	240
2161W92	1275	1183	226	262
DAY 366				
zolmitriptan	1413	1324	367	429
183C91	NE	NE	<12	<26
1652W92	438	531	98	176
2161W92	537	554	113	161

311C90 at 25 mg/kg/day: Mean C_{max} and $AUC_{0-\tau}$ for 311C90 and metabolites

DAY 23				
	$AUC_{0-\tau}$ (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	22784	21383	5047	3527
183C91	1106	1114	212	228
1652W92	7926	9439	1560	2087
2161W92	4586	3845	734	651
DAY 366				
zolmitriptan	22620	33259	6388	7977
183C91	925	1254	175	215
1652W92	8123	15915	1686	4130
2161W92	6741	7977	1142	1760

311C90 at 100 mg/kg/day: Mean C_{max} and $AUC_{0-\tau}$ for 311C90 and metabolites

DAY 23				
	$AUC_{0-\tau}$ (ng.h/ml)		C_{max} (ng/ml)	
	Males	Females	Males	Females
zolmitriptan	94910	118761	16228	22752
183C91	5342	6349	1029	1052
1652W92	47137	64722	9970	13037
2161W92	19011	20098	2506	3064
DAY 366				
zolmitriptan	118707	121352	25591	32121
183C91	5589	5220	970	767
1652W92	58853	57262	11202	10948
2161W92	20866	22716	3232	4014

Toxicokinetics data show that 311C90 exposure (AUC) increases approximately proportionally with dose, as do AUCs for the metabolites 183C91 and 2161W92. However, metabolite 1652W92 increases greater than dose-proportionally from 25 to 100 mg/kg/day. Over the 4-fold increase in drug (25 to 100 mg/kg/day), this metabolite increases about 6- to 7-fold. There does not appear to be any difference between the two genders, nor does there appear to be any accumulation of drug or metabolites with time.

T_{max} for 311C90 appeared to be about 0.75 h, with a $T_{1/2}$ of about 2.4 hours (monophasic). The T_{max} for the metabolites ranged from 0.75 to 4 h, with the average $T_{1/2}$ being 3.8 h for 183C91, 2.5 h for 1652W92 and 3.4 h for 2161W92.

With respect to the metabolites, 1652W92 had the highest plasma levels. The average ratio of 311C90:1652W92 AUCs was about 2.5 over the dose range. The ratio of 311C90:2161W92 increased slightly in a dose-dependent fashion from about 2.8 at 5 mg/kg/day to 6.5 at 100 mg/kg/day, suggesting that the formation of this metabolite may have been approaching saturation. Exposure to 183C91 was the lowest, with a 311C90:183C91 average ratio of about 25.

The average AUC and C_{max} for 311C90 parent and the metabolites over the course of the 1 year study are given in the following sponsor's table:

The average exposures to 311C90 were as follows:

	5mg/kg/day		25mg/kg/day		100mg/kg/day	
	Day 23	Day 366	Day 23	Day 366	Day 23	Day 366
AUC_{0-t} (ng/ml x h)	3069	1362	22183	27180	106836	120150
Cmax (ng/ml)	827	398	4395	7069	19490	29153

The average exposures to 183C91 were as follows:

	5mg/kg/day		25mg/kg/day		100mg/kg/day	
	Day 23	Day 366	Day 23	Day 366	Day 23	Day 366
AUC_{0-t} (ng/ml x h)	NE	NE	1109	1066	5845	5388
Cmax (ng/ml)	34	<26	219	192	1040	859

The average exposures to 1652W92 were as follows:

	5mg/kg/day		25mg/kg/day		100mg/kg/day	
	Day 23	Day 366	Day 23	Day 366	Day 23	Day 366
AUC_{0-t} (ng/ml x h)	1047	491	8575	11462	55929	57985
Cmax (ng/ml)	234	137	1786	2733	11504	11063

The average exposures to 2161W92 were as follows:

NOEL and dose comparison to human (maximum recommended daily dose)

While the sponsor does not specifically propose a NOEL for this study, based on the clinical symptoms (excluding aggression), one could designate 5 mg/kg/day as the NOEL. This provides a margin of safety of about 9-fold by AUC (1362 ng.h/ml) compared to the maximum recommended daily dose of 15 mg (AUC 160 ng.h/ml) in humans. With respect to convulsions (100 mg/kg/day; AUC 27180 ng.h/ml for 311C90), the margin of safety with respect to AUC is about 170-fold compared to the AUC at the maximum recommended human dose. It should also be noted that AUC comparison of parent compound may or may not be the most important factor with respect to convulsions and clinical symptoms, as at least the 183C91 metabolite is know to also be active.

Summary and Conclusions for 1-year dog study

A single high dose (100 mg/kg/day) animal was found dead (study day 275), with no cause of death determined. Clinical symptoms appeared to be consistent with increased sympathetic outlflow (through $5HT_{1A}$ receptor?) and included body tremors, aggressive behavior, and convulsions, among others. The clonic convulsions were the symptom of greatest concern, and this occurred in 3 of 12 high dose animals (100 mg/kg/day). The decrease in monocyte and neutrophil numbers during treatment, followed by increases in the recovery animals, are consistent with some sort of inflammatory response. Histopathology data reveal a number of seemingly random sites of "focal inflammation" and "mononuclear cell infiltration" in a number of different organs and tissues. These data, along with the known effect of serotonin to activate monocytes, raises the question as to whether or not the 5HT agonistic activity of the drug is resulting in mononuclear cell activation, which in turn is causing a low level of focal inflammatory response in these animals. Since serotonin is also known to act as an attractant in an inflammatory response, it is feasible that these inflammatory sites might correspond to the tissue distribution of the drug. Unfortunately, results of this animal study do not provide sufficient information to allow formation of any concrete conclusions in this regard. Finally, a number of these inflammatory lesions were also seen in control animals, although in most cases where this occurred, the incidence or severity of the lesions increased in treated animals.

Clinical chemistry results indicated some increase in ALP and SGPT, enzymes that can be indicators of effects on liver. While the sponsor included the conclusion that no effects were seen on the cardiovascular system, no ECG tracings, heart rate or blood pressure data were included in the submission. A dose-dependent increase in adrenal weights was observed in female animals.

Toxicokinetics data revealed that exposure (AUC) to 311C90 parent drug increased in a dose-dependent manner in this study, with no apparent gender difference or time-related effect. With respect to metabolites, the order or exposure was 1652W92>2161W92>183C91. Exposure to 1652W92 appeared to increase at a greater rate than dose-proportionality. 183C91 is a known active metabolite, that

With respect to clinical symptoms, the NOEL for this study would be about 5 mg/kg/day (average AUC 1362 ng.h/ml for 311C90 parent drug), giving a margin of safety (AUC) compared to the maximum recommended daily dosing (AUC 160 ng.h/ml) of about 9-fold. With respect to convulsions, the NOEL is about 25 mg/kg/day (average AUC 27180 ng.h/ml for 311C90), which provides a margin of safety (AUCs) of about 170-fold. This is an adequate margin of safety for human use.

Carcinogenicity Studies

The following sponsor's Table 2.5 provides a summary of the carcinogenicity studies.

2.5 **Carcinogenicity studies**

Species	No./Group (M/F)	Doses (mg/kg)	Route	Duration	Ref No
CD1 Mouse	51 60	0, 0 6, 60,400	Oral	85 wks male 92 wks female	52
Wistar Rat	50 60	0 0, 25, 100, 400	Oral	104 weeks[a]	53

[a] 400 mg/kg females sacrificed in week 86 and 400 mg/kg males at week 101

Oral Mouse Carcinogenicity Bioassay

1. **Report of an oral carcinogenicity study of 311C90 in the CD_1 mouse** (study number BDRE/94/0010), Glaxo Wellcome R&D, in-life phase of study done at Pharmakon Europe, 311C90 batch no. Q, September, 1996, GLP.

Study description: Sponsor's table 1.2 summarizes the mouse carcinogenicity study.

Animals:

CD1 mice (60/sex/group; 51/sex/group in Controls; two Control groups); 3 treated satellite groups for periodic toxicokinetics evaluations (18/sex/group); Charles River (France); age 5 weeks at study initiation; body weights (M 26.3-36.7 g; F20.0-29.7 g); housed 3/cage by sex.

Drug:

3111C90 (batch no. Q6 (AN42351)) supplied by The Wellcome Foundation

Treatment:

4.3.1. EXPERIMENTAL DESIGN

Group number	Group designation	Dose level (mg/kg/day)	Dose volume (ml/kg/day) (b)	Dose concentration (mg/ml)	Number of animals* Males	Females
1	Control I	0	20/10	0	51	51
2	Low dose	6	20/10	0.3/0.6 (b)	60(9)	60(9)
3	Intermediate dose	60	20/10	3.0/6.0 (b)	60(9)	60(9)
4 (a)	High dose	600/400 (a)	20/10	30/20 (a)/40 (b)	60(9)	60(9)
5	Control II	0	20/10	0	51	51

Group 1 and 5 animals (control) received the control article (sterile water for injection).
On day 223 (week 32), female nos. 304, 305 and 306 (group 1 - cage no. 102) were inadvertently treated with the low dose (technical error - see section 8.).
On day 418 (week 60), female no. 362 (group 3) was not treated because of transitory poor clinical condition.

(a) : high dose animals (group 4 - 600 mg/kg/day) were not treated on the 12 and 13 March 1994 (days 11 and 12 for males and days 10 and 11 for females and days 12 and 13 for animals from satellite group).
From 14 March 1994 (day 13 for males and day 12 for females and day 14 for animals from satellite group) the group 4 high dose level was reduced to 400 mg/kg/day (dose volume of 20 ml/kg and dose concentration of 20 mg/ml).

(b) : from 23 March 1994 (day 22 for males and day 21 for females and day 23 for animals from satellite group), the dose volume administered to all groups was reduced to 10 ml/kg and the concentrations of the test article solutions were adjusted accordingly.

*** : number in parentheses show satellite group animals which had not been bled on day 1 of treatment (9 mice per sex per treated group) and which were integrated into the main study groups from the beginning of week 6 (day 36).**

Daily oral (gavage) administration of 311C90 (0, 6, 60 or 600 mg/kg/day) for the purpose of evaluating the effect of the drug on incidence and morphology of tumors following prolonged administration for a minimum of 80 weeks. Control animals in Groups 1 and 5 received Control article (sterile water for injection).

Doses were selected based on PK and toxicity data from previous toxicology studies in the mouse.

Due to the number of early deaths in the high dose group (6 main group males and 6 satellite group males; probably due to drug toxicity) a number of unbled satellite animals (9/sex/treated group) were assigned to the main study groups from the beginning of week 6 (Day 36). The inclusion of these animals is shown in Table 4.3.1 above in parentheses. Remaining satellite animals that had been bled at the beginning of the study were discarded and a second satellite study (no. 332/516) was started to provide PK data for long-term oral administration of the drug

Observations:

Morbidity/mortality, clinical observations, body weights, food consumption, hematology, macro- and micropathology. No organ weights were taken. Animals were sacrificed by carbon dioxide inhalation and exsanguination. The following organs/tissues were sampled and preserved for histopathology: adrenals, aorta, bone (femur), bone marrow smears, brain, caecum, colon, duodenum, eyes, gall bladder, Harderian gland, heart, ileum, jejunum, kidneys, larynx, liver, lungs and mainstem bronchi, lymph nodes (submaxillary and mesenteric), mammary gland, oesophagus, optic nerves, ovaries, pancreas, parathyroids, pituitary, prostate, rectum, salivary gland, sciatic nerves, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes and epididymides, thymus, thyroids, tongue, trachea, urinary bladder, uterus, vagina, Zymbal glands, all gross lesions. Bone marrow smears were apparently prepared, but due to lack of significant effects for other parameters the sponsor did not consider examination of bone marrow to be necessary.

All animals (including earth deaths or moribund sacrifices) were submitted to full necropsy procedures, including examination of external surfaces for palpable masses, all orifices, the cranial cavity, carcass, external surface of the brain and samples of spinal cord, thoracic and abdominal cavities and organs, and the cervical tissues and organs.

With respect to histopathology examination, all tissues/organs for all animals in all groups (including early deaths and moribund sacrificed) were prepared for histopath examinatinon by Prepared slides were shipped to Dr. W.A. Macklin (Burroughs Wellcome Co., Research Triangle, N.C.) For histopathology examination. . performed histopathological examination for animals dying during the first 6 weeks of the study, to evaluate the possible cause of death and whether or not it was related to gavage accident.

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ON ORIGINAL

Sacrifice

The study was initially planned to continue for 80 weeks with the possibility of prolongation depending on survival. Actually sacrifice of males occurred after 85 weeks and females after 92 weeks.

Group 4 (high dose) animals had a higher mortality than controls. It was decided to sacrifice all animals of one sex at the same time as all groups were approaching 50% survival and the death rate was increasing as the end of life span was being reached. The following sponsor's table shows survival at termination for each study group:

Group	Males	Females
1. Control	38/51 75%	30/51 57%
2. 6 mg/kg	28/60 47%	38/60 63%
3. 60 mg/kg	32/60 53%	34/60 57%
4. 400 mg/kg	21/60 35%	26/60 43%
5. Control	29/51 57%	32/51 63%

Results

Mortality

During the treatment period, a total of 133 male and 122 female animals were found dead or killed moribund, with the following distribution:

2.1. MORTALITY

(Table 1 - Figures 1 and 2 - Appendix 1)

During the treatment period a total of 133 males and 122 females were found dead or killed moribund, distributed as follows :

		Total deaths *			
Group/Treatment		Males		Females	
1	control	13/51	25 %	21/51	41 %
2	6 mg/kg/day	31/60	52 %	22/60	37 %
3	60 mg/kg/day	28/60	47 %	26/60	43 %
4 +	600/400 mg/kg/day	39/60	65 %	34/60	57 %
5	control	22/51	43 %	19/51	37 %

During the first 4 weeks of treatment an unexpectedly high percentage of the animals died in groups 4 and 5. Those deaths were distributed as follows:

During the first approximately 4 weeks of treatment an unexpectedly high mortality was observed in groups 4 and 5 male. Deaths were distributed as follows

Group/Treatment		Number of deaths			
		Main groups *		Satellite groups **	
		Males	Females	Males	Females
1	control	3(2)	2(1)	-	-
2	6 mg/kg/day	2(1)	0	1'	0
3	60 mg/kg/day	3(1)	0	0	0
4 +	600/400 mg/kg/day	6(0)	1(0)	6(5)	1(1)
5	control	4(0)	2(1)	-	-

+ dose level reduced from day 13/12 (male/female).

*** original group size 51 animals/sex/group.**

**** original group size 18 animals/sex/treated group.**

' satellite animal in the subgroup reassigned to the carcinogenicity phase of the study (unbled).

It is clear that the sponsor used some rationale for replacing some of these early decedents to maintain the total number/sex/group at 60. However, the way in which the animals were replaced is somewhat confusing. Apparently, the numbers in parentheses in the above table indicate the number of animals that were replaced because clinical or macropath findings were consistent with possible gavage accident or as the result of blood sampling (satellite animals). Males replaced because of the death of a cage mate are not included in the table.

Therefore, it would appear that, in the first 4-5 weeks of the study, the sponsor replaced early decedents that were diagnosed as deaths due to gavage or bleeding trauma with healthy animals. It is unclear what animals were used to replace these early decedents in the first 4 weeks. However, the sponsor also states that there was no clear evidence of trauma as the cause of death for the 6 males and 1 female from the high dose group (group 4) which were found dead within 2 hours of dosing between days 1 and 9 of treatment. Apparently these animals did not show abnormal clinical signs immediately before dying. The sponsor considered these deaths to be related to a toxic effect of the drug, and therefore did not replace them, presumably so they would be reflected in the final mortality data at the end of the study. The sponsor was unable to determine the cause of death of the animals. They did state that there were no premonitory signs before the deaths.

Also, in the "Experimental Design" section of the study, the sponsor stated that due to the high mortality in the high dose group the high dose was reduced from 600 mg/kg/day to 400 mg/kg/day at the end of week 2 of treatment. They also state that satellite group animals which had not been bled on day 1 of treatment (9/sex/group) were integrated into the main study groups from the beginning of week 6 (day 26) of the study. However, this is also confusing because histopathology data tables and the experimental design table all reflect 60 animals/sex/group as the final number of

Excluding the early deaths, the distribution of the mortalities from week 5 of treatment was as follows:

Excluding these early deaths the distribution of mortalities from week 5 of treatment was as follows :

Group/Treatment		Total deaths *	
		Males	Females
1	control	12/50 24%	20/50 40%
2	6 mg/kg/day	29/58 51%	22/60 37%
3	60 mg/kg/day	26/58 46%	26/60 43%
4	400 mg/kg/day	33/54 61%	33/59 56%
5	control	18/47 37%	18/50 36%

* at the end of the treatment period.

Pharmacologist's comments:

It is very difficult to clearly understand how the sponsor dealt with the replacement of early decedent animals. It would appear that, in the first 4 weeks of the study, they replaced dead animals that were diagnosed as dying from gavage or bleeding trauma but they did not replace animals that they concluded had died from drug toxicity (animals in the high dose group). Presumably the purpose of this was to allow the death of these animals due to drug toxicity to be reflected in the final mortality data. However, this would appear to me to constitute rather subjective data manipulation.

Then on or about week 6 they apparently added 9 unbled animals/sex/group from the treated satellite group to the carcinogenicity treatment groups. However, it is very difficult to figure out how this all adds up to the total animals per group reflected either in the above table or any of the other data tables that simply reflect either 51 (controls) or 60 (treated) per group.

According to the sponsor's interpretation of the data, up to 56(M)-61%(F) of the animals were early decedents in the high dose group from week 5 until the end of the study (after the dose was decreased to 400 mg/kg/day). Over the total study period 57-65% of the high dose animals were early decedents or were killed moribund.

No cause of death was determined by the sponsor. They did state that there were no premonitory signs before the deaths.

Clinical signs:

General Clinical Signs

Apparently clinicals signs related to treatment were only observed in group 4 (high dose) animals that received 600 mg/kg/day of 311C90. These clinical signs included:

Lethargy, dorsal decubitus, abnormal respiration (labored or jerky breathing) in some animals, and aggressive behavior in males. Some male animals had to be housed individually due to agressive behavior. Some males also had symptoms resulting from aggressive behavior, such as fur loss/thinning fur, particularly in the dorsal regions, as well as sores or areas of scab formation, abscesses or swelling in the urinogenital/abdominal or dorsal reginos or involving a limb or head/neck. Female animals in this high dose group also showed fur loss/thinning fur as well as a low incidence of sores or scab formation involving ears, periorbital and cervical regions and occasionally the back or limbs.

These signs and symptoms were noted within 1 hour of dosing on day 1 for two females that survived to study termination and in one female before death on day 8 and one male on day 9 before death. The sponsor states that throughout the treatment period the majority of group 4 decedents were found dead without treatment related clinical signs or evidence of poor general condition.

Pharmacologist's comments:

It may be of interest that some of the early decedent animals (first 4 weeks of study) that received the 600 mg/kg/day dose showed labored or abnormal breathing before they died. In a previously reviewed (IND 45147) safety pharmacology examining the effects of 311C90 (0.09, 0.9 and 9 mg/kg/day i.v.) on respiration in the cat, the animals demonstrated a dose-related inhibition of respiration, including decreased tidal volume, increased respiratory rate, hypoxia and death in 2 of 6 animals receiving up to 9 mg/kg/day of drug. The sponsor stated that this effect did not appear to be due to constriction the smooth muscle of the airways, but rather was probably due to a central effect on respiration. One could speculate that this mechanism had something to do with the death of the high dose (600 mg/kg/day) animals in this mouse carcinogenicity study, although without the appropriate data this is admittedly purely speculation.

Body Masses

A low incidence of clinically observed masses were either first noticed or confirmed at necropsy. The sponsor concluded that these masses were not treatment-related based on their distribution throughout both control and treatment groups as follows:

Group/Treatment		Number of mass-bearing animals *	
		Males	Females
1	control	3/51 6%	2/51 4%
2	6 mg/kg/day	4/60 7%	3/60 5%
3	60 mg/kg/day	4/60 7%	6/60 10%
4 +	600/400 mg/kg/day	2/60 3%	3/60 5%
5	control	2/51 4%	3/51 6%

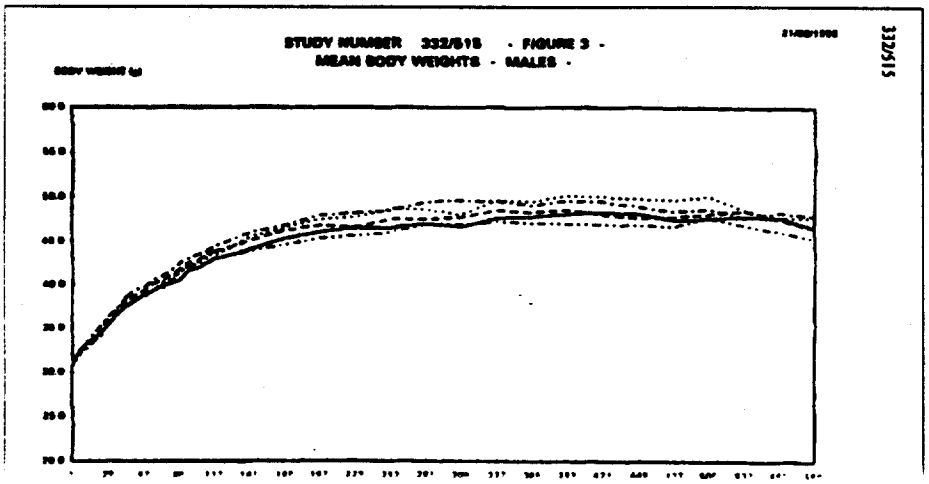
+ dose level reduced from day 13/12 (males/females)

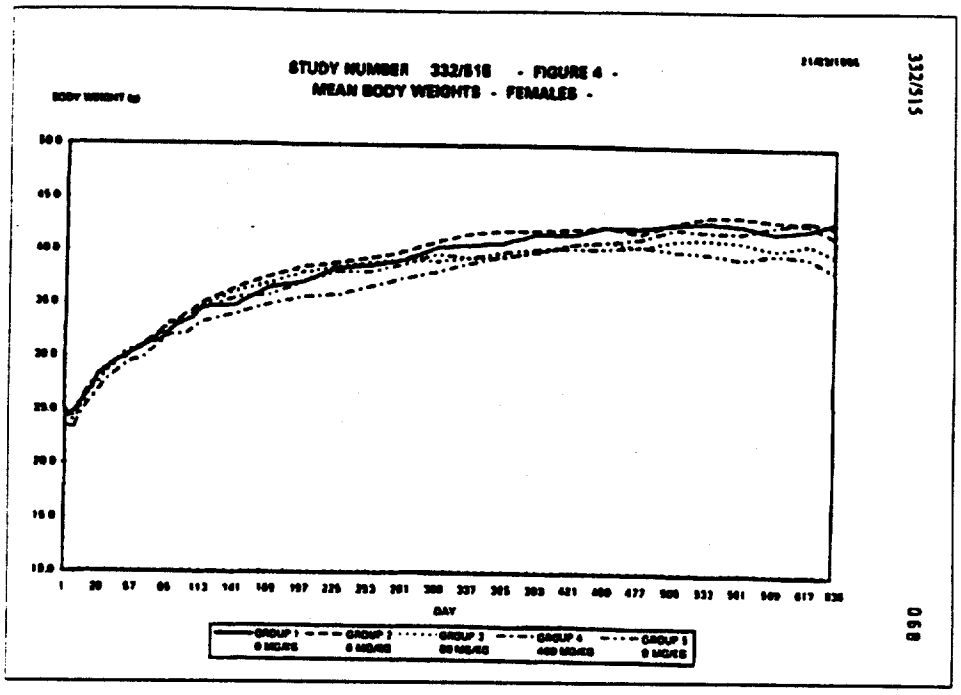
* excluding animals where clinically observed masses were found to be due to enlarged or abscessed glands or lymph nodes, or local fat accumulation.

The most common site for these masses in both males and females (still present at necropsy) were in dorsal regions (cervical, thoracic and abdominal) and in the urogenical and ventral abdominal regions.

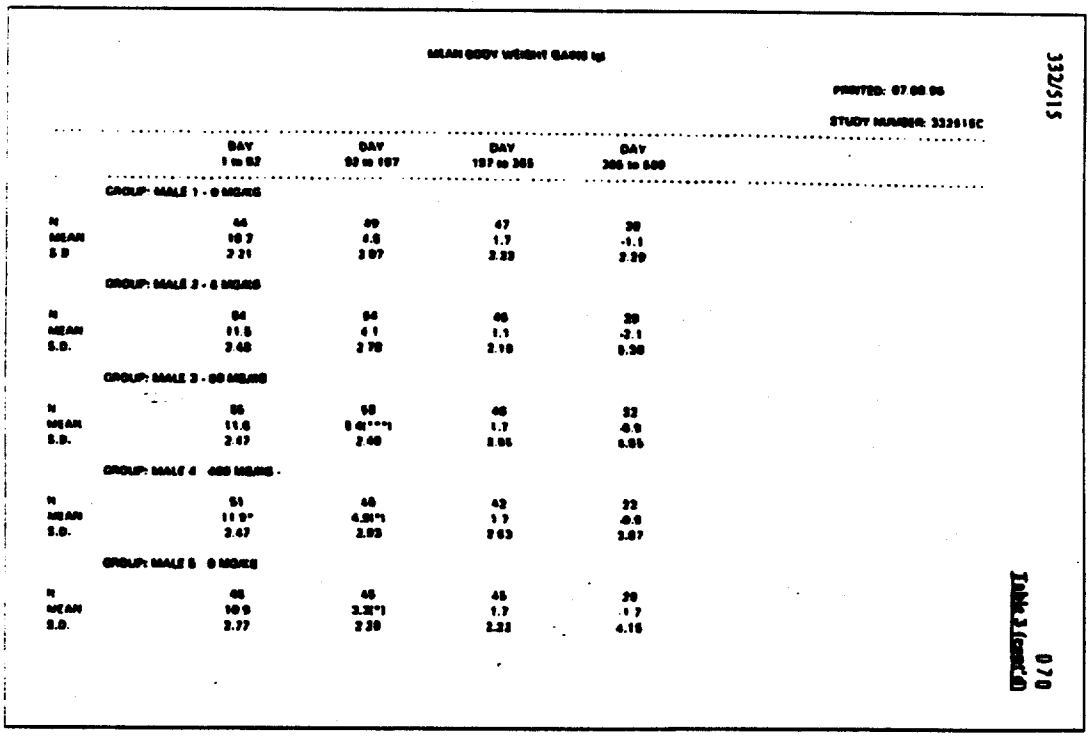
Body weight:

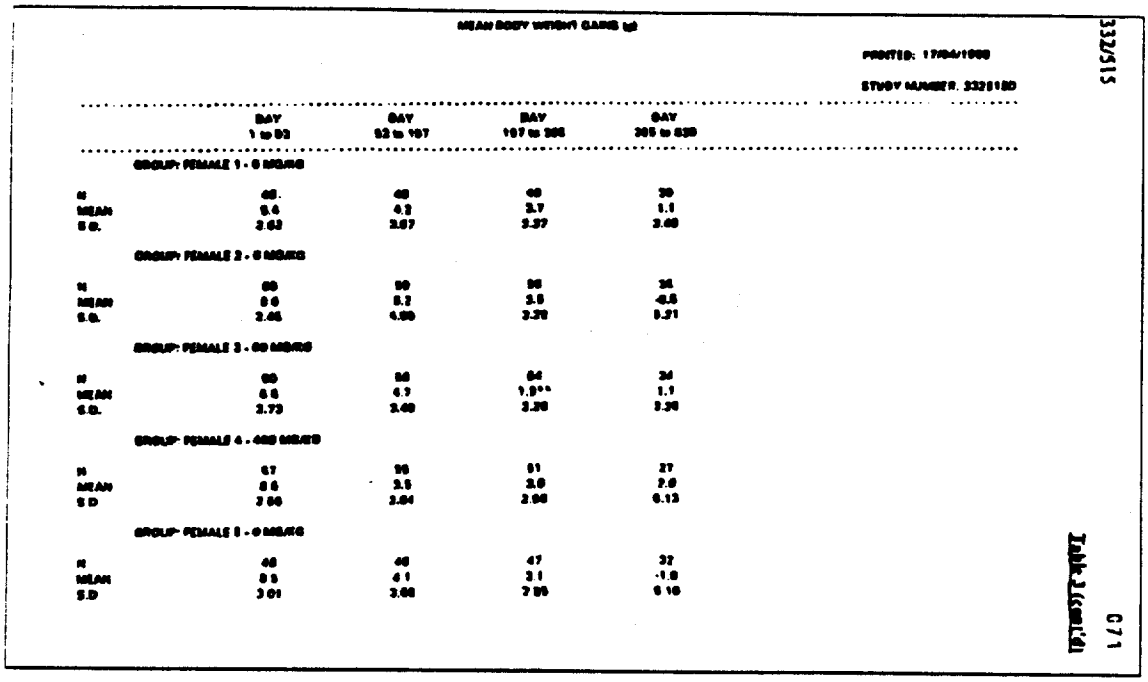
There was no effect on body weight (see sponsor's figure below). If anything, the body weights of the high dose animals were very slightly (1 or 2 grams) higher than the Controls throughout the study. This trend was not statistically significant ($p<0.05$).





There were no effects of drug on mean body weight gain as shown in Table 3 below:





MEAN BODY WEIGHT GAINS (g)

PRINTED: 17/04/1990

STUDY NUMBER: 332515D

	DAY 1 to 92	DAY 92 to 197	DAY 197 to 365	DAY 365 to 639
GROUP: FEMALE 1 - 0 MG/KG				
N	48	48	48	39
MEAN	5.6	4.2	3.7	1.1
S.D.	2.62	3.07	3.37	3.48
GROUP: FEMALE 2 - 6 MG/KG				
N	50	50	50	36
MEAN	5.6	5.2	3.5	-0.5
S.D.	2.46	4.09	3.70	3.71
GROUP: FEMALE 3 - 60 MG/KG				
N	50	50	50	34
MEAN	5.5	4.7	1.9**	1.1
S.D.	2.73	3.48	3.30	3.36
GROUP: FEMALE 4 - 400 MG/KG				
N	47	50	51	37
MEAN	5.6	5.5	3.8	2.8
S.D	2.66	3.84	2.98	6.13
GROUP: FEMALE 5 - 0 MG/KG				
N	48	48	47	32
MEAN	5.5	4.1	3.1	-1.8
S.D	3.01	3.68	7.95	9.10

332515

Table 3 (cont'd)

071

Food consumption:

Group 4 males: slightly higher food consumption values than controls throughout the study; statistically significant for mean average for each analysis period (see below).

Group 4 females: slightly higher food consumption values after the first year of treatment (statistically significant for the last analysis period (weeks 56 through 92)).

MEAN AVERAGE FOOD CONSUMPTION (g/animal/day)

	WEEK 1 TO 13	WEEK 14 TO 26	WEEK 32 TO 52	WEEK 56 TO 84
GROUP: MALE 1 - 0 MG/KG				
N	15	17	17	16
MEAN	5.8	5.9	5.8	5.9
S.D.	0.37	0.46	0.39	0.45
GROUP: MALE 2 - 6 MG/KG				
N	16	20	19	16
MEAN	6.1	6.2	6.0	6.2
S.D.	0.38	0.59	0.53	0.69
GROUP: MALE 3 - 60 MG/KG				
N	13	20	19	16
MEAN	6.0	6.0	5.8	6.2
S.D.	0.40	0.42	0.43	0.53
GROUP: MALE 4 - 400 MG/KG				
N	14	20	20	16
MEAN	6.6***	6.7***	6.4***	7.0***
S.D	0.46	0.59	0.53	0.92
GROUP: MALE 5 - 0 MG/KG				
N	17	17	16	14
MEAN	5.9	5.9	5.7	6.0
S.D.	0.44	0.32	0.47	0.61

MEAN AVERAGE FOOD CONSUMPTION (g/animal/day)

	WEEK 1 TO 13	WEEK 14 TO 28	WEEK 32 TO 52	WEEK 56 TO 91
GROUP: FEMALE 1 - 0 MG/KG				
N	16	17	17	14
MEAN	5.3	5.6	5.3	5.3
S.D.	0.36	0.30	0.39	0.32
GROUP: FEMALE 2 - 6 MG/KG				
N	17	20	20	19
MEAN	5.1	5.3	5.1	5.3
S.D.	0.31	0.39	0.38	0.42
GROUP: FEMALE 3 - 60 MG/KG				
N	17	20	20	18
MEAN	5.1	5.3	5.2	5.4
S.D.	0.24	0.39	0.48	0.54
GROUP: FEMALE 4 - 400 MG/KG				
N	17	20	20	17
MEAN	5.3	5.6	5.4	5.9**
S.D.	0.31	0.43	0.44	0.64
GROUP: FEMALE 5 - 0 MG/KG				
N	16	17	14	16
MEAN	5.3	5.2	5.1	5.4
S.D.	0.42	0.48	0.50	0.52

Hematology:

Sponsor concluded that there were no treatment-related effects on hematology parameters. However, a small number of animals at study termination and of those killed moribund that had very high total WBC counts (≥50,000 per mm^3) compared to controls (≤10,000 per mm^3) as follows:

Animals killed at termination:

one group 1 male; one group 2 male, one group 5 male, one group 3 female.

Animals killed moribund before study termination:

5 of 15 group 2 males
5 or 10 group 3 males
1 of 7 group 4 males
2 of 5 group 5 males
2 of 12 group 1 females
1 of 13 group 2 females
5 of 14 group 3 females
1 of 6 group 4 females
2 of 12 group 5 females

Pharmacologist's comment:

The sponsor concluded that this increase in WBC counts was not a treatment-related phenomenon. I would agree based on the fact that the number of animals with increased WBC counts was failry evenly distibuted between control and treated animals.

The sponsor did not do differentials on the animals with the high WBC counts. It would have been preferable if they had completed differentials, as it would be helpful to know whether or not this increased WBC number was due mainly to an increase in the number of macrophages or neutrophils. These cells are known to be activated by 5HT agonists.

Macropathology:

All Animals

No treatment-related findings were reported, although there were miscellaneous gross findings reported in various organs in both control and treated mice. There were apparently somewhat more masses reported in the uterus in group 2 and 4 females.

Unscheduled Deaths

Somewhat more masses were reported in the uterus of group 2 and 4 females. Other findings that were reported included plae livers, skin sores, enlarged spleens or lymph nodes, and enlarged uteri. A few other findings were more common in treated animals, such as pale livers, skin sores, enlarged spleens and lymph nodes and teri. However, no dose-response relationship was apparent with these effects.

Terminal Sacrifice

Only cystic kidney was noted more frequently in group 2 females than controls, and this was not evident in males. Other commonly reported changes seen in both control and treated animals included dysmorphia of sternum, pale livers, liver masses, nodules or masses in the lungs, enlarged seminal vesicles, soft testes, cystic ovaries, and enlarged spleens. These effects did not appear to be treatment-related.

Conclusions related to macropathology

The sponsor concluded that there were no macroscopic changes indicative of a tumorigenic effect of treatment. They stated that all the changes noted were part of the normal spectrum of changes seen in old mice. The only treatment-related effect of note was the increase in the number of masses reported in the uteri from group 2 and 4 mice.

In the histopathology report, the pathologist concluded that there were no treatment-related neoplasias apparent in this study (see "histopathology; neoplasias" section of my review). However, the non-neoplastic histopathology report revealed a higher incidence of cystic endometrial hyperplasia in the uterus of some treatment animals than controls, which may or may not explain the masses in the uteri of treated

animals. The study pathologist did not comment on this issue in the final report, nor did the sponsor make further comment on this issue anywhere else in the NDA.

Histopathology:

Non-neoplastic histopathological findings

The following is a summary of the non-neoplastic histopathological findings of note:

Study Group	Organ/lesion	Incidence --group*-- 1 M F	2 M F	3 M F	4 M F	5 M F
Terminal sacrifice	none	– –	– –	– –	– –	– –
Found dead	*kidney*					
	aggregates of mononuclear cells	1 1	2 0	2 2	10 8	2 4
	heart					
	myocardial degeneration and fibrosis	1 0	2 0	1 2	7 1	2 1
	adrenals					
	adrenal hyperplasia, subcapsular cell	1 5	1 2	2 8	1 14	1 4
	uterus					
	cystic endometrial hyperplasia	0 4	0 1	0 1	0 8	0 0
Sacrificed moribund	*uterus*					
	cystic endometrial hyperplasia	0 6	0 8	0 4	0 4	0 3

*Doses for Groups 1,2,3,4,5 are 0, 6, 60, 400 and 0 mg/kg/day, respectively

The sponsor concluded that there were no treatment-related non-neoplastic findings. I agree that with the majority of the histopathological lesions reported, the incidence was fairly equally distributed between control and treatment groups. However, in the above summary table are listed findings in kidney, heart, adrenals and uterus in which there appears to be somewhat of a dose-related effect. However, even with these findings, there were lesions in some of the control animals as well.

The sponsor states that "there were no other findings suggesting a possible effect on the bone marrow (and not evident in sternum and bone sections containing bone marrow) and thus the study pathologist elected not to examine them" (the bone marrow smears)..."which was consistent with the protocol." Therefore, bone marrow smears were not examined as part of the histopathological evaluation.

Neoplasias

There were no treatment-related neoplasias found in this mouse carcinogenicity study. The few neoplasias that were reported in the "neoplasia morphology summary table" of the histopath report were of very low incidence and equally distributed between control and treated animals.

Factors contributing to animal death

The sponsor states that the only treatment-related factor in this study was a dose-related (high dose only) decrease in survival time, which was not associated with any histopathological findings.

Pharmacokinetics (PK) studies

Study Description

Due to the advent of an unexpectedly high mortality rate for mice in the high dose (600 mg/kg/day), there were actually two PK studies associated with this 80-week mouse carcinogenicity study, the first spanning the first 6 weeks of the mouse carcinogenicity study and the second spanning the last 74 weeks of the study.

1st Six Weeks

At initiation of the mouse carcinogenicity study, a satellite group of 18 mice/sex/group was established to examine the PK of 311C90 during the intended 80 weeks of treatment. Animals (CD1 mice) were treated by oral gavage with 0, 6, 60 or 600 mg/kg/day drug. However, due to the toxicity of the drug at the high dose, at Day 10 the high dose was reduced to 400 mg/kg/day. Decedent animals were replaced by spare animals at this point. During this phase of the study, blood samples (0.5 ml) were collected from a retro-orbital sinus under light ether anesthesia at 2, 6, or 12 hours after dosing on Days 1 and at Week 6 (in the high dose group only). The sponsor determined plasma levels of 311C90 parent drug, and the metabolites 183C91 (active), 1652W92, and 2161W92.

At week 6 of the study, treated satellite PK animals that were unbled (9/sex/group) were added to each of the three dosing groups in the mouse carcinogenicity study. Therefore, exposure data for this early phase of the study were available for all groups on Day 1, but for only the high dose group on Week 6.

Results of this study are shown on the next page in sponsor's Appendix E2 and discussed below:

311C90

Increased approximately proportionally with dose. AUCs not estimated in low and mid-dose groups because plasma levels below LOQ (limit of quantification) of assay. $T_{1/2}$ of elimination was 2.6 h.

183C91

Highest levels of the metabolites. No low or mid-dose AUC estimations because plasma levels below LOQ. $T_{1/2}$ of elimination was 4.3 h.

1652W92

Second highest levels of the metabolites. Concentrations at low and mid-doses below LOQ on Day 1. $T_{1/2}$ of elimination was 2.7 h.

2161W92

Lowest levels of the metabolites. Most values below LOQ. $T_{1/2}$ of elimination was 3.7 h.

Appendix E2: The exposures to 311C90 and metabolites

311C90								
	Day 1						Week 6	
	6mg/kg/day		60mg/kg/day		600mg/kg/day		400mg/kg/day	
	male	female	male	female	male	female	male	female
AUC	NE	NE	NE	25856	147333	151780	69309	62910
Cmax	124	124	1455	3818	18118	28558	19199	13418
Tmax	2	2	2	6	2	2	2	2
t½	NE	NE	NE	NE	4.7	1.8	1.6	2.1

183C91								
	Day 1						Week 6	
	6mg/kg/day		60mg/kg/day		600mg/kg/day		400mg/kg/day	
	male	female	male	female	male	female	male	female
AUC	NE	NE	NE	NE	6696	6312	3230	4541
Cmax	12.3	<10.0	126	184	680	1021	545	615
Tmax	2	NE	2	6	2	2	2	2
t½	NE	NE	NE	NE	6.1	2.7	3.4	4.9

1652W92								
	Day 1						Week 6	
	6mg/kg/day		60mg/kg/day		600mg/kg/day		400mg/kg/day	
	male	female	male	female	male	female	male	female
AUC	NE	NE	NE	NE	5676	8055	NE	3174
Cmax	<9.6	<9.6	103	208	809	1173	523	520
Tmax	NE	NE	2	6	2	2	2	2
t½	NE	NE	NE	NE	3.6	2.1	NE	2.5

2161W92								
	Day 1						Week 6	
	6mg/kg/day		60mg/kg/day		600mg/kg/day		400mg/kg/day	
	male	female	male	female	male	female	male	female
AUC	NE	NE	NE	NE	1990	NE	NE	2144
Cmax	<13.5	33.4	145	165	219	580	165	262
Tmax	NE	2	2	2	2	6	2	2
t½	NE	NE	NE	NE	3.6	NE	NE	3.7

AUC (ng/ml x h) - area under the plasma concentration-time curve ($AUC_{0-\infty}$ - Day 1; AUC_{0-24} - Week 6)

Cmax (ng/ml) - maximum observed plasma concentration

Tmax (h) - time at which Cmax was observed

t½ (h) - half-life of elimination

Last 74 Weeks

Since unbled treated satellite PK animals were incorporated into the main mouse carcinogenicity study at Week 6, at that point in the study the sponsor established a new set of satellite animals for PK determination for the last 74 weeks of the study. The new PK satellite group, consisting of 18 mice/sex/group, received oral gavage treatment of 0, 6, 60 or 400 mg/kg/day (dose volume of 10 ml/kg/day) for the last 74 weeks of the study. Blood samples were collected from three animals/sex/time point at 2, 6, and 12 hours after dosing during Weeks 26, 52 and 74 and analzed for 311C90 parent drug, and 183C91, 1652W92 and 2161W92 metabolites.

The following sponsor's appendices B4, B5 and B6 show the results of this study at Weeks 26, 52 and 74:

Appendix B4: The exposures to 311C90 and metabolites in Week 26

311C90						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	7804	NE	71773	74080
Cmax	241	93	2286	1814	15259	16997
183C91						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	3395	4279
Cmax	30	<20	139	109	528	582
1652W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	NE	NE
Cmax	<20	<20	80	73	329	726
2161W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	3657	5089
Cmax	64	18	302	177	827	972

n=3

$AUC_{0-\tau}$ (ng/ml x h) – area under the plasma concentration–time curve (extrapolated to the LOQ) for the dosing interval

Cmax (ng/ml) – maximum plasma concentration measured at 2h after dosing.

NE – not estimated due to insufficient data.

Appendix B5: The exposures to 311C90 and metabolites in Week 52

311C90						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	9617	8149	49166	106166
Cmax	115	121	2965	2313	11474	19198
183C91						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	2228	4897
Cmax	<20	<20	129	94	362	692
1652W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	NE	4669
Cmax	<10	<10	89	81	244	655
2161W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	4049	7055
Cmax	<20	<20	249	154	783	1259

n=3

$AUC_{0-\tau}$ (ng/ml x h) – area under the plasma concentration–time curve (extrapolated to the LOQ) for the dosing interval

Cmax (ng/ml) – maximum plasma concentration measured at 2h after dosing.

NE – not estimated due to insufficient data.

Appendix B6: The exposures to 311C90 and metabolites in week 74

311C90						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	6497	10724	74670	104716
Cmax	89	139	1821	2841	14615	22207
183C91						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	3891	5101 [a]
Cmax	<10	<10	92	142	404	819
1652W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	NE	8337
Cmax	<50	<50	73	119	344	803
2161W92						
	6mg/kg/day		60mg/kg/day		400mg/kg/day	
	Males	Females	Males	Females	Males	Females
$AUC_{0-\tau}$	NE	NE	NE	NE	3504	9821

Exposures at the high dose level of 400mg/kg/day after dosing for 74 weeks were as follows:

	males		females	
	AUC (ng/ml x h)	Cmax (ng/ml)	AUC (ng/ml x h)	Cmax (ng/ml)
311C90	74670	14615	104716	22207
183C91	3891	404	5101	819
1652W92	not estimated	344	8337	803
2161W92	3504	467	9821	1442

AUC Comparison with Human MRDD (AUC ratios)

At the high dose of 400 mg/kg/day, the total exposure (AUC) to 311C90 was on the order of 65,000 to 95,000 ng.h/ml over the 80 week study. The AUC for the mrdd (maximum recommended daily dose) in humans for zolmitriptan is 15 mg (0.25 mg/kg for a 60 kg patient), which was reported to result in an exposure (AUC) in humans of about 160 ng.h/ml. Therefore, at the high dose (400 mg/kg/day) the mice were exposed to about a 406- to 594-fold greater AUCs than humans at the mrdd, or the AUC ratio at the high dose was about 460:1 to 594:1 for mouse:human.

Determination of an MTD or Other Basis to Justify 400 mg/kg as the High Dose

The sponsor did not complete the typical 90-day dose-ranging studies in mice to determine the appropriate dose range for the mouse carcinogenicity study. Therefore, it is necessary to evaluate the results of the carcinogenicity study itself to determine whether or not the sponsor's dose selection was appropriate.

According to the "ICH Guidelines for Industry for Dose Selection for Carcinogenicity Studies of Pharmaceuticals", dose selection can be based on a number of factors, including toxicity-based endpoints, pharmacokinetic endpoints, saturation of absorption, pharmacodynamic endpoints, maximum feasible dose, or additional endpoints.

With respect to a PK endpoint, at the high dose (400 mg/kg/day) there is a minimum of a 460:1 AUC ratio between the mice and the humans at the mrdd, which would easily satisfy the 25:1 AUC ratio specified in the aforementioned ICH guideline for dose-selection. However, in order to use the 25:1 AUC ratio the drug must be shown to be non-genotoxic. Zolmitriptan tested positive in the human lymphocyte assay, indicating that it has some clastogenic potential. Therefore, the use of the 25-fold AUC ratio would not be useful for dose-selection in this case.

PK revealed no saturation of absorption, nor were there any pharmacodynamic endpoints in mice that might prove useful for dose-selection. It is fairly common to calculate an MTD based on at least a 10% decrease in body weight change, but in this

there were no other notable toxic effects on which to base an MTD.

However, there were a number of unexpected early deaths in the study at the high dose, which the sponsor concluded were due to drug toxicity. Also, both in the first 6 weeks of the study and at the time of study termination, the percentage of unplanned animal deaths was consistently higher in the high dose animals than in the controls or other treatment groups (see tables below). While the cause of death was not determined for these animals, some of the early decedent animals that received the high dose (600 mg/kg/day) showed labored or abnormal breathing before they died. This is consistent with a previous safety pharmacology study for 311C90 in cats, in which 0.09, 0.9 or 9 mg/kg/day of drug (i.v.) resulted in a dose-related inhibition of respiration and death in 2 of 6 animals at the high dose.

Irrespective of the cause of death, it is my conclusion that the data demonstrating animal mortality (see below) support the dose-selection for this mouse carcinogenicity study. It is apparent from these data that the per cent mortality was similar in controls and animals receiving 6 and 60 mg/kg/day, while animals in the high dose group had a higher percentage of animal deaths. The data indicate that administration of zolmitriptan at a range of 400 to 600 mg/kg/day (oral) resulted in excessive mortality (due to toxicity). Therefore this represents a dose-limiting level of drug administration by the oral route and indicates that at the highest dose the sponsor has exceeded the MTD (maximum tolerated dose). Since the highest dose used in a carcinogenicity study is usually the MTD, these data support the choice of 400-600 mg/kg/day as the high dose for the mouse carcinogenicity study.

2.1. MORTALITY

(Table 1 - Figures 1 and 2 - Appendix 1)

During the treatment period a total of 133 males and 122 females were found dead or killed moribund, distributed as follows :

Group/Treatment		Total deaths *			
		Males		Females	
1	control	13/51	25 %	21/51	41 %
2	6 mg/kg/day	31/60	52 %	22/60	37 %
3	60 mg/kg/day	28/60	47 %	26/60	43 %
4 +	600/400 mg/kg/day	39/60	65 %	34/60	57 %
5	control	22/51	43 %	19/51	37 %

+ dose level reduced from day 13/12 (male/female).

* including animals found dead during the terminal period (weeks 85 to 86 for males and weeks 92 to 93 for females).

Excluding these early deaths the distribution of mortalities from week 5 of treatment was as follows :

Group/Treatment		Total deaths *	
		Males	Females
1	control	12/50 24%	20/50 40%
2	6 mg/kg/day	29/58 51%	22/60 37%
3	60 mg/kg/day	26/58 46%	26/60 43%
4	400 mg/kg/day	33/54 61%	33/59 56%
5	control	18/47 37%	18/50 36%

* at the end of the treatment period.

The lowest dose used in a carcinogenicity study is usually one that results in either plasma C_{max} or AUC levels similar to those shown in patients at the mrdd, in order to allow some relevance of the study to patients receiving the drug. As previously stated, the mrdd for zolmitriptan (15 mg/day) has been reported to result in AUCs of about 160 ng.h/ml. Administration of a single oral dose of 50 mg/day to patients has been reported to result in a plasma C_{max} of about 68 ng/ml. Since the PK for zolmitriptan appears to be fairly linear and dose-dependent, extrapolation to a 15 mg dose would predict plasma C_{max} levels on the order of about 20 ng/ml.

PK data from this mouse carcinogenicity study for weeks 26, 52 and 74 reveal that the low dose (6 mg/kg/day) results in AUC levels for parent 311C90 drug in the range of about 800 ng.h/ml (extrapolated from an average AUC at 60 mg/kg/day of about 8000 ng.h/ml). This AUC is about 5-fold greater than the AUC (160 ng.h/ml) at the human mrdd. Therefore, in terms of AUC comparison, the low dose in the mouse carcinogenicity study was a bit too high, and could have been as much as 5-fold less.

In terms of plasma C_{max}, PK data in mice revealed that levels at the 6 mg/kg/day dose are in the 100-200 ng/ml range. This is about 5-10-fold higher than the predicted plasma C_{max} in humans at the mrdd. Therefore, based on data for either AUC or plasma C_{max}, the low dose would appear to be 5-10-fold higher than would be preferred based on providing drug exposure similar to that found at the mrdd in humans.

Summary and Conclusions for Mouse Carcinogenicity Study

Animals initially received 6, 60 or 600 mg/kg/day 311C90 by oral gavage, but there were several unexpected deaths at the high dose in the first 4 weeks of the study. Therefore, on day 10 the high dose was reduced to 400 mg/kg/day, and animals were replaced with spare animals as described in the review. Then at week 6, unbled treated satellite PK animals were added (9/sex/group) to the main carcinogenicity study, and a new satellite PK group was established. While cause of death was undetermined, some of the early decedents demonstrated labored respiration, which is consistent with a cat safety pharmacology study in which animals presented with labored respiration and died at 9 mg/kg/day i.v. administration of the drug. While the sponsor concluded that there were no notable toxic effects of the drug other than early deaths, a low incidence of histopathological lesions in these early decedents included effects in kidney, heart, adrenals and uterus. PK data showed that 311C90 parent drug was absorbed in a linear, dose-dependent manner, with no apparent accumulation with dose or time. Plasma exposure to metabolites was, from greater to lesser exposure, 183C91, 1652W92 and 2161W92. Exposure to 311C90 parent drug at the high dose (400 mg/kg/day) was 406-594-fold greater in the mice than in patients at the mrdd.

With respect to appropriateness of the doses used in this study, it is my conclusion that the high dose (400-600 mg/kg/day range) exceeds the MTD based on excessive mortality at this dose. The low dose is about 5-10-fold higher than we would prefer, based on the precept that exposure levels at the low dose in a carcinogenicity should approximate the levels found in humans at the mrdd.

Overall, there were no treatment-related neoplasias found in this mouse study. The few neoplasias reported were of a very low incidence and equally distributed between control and treated animals.

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