BEST POSSIBLE COPY

Study #2 Repeat of Ames test and Yahaghi modification (9/3/92)

Results of the repeat studies are shown in sponsor's tables 15 (vol. 49, pg. 30) and 19 (vol. 49, pg. 34) below:

Table 15

Laboratory Ref: 1680 Strain: TA 1538
Test Type: Ames Test Reading Date: 09/03/92
S9Batch: 66 Robens Reading Time: 09:47
Test Date: 6.3.92
Direct Control : Hycanthone (HYC)
Indirect Control: 2-Aminoanthracene (2AA)

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

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	1	22	P	31	45	P
		2	23	P	32	38	P
		3	29	P	33	40	P
		MEAN	25		MEAN	41	
.	1000	4	33	P	34	45	P
		5	21	P	35	33	P
		6	35	P	36(M)	32	P
		MEAN	30		MEAN	37	
.	1778	7	28	P	37	52	P
		8	26	P	38	36	P
		9	26	P	39	49	P
		MEAN	27		MEAN	46	
.	3160	10	26	P	40	39 107	P
		11	16	P	41	42	P
		12	19	P	42	35	P
		MEAN	20		MEAN	48 46	
.	5620	13	4	R	43	27	R
		14	11	R	44	22	R
		15	18	R	45	21	R
		MEAN	10		MEAN	23	
.	10000	16	17	R	46	14	R
		17	1	R	47	11	R
		18	12	R	48	20	R
		MEAN	10		MEAN	15	
	HYC	19	213	P	49	154	P
		20	230	P	50	185	P
		21	229	P	51	201	P
		MEAN	223		MEAN	180	
	2AA	22	32	P	52	2079	P
		23	27	P	53	1373	P
		24	27	P	54	1659 [illegible]	P
		MEAN	29		MEAN	1847 1511	
CONTAMINATION		25(M)	0		55(M)	0	
		26(M)	0		56(M)	0	
HISTIDINE		27(M)	0		57(M)	0	
		28(M)	+		58(M)	+	
		29(M)	+		59(M)	+	
		30(M)	+		60(M)	+	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
S - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 9/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

Table 19

Laboratory Ref: 1682 Strain: TA 1538
Test Type: Yahagni Modification Reading Date: 09/03/92
S9Batch: 66 Robens Reading Time: 11:32
Test Date: 6.3.92.
Direct Control : Hycanthone (HYC)
Indirect Control: 2-Aminoanthracene (2AA)

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

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	1	37	P	31(M)	21	P
		2	35	P	32	40	P
		3	23	P	33	32	P
		MEAN	32		MEAN	31	
.	1000	4	30	P	34	29	P
		5	13	P	35	27	P
		6	20	P	36	28	P
		MEAN	21		MEAN	28	
.	1778	7	30	P	37	37	P
		8	18	P	38	39	P
		9	25	P	39	30	P
		MEAN	24		MEAN	35	
.	3160	10	22	P	40	43	P
		11	10	P	41	57	P
		12	10	P	42	57	P
		MEAN	14		MEAN	52	
.	5620	13	13	R	43	43	R
		14	13 [illegible]	R	44	52	R
		15	17	R	45	35	R
		MEAN	14 [illegible]		MEAN	44	
.	10000	16	367	R	46	20	R
		17	12	R	47	29	R
		18(M)	174	R	48	18	R
		MEAN	184		MEAN	22	
	HYC	19	328	P	49	368	P
		20	333	P	50	355	P
		21	247	P	51	333	P
		MEAN	303		MEAN	352	
	2AA	22	21	P	52	2004	P
		23	19	P	53	2295	P
		24	16	P	54	1917	P
		MEAN	19		MEAN	2072	
CONTAMINATION		25(M)	0		55(M)	0	
		26(M)	0		56(M)	0	
HISTIDINE		27(M)	0		57(M)	0	
		28(M)	+		58(M)	+	
		29(M)	+		59(M)	+	
		30(M)	+		60(M)	+	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
S - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 9/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

There was quite a substantial positive response in the first Ames test, with a dose-related increase in revertant colonies of 2.9- and 32-fold over controls at 1000 and 3160 µg per plate, respectively +S9. However, these results did not repeat in the second study (see sponsor Table 15 above). Even though the results did not repeat, such a strong positive response is still disconcerting, and perhaps the sponsor should have repeated the test with strain 1538 a third time. It is even more disconcerting because this result was found in the presence of S9, and suggests that the effect may be due to a metabolite. 311C90 is metabolized to a number of other compounds that are present at about half the plasma levels at the parent drug in humans.

While there was no repeat of the positive response in the Ames test (strain 1538), there was a repeat positive response in the Yahaghi modification test, at 10000

cytotoxicity apparently occurs (sponsor's Table 19 shows reduced bacterial lawn at this concentration). However, in this type of assay, some cytotoxicity is desired at the high dose, and does not necessarily negate a positive finding. Therefore, it is my opinion that it is inappropriate to negate this positive finding at 10000 µg in the Yahaghi modification test, just because there is some cytotoxicity at this concentration.

Pharmacologist's comments: Data for strain 1538 are disconcerting, in that in the first Ames test there was a strong, dose-related increase in the number of revertant colonies at 1000 (2.9 fold increase over controls) and 3160 µg (32-fold increase) concentrations in the presence of S9, which could indicate involvement of a metabolite. This positive response did not repeat in the standard Ames test, but in my opinion it would be inappropriate to ignore such a strong finding, even though it did not repeat.

Substantial positive results were seen in both Yahaghi modification tests at the 10000 µg concentration +S9 (first study) and -S9 (repeat study). While the sponsor indicates that this concentration shows cytotoxicity (reduced background bacterial lawn), some cytotoxicity is desired at the highest concentration, and does not justify completely ignoring this positive finding.

Strain TA98

Testing in strain TA98 was also carried out by both standard Ames test and by the Yahaghi (pre-incubation) modification test, and a repeat of each test was included. Controls responded appropriately in all tests. In the first Ames test with strain TA 98, a strong positive result (12-fold increase over control; 405 for treated versus 33 for controls) was found at the 3160 µg concentration in the presence of S9 (see sponsor's Table 4 below). A positive response was also found at the 10000 µg concentration (3-fold increase over controls; 107 for treated versus 33 for controls) +S9. Sponsor's Table 4 (below) indicate that the background was reduced at the 10000 µg dose, indicating that cytotoxicity may have been present at this concentration.

In the repeat of the Ames test (see sponsor's table 16 below) , there was no positive response, although the control responses both +S9 and -S9 were at least 2-fold higher than those in the first study. It is unknown why this was the case.

In the first Yahaghi preincubation study a 1.8-fold increase in revertant colonies was seen at the 10000 µg concentration (see sponsor's Table 11 below). Again, the background was reduced at this concentration. In the repeat Yahaghi test, no positive response was found, but again the background colony count was at least 2-fold that of the first study (see sponsor's Table 20 below).

So again with strain TA 98, the first experiment gave strong positive responses for both standard Ames test and Yahaghi modification, while repeat studies were negative. With strain TA 98, backgrounds were 2-fold higher in the repeat for some unknown reason.

BEST POSSIBLE COPY

Table 4

Laboratory Ref: 1658
Test Type: Ames Test
S9Batch: 66 Robens
Test Date: 28.2.92
Direct Control : Daunomycin (D)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 98
Reading Date: 02/03/92
Reading Time: 09:44

Compound Batch Number: WOCP 91/0017/104/1 BIR
Compound Source: Mr.G. Salmon
DSE
Beckenham.
Compound Name: 311C90
Comment: No comment

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	181	37	P	211(M)	27	P
		182	30	P	212(M)	30	P
		183	26	P	213(M)	13	P
		MEAN	31		MEAN	23	
.	100	184	30	P	214(M)	35	P
		185	36	P	215(M)	22	P
		186	27	P	216	62	P
		MEAN	31		MEAN	40	
.	316	187	22	P	217(M)	51	P
		188	37	P	218(M)	58	P
		189	36	P	219(M)	31	P
		MEAN	32		MEAN	47	
.	1000	190	41	P	220(M)	48	P
		191	43	P	221(M)	49	P
		192	51	P	222(M)	49	P
		MEAN	45		MEAN	49	
.	3160	193	22	P	223	368	P
		194	23	P	224	430	P
		195	16	P	225	417	P
		MEAN	20		MEAN	405	
.	10000	196	12	R	226	101	R
		197(M)	8	R	227	118	R
		198	11	R	228	101	R
		MEAN	10		MEAN	107	
	D	199	1095	P	229	1713	P
		200	1038	P	230	1561	P
		201	984	P	231	1782	P
		MEAN	1039		MEAN	1685	
	2AA	202(M)	17	P	232	2496	P
		203(M)	13	P	233	2192	P
		204(M)	34	P	234	2062	P
		MEAN	21		MEAN	2250	
CONTAMINATION		205(M)	0		235(M)	0	
		206(M)	0		236(M)	0	
		207(M)	0		237(M)	0	
HISTIDINE		208(M)	-		238(M)	-	
		209(M)	-		239(M)	-	
		210(M)	-		240(M)	-	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
X - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 2/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

Report No. : BPAT 91/0048

Table 11

Laboratory Ref: 165C
Test Type: Yahagi Modification
S9Batch: 66 Robens
Test Date: 28.2.92.
Direct Control : Daunomycin (D)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 98
Reading Date: 02/03/92
Reading Time: [illegible]

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

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	181	25	P	211	32	P
		182	32	P	212	37	P
		183	23	P	213	30	P
		MEAN	27		MEAN	33	
.	100	184	31	P	214	43	P
		185	30	P	215(M)	27	P
		186	38	P	216	33	P
		MEAN	33		MEAN	34	
.	316	187	22	P	217	39	P
		188	25	P	218	37	P
		189	20	P	219	32	P
		MEAN	22		MEAN	36	
.	1000	190	35	P	220	40	P
		191	29	P	221	31	P
		192	32	P	222	37	P
		MEAN	32		MEAN	36	
.	3160	193	22	P	223	41	P
		194	19	P	224	47	P
		195	23	P	225	35	P
		MEAN	21		MEAN	41	
.	10000	196	21	R	226	48	R
		197	4	R	227	46	R
		198	10	R	228	47	R
		MEAN	12		MEAN	40	
	D	199	1315	P	229	990	P
		200	1309	P	230	892	P
		201	1439	P	231	909	P
		MEAN	1354		MEAN	930	
	2AA	202	26	P	232	2066	P
		203	35	P	233	1619	P
		204	30	P	234	1289	P
		MEAN	30		MEAN	1691	
CONTAMINATION		205(M)	0		235(M)	0	
		206(M)	0		236(M)	0	
		207(M)	0		237(M)	0	
HISTIDINE		208(M)	-		238(M)	-	
		209(M)	-		239(M)	-	
		210(M)	-		240(M)	-	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
X - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 2/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

Report No. : BPAT 91/0048

Table 16

Laboratory Ref: 1650
Test Type: Ames Test
S9Batch: 66 Robens
Test Date: 6.3.92
Direct Control : Daunomycin (D)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 98
Reading Date: 09/03/92
Reading Time: 09:28

Compound Batch Number: BIR WOCP 91/0017/104/1
Compound Source: Mr.G.Salmon
DSE
Beckenham.
Compound Name: 311C90
Comment: No comment

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	61	88	P	91	78	P
		62	92	P	92	83	P
		63	78	P	93	67	P
		MEAN	87		MEAN	76	
.	1000	64	60	P	94	80	P
		65	75	P	95	103	P
		66	79	P	96	77	P
		MEAN	71		MEAN	87	
.	1778	67	90	P	97	91	P
		68	77	P	98	83	P
		69	79	P	99	79	P
		MEAN	82		MEAN	84	
.	3160	70	67	P	100	79	P
		71	93	P	101	70	P
		72	68	P	102	80	P
		MEAN	76		MEAN	76	
.	5623	73	27	R	103	58	R
		74	12	R	104	59	R
		75	16	R	105	28	R
		MEAN	18		MEAN	48	
.	10000	76	16	R	106	17	R
		77	18	R	107	18	R
		78	11	R	108	19	R
		MEAN	15		MEAN	18	
	D	79	808	P	109	2120	P
		80	381	P	110	2136	P
		81	629	P	111	2354	P
		MEAN	606		MEAN	2210	
	2AA	82	80	P	112	2941	P
		83	68	P	113	2287	P
		84	82	P	114	1476	P
		MEAN	77		MEAN	2235	
CONTAMINATION		85(M)	0		115(M)	0	
		86(M)	0		116(M)	0	
		87(M)	0		117(M)	0	
HISTIDINE		88(M)	-		118(M)	-	
		89(M)	-		119(M)	-	
		90(M)	-		120(M)	-	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
X - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 9/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

Report No. : BPAT 91/0048

Table 20

Laboratory Ref: 165E
Test Type: Yahagi Modification
S9Batch: 66 Robens
Test Date: 6.3.92.
Direct Control : Daunomycin (D)
InDirect Control: 2-Aminoanthracene (2AA)

Strain: TA 98
Reading Date: 09/03/92
Reading Time: 12:03

Compound Batch Number: BIR WOCP 91/0017/104/1
Compound Source: Mr.G.Salmon
DSE
Beckenham.
Compound Name: 311C90
Comment: No comment

Prec	Dose (ug) per plate	S9 ABSENT Plate No.	Colonies	Bkgd	S9 PRESENT Plate No.	Colonies	Bkgd
.	0	61	56	P	91	79	P
		62	66	P	92	82	P
		63	70	P	93(M)	66	P
		MEAN	64		MEAN	76	
.	1000	64	67	P	94	78	P
		65	67	P	95	103	P
		66	76	P	96	69	P
		MEAN	70		MEAN	83	
.	1778	67	55	P	97	109	P
		68	90	P	98	93	P
		69	43	P	99	71	P
		MEAN	63		MEAN	91	
.	3160	70	79	P	100(M)	71	P
		71	31	P	101	58	P
		72	50	P	102	59	P
		MEAN	53		MEAN	63	
.	5623	73	75	R	103	62	R
		74	26	R	104	68	R
		75	13	R	105	66	R
		MEAN	38		MEAN	65	
.	10000	76	14	R	106	9	R
		77	27	E	107	20	R
		78	21	R	108	18	R
		MEAN	21		MEAN	16	
	D	79	1139	P	109	868	P
		80	1169	P	110	686	P
		81	1319	P	111	670	P
		MEAN	1209		MEAN	741	
	2AA	82	54	P	112	737	P
		83(M)	55	P	113	797	P
		84	61	P	114	817	P
		MEAN	57		MEAN	784	
CONTAMINATION		85(M)	0		115(M)	0	
		86(M)	0		116(M)	0	
		87(M)	0		117(M)	0	
HISTIDINE		88(M)	-		118(M)	-	
		89(M)	-		119(M)	-	
		90(M)	-		120(M)	-	

KEY for BACKGROUND GROWTH

P - present
R - reduced
A - absent
E - enhanced
X - plate skipped

Signed: [signature]
Witnessed: [signature]
Date: 9/3/92

NB: @ denotes precipitation at specified dose level
. denotes no precipitation at specified dose level
(M) next to a plate number indicates a manual reading

Report No. : BPAT 91/0048

Strain TA100

Both an Ames test and a Yahaghi modification were completed in strain TA100. The appropriate controls were used and responded correctly. There were no notable increases in revertant colonies at any concentration up to 10000 per plate.

Summary and Conclusions:

The sponsor included test strains *S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100. The OECD guidelines (1994) for Genetic Toxicology testing also recommend the use of one of the *E. Coli* strains of *S. typhimurium* strain TA102, that detect certain oxidizing mutagens, cross-linking agents, and hydrazines.

The sponsor states that they completed a cytotoxicity screen, and that 10000 µg was the concentration at which limiting cytotoxicity occurred as reflected by reduced backgrounds in their various data tables. 5623 µg also gave a reduced background in the one experiment that included this concentration.

Strains TA1535, TA1537 and TA100 all tested negative, both in the standard Ames test and in the Yahaghi modification (preincubation). However, strain 1538 showed a very substantial positive response at 1000 µg (increased 2.9-fold over control) and 3160 µg (32-fold increased over controls) concentrations +S9 in the Ames test. While this result did not repeat in a second study, it is still of concern since it was such a large dose-related positive response. With the Yahaghi modification, strain 1538 gave a positive result at 10000 µg, +S9 in the first study and -S9 in the repeat study. The background was apparently reduced at this concentration suggesting cytotoxicity, but this does not necessarily negate the positive finding since some cytotoxicity is desirable at the highest dose in the Ames test.

Tests with strain TA98 revealed a strong positive response (12-fold increase over controls) at 3160 µg/plate in the presence of S9 in the Ames test. This effect did not repeat, but control values in the repeat study were at least two-fold higher than in the original. A positive response (3-fold>controls) was also seen at 10000 µg, the dose at which the background was reduced indicating cytotoxicity. In the Yahaghi modification, a 1.8-fold increase was seen at 10000 µg, but this also did not repeat. Again, the background was apparently reduced at this concentration in the repeat study.

It is my opinion that it would be inappropriate to ignore the positive findings in strains TA 1538 and TA 98. With both strains, the initial experiments demonstrated strong, dose-related, positive findings with standard Ames and Yahaghi modification tests. While it is true that these findings did not repeat in the repeat experiments with either strain, the initial positive results were of too large a magnitude to ignore. Furthermore, the fact that some of the positive responses (Yahaghi modification) occurred at 10000 µg concentration where the background lawn was reduced does not necessarily negate the positive findings, as some cytotoxicity is actually desirable at the highest dose in an Ames test to demonstrate one is testing in the appropriate concentration range. Finally, with strain 98, the background counts were 2-fold higher in the Ames and Yahaghi tests than in the first set of experiments, which may have

2. Evaluation of 311C90 for clastogenicity using metaphase analysis of human lymphocytes, study BPAT/92/0025, The Wellcome Foundation, Kent, UK, February 4, 1993, GLP.

Cells: human blood from healthy male volunteers

Study design: Lymphocytes stimulated by PHA (phytohemagglutinin), cultured 48h incubation, treated with 311C90, solvent control or positive control for 24 hrs (-S9) or 3 hrs (+S9; washed, resuspended in fresh culture media, and reincubated for 21 additional hrs). Cell cycle was halted at metaphase by treatment with colchicine, 3 hrs before end of incubation period. Approximately 100 cells in metaphase were scored from each culture and aberrant cells recorded. Cytotoxicity was determined in the main experiment. Positive controls included ethyl methanesulphonate (-S9) and cyclophosphamide (+S9).

Drug concentrations/cytotoxicity screen:
In the first study without S9, the mitotic index was reduced to 39% at 1000 µg/ml, with extreme toxicity at 2000 and 4000 µg/ml, as evidenced by absence of live cells. The concentrations selected for study were 125, 500 and 1000 µg/ml. With S9, 4000 µg/ml was extremely toxic, and 2000 µg/ml reduced the mitotic index to 47% of solvent control. The three concentrations chosen for study were 250, 1000 and 2000 µg/ml.

Pharmacologist's comment: The study protocol is consistent with the OECD recommendations for *In vitro* mammalian chromosomal aberration tests, with the exception that the sponsor only examined 100 cells in metaphase. The OECD guidelines recommend that they examine 200 cells per culture.

The sponsor used the appropriate positive and negative controls, and responses were appropriate in the studies. The choice of study concentrations were also appropriate based on cytotoxicity results.

Results:

First study

Absence of S9: Results of the first study in absence of S9 are shown in sponsor's Table 3 (vol. 49, pg. 33-35) shown below:

TABLE 3

The effect of 311C90 on the chromosomes of cultured human lymphocytes, 24 hour harvest - first test

(a) Without S-9 mix

Test agent	Concentration µg/ml	No. cells examined	Aberrations: Chromatid type csb	csa	Chromosome type csb	csa	Others	Gaps csg	csg	No. of aberrant cells: Exc. gaps	Mean %	Inc. gaps	Mean %
Dimethyl sulphoxide (solvent control)	10 µl/ml	100								0		0	
		100			2					2	1.25	2	1.25
		100	1		2					2		2	
		100								1		1	
311C90	125	100	2		2					4	5.0**	4	5.0**
		100	2		3					6		6	
	500	100	2		4					9	9.5***	9	9.5***
		100	7		4					10		10	
	1000	100	10		3			1		10	14.0***	11	14.5***
		100	10		10					18		18	
Ethyl methanesulphonate (positive control)	750	100	27	3	9					27	30.0***	27	30.0***
		100	34	3	30					33		33	

*** P<0.001 csb Chromatid break csa Chromatid exchange
** P<0.01 csb Chromosome break csa Chromosome exchange
* P<0.05 csg Chromatid gap csg Chromosome gap
Otherwise P>0.05 others Cells with greater than 10 aberrations, pulverized cells and pulverized chromosomes

TABLE 3

(continued)

(a) Without S-9 mix, additional concentrations analyzed

Test agent	Concentration µg/ml	No. cells examined	Aberrations: Chromatid type csb	csa	Chromosome type csb	csa	Others	Gaps csg	csg	No. of aberrant cells: Exc. gaps	Mean %	Inc. gaps	Mean %
Dimethyl sulphoxide (solvent control)	10 µl/ml	100								0		0	
		100								0	0.5	0	0.5
		100	1		1					2		2	
311C90	31.3	100			4					3	2.5*	3	2.5*
		100			2					2		2	
	62.5	100	2		3					5	4.5**	5	4.5**
		100	1		4					4		4	
	250	100			4					4	6.5***	4	6.5***
		100	3		6					9		9	

*** P<0.001 csb Chromatid break csa Chromatid exchange
** P<0.01 csb Chromosome break csa Chromosome exchange
* P<0.05 csg Chromatid gap csg Chromosome gap
Otherwise P>0.05 others Cells with greater than 10 aberrations, pulverized cells and pulverized chromosomes

TABLE 3

(continued)

(a) With S-9 mix

Test agent	Concentration µg/ml	No. cells examined	Aberrations: Chromatid type csb	csa	Chromosome type csb	csa	Others	Gaps csg	csg	No. of aberrant cells: Exc. gaps	Mean %	Inc. gaps	Mean %
Dimethyl sulphoxide (solvent control)	10 µl/ml	100	1		3					3		2	
		100								0	0.75	0	1.25
		100	1							1		1	
		100						2		0		2	
311C90	250	100								0	0.5	0	0.5
		100			1					1		1	
	1000	100			1					1	3.5*	1	3.5
		100			7					6		6	
	2000	100			15					11	12.0***	11	12.5***
		100	2		19			5		15		14	
Cyclophosphamide (positive control)	15	50	4	4	31			4		19	27.3***	20	28.0***
		100	5	1	22		2			22		22	

Absence of S9: clastogenic effects were seen at all three concentrations (125, 500 and 1000 µg/ml) (Table 3a above). Sponsor examined three additional concentrations (Table 3b above), and reported the NOEL to be 31.3 µg/ml.

Pharmacologist's comment: Actually the effect appears to be statistically ($p<0.05$) significant even at the 31.3 µg/ml concentration, so it is unclear why the sponsor designated this as the NOEL. Positive controls responded appropriately in both +S9 and -S9 studies.

Presence of S9: clastogenic effects were seen at 2000 µg/ml, but not lower concentrations (Table 3c above).

Second study

Cytotoxicity

In the second study, cytotoxicity occurred at 1000 and 1250 µg/ml and mitotic index was decreased to 22% at 750 µg/ml and 55% at 500 µg/ml. Concentrations for analysis were 19.5, 39.1, 156 and 500 µg/ml.

Absence of S9: Study results of the second study in the absence of S9 are shown in the following sponsor's Table 4a (vol, 49, pg 36).

TABLE 4

The effect of 311C90 on the chromosomes of cultured human lymphocytes, 24 hour harvest - second test

(a) Without S-9 mix

Test agent	Concentration µg/ml	No. cells examined	Abnormalities: Chromatid type csb	Abnormalities: Chromatid type csg	Abnormalities: Chromosome type csb	Abnormalities: Chromosome type csg	Abnormalities: Others	Abnormalities: Gaps	No. of abnormal cells: Exc. gaps	No. of abnormal cells: Mean %	Inc. gaps	Inc. Mean %
Dimethyl sulphoxide (solvent control)	10 µl/ml	100			1				1		1	
		100			2				2	2.5	2	2.5
		100			6				3		3	
		100			2				2		2	
311C90	19.5	100			2				2	2.5	2	2.5
		100			3				3		3	
	39.1	100			2				2	1.5	2	1.5
		100			1				1		1	
	156	100	2		2				4	3.0	4	3.0
		100			3			1	2		2	
	500	99	1		8				8	9.7***	8	10.3***
		76			12		1	3	9		9	
Ethyl methanesulphonate (positive control)	750	100	1		43			3	25	25.0***	25	25.0***
		100	3	2	41		1	3	25		25	

*** P<0.001 csb Chromatid break csb Chromosome exchange
** P<0.01 csb Chromosome break csb Chromatid exchange
* P<0.05 csg Chromatid gap csg Chromosome gap
Otherwise P>0.05 others Cells with greater than 10 abnormalities, pulverised cells and pulverised chromosomes

Absence of S9:

Clastogenicity was seen at 500 µg/ml (Table 4a above).

Presence of S9:

Results of second study +S9 are shown in Table 4b (volume 49, pg. 37) shown below:

TABLE 4

(continued)

(b) With S-9 mix

Test agent	Concentration µg/ml	No. cells examined	Aberrations: Chromatid type csb	csb	Chromosome type csb	csb	Other	Gaps csg	csg	No. of aberrant cells: Exc. gaps	Mean %	Inc. gaps	Mean %
Dimethyl sulphoxide (solvent control)	10 µl/ml	100			1					1		1	
		100			2					1		1	
		100			3			2		3	1.5	5	2.0
		100			1					1		1	
	156	100			3					3	1.5	3	1.5
		100								0		0	
311C90	313	100	1	1	1		2			4	2.0	4	2.5
		100						1		0		1	
	1000	100	1		2			3		3	5.5**	5	7.5**
		100	4		6			2		8		10	
Cyclophosphamide (positive control)	15	100	19	2	41		6	12		39	38.0***	40	39.5***
		100	14	5	44		5	18		37		39	

*** P<0.001 csb Chromatid break csx Chromatid exchange

** P<0.01 csb Chromosome break csx Chromosome exchange

* P<0.05 csg Chromatid gap csg Chromosome gap

Otherwise P>0.05 other Cells with greater than 10 aberrations, pulverized cells and pulverized chromosomes

Presence of S9: clastogenicity was seen at 1000 µg/ml.

Summary and Conclusion for Human Lymphocyte Assay

311C90 gave a positive response both in absence and presence of S9. The study design was consistent with the OECD guidelines, with the exception that the sponsor only examined 100 instead of 200 cells in metaphase. However, the OECD guidelines do allow that the number of cells examined can be reduced in the presence of "high numbers of aberrations." The positive controls used were appropriate and gave an appropriate response in the assays. The choice of concentrations based on cytotoxicity were also appropriate.

Without S9, the first study shows positive results at concentrations all the way down to 31.3 µg/ml, while in the second study positive results are seen at concentrations of 500 µg/ml and greater. The sponsor contends that the variability in the background (control) response resulted in the much lower concentration (31.3 µg/ml) maintaining statistical significance in the first study, and they contend that "biologically significant" increases in aberrant cells should be considered to be seen at ≥250 µg/ml without S9. I would disagree, and my read of the data would be that significant aberrations are represented at concentrations probably ≥156 µg/ml.

With S9, data are consistent with the sponsor's conclusion that increases in aberrant cells are shown at concentrations ≥1000 µg/ml.

3. **Mutagenicity test on 311C90 in the CHO/HGPRT forward mutation assay, TTEP/93/0007, Burroughs Wellcome, Co.** **GLP.**

Cells: Chinese hamster ovary (CHO) cells
Drug: 311C90 Batch W1 (ref. no. 91/0017-108-2)
Study design: Treatment was for four hours in presence or absence of S9. Positive controls were 5-bromo-2'-deoxyuridine (BrdU) for cells without S9 and 3-methylcholine (3-MCA) for cells +S9. 311C90 was dissolved into DMSO. An appropriate cytotoxicity screen was done. Seven doses from 500 to 3600 µg/ml were utilized, a range that included a concentration with no cytotoxicity and a dose with slight cytotoxicity and limited solubility in F12 culture medium.

Cytotoxicity screen: 311C90 in DMSO (drug soluble up to 350 mg/ml; diluted 1:100 into F12 culture medium). Treatments above 878 µg/ml were basic. Cytotoxicity assay was first performed without pH adjustments, and toxicity was seen only in basic cultures. In mutation assay, pH was adjusted so all cultures were between pH of 7.0 and 7.2.

Rangefinding studies included concentrations from 6.36-3510 µg/ml. There was no cytotoxicity up to 1755 µg/ml in presence or absence of S9. Without activation severe cytotoxicity (cell lethality) occurred at 3510 µg/ml (0% survival relative to control). Weak cytotoxicity was obtained at this concentration +S9 (51.8% survival relative to control). Doses chosen were from 500 to 3600 µg/ml.

Pharmacologist's comments: The study design was consistent with the OECD guidelines for *in vitro* mammalian cell gene mutation test.

Results:

Mutagenicity study without S9:

Absence of S9: Results of mutagenicity testing in absence of S9 are shown in the following sponsor's Tables 3 and 4 (volume 49, pages 29 and 30); experiments 1 and 2, respectively):

TABLE 3

MUTAGENICITY ASSAY WITHOUT METABOLIC ACTIVATION - Trial 1

SAMPLE NAME: 311C88 SPONSOR: Burroughs Wellcome ASSAY NO. 15418 TEST DATE February 5, 1991
VEHICLE DMSO Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E.
Expression Time: 7 days 2x10⁵/dish for mutants

MONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT MEAN COLONY NUMBER±S.D.	PERCENT VEH. CONTROL	RELATIVE POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1 2 3 4 5 6 7 8 9 10 11 12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ IN 10⁻⁶ UNITS*
Vehicle Control	183.0 ± 9.8	104.6	100.6	2 1 1 0 1 2 0 0 0 0 0 1	8	118.7 ± 1.2	2.8
Vehicle Control	187.0 ± 4.6	95.4	99.2	1 0 1 0 1 0 0 1 0 0 1 0	5	83.7 ± 5.7	2.5
Positive Control (50 µg/ml BrdU)[b]	94.7 ± 10.4	54.1	39.3	6 11 9 14 16 9 8 15 14 4 14 13	132	91.7 ± 5.8	60.8**
TEST ARTICLE							
500 µg/ml	190.0 ± 8.1	96.7	202.0	0 1 0 0 0 0 1 0 0 1 1 0	4	69.7 ± 4.8	2.4
1000 µg/ml	209.7 ± 19.4	119.8	170.2	0 0 0 0 0 0 1 1 0 0 0 0	2	98.7 ± 6.5	0.8
1500 µg/ml	187.0 ± 10.4	106.9	157.3	0 1 0 0 0 1 0 0 0 0 0 0	2	86.7 ± 13.0	0.9
2000 µg/ml	161.0 ± 4.4	92.0	132.0	1 0 0 0 1 2 0 0 1 0 0 0	5	80.0 ± 2.5	2.4
2500 µg/ml	140.0 ± 38.2	80.0	91.0	1 0 3 2 2 2 1 0 1 2 0 6	20	99.2 ± 9.0	8.4*
3000 µg/ml	154.3 ± 15.0	88.2	70.4	1 2 1 0 1 0 1 2 2 2 1 1	14	93.4 ± 7.7	6.2
3600 µg/ml	124.0 ± 2.6	70.9	112.4	2 2 0 0 1 0 2 1 1 0 2 0	11	105.0 ± 17.0	4.4

[a]Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.)
[b]BrdU = 5-Bromo-2'-deoxyuridine

*Significant increase: Kastenbaum Bowman test p ≤ 0.01 but mutant frequency ≤ 15 x 10^{-6}.
**Significant increase: Kastenbaum Bowman test p ≤ 0.01 and mutant frequency ≥ 15 x 10^{-6}.

TABLE 4

MUTAGENICITY ASSAY WITHOUT METABOLIC ACTIVATION - Trial 2

SAMPLE NAME: 311C88 SPONSOR: Burroughs Wellcome ASSAY NO. 15438 TEST DATE February 24, 1991
VEHICLE DMSO Selective Agent: 4 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E.
Expression Time: 7 days 2x10⁵/dish for mutants

MONACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT MEAN COLONY NUMBER±S.D.	PERCENT VEH. CONTROL	RELATIVE POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1 2 3 4 5 6 7 8 9 10 11 12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ IN 10⁻⁶ UNITS*
Vehicle Control	178.3 ± 13.2	98.6	80.1	0 1 2 0 1 0 2 1 0 2 0 5	14	94.2 ± 3.3	6.2
Vehicle Control	183.3 ± 7.8	101.4	119.9	0 1 1 0 1 1 0 0 0 2 3 1	10	86.4 ± 4.5	4.8
Positive Control (50 µg/ml BrdU)[b]	145.7 ± 13.5	80.6	85.6	12 11 15 17 15 12 19 21 14 19 15 18	188	85.9 ± 6.4	91.2**
TEST ARTICLE							
500 µg/ml	181.3 ± 5.7	100.3	79.4	0 0 0 0 0 0 0 0 0 0 0 0	0	84.2 ± 7.3	0.0
1000 µg/ml	172.7 ± 2.1	95.5	76.2	0 1 0 0 0 0 0 0 0 1 1 0	3	94.4 ± 2.0	1.3
1500 µg/ml	191.3 ± 17.0	105.8	77.9	1 1 1 2 1 1 4 0 3 1 1 1	17	98.4 ± 7.3	7.9
2000 µg/ml	156.3 ± 2.5	86.4	93.7	1 0 1 0 0 0 2 0 2 1 0 0	7	83.5 ± 7.4	3.5
2500 µg/ml	148.3 ± 2.5	82.0	67.6	0 0 0 1 0 1 0 0 0 0 0 1	3	87.7 ± 10.4	1.4
3000 µg/ml	183.0 ± 17.3	101.2	70.8	1 1 0 0 1 0 1 0 0 0 1 0	5	83.0 ± 3.1	2.5
3600 µg/ml	167.0 ± 14.0	92.4	66.9	0 1 0 1 0 0 0 0 0 1 0 0	3	78.2 ± 3.1	1.6

[a]Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.)
[b]BrdU = 5-Bromo-2'-deoxyuridine

**Significant increase: Kastenbaum Bowman test p ≤ 0.01 and mutant frequency ≥ 15 x 10^{-6}.

Absence of S9: In both experiments (Tables 3 and 4 above) the positive controls responded appropriately. There was somewhat less toxicity at the high concentration, probably because the pH was adjusted at the higher doses in the studies (was not done in the initial cytotoxicity screens). There were no mutagenic effects of the drug at any of the concentrations tested. These concentrations were appropriate

Presence of S9: Mutagenicity study results are shown in Tables 5 and 6 (vol 49, pages 31 and 32, respectively) below:

TABLE 5

MUTAGENICITY ASSAY WITH METABOLIC ACTIVATION - Trial 1

SAMPLE NAME: 311C80 SPONSOR: Burroughs Wellcome ASSAY NO. 18418 TEST DATE February 5, 1993
VEHICLE DMSO Selective Agent: 6 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E.
Expression Time: 7 days 2x10⁵/dish for mutants

ACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT MEAN COLONY NUMBERS±S.D.	SURVIVAL TO TREATMENT PERCENT VEH. CONTROL	RELATIVE POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1 2 3 4 5 6 7 8 9 10 11 12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ IN 10^{-6} UNITS[a]
Vehicle Control	164.7 ± 17.6	100.4	112.8	0 0 0 1 2 1 0 0 0 0 0 1	5	102.5 ± 6.1	2.0
Vehicle Control	139.3 ± 17.6	81.6	87.8	1 2 2 0 1 3 1 0 1 0 0 0	11	107.7 ± 7.2	4.3
Positive Control (6 µg/ml 3-MCA)[b]	126.3 ± 14.2	83.7	63.0	21 15 25 16 14 9 21 12 17 22 23 16	211	82.2 ± 4.6	107.0**
TEST ARTICLE							
500 µg/ml	137.0 ± 19.3	99.1	207.2	0 0 1 1 0 1 0 1 1 1 0 0	6	99.2 ± 7.6	2.5
1000 µg/ml	138.7 ± 19.3	91.3	218.0	1 0 1 2 1 0 0 0 2 2 2 1	12	97.2 ± 8.2	5.1
1500 µg/ml	137.0 ± 20.0	90.1	86.6	0 0 1 0 1 0 1 0 2 0 2 1	8	98.0 ± 7.0	3.4
2500 µg/ml	110.7 ± 3.7	72.8	160.6	1 0 1 0 0 1 1 0 0 3 1 0	8	86.2 ± 14.8	3.9
3000 µg/ml	119.7 ± 8.1	78.8	94.4	1 2 0 0 0 1 0 0 0 1 0 0	5	99.0 ± 6.1	2.3
3600 µl/ml	141.7 ± 19.4	93.2	90.7	1 1 1 0 2 1 0 1 0 1 0 1	9	82.5 ± 4.3	4.5

[a]Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.)
[b]3-MCA = 3-Methylcholanthrene

**Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10^{-6}$.

TABLE 6

MUTAGENICITY ASSAY WITH METABOLIC ACTIVATION - Trial 2

SAMPLE NAME: 311C80 SPONSOR: Burroughs Wellcome ASSAY NO. 18418 TEST DATE February 24, 1993
VEHICLE DMSO Selective Agent: 6 µg/ml 6-thioguanine Cells seeded for analysis: 200/dish for C.E.
Expression Time: 7 days 2x10⁵/dish for mutants

ACTIVATION TEST CONDITION	SURVIVAL TO TREATMENT MEAN COLONY NUMBERS±S.D.	SURVIVAL TO TREATMENT PERCENT VEH. CONTROL	RELATIVE POPULATION GROWTH (% OF CONTROL)	MUTANT COLONIES DISH NUMBER 1 2 3 4 5 6 7 8 9 10 11 12	TOTAL MUTANT COLONIES	ABSOLUTE C.E.±S.D. (%)	MUTANT FREQ IN 10^{-6} UNITS[a]
Vehicle Control	142.0 ± 16.5	79.2	117.5	0 0 3 0 1 1 1 0 1 0 1 0	8	91.5 ± 9.9	3.6
Vehicle Control	216.7 ± 15.0	120.8	82.5	1 1 0 1 1 2 1 1 0 2 3 5	18	83.2 ± 13.0	9.0
Positive Control (5 µg/ml 3-MCA)[b]	173.3 ± 15.6	96.6	67.6	41 29 26 33 24 31 23 25 33 29 26 32	352	95.5 ± 8.7	153.6**
TEST ARTICLE							
500 µg/ml	152.7 ± 6.8	85.1	74.8	0 0 2 1 1 0 0 1 0 2 0 0	7	86.4 ± 4.1	3.4
1000 µg/ml	165.7 ± 8.8	92.4	66.8	2 1 0 2 1 2 0 0 1 3 4 2	18	88.4 ± 13.3	8.5
1500 µg/ml	174.0 ± 12.8	97.0	71.6	0 0 0 0 0 0 0 0 0 0 0 1	1	85.2 ± 2.8	0.5
2000 µg/ml	178.0 ± 18.0	99.2	68.8	1 1 2 1 1 0 2 1 0 0 1 0	10	79.7 ± 7.3	5.2
2500 µg/ml	185.0 ± 27.9	103.2	95.1	0 0 0 1 1 1 2 2 0 1 1 1	12	83.2 ± 11.4	6.0
3000 µg/ml	136.0 ± 14.5	75.8	119.0	0 1 1 0 1 2 0 0 2 1 2 0	10	80.5 ± 6.6	5.2
3600 µg/ml	163.7 ± 14.5	91.3	95.7	1 1 0 1 0 0 1 2 1 0 0 2	9	86.7 ± 1.6	4.3

[a]Mutant Frequency = Total mutant colonies/(No. of dishes x 2 x 10^5 x absolute C.E.)
[b]3-MCA = 3-Methylcholanthrene

**Significant increase: Kastenbaum Bowman test $p \leq 0.01$ and mutant frequency $\geq 15 \times 10^{-6}$.

Presence of S9: Positive controls responded appropriately. There was little evidence of cytotoxicity even at the highest concentration. The sponsor states that this was the limiting concentration based on drug solubility in DMSO and diluted into culture medium. There was no evidence of mutagenicity at any of the concentrations tested in these two studies +S9.

Summary and conclusion for mutagenicity testing (CHO/HGPRT test)

The study design was consistent with the OECD guidelines for *in vitro* mutagenicity testing of this kind. The correct positive controls were used and responded appropriately. The dose range was chosen based on an initial cytotoxicity screen (both + and - S9), and ultimately on the solubility limitations of the drug dissolved into DMSO and diluted into culture medium.

There were no signs of mutagenicity at any of the doses tested ± S9 in either the initial or repeat studies.

4. 311C90: a micronucleus assay in mice with 311C90, study #TTEP/93/0006, Burroughs Wellcome. March 31, 1993, GLP. (Also includes review of 311C90: evidence of exposure to 311C90 and metabolites during a micronucleus assay in mice with oral 311C90, study #BPAT/93/0118, The Wellcome Foundation, Kent, UK, July 30, 1993, GLP).

Drug: 311C90Wi (ref. #91/0017-108-2); positive control was cyclophosphamide; vehicle 0.5% methylcellulose.

Test system: CD-1 mice (Charles River); 9 groups of 5/sex/group for study; 6 satellite groups of 9/sex/group for toxicokinetics.

Dosing: three daily oral doses of 311C90 at 50, 100, 200, 300, and 500 mg/kg/day or three daily oral doses of vehicle control. The highest dose of 500 mg/kg/day was chosen based on results of an acute oral study in which several deaths were reported in male and female mice receiving 1500 or 1000 mg/kg, but not 500 mg/kg. 5 males and 5 females were sacrificed 24 hours after dosing. Since no excessive toxicity was found, the sponsor chose 100, 300 and 500 mg/kg animals for micronucleus analysis. The mean number of micronucleated polychromatic erythrocytes (MN-PCEs) was calculated for each sex within each test group.

Blood samples were collected from satellite animals 90 minutes after the last dose in the high dose group. 500 mg/kg/day mice had plasma levels of about 13.8 µg/ml (males) to 16.5 µg/ml (females).

Micronuclei analysis: 1000 polychromatic erythrocytes (PCEs) per animal were scored for the incidence of micronuclei. The ratio of PCEs to normochromatic erythrocytes (NCEs) was determined for each animal by counting a total of 1000 erythrocytes. Bone marrow was taken from all groups, but micronuclei were only analyzed for vehicle controls, three dose levels (100, 300 and 500 mg/kg) and positive controls.

Pharmacologist's comments: The study design was consistent with the OECD guidelines for the mouse micronucleus test, with the exception that those guidelines recommend that at least 2000 immature erythrocytes per animal be scored for the incidence of micronuclei, and the sponsor only scored 1000 per animal.

Results:

Mortality and body weights

No premature deaths were seen, and a small decrease (3-4%) in body weight was noted in females at the high dose (500 mg/kg/day). Apparently no clinical signs of toxicity were observed.

Analysis of micronuclei

Results of the analysis of micronuclei for male and female animals are shown below in sponsor Tables 5 and 6, respectively.

MUT 208: A Micronucleus Assay in Mice with 311C90

Table 5: Summary Data for Male Mice

Dose (mg/kg)	Sacrifice Time (hrs)	Group No.	No. of Animals	%PCEs (1) (Mean +/- S.D.)	MN-PCEs (2) (Mean +/- S.D.)
Vehicle Control	24	1,2,3	15	42.8 +/- 8.12	0.8 +/- 0.77
100	24	5	5	34.1 +/- 2.76	0.4 +/- 0.89
300	24	7	5	47.2 +/- 9.82	0.6 +/- 0.55
500	24	8	5	40.7 +/- 2.39	1.2 +/- 1.64
CP-25 (mg/kg)	24	9	5	35.2 +/- 11.56	19.0 +/- 9.03*

(1) %PCE = 100 x PCE/(NCE + PCE). Values are based on 1000 erythrocytes per animal.
(2) MN-PCEs = Micronucleated polychromatic erythrocytes. Values are based on 1000 PCEs per animal.
* $p < 0.05$; each test dose group was compared with the pooled vehicle control males for statistical significance (See Appendix 3).

MUT 208: A Micronucleus Assay in Mice with 311C90

Table 6: Summary Data for Female Mice

Dose (mg/kg)	Sacrifice Time (hrs)	Group No.	No. of Animals	%PCEs (1) (Mean +/- S.D.)	MN-PCEs (2) (Mean +/- S.D.)
Vehicle Control	24	1,2,3	15	41.3 +/- 9.58	0.7 +/- 1.11
100	24	5	5	29.6 +/- 7.26*	0.6 +/- 1.34
300	24	7	5	45.7 +/- 6.15	0.8 +/- 0.84
500	24	8	5	52.6 +/- 5.83*	1.0 +/- 1.22
CP-25 (mg/kg)	24	9	5	32.7 +/- 13.16	12.0 +/- 4.74*

(1) %PCE = 100 x PCE/(NCE + PCE). Values are based on 1000 erythrocytes per animal.
(2) MN-PCEs = Micronucleated polychromatic erythrocytes. Values are based on 1000 PCEs per animal.
* $p < 0.05$; each test dose group was compared with the pooled vehicle control females for statistical significance (See Appendix 3).

The positive control (cyclophosphamide) gave a statistically significant ($p<0.05$) response in both males and females, and there was no increase in micronuclei at any dose in either males or females.

Plasma levels of 311C90 parent and metabolites

Plasma levels of 311C90 parent drug and metabolites 183C91, 1652W92 and 2161W92 at the highest dose level (500 mg/kg/day) are shown in the sponsor Table (vol 49, pg 191) below:

The concentrations at the highest dose level of 500mg/kg/day were as follows:

Plasma concentrations (ng/ml) - at 90 minutes post dose after three doses of 311C90 at 500mg/kg/day		
	MALES	FEMALES
311C90	13755 ± 4369	16512 ± 5824
183C91	1079 ± 263	1212 ± 442
1652W92	190 ± 55	366 ± 127
2161W92	357 ± 195	920 ± 300

Mean ± S.D.; n=3 (of pooled samples containing 3 samples each).

Plasma concentrations of the parent and metabolites increased in a dose-related manner, with concentrations appearing to be higher in females than in males. Plasma levels of 183C91 were next highest to parent drug, followed by 2161W92 and then 1652W92. Plasma levels of 183C91 were about 7-8% of parent drug levels, whereas in humans levels of this metabolite are apparently about 50% of parent drug at a given dose.

Plasma levels compared to humans at the mrdd.

Humans receiving a single oral dose of zolmitriptan of 50 mg attained plasma C_{max} levels of 311C90 parent drug of about 68 ng/ml. Since human PK show fairly dose-related increases in plasma levels, one can extrapolate a human plasma C_{max} of about 20 ng/ml for a 15 mg dose (the mrdd in humans). Plasma C_{max} levels in these mice (13755-16512 ng/ml) at the high dose (500 mg/kg/day) were about 687-825-fold greater than plasma C_{max} levels in humans receiving the mrdd. Compared on a mg/m^2 surface area basis, a dose of 500 mg/kg/day in mice is equivalent to a dose in humans of about 42 mg/kg/day. The mrdd for humans is 15 mg, or about 0.25 mg/kg/day for a 60 kg patient. Therefore, on a surface area basis, the high dose mice received a dose of drug about 168-fold greater than the human mrdd.

Summary and conclusion for mouse micronucleus study

The study protocol was consistent with the OECD guidelines for micronucleus testing *in vivo.* Dose selection was appropriate. No increase in micronuclei over control levels was found at any dose in either male or female animals. Toxicokinetics data revealed a dose-dependent increase in plasma C_{max} and higher plasma levels in female than male mice. High dose animals were exposed to plasma C_{max} levels 687-825-fold greater than in humans at the mrdd. By surface area extrapolation of dose, mice at the high dose received a dose of drug about 168-fold greater than the human mrdd.

5. **311C90: report of a study to assess 311C90 for unscheduled DNA synthesis using rat livers in vitro/in vivo procedure, study number BDRE/93/0170, The Wellcome Foundation, Kent, UK, April 8, 1994, GLP.**

Animals: Male Wistar Rats ( ), 207-320 grams, 44-58 days old at treatment, 5/sex/group.

Drug: 311C90 batch number Q5

Dosing: Doses were chosen from preliminary acute toxicity study, and were 500, 750 and 1000 mg/kg. Also previous single oral dose toxicology studies in Wistar rats reported 1000-1500 mg/kg as the LD_{50}. Therefore, this was an appropriate high dose.

Study description: Rats received single oral dose of 311C90 at 0, 500, 750 or 1000 mg/kg. Rats were sacrificed 12-14 hours after oral dosing, with a second experiment carried out with an earlier time point (2-4 hours after dosing). Rat livers were perfused with collagenase to prepare primary hepatocyte cultures, cultures treated with (^{3}H)-thymidine to label DNA synthesis, slides prepared from fixed hepatocytes, and examined for non-S-phase (unscheduled) DNA synthesis by autoradiography. Positive controls included dimethylnitrosamine (DMN) and 2-acetamidofluroene (2-AAF), which are known to cause the type of damage that induces unscheduled DNA synthesis.

Pharmacologist's comments: The study design was consistent with the OECD guidelines (1994) for the UDS test.

Results: Results of the UDS test are whoen in sponsor's Table 1 (vol. 49, pg. 29) below:

TABLE 1: Group mean net grain count values

12 hours sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD
0 d.HCl	-1.3	0.3	0	-	-	-
500	-1.2	0.3	5.0	0.0	0.5	0.6
750	-1.4	0.3	5.8	0.2	0.5	0.6
1000	-1.0	0.3	5.8	0.2	0.5	0.6
75 2-AAF	11.8	2.1	12.9	1.6	87.5	7.0

2 hours sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≥ 5)	
	mean	SD	mean	SD	mean	SD

Positive controls responded appropriately. There was no indication of unscheduled DNA synthesis at any dose of 311C90, indicating that there was no genotoxicity associated with the drug in this assay.

Toxicokinetics/drug exposure

After single oral doses of 311C90 of 400 or 1600 mg/kg, plasma concentrations at 4 and 12 hours were 4592 and 3534 ng/ml (400 mg/kg) and 9201 and 10965 ng/ml (1600 mg/kg), respectively. Compared to the plasma C_{max} (20 ng/ml) at the human mrdd (15 mg/kg; plasma level extrapolated from 25 mg/kg oral dose), the animals in this UDS study were exposed to plasma levels of 311C90 parent drug about 225-342-fold greater than at the human mrdd.

Miscellaneous Toxicology Studies

1. **A 14-day oral toxicity study in the Wistar rat of zolmitriptan containing 0.2% (w/w) 420C90 or 0.5% (w/w) 439C90, Glaxo Wellcome R&D, BDPR/95/0040, March 22, 1996, GLP.**

Study purpose: Assess any change in toxicological profile of 311C90 due to presence of a degradation product (420C90) or an isomer (439C90), either of which may occur in final product.

Drug: 311C90 (Batch Q5); 420C90 (Batch WDCL/94/6/184) and 439C90 (Batch WDCL/95/14/53) supplied by N Thacker (ADL). See chemical structures below:

Study description: 311C90 administered orally once daily for 14 days to Wistar rats, (10/sex/group) at dose of 100 or 400 mg/kg/day, containing 0.2% (w/w) 420C90 or 0.5% (w/w) 439C90. Animals were evaluated for clinical symptoms, body weight, food and water consumption, ophthalmological changes, hematology, clinical chemistry, macroscopic and microscopic exam.

Dosing groups were as follows:

Group 1 deionized water
Group 2 100 mg/kg 311C90
Group 3 400 mg/kg 311C90
Group 4 100 mg/kg 311C90 with 0.2% (w/w) 420C90
Group 5 400 mg/kg 311C90 with 0.2% (w/w) 420C90
Group 6 100 mg/kg 311C90 with 0.5% (w/w) 439C90
Group 7 400 mg/kg 311C90 with 0.5% (w/w) 439C90

Results:

Early deaths were as shown in following sponsor's table (vol 15, pg 18):

Group/Sex	Animal number	Termination status	Pre-death clinical signs	Necropsy lung findings
1/F	951568	found dead	respiratory distress	yes
3/M	951521	found dead	NAD	yes
4/M	951522	moribund kill	respiratory distress	yes
4/F	951601	found dead	died immediately post-dose	yes (fluid-filled)
5/F	951604	found dead	died post-dose	yes
6/F	951613	moribund kill	respiratory distress	yes
7/F	951631	found dead	cannibalised	not taken

With exception of Groups 3 and 7 females, apparently all animals exhibited signs of respiratory distress consistent with accidental dosing of the lungs (gavage accident).

No effects on body weight, food or water consumption or ophthalmoscopy.

Hematology: slight decrease in lymphocyte count in all treated male animals (33% decrease in Group 7 males).

Clinical chemistry: Slight decrease plasma urea levels group 3 and 5 males compared to controls; slight increase in treated female groups of alkaline phosphatase. Increase in plasma LDH in Group 2, 3, 4 and 5 females.

Organ weights: no effects.

Macroscopic findings: no treatment-related findings; 5 of 7 animals killed moribund or found dead showed gross changes in lungs, possibly due to gavage accident.

Microscopic findings:

Table of the histopathological incidence of hypertrophy of the thyroid follicular epithelium

Severity of Finding	Control	311C90		311C90 + 420C90		311C90 + 439C90	
		100	400	100	400	100	400
		mg/kg/day		mg/kg/day		mg/kg/day	
Number of Males examined	10	0	1	1	10	0	10
- minimal	7	-	0	0	3	-	6
- slight	2	-	0	0	3	-	2
Number of Females examined	10	10	10	10	10	10	10
- minimal	2	2	1	0	8	0	4

Increased incidence of minimal hypertrophy of the thyroid follicular epithelium in females treated with 400 mg/kg/day 311C90 containing 0.2% (w/w) 420C90 compared to controls (see above sponsor's table, volume 15, pg. 21).

Sponsor states that effects on thyroid can be either direct (interfering with hormone production), or indirect, being secondary to hepatic or renal changes. The sponsor goes on to say that in the absence of more severe microscopic findings and TSH assays, a mechanism for the thyroid change remains unclear.

Pharmacologist's comment: These data are consistent with a minor histopathological change in thyroid (hypertrophy of thyroid follicular epithelium). These data may be consistent with the results of the carcinogenicity studies, in which the sponsor reported an increase in thyroid follicular cell hyperplasia and increased thyroid follicular cell neoplasms in the rat.

Since this thyroid effect in this study occurred mainly in the presence of high dose 311C90 (400 mg/kg/day) plus 420C90 only, data are consistent with a role of this degradation product in the thyroid hypertrophy. However, this is only a single study, and should have been repeated to allow for a more definitive conclusion.

The sponsor carried out an additional toxicology study to examine effects of 311C90 on thyroid function.

2. **311C90: 2-week oral (gavage) toxicity study in the Wistar rat, report #WPT/96/044, study #E95376, Glaxo Wellcome R&D, 10/28/96, GLP.**

Purpose: assess possible changes in toxicological profile of a proposed intranasal formulation of zolmitriptan, using a solution that had been subjected to accelerated degradation process.

Pharmacologist's comment: Due to time constraints I only looked very briefly at this study, as it pertains to an intranasal formulation to be submitted in the future, and not to the formulation utilized in this NDA.

Study Summary: 125 mg/kg/day of either undegraded or degraded intranasal formulation of zolmitriptan was administered to Wistar rats (10/sex/group) by oral gavage for two weeks. A control group received vehicle. The usual observations were made (clinical symptoms, body weight, food consumption, urinalysis, hematology, clinical chemistry, organ weights, macro- and micropathology).

Summary of Results: Treated males gained slightly less weight (7-8% less) than vehicle controls, with both degraded and undegraded drug formulation, with no effects in females. Food consumption was unaffected. Treated males had slightly higher haemoblobin concentrations, RBC counts and packed cell volumes compared to controls, as well as lower potassium concentrations and calcium concentrations (Group 3 males only). Group 2 males produced smaller quantities of urine with higher specific gravity than controls. Treated males also had lower kidney weights than controls. There were no treatment-related macroscopic or microscopic findings.

There were two different degradants detected by HPLC. The first was designated GW320486, at a level of 3.4%. The second was analyzed by capillary electrophoresis with a relative migration time of 0.92, at a level of 2.1%.

Pharmacologist's comments: I only did a cursory review of this study due to time constraints, but there did not appear to be any difference in the toxicological profile between the degraded and undegraded nasal formulation of the drug, when administered orally.

3. **311C90: Assessment of thyroid function in rats, study #TKR/2580, Glaxo Wellcome R & D, August 29, 1996, GLP.**

Study purpose: The rat carcinogenicity study results revealed histopathological findings of thyroid follicular hyperplasia and thyroid neoplasia. The sponsor carried out this study in an attempt to determine the possible mechanism for this effect. There are basically two ways in which a compound can result in thyroid dysfunction, either via a direct effect on the thyroid gland or indirectly through alterations in thyroid hormone clearance by the liver, for example. The purpose of this study was to determine which might be operative in the case of 311C90.

Study Description: The study was set up as shown in the following table:

Group (# animals)	Dose (mg/kg/day)	Purpose
I (10M)	0	clin. path., thyroid hormone measure, histopath., liver enzymes
II (15M)	1000	clin. path., thyroid hormone measure,histopath., liver enzymes
III (20M)	0	thyroxine clearance
IV (30M)	1000	thyroxine clearance
V (28M)	1000	pharmacokinetics

Wistar rats were dosed for 30 days with either 0 or 1000 mg/kg/day by oral gavage; dose chosen to maximize the chances of observing thyroid changes after 1 month of treatment.

Results:

Mortality and clinical symptoms

9 treated animals died prematurely; cause of death unknown. Transient salivation was only clinical symptom reported.

Body weight/food consumption: No effect.

Thyroid hormones: No effect on plasma levels of thyroid stimulating hormone (TSH), thyroxine (T_4) or free T_4. Plasma radioactivity associated with T_4 clearance decreased over the 30 hour period of measurement. Animals treated with zolmitriptan had significantly higher ($p<0.05$; approximately 28%) rate of clearance and significant reduction ($p<0.05$; 15%) in terminal half-life compared to controls (see sponsor's Tables 8 and 9 below; volume 44, pg 36 and 37).

Group : Dose ID8250 (mg/kg/day)			III 0		IV 1000		LSD
	Hour	Sex	N	Mean	N	Mean p	
T4 (cpm/ml)	0.5	M	19	11780	19	10190 **	1160
	1	M	19	10610	19	9310 **	900
	2	M	19	8720	19	7380 ***	710
	4	M	19	6910	19	6020 **	590
	8	M	19	5600	19	4910 **	500
	12	M	19	4710	19	3710 ***	660
	24	M	19	3000	19	2100 ***	388
	30	M	19	2488	18	1700 ***	328

Notes to table:

p = statistical significance of difference from control mean

Significance levels - * p<0.05 ** p<0.01 *** p<0.001

LSD is the Least Significant Difference from control mean

Group : Dose ID8250 (mg/kg/day)		III 0		IV 1000		LSD
	Sex	N	Mean	N	Mean p	
Plasma T4 clearance (ml/min/kg)	M	19	5.35	19	6.84 ***	0.61
Half life (hr)	M	19	14.12	19	11.95 ***	0.87

Notes to table:

p = statistical significance of difference from control mean

Significance levels - * p<0.05 ** p<0.01 *** p<0.001

LSD is the Least Significant Difference from control mean

Liver enzymes

Statistically significant ($p<0.05$) increases in ECOD (ethoxycoumarin O-dealkylase), EROD (ethoxyresorufin O-dealkylase) and PROD (pentoxyresorufin O-dealkylase) were seen with 1000 mg/kg/day 311C90. However, there were no changes in levels of cytochrome p450, testosterone 6ß-hydrolase activity, testosterone 16-ß hydrolase activity, or T_4 UDPGT activity. The sponsor concluded, and I agree, that there was little potency for 311C90 for producing enzyme induction in the rat.

Histopathology

All Group II animals showed mild to moderate thyroid follicular epithelial hypertrophy. One Group II animals showed moderate focal ulceration of the urinary bladder.

PK

At 1000 mg/kg/day oral gavage, on Day 28, AUCs were in the range of 742,000 ng.h/ml. The human AUC at the mrdd (15 mg/day) is about 160 ng.h/ml, so these animals received exposures 4637-fold greater than at the mrdd.

Summary and conclusions: thyroid function study in rat

These animals received 1000 mg/kg/day 311C90, which was 2.5-fold larger dose than given to the animals in the rat carcinogenicity study (400 mg/kg/day). High dose animals (400 mg/kg/day) in the rat carcinogenicity study were exposed to up to 301,811-445,719 ng.h/ml 311C90 (males and females, respectively) by Week 102 of the study. The animals in this thyroid function study receiving 1000 mg/kg/day also received about 2-fold greater exposure to 311C90 than in the carcinogenicity study.

In this study, animals demonstrated thyroid follicular epithelial hypertrophy.

similar to animals in the rat carcinogenicity study. One month is insufficient time to expect the appearance of thyroid neoplasias. In this study, thyroxine (T_4) clearance increased and thyroxine half-life decreased, while levels of TSH and other liver enzymes, including T_4UDPGT remained fairly constant. These data are consistent with the sponsor's conclusion that 311C90 is somehow affecting the thyroid through an indirect mechanism, which may involve an ehnanced thyroxine clearance. That indirect mechanism further does not appear through induction of liver enzymes resulting in an increased biliary T_4 turnover. Therefore, the indirect mechanism remains unclear.

4. 311C90: Telemetry and respiration study with ZD8250 (311C) in the rat, study #TKR/2608 (9/IF/1022914),
September 9, 1996, GLP.

Purpose: The purpose was to study effects of single dosing of 311C90 on blood pressure, ECG, core temperature and activity and respiration rate in conscious rat to examine the possible causes of early deaths ("sudden deaths") observed in a number of toxicological studies in this species.

Study description:

12 male Wistar rats (343-441 g) surgically implanted with telemetry transmitters to measure aortic blood pressure, lead II ECG, heart rate, core temperature and locomotor activity. 2-3 weeks later rats were given i.v. injection of 0.9% saline w/v sterile saline (1ml/kg) via tail vein, and transferred to whole body plethysmography chamber for 1 h to monitor respiration. 24 hours later the procedure was repeated, with rats receiving i.v. injection of zolmitriptan, 100 mg/kg, infused via tail vein over 15-60 seconds (see below for treatment groups). The experiment was considered to be done in two phases, 0-60 minutes and 2-16 hours.

Group	Treatment
A	Saline vehicle control: n=12
B	Zolmitriptan 100 mg/kg i.v., injected over 60 s or longer; n=5
C	Zolmitriptan 100 mg/kg i.v., injected over 45 s; n=4
C	Zolmitriptan 100 mg/kg i.v., injected over ≤45 s; n=3 (1 over 15 s, 1 over 30 s and 1 over 45 s

The second phase (2-16 h) comprised 2 treatment groups: group A (as above) and group E (groups B and C pooled: n=9).

Results:

Pharmacologist's comment: For purposes of this NDA, the information regarding probable cause of death is the most important. The NDA stipulates the oral route, while this rat study included i.v. administration. Therefore, actual duration of i.v. administration is not relevant to the NDA. Plasma levels may be important, and will be mentioned in my review.

Cause of death

Brief convulsions occurred in 10 of 12 treated rats, lasting a few seconds at end of the injection period (100 mg/kg, i.v.). Death occurred as follows: one rat dosed over 15 s, one rat dosed over 30 s, one rat doses over 45 s. These rats stopped breathing either during or at end of the injection, and respiration did not return. Respiratory arrest was accompanied by loss of consciousness, hypotension, and bradycardia. The sponsor states that the cause of death was respiratory arrest, as the hearts of these animals apparently continued to beat for several minutes after respiratory arrest. Apparently two rats dosed over 45 s also stopped breathing, blood pressure and heart rate fell, but breathing restarted spontaneously and both rats recovered fully within a few minutes. None of the 5 rats dosed over 60 s experienced apnea or loss of consciousness. Therefore, the sponsor concluded that death due to respiratory arrest only occurred at 100 mg/kg, i.v. when duration of the infusion was 45 s or less.

The mechanism for the respiratory arrest is unclear. This could occur through effects of the drug on the respiratory center of the CNS or on "J-receptors" (C-fiber afferents located in the pulmonary capillary beds and activated by a number of chemical irritants and agents, including 5-HT).

Symptoms in surviving rats

Prolonged tachycardia: (from 30 min to 4 hours post-dose) that was maximal 2 hours post-dose (Controls H.R. 346 bpm; Group E animals H.R. 487 bpm; significantly different $p<0.01$).

Mild hypotension: two states, 1-30 min post-dose and 2-4 hours post-dose; maximal at 15 min post-dose; Controls MBP 110.5 mmHg, group B MBP 95 mmHg, group C MBP 90 mmHg; $p<0.01$.

Lowered respiratory rate:

- 5 minutes post-dose: group A controls resp. rate 217 breaths/min; group B 158 ($p<0.05$).
- 15 min post-dose: group A controls 187 breaths/min; group C 151 ($p<0.05$).

ECG changes: small, reversible change in PR interval, QTS interval, QT intervals.

Core temperature: significantly lower than the controls at 1, 4 and 8 h after dosing; greatest difference 60 min after dosing (group A controls Tc 37.9

Exposure to 311C90

Sponsor predicted the plasma concentration one would expect in Wistar rats receiving 100 mg/kg i.v. bolus by assuming all the drug entered the plasma at once (C_{p0}), with rat plasma volume of 40ml/kg. This would result in 100 mg/kg dose divided by 40 ml/kg plasma volume, to give a predicted plasma concentration of about 2.5 mg/ml, or about 2500 µg/ml, or aboit 2500000 ng/ml. The predicted plasma C_{max} in humans at the mrdd (15 ml) is about 20 ng/ml, and therefore one could predict that these rats receiving 100 mg/kg i.v. should experience plasma levels about 125,000-fold greater than in patients receiving the mrdd. Therefore, these data would indicate that there is little cause for worry with respect to respiratory arrest in patients receiving the oral mrdd for this drug.

The sponsor also pointed out that in real terms, the plasma C_{max} for these rats (100 mg/kg, i.v.) would probably constitute a range of plasma levels dependent on the weight of the animal and the cardiac output (10-80 ml/min/100 g body weight for the rat). Using this figure, the sponsor calculated that plasma C_{max} in these rats probably ranged from 1.3-16.7 mg/ml, depending on the weight of the rat.

If one compares a dose of 100 mg/kg to the rat in terms of a human dose using surface area comparison (mg/m^2), this would be equivalent to a human dose of about 14.3 mg/kg. This is about 57-fold greater than the human dose at the mrdd (15 mg or 0.25 mg/kg for a 60 kg patient).

SUMMARY AND EVALUATION

Pharmacology

Mechanism of Action: Receptor Binding and Functional Assays

311C90 is a 5-HT_{1D} receptor agonist, with high affinity for 1D∝ (pKi 8.7) and 1Dß (pKi 9.2) receptors in particular. However, the drug also has reasonably high affinity for the 5-HT_{1A} receptor (pKi 7.0), which is thought to mediate CNS side effects, among others. 311C90 (p[A_{50}] 6.8) has a somewhat higher potency than sumatriptan (p[A_{50}] 6.5) in the rabbit saphenous vein contraction assay. It is 2-fold more potent than sumatriptan at reducing carotid arterial blood flow in the anesthetized dog and 5-fold more potent than sumatriptan at reducing intra-cranial AVA blood flow in the anesthetized cat. Finally, it is more potent than sumatriptan at inhibiting neurally-evoked plasma protein extravasation into the dura mater of anesthetized guinea pigs.

Overall, the scientific rationale for use of this drug for treatment of migraine is sound, and based on the nonclinical pharmacology data one might predict zolmitriptan to be at least 2-fold more potent than sumatriptan for the treatment of migraine on a dose-to-dose basis.

Safety Pharmacology

With respect to safety pharmacology, 311C90 appears to result in cardiovascular side effects (an increase in blood pressure and heart rate), also with a 2-3-fold increase in potency over sumatriptan (conscious dog). There appear to be no effects on coronary blood flow in animals. However, the drug did cause a dose-related contraction of human coronary artery *in vitro*. This may be of some concern, as coronary vasospasm is one of the undesirable side effects of sumatriptan. There were no effects on blood flow to subregions of the brain or pulmonary vascular conductance. There did appear to be some decrease in renal blood flow, as well as ocular, splanchnic and stomach vascular conductance. Other effects included CNS and autonomic effects, as well as an inhibition of respiration (mainly in conscious rat and anesthetized dog and cat).

Overall, any potential advantage of the drug gained by an increase in potency with respect to efficacy would appear to be offset by a similar increase in potency with respect to undesirable cardiovascular side effects.

183C91 Active Metabolite: Pharmacology and Safety Pharmacology

183C91 metabolite has similar pharmacological activity to parent 311C90, but is more potent in a dose-to-dose comparison. It was reported to be 2-fold more potent than parent drug in the rabbit saphenous vein assay and in mediating a transient reduction in renal blood flow in the anesthetized dog and 6-fold more potent at reducing carotid arterial blood flow in the anesthetized dog. 183C91 also demonstrates a slighly higher binding affinity at 5-$HT_{1D∝}$ (p[A_{50}] 8.0) and 5-$HT_{1Dß}$ (p[A_{50}] 10.8) receptors than parent zolmitriptan, with a somewhat lower affinity to "5HT-1_D-like"-receptors and 5-HT_{1A} receptors (p[A_{50}] 6.8) similar to zolmitriptan. Finally, 183C91 mediates a similar

and has little effect on coronary blood flow. Therefore, it is important that the sponsor included levels of this metabolite in their carcinogenicity, reproductive toxicology, and other toxicology studies.

Overall, the sponsor has met the requirements for examination of the pharmacology and safety pharmacology of 311C90, and resultant data support the approval of this drug for treatment of migraine.

ADME

Absorption

PK studies showed that zolmitriptan was rapidly absorbed by the oral route of administration, with T_{max} of 0.25, 3, 0.75, and 0.5 h in mouse, rat, rabbit and dog, respectively. This compares to a T_{max} in humans of 2-6 h by the oral route.

The $T_{1/2}$ for zolmitriptan was fairly consistent for the animal species studied as well as for man. $T_{1/2}$s were 1.3, 1.3, 2.0 and about 2.0 hours for mouse, rat, rabbit and dog, respectively. The $T_{1/2}$ in humans was slightly longer, at about 2.5-3.0 h.

Oral bioavailability varied somewhat, with bioavailability in the mouse, rat, rabbit and dog reported as 50%, about 40%, 25%, and about 75%, respectively. Bioavailability by the oral route in humans is reported to be about 40%.

Similar metabolites were found in plasma for all animal species and humans, although the ratio of the various metabolites to parent drug varied considerably with species. The major metabolites found in all animal species studied as well as in human were 183C91 (a desmethyl metabolite; pharmacologically active), 2161W92 (an indole acetic acid), and 1652W92 (an N-oxide). These metabolites were found at fairly high plasma levels in humans, and were therefore measured in most of the animal studies (See "Metabolism" section of this review for further discussion).

Toxicokinetics data for the various animal species studied revealed some evidence of parent drug (zolmitriptan) accumulation with time and some differences in exposure levels in male and female animals. In the mouse, plasma exposure (AUC) levels remained fairly constant over time in the males, while in females (40 mg/kg) AUCs increased from about 62,000 ng.h/ml on Week 6 to about 105, 000 ng.h/ml by Week 74. In the rat, exposure levels were generally linear with dose, but increased (2-3-fold) with time up to 52 Weeks in both males and females. Levels subsequently dropped again on weeks 78 and 104. One explanation for an accumulation of drug with time is a saturation of the pathway of elimination. There was no apparent accumulation of drug with time in the dog when drug was administered by the oral route for up to 1 year. Apparently in humans the PK of zolmitriptan was linear following single doses from 2.5 to 10 mg (0.04-0.17 mg/kg for 60 kg patient) and dose proportional with oral doses of 2.5 to 50 mg (0.04-0.83 mg/kg). The sponsor reported that there was no accumulation of drug following multiple doses of zolmitriptan.

With respect to gender differences, in mice the female animals demonstrated a higher exposure level than males at the 10 mg/kg dose (848 ng.h/ml female; 586 ng.h/ml males). AUCs in female mice also increased with time up to Week 74 (40

in rats or dogs. The sponsor reported that humans were similar to mice in that young females experienced plasma concentrations approximately double those of males with resulting greater Cmax and AUC values for females. Apparently females also excreted a greater proportion of the dose unchanged in the urine.

The sponsor did an adequate job of examining the oral absorption of zolmitriptan. The PK for this drug is fairly well adapted for a migraine drug, in that it is rapidly absorbed by the oral route and is rapidly eliminated. The most important PK findings reported with respect to any potential problems with drug administration are 1) the potential for accumulation of drug with repeat exposure (although this is unlikely with the chronic intermittent exposure that is more likely in migraine patients) and 2) the higher exposure levels in females, especially in light of the fact that a fairly high proportion of migraine patients are female.

Plasma Protein Binding

Plasma protein binding should not be a problem with zolmitriptan, as the drug binds (*in vitro* studies) only at low levels (ranging from 11-27% in animal species), including about 18-26% in humans.

Tissue Distribution

The sponsor carried out the appropriate tissue distribution studies in rats. A study in male albino and pigmented rats demonstrated that a slight amount of radioactive material (drug or metabolites) crossed the blood/brain barrier at 2 hours after oral dosing. In this same study, high levels of radioactivity were still seen in the eyes of pigmented animals, indication that 311C90 and/or its metabolites probably bind to melanin. In an additional study in pigmented Lister Hooded rats, 168 h post-dose drug (radioactivity) levels above background were found in the eye (75% retention), thyroid, liver and testes (only 1-2% retention). Small amounts of drug reached the brain at 4 h only. In addition to the eye, high levels of radioactivity were retained in the skin up to 72 hours, suggesting that the drug may be associated with melanin.

Drug was secreted into the milk of lactating female Wistar rats dosed orally with radiolabelled zolmitriptan. Milk levels of radioactivity were equivalent to those in plasma at 1 hour and 4-fold higher than in plasma at 4 hours. Radiolabelled drug showed general distribution in pregnant Dutch rabbits, with highest levels in liver and minimal levels in the CNS. Low levels of drug were still detectable in liver, kidney and maternal eye at 24 h. At 2 and 6 h after dosing, low levels of radioactivity were found in the fetuses, evenly distributed throughout the fetal tissue. At 24 h, low levels of radioactivity were found in placenta and intestinal contents of fetus only.

Metabolism

The same three major metabolites were found in plasma of mice, rats, dogs and man; 2161W92 (indole acetic acid), 183C91 (desmethyl metabolite, active metabolite), and 1652W92 (N-oxide). Chemical structures are found in sponsor's figure 2, page 22

Following is a summary of the plasma metabolite concentration findings for each animal species examined as well as for humans, with metabolites arranged in descending order according to plasma exposure levels:

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

In humans, 2161W92 plasma levels were similar to those of parent drug, while 183C91 and 1652W92 were present in levels about 50% of parent. In mouse, all three metabolites were found in plasma at concentrations about 5-10% of parent. In rat, 2161W92 and 1652W92 were found in levels about 5-10% of parent, while 183C91 was present at levels of 2-5% that of parent. In rabbit, 1652W92 was present at levels up to 3 times the parent level, 21671W92 at levels simlare to parent and 183C91 at low levels only. And finally in dog, 1652W92 was found at about 30-50% of parent drug levels, with 2161W91 and 183C91 being reported at 20% and 5%, respectively , of parent drug.

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. The known active metabolite, 183C91, was the major metabolite found at the highest level in mouse plasma, while it was at the lowest level of the major metabolites in the other animal species. It was found at intermediate levels in humans.

In *in vitro* studies using rat and human liver enzymes, only very low levels of metabolites were produced, and therefore the specific enzymes responsible for metabolism of zolmitriptan have not been determined. The major urinary metabolite of zolmitriptan, 2161W92, is structurally similar to a major metabolite of sumatriptan, and sumatriptan metabolism is known to be mediated through the MAO (monoamine oxygenase) system. Therefore, a study of possible interaction of zolmitriptan with the MAO system was completed, and no interactions were found between the drug and MAO-A or MAO-B.

With respect to possible drug interactions, zolmitriptan did not appear to induce liver enzymes and had no effect on barbiturate sleep times in rats or mice. Apparently zolmitriptan is a weak inhibitor of isozyme $2D_6$ amd $1A_2$, but only at concentrations well

The sponsor did a good job of examining the levels of the major metabolites in the various animal species used in the toxicology studies.

Excretion

Following oral dosing, the major excretory route was fecal in mice and rats and urinary in rabbits and dogs. In humans, the majority of an oral dose was found in the urine.

Toxicology

Acute Toxicology Studies

The acute toxicology studies were reviewed in the original submission of IND , reviewed June 10, 1994 and attached to this NDA review.

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.

The sponsor did an adequate job of evaluating the acute toxicological aspects of this drug.

Subchronic Toxicology Studies (28-day)

Two subchronic toxicology studies (rat and dog) were reviewed in the original IND submission (IND reviewed June 10, 1994; attached to this NDA review). Following is a summary of results of those studies.

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-fold greater than AUC at maximum daily recommended human oral dose).

Chronic Toxicology Studies

The chronic toxicology studies included a 26-week rat (Wistar) study, a 28-week dog (Beagle) study and a 1-year dog (Beagle) study, all using the oral route of administration.

26-week rat study

In the 26-week rat study, the main effect of note was the large number of unscheduled deaths at the high dose of 400 mg/kg/day (10 of 30 males; 13 of 30

animals was due to dosing or bleeding procedures. 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 (high dose). Of some concern was the fact that these deaths occurred without any apparent preemptory signs.

Other effects included some scattered evidence of effects on kidney, including increased creatinine levels (High dose males), plasma urea (males, 100 mg/kg/day) and urine volume, but these effects occurred only in males. There was also an 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 the "interim sacrifice", "final sacrifice" or "unscheduled death" animals. This increase in thyroid hyperplasia is consistent with the findings in the rat carcinogenicity study, in which thyroid follicular cell hyperplasia and rat thyroid follicular cell adenoma and carcinoma were reported.

With respect to safety margins in the context of the mrdd (maximum recommended daily dose) in humans, if one accepts the sponsor's NOEL of 100 mg/kg/day for this study, there would be an 880-1215-fold margin of safety with respect to the human AUC (160 ng.h/ml). I would take a more conservative approach to these results, and designate the 25 mg/kg/day dose as the NOEL. However, even at this lower dose, data still support a margin of safety of about 242-353-fold greater than the AUC reported for humans at the mrdd.

Toxicokinetics data revealed that exposure to 311C90 parent drug (zolmitriptan) increased 6-10-fold with time, while exposure (AUC) to the metabolites 183C91, 1632W92 and 2161W92 also increased with time, but at a slower rate than the parent. Therefore, there was an overall increase in the zolmitriptan:metabolite ratio for all three metabolites over time, which could be interpreted to indicate a saturation of the metabolic pathways.

28-week and 1-year Dog Studies

With respect to mortality, a single high dose male (100 mg/kg/day) was found dead in the 28-week study, but death was not related to drug administration (surgery to repair strangulated hernia). A single animal (25 mg/kg/day) was also killed moribund after a number of convulsions, but none of the high dose (100 mg/kg/day) animals were reported to convulse. In the 1-year dog study, a single high dose (100 mg/kg/day) male was found dead on study day 275, with no cause of death determined.

Clinical symptoms in the 28-day study were consistent with exaggerated pharmacological effects and included pupillary dilation, vasodilation, trembling, and difficulty awakening at all treatment groups, with a dose-dependent pattern. The majority of these symptoms disappeared over time. In the 1-year dog study, clinical symptoms appeared to be consistent with increased sympathetic outflow (through $5HT_{1A}$ receptor?) and included body tremors, aggressive behavior, and convulsions.

With respect to convulsions, as previously stated, a single male animal in the 28-

clonic convulsions occurred in 3 of 12 high dose (100 mg/kg/day) animals. The NOEL for these convulsions was about 25 mg/kg/day (average AUC 27,180 ng.h/ml for 311C90 parent drug), giving a margin of safety (AUC) compared to mrdd in humans (AUC 160 ng.h/ml) of about 170-fold, which is certainly an adequate margin of safety for administration to humans.

Histopathology evaluation of animals in the 28-day study 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. In the 1-year study, the decrease in monocyte and neutrophil numbers during treatment, followed by increased in the recovery animals, was consistent with some sort of inflammatory response. In that study, histopathology data also revealed a number of seemingly random sites of "focal inflammation" and "mononuclear cell infiltration" in a number of different organs and tissues. Since serotonin is known to activate monocytes and act as a chemoattractant to this immune cell population, it is feasible that these inflammatory sites might be due to the presence of zolmitriptan and might correspond to the tissue distribution of the drug. Unfortunately, results of these animal studies do not provide sufficient information to allow any conclusions in this regard as the experiments were not designed to answer examine any correlation between tissue distribution of parent drug or metabolites and local inflammatory lesions. Furthermore, a number of these inflammatory lesions were also seen in control animals, although when this occurred the incidence and severity of the lesions increased in the treated animals.

Clinical chemistry results in the 28-day study showed that SGPT levels increased at the high dose (100 mg/kg/day) at the 4-week recovery period. SGPT and ALP levels also increased in the high dose animals in the 1-year study. These data are consistent with a seemingly minor effect on the liver. There were no histopathological sequelae associated with these liver enzyme effects.

With respect to cardiovascular effects, heart rates increased about 5-12% at the high dose on Day 28 of the 28-week study, but heart rates decreased by the 4-week recovery period. The sponsor concluded that there were no effects seen on the cardiovascualar system in the 1-year dog study, but no ECG tracings, heart rate or blood pressure data were included for this study in the submission.

Toxicokinetics data in the 28-week dog study revealed that parent 311C90 plasma exposure increased in a supra-proportional dose-dependent manner, especially at the 100 mg/kg/day dose. This could be due, in part, to a saturation of metabolic pathways for 183C91 and 1652W92 metabolites. The active metabolite 183C91 occurred at AUCs in the range of 6-10% of the parent drug, whereas in humans this metabolite occurs at about 50% of the parent drug level. In the 1-year dog study, there was no apparent time-dependent increase in 311C90 parent drug exposure. There was also no differences in AUCs with respect to animal gender. With respect to metabolites, in this study the exposure to 1652W92 appeared to increase at a greater than dose-proportionality. The order of exposure to metabolites included, in descending order, 1652W92>2161W92>183C91.

mg/kg/day, based on the premise that there were no toxicities found. Their contention was that all the drug-related effects observed were exaggerated pharmacolgical effects of zolmitriptan. I would take a more conservative approach, and set the NOEL at about 25 mg/kg/day based on effects on GPT levels and APTT levels. However, this still leaves a margin of safety of about 141-fold by AUC compared to the mrdd in humans, which is certainly an adequate margin of safety for administration to humans. In the 1-year dog study, the NOEL (based on clinical symptoms of body tremor, aggressive behavior) was 5 mg/kg/day, which gives about a 9-fold safety margin by AUC over the mrdd. This is still an adequate margin of safety, considering that the clinical symptoms (with the exception of convulsions) were not very serious and disappeared with time.

With respect to convulsions, the NOEL in the 1-year dog study was about 25 mg/kg/day, which gives a margin of safety (AUCs) of about 170-fold over the mrdd in humans. This is also a satisfactory margin of safety.

Adequacy of the Nonclinical Toxicology Program for Zolmitriptan

With respect to general toxicology studies, the requirements for an NDA for a chronic, intermittent administration of a drug such as proposed for zolmitriptan include a 6-month rodent and 1-year non-rodent study, in which drug is administered by the same route of administration as proposed in the clinic. The sponsor has satisfactorily completed such toxicology studies for zolmitriptan. Furthermore, while the results of these studies do indicate a number of findings of some concern, including induction of convulsions and the occurrence of a number of inflammatory lesions, the margins of safety with respect to the various findings are quite adequate to support the administration of the drug to humans.

Carcinogenicity Studies

Mouse Carcinogenicity Study

A sufficient number of animals (60/sex/group) were included in the 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 previously described in this review (Mouse Carcinogenicity Study section). 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. In this cat study, the effect on respiration appeared to be due to a central effect rather than to constriction of the smooth muscles of the airways.

While the sponsor concluded that there were no notable toxic effects of the drug

lesions) in these early decedents included effects in kidney (aggregates of mononuclear cells), heart (myocardial degeneration and fibrosis), adrenals (adrenal hyperplasia) and uterus (cystic endometrial hyperplasia). See page 79 of this review for incidences. There were no dose-related neoplastic lesions found in any of the animals in this study.

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 opinion that the high dose (400-600 mg/kg/day range) exceeded the MTD based on excessive mortality at this dose. Therefore, this is probably an appropriate high dose. The high dose also gave an AUC ratio of mouse:human of about 460:1, which would comply with the 25:1 ratio that can also be used to determine appropriate dosing in a carcinogenicity study. However, this precept only works if the drug is not genotoxic, and zolmitriptan tested positive in the human lymphocyte assay as well as the Ames test. The low dose (6 mg/kg/day) is about 5-10-fold higher than we would prefer, based on the precept that exposure levels at the low dose in a carcinogenicity study should approximate the levels found in humans at the mrdd.

Overall, the mouse carcinogenicity study was carried out appropriately with respect to the number of animals, study protocol, and choice of dose (although the sponsor chose not to submit their carcinogenicity study protocols to the Agency for review and concurrence by the Carcinogenicity Assessment Committee). While there were a number of non-neoplastic lesions associated with administration of zolmitriptan, 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.

Rat Carcinogenicity Study

As with the mouse study, the rat carcinogenicity study also included an adequate number of animals (60/sex/group). Animals received 5, 25, 100 or 400 mg/kg/day drug. As with the mouse study, rat carcinogenicity study results also demonstrated excessive mortality at the high dose (400 mg/kg/day; 58.3% mortality in males; 51.6% of females; see pg. 90-91 of this review). There was some increase in liver enzymes (see page 95 of this review) in the high dose animals, but there was no effect on TSH (thyroid stimulating hormone). There were also notable histopathological findings in the rat, including lesion in the thyroid (follicular cell hyperplasia), lungs (edema) and thymus (acute thymic hemorrhage). For the most part these effects appeared to be dose-related in incidence (see page 96 of this review for details). There was also a dose-related increase in a neoplastic lesion, that being rat thyroid follicular cell adenoma and carcinoma. Those results are summarized in the following sponsor's Table:

Incidence of rat thyroid follicular cell neoplasms

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

These data demonstrate a significant ($p<0.05$) increase in the incidence of thyroid follicular cell neoplasms in 400 mg/kg males. According to the sponsor, statistical significance was attained both for adenoma alone and by combining the adenoma and adenocarcinomas, while the incidence of carcinomas alone was actually about the same in treated and control animals.

The sponsor stated that the mechanism for this increase in neoplasms may be due to an increased rate of clearance of thyroid hormones causing trophic feedback. As evidence they cite an increase in thyroid weight at 1000 mg/kg/day 311C90 in a 1-month oral rat study and a slight increase in the incidence of thyroid follicular hyperplasia together with an increase in thyroid and liver weights at 400 mg/kg/day in a 6-month rat study.

Apparently such thyroid neoplasms are thought to often be the result of overstimulation of the thyroid. However, results of this study did not demonstrate any clear increase in TSH in treated animals, indicating that this is probably not the mechanism involved in this case. The sponsor contends that the absence of a clear effect on TSH in their opinion does not rule out the possibility of mild stimulation of the thyroid as a possible mechanism.

The NOEL for this study (based on statistical significance) is 100 mg/kg/day, which gave an AUC for parent drug of 65-80,000 ng.h/ml, on the order of 406-500-fold greater than the AUC at the mrdd. The AUC for parent drug at the high dose (400 mg/kg/day) was about 300,000 ng.h/ml, about 1875-fold greater than the AUC at the mrdd in patients. The sponsor concluded, based on this information, that these tumors were not relevant to the clinic. However, no evidence is provided to establish the fact that it is the parent drug that is responsible for formation of these adenomas and carcinomas. In fact, in this rat carcinogenicity study, metabolites 183C91, 1652W92

parent drug levels at the high dose (average at 52 weeks of the study; 400 mg/kg/day).

Toxicokinetics data in the rat revealed that exposure levels of 311C90 parent drug and metabolites increased fairly dramatically with time up until about Week 52 of the study. From week 52 on, levels decreased somewhat and then satabilized (see pg. 99 of this review for more details). Data were consistent with a saturation of metabolic pathways for 183C91 and 1652W92 metabolites over time.

With respect to the chosen dosing range for the rat study, the data for excessive mortality (see Table below) indicate that 400 mg/kg/day is somewhat above the MTD, and therefore, support the use of this as the high dose in the study. The low dose of 5 mg/kg/day was probably about 10-fold higher than would have been desirable in order to show relevance to the AUC in patients at the mrdd.

Mortality data for rat carcinogenicity study expressed as per cent are shown in the following table:

% Mortality

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

Reproductive Toxicology

Reproduction/Fertility Study in Rats

The study design was consistent with the ICH guidelines ("Detection of toxicity to reproduction for medicinal products") for a reproduction/fertility study and an adequate number of animals were utilized. The sponsor used the same doses for this study as they used in the Wistar rat subchronic toxicology studies (25, 100, and 400 mg/kg/day; daily oral gavage), and in which they saw some mortality (3 of 15 animals) at the high dose.

While there was no effect on body weight gain in the females, male body weight gain at the high dose decreased 12-64% compared to controls at various time intervals during this study. The overall decrease in body weight gain for the males was about 6% at the high dose. These data could be interpreted to support an MTD, which might further support the use of 400 mg/kg as the high dose in this reproductive toxicology study.

reported in 28-day and 26-week oral gavage studies in Wistar rats would predict that these animals in the reproduction/fertility study experienced exposure levels (AUC) of parent drug on the order of 1250- to 2500-fold higher at the high dose (400 mg/kg/day) than humans at the mrdd, and about 20-30-fold greater (at a minimum) at the low dose (25 mg/kg/day).

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

Oral Teratology Studies

Rats

The study design was consistent with the ICH guidelines for a teratology study. A sufficient number of animals (30/sex/group) were included in the study. Animals received 0, 100, 400 or 1200 mg/kg/day zolmitriptan (oral gavage), a single dose per day for Days 6-15 of gestation. Plasma levels of parent drug and metabolites were determined, and in descending order according to AUC were as follows: 311C90>2161W92>1652W92>183C91. 2161W92, 1652W92 and 183C91 were 9-14-fold, 30-fold, and 19-29-fold lower, respectively, than zolmitriptan parent drug.

Mean body weights were decreased (6% versus controls overall) at the high dose, indicating the possibility of maternal toxicity at this dose (this translates into a decrease in body weight gain at the high dose (1200 mg/kg/day; Days 6-15) of about 20.8%). There was also a decrease in mean body weight gain on Days 9-12 (30%) and Days 6-15 (21%) that were statistically significant ($p<0.05$). Toxicokinetics data indicated a time-dependent increase in 311C90 parent drug as well as at least two of the metabolites (1652W92 and 183C91).

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

New Zealand White Rabbit: 1st Study

The study design was consistent with the ICH guidelines for a teratology study, with female animals (20/sex/group) receiving 10, 30 or 100 mg/kg/day zolmitriptan as a single oral dose per day for days 6-18 of gestation. In this study there were excessive maternal deaths (12/20) at the high dose (100 mg/kg/day) in addition to a dramatic decrease in the number of litters with live fetuses (2 versus 11 in controls). There was also a decrease in maternal body weight gain during gestation days 6-18 as shown below:

Mean maternal body weight changes (grams) during gestation

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

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

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

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

New Zealand White Rabbits: 2nd Study

The study consisted of 20 female rabbits/sex/group, receiving 0, 10, or 30 mg/kg/day zolmitriptan by daily oral gavage during Days 6-18 of gestation. Study design was again consistent with the ICH guidelines for a teratology study. Clinical signs included ataxia and labored breathing, mainly in high dose animals. Mean body weight changes were less in mid-and high-dose pregnant females during various time intervals, supporting an MTD somewhere between 10 and 30 mg/kg/day (only the decreased body weight gain at the high dose was statistically significantly different ($p<0.05$) from controls). These data indicated that 30 mg/kg/day may have been a little too high, and 10 mg/kg/day ay have been sufficiently high for rabbits for this study with respect to an MTD based on decreased body weight gain. A dose of 10 mg/kg/day gave AUC of about 8-fold higher than in humans at the mrdd, and 30 mg/kg/day gave AUCs about 31-fold greater.

With respect to fetal effects, the number of early resorptions (and thus implantation losses) increased at the mid- and high-doses. However, the majority of these early resorptions (9/12 mid dose; 8/11 high dose) occurred in a single pregnant female. This effect could be explained by maternal toxicity in the high dose female, as her body weight was considerably less than the mean weight of either control or other high dose animals. However, this was not the case with the mid-dose animal. The NOEL for early resorptions was 3 mg/kg/day, which gave an AUC of about 100 ng.h/ml for 311C90 parent drug. This in the same range of exposure as in humans at the mrdd (160 ng.h/ml).

There were also a number of fetal skeletal abnormalities, but only at the high dose (30 mg/kg/day). The sponsor pointed out that 3 of these skeletal abnormalities were from a single high dose doe, and that there was considerable (90%) and [redacted]

the high dose group. The sponsor pointed out that these data suggest the fetal skeletal abnormalities could be due to maternal toxicity. I concur, with this, especially since the overall body weight data suggest that the MTD for this study is probably 10 mg/kg/day (base on decreased body weight change), and the 30 mg/kg/day dose may be well above MTD.

There was also a trend toward in increase in the incidence of fetal major vessel variation and irregular ossification pattern of the rib(s) (see Table 7, pg. 124 of this review), although the sponsor pointed out that this trend was not statistically significant. The NOEL for skeletal abnormalities, major vessel variations and irregular ossification pattern of ribs is 10 mg/kg/day, which gave an AUC for zolmitriptan parent drug of about 1300 ng.h/ml, which is about 8-fold greater than in humans at the mrdd.

Toxicokinetics studies revealed that AUCs for parent drug and two metabolites (1652W92 and 183C91) increased proportionally with dose on Day 18 of gestation. Metabolite 2161W92 appeared to increase in a supra-proportional manner when the dose was increased from 10 to 30 mg/kg/day.

Overall, the only effect of major concern in this study is the early fetal resorptions, with a NOEL of 3 mg/kg/day, and at the high dose these could be at least partially explained by maternal toxicity (decreased body weights in pregnant animals). However, at the mid dose (10 mg/kg/day), while the majority of the early resorptions (8 of 11) did occur in a single pregnant female, the effect does not appear to be explainable by maternal toxicity.

Teratology Results Summary

Mean body weight change was decreased at the high dose (rats 1200 mg/kg/day; rabbits 30 mg/kg/day), indicating the possibility of drug-related maternal toxicity. Clinical signs included post-dose salivation in rats and ataxia and labored breathing in rabbits. Fetal effects included early fetal resorptions in both rat and rabbit. The NOEL for this effect in rats may have been about 400 mg/kg/day (based on mean number early resorptions per litter). 400 mg/kg/day gave an AUC for zolmitriptan of about 114,000 ng.h/ml, giving a safety margin of about 712-fold greater than in humans at the mrdd. Furthermore, based on the decrease in body weight gain seen in the high dose (1200 mg/kg/day) rats, this effect could be due to maternal toxicity. The NOEL for increased fetal resorptions in rabbits was about 3 mg/kg/day, which gave an AUC (100 ng.h/ml) for zolmitriptan in the same range as that for humans at the mrdd, thus indicating no margin of safety for the rabbit data.

Zolmitriptan administration also resulted in fetal skeletal abnormalities, increased incidence of fetal major vessel variation, and irregular ossification pattern of the rib(s) in rabbits at the high dose. Again, based on pregnant female body weight changes, these effects may be associated with maternal toxicity. The NOEL for these effects in rabbits was 10 mg/kg/day, with an AUC for zolmitriptan of about 1300 ng.h/ml, a safety margin of about 8-fold greater than in humans at the mrdd.

Teratology Results Evaluation

The effect of greatest concern in these reproductive toxicology studies is the increase in fetal resorptions that occurred in both rats and rabbits. The sponsor is of the opinion that, since the majority of the early resorptions at the intermediate and high doses occurred in the rabbit in individual pregnant does (9 of 12 from single mid-dose doe; 8 of 11 from single high-dose doe), that this effect was not drug-related. Furthermore, they were of the opinion that the early resorptions at the high dose in both rats and rabbits could be explained by maternal toxicity. Additionally, the NOEL in the rat was 400 mg/kg/day, with a safety margin of about 712-fold over the human mrdd, which they considered to be an adequate safety margin. Overall, they concluded that early resorptions in both species were not drug-related and not of concern with respect to drug toxicity.

In my opinion, it is not a good idea to entirely ignore these findings involving early fetal resorptions. Based on the fact that there was a decrease in body weight gain of about 20% in the high dose pregnant female rats (mean for Days 6-15) and about 90% in the high dose pregnant female rabbits (mean for Days 6-18), I agree that these data are consistent with considerable maternal toxicity in the high dose animals. However, it is difficult to completely ignore a given result of animal toxicology studies that occurs in two different species (rat and rabbit in this case). Furthermore, in the rabbit study, while the medium dose rabbits (10 mg/kg/day) demonstrated a mean decrease in body weight gain compared to control animals, the individual pregnant female demonstrating the majority of the early resorptions (8 of 11 total) had a body weight greater than the mean control value. Therefore those data did not support the concept of maternal toxicity. These results would tend to indicate that early resorptions can occur with drug administration in pregnant females for reasons other than maternal toxicity. At the NOEL for early fetal resorptions in the pregnant rabbits (3 mg/kg/day), the AUCs were on the order of 100 ng.h/h, which is in the same range as AUCs in patients receiving the mrdd (160 ng.h/ml). At the 10 mg/kg dose (at which increased fetal resorptions occurred), the AUC levels were about 1,300 ng.h/ml for parent drug, which is about 8-fold greater than at the mrdd in patients. (In rats, at the NOEL the data indicated a safety margin of 712-fold (based on AUC) over patients at the mrdd).

Finally, zolmitriptan is a drug for the treatment of migraine, and as such a major patient population would consist of women of childbearing age. The sponsor's draft labelling lists the drug as "category B", which indicates that there were no adverse findings in the animal reproductive toxicology studies. It is my opinion that the data for early resorptions in animals creates enough of a doubt as to the complete safety of zolmitriptan administration to women of childbearing age that we should require that these results be placed in the labelling. Furthermore, it is my opinion that based on these results the drug should be labelled "category C" rather than "category B" as per the sponsor's draft labelling submitted to the NDA.

Pre- and Postnatal Reprotoxicology Studies in the Rat

The study design was consistent with the recommendations in the ICH guidelines for studies of effects on pre- and postnatal development. Animals were administered 0, 25, 100 or 400 mg/kg/day zolmitriptan from Day 6 of pregnancy until lactation day 20. Animal weights were transiently decreased (46%) in F0 dams in mid and high dose animals on gestation days 6-9, supporting an MTD of 100 mg/kg/day (AUC 29,000 ng.h/ml; 181-fold higher than in humans at mrdd; predicted from the rat oral teratology study). Results included three F1 pups (postpartum) from the same pregnant mid-dose (100 mg/kg) dam presenting with splayed hindlimbs, abnormal gate, and dragging hindlimbs so severely that they were sacrificed moribund. This was probably not a significant toxicological finding since it did not occur at the high dose as well. Also, hydronephrosis was reported in F1 generation pups, mainly at the high dose. It is unclear how many animals were included in these sacrifice groups, but the incidence of hydronephrosis was probably fairly low (11 animals in a total of about 140 at the high dose). With respect to these two effects, the NOEL for this study was about 25 mg/kg/day, which would give a predicted (from oral rat teratology/toxicokinetics data) AUC of about 7,500 ng.h/ml, about 47-fold higher than in humans at the mrdd. The hydronephrosis effect was clearly dose-related, although this effect is apparently known to occur to in some rat strains in the absence of treatment.

Overall Summary of Reproductive Toxicology Study Results

There were no apparent effects of zolmitriptan on reproductive performance or fertility when drug was administered to rats at daily oral doses of up to 400 mg/kg/day (AUCs 1250-2500-fold greater than at the mrdd in humans). Teratology studies in rats and rabbits revealed that oral administration of zolmitriptan may be associated with the occurrence of early fetal resorptions, although maternal toxicity (decreased body weight gain in pregnant females) somewhat confounded data interpretation at the highest dose in both species. Some increase in fetal resorptions did occur at the mid dose in the absence of signs of maternal toxicity, suggesting that this may be a drug-related finding. Hydronephrosis in the F1 generation pups was reported in the pre- and postnatal study in rats, and this occurred in rats with a NOEL of about 25 mg/kg (47-fold greater AUC than in humans at the mrdd).

The sponsor concluded that the findings in the teratology studies (early resorptions) were not drug-related and therefore not of toxicological significance. They did not discuss the hydronephrosis in the pre- and postnatal development study. The sponsor's draft labelling lists zolmitriptan as a "Category B" drug with respect to reproductive toxicology, which indicates that there were no animal findings of concern. I would recommend that zolmitriptan be listed as a "Class C" drug, indicating that there were animal findings and "There are no adequate and well-controlled studies in pregnant women"...and that "the drug should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus."

Genetic Toxicology

Genetic Toxicology Summary and Evaluation

(Note: for specifics, see Genetic Toxicology section of this review, page 136).

Summary of Genetic Toxicology Study Results

Study	Results
Ames Assay	Positive in strains *S. typhimurium* TA 1538 and TA98
Human lymphocyte clastogenicity test	Positive both ±S9
Mammalian gene cell mutation assay (CHO/HGPRT)	Negative
Mouse micronucleus test	Negative
UDS (unscheduled DNA synthesis) test	Negative

The sponsor carried out the required genetic toxicology testing, including an Ames test, mammalian gene cell mutation assay (CHO/HGPRT test), human lymphocyte chromosomal aberration test, mouse micronucleus test, and unscheduled DNA synthesis test in rat liver. With the exception of couple of minor points, the study protocols were all consistent with the OECD guidelines (1994) for the various genetic toxicology tests.

Ames Test

In the Ames test, the sponsor failed to examine the effects of 311C90 on one of the *E. coli* strains or the *S. typhimurium* strain that detects certain oxidizing mutagens, cross-linking agents and hydrazine. According to the sponsor, cytotoxicity screening was carried out and results are indicated in the various data tables as reduced background bacterial "lawns". Dose-selection was probably adequate, since backgrounds were decreased at the high dose in all assay systems both ±S9.

The sponsor contends that Ames test results were negative for all bacterial strains tested. However, results from both standard Ames tests and Yahaghi modification tests including strain TA1538 indicated a positive response (see page 137 of this review for details). The first Ames test with this strain gave a strong, dose-related, positive response in the presence of S9, which was not repeated in the second test. Both Yahaghi modification tests gave a positive response at the 10000 µg/plate concentration (first time +S9, repeat -S9), the concentration at which backgrounds were reduced. Similar results were found with strain TA98 (see page 140 of this review), in which a strong positive response was found with the first standard Ames test, but not the repeat. With this strain, background levels in the repeat experiments were 2-fold higher than in the first set of experiments, which may have biased the data interpretation.

Human Lymphocyte Clastogenicity Study

The study protocol for the human lymphocyte clastogenicity study was consistent with the OECD guidelines (1994). The sponsor examined 100 cells in metaphase instead of 200, but the guidelines state that this is acceptable in the presence of a "high number of aberrations", and it is my opinion that the results are consistent with this exception. Results are positive in the presence of S9, with significant aberrations represented at concentrations probably ≥156 µg/ml (see page 143 of this review). In the absence of S9, the results were positive at concentrations ≥1000 µg/ml.

Mammalian gene cell mutation assay, mouse micronucleus test and UDS test

Results of the mammalian gene cell mutation assay (CHO/HGPRT), mouse micronucleus test, and UDS (unscheduled DNA synthesis) test were all negative. Study protocols for these assays were consistent with the OECD guidelines (1994), with the exception that in the mouse micronucleus test the sponsor scored only 1000 immature erythrocytes per animal for micronuclei instead of the recommended 2000. Dose-selection was appropriate in these tests. Finally, toxicokinetics data revealed that the high dose animals in the mouse micronucleus test were exposed to plasma C_{max} levels 687-825-fold greater than in humans at the mrdd.

Overall Summary of Genetic Toxicology Testing

The sponsor submitted the required battery of genetic toxicology studies, as per the OECD guidelines (1994). Results were negative in the mammalian gene cell mutation assay, mouse micronucleus test and UDS test. While the sponsor also concluded that results of the Ames test were negative, it is my opinion that results with respect to *S. typhimurium* bacterial strains TA1538 and TA98 were positive (both in the standard Ames test and the Yahaghi modification test). Results of the human lymphocyte assay were also positive, indicating that zolmitriptan is clastogenic.

Miscellaneous Toxicology Studies

The sponsor carried out a number of additional toxicology studies, to examine potential toxic effects of a number of breakdown products and contaminants of zolmitriptan. Those studies are summarized in the following:

1. Study of zolmitriptan containing 0.2% (w/w) 420C90 and 0.5% 439C90 (w/w) (see page 155 of this review for details)

The sponsor carried out a 14-day oral toxicology study in the Wistar rat to examine the toxicological profile of zolmitriptan contaminated with two known degradation products, 420C90 (0.2% w/w) and 439C90 (0.5% w/w). Wistar rats (10/sex/group) were treated with 100 or 400 mg/kg of the contaminated zolmitriptan by daily oral dosing for 14 days and evaluated for the usual parameters. Six animals in the various treatment groups (311C90, 311C90 with 420C90, 311C90 with 439C90) exhibited signs of respiratory distress as well as gross changes in the lungs

However, this must also be considered in light of the safety pharmacology results in the anesthetized cat, that demonstrated inhibition of respiration resulting in death at 9 mg/kg (i.v.). The effect in the cat appeared to be due to a central effect of the drug on respiration. In light of those data, it is possible that these effects in the rat are the result of drug administration rather than gavage accident.

The other effect of note reported in this study was concerning the histopathology results. Administration of zolmitriptan resulted in an increase in the incidence of minimal hypertrophy of the thyroid follicular epithelium in females treated with 400 mg/kg/day 311C90 with 0.2% (w/w) 420C90 contaminant. The sponsor stated that effects on the thyroid can be either direct (interfering with hormone production) or indirect (secondary to hepatic or renal changes). They concluded that an additional study, in which thyroid stimulating hormone (TSH) levels are measured, should be completed to further examine the possible mechanism for this effect.

These results are consistent with the conclusion that the contaminant (420C90) may play a role in the thyroid hypertrophy, since no such effect was seen in animals receiving 311C90 alone. The results of this study are also consistent with the results of the rat carcinogenicity study, in which thyroid follicular cell hyperplasias and neoplasms were reported. Finally, the combination of results from this degradant study and the rat carcinogenicity study raise the question of whether or not this degradant (420C90) might play a role in the formation of thyroid follicular cell neoplasms. Chemistry specifications should be set to include the lowest amount of this contaminant possible.

2. 2-week oral gavage study in Wistar rat to examine the toxicological effects of a zolmitriptan nasal preparation subjected to an accelerated degradation process

Animals (10/sex/group) received daily oral dosing of 125 mg/kg undegraded or degraded intranasal formulation of zolmitriptan. The identified degradant in this study was designated GW320486, and was at a level of 3.4%. Due to time constraints, I only did a very cursory review of this study, since this is not the formulation proposed for use in this NDA. There did not appear to be any difference in the toxicological profile between the degraded and undegraded nasal formulation of the drug.

3. Assessment of the effects of oral zolmitriptan administration on thyroid function in rats.

In the rat carcinogenicity study, oral administration of zolmitriptan resulted in thyroid follicular cell hyperplasia and neoplasia. The sponsor carried out this study to try to determine the mechanism for this effect. There are apparently two possible mechanisms by which a drug can induce thyroid dysfunction, either by a direct effect on the thyroid gland or indirectly through alterations in thyroid hormone clearance by the liver. A direct effect might involve overstimulation of the thyroid, inducing hyperplasia and evidenced by an increase in TSH levels.

Wistar rats were dosed for 30 days with 0 or 1000 mg/kg daily oral dose of zolmitriptan. This is over twice the dose given at the high dose in the rat carcinogenicity

study (400 mg/kg/day) (see page 158 of this review for details). In this study, animals demonstrated thyroid follicular epithelial hypertrophy, with an increase in thyroxine (T_4 clearance), a decrease in thyroxine half-life, and no effect on TSH levels or levels of other liver enzymes including T_4UDPGT. These data indicated that the mechanism for zolmitriptan's effect on the thyroid was probably indirect, since there was no effect on TSH levels. Results are consistent with a mechanism involving alteration of thyroxine clearance, but this does not appear to be due to induction of liver enzymes. Therefore the mechanism for the indirect effect remains unclear.

4. Study of the effects of zolmitriptan on respiration in the rat.

As previously stated, in the safety pharmacology study in the cat as well as in a previous toxicology study in the rat, there was some evidence that zolmitriptan might inhibit respiration (probably through a centrally mediated mechanism in the cat). Furthermore, a number of early deaths reported in the various toxicology studies in the rat remained unexplained. Therefore, the sponsor implemented this study to evaluate whether or not those early animal deaths may be due to effects of zolmitriptan on respiration.

Telemetry transmitters were used to monitor aortic blood pressure, lead II ECG, heart rate, core temperature and locomotor activity in male Wistar rats administered i.v. injections of zolmitriptan, 100 mg/kg, infused via tail vein over 15-60 seconds. Whole body plethysmography was used to monitor respiration during drug administration. Brief convulsions occurred in 10 of 12 treated rats, lasting a few seconds at the end of the injection period. Death occurred in single rats dosed over 15, 30 and 45 seconds. These rats apparently stopped breathing during or at the end of the injection, and respiration did not return. Apparently the hearts of these animals continued to beat for several minutes after respiratory arrest, indicating the cause of death involved respiratory arrest and not a cardiac event. Dosing over ≤45s appeared to result in death, while none of the 5 animals dosed over a 60 second interval died, experienced apnea nor loss of consciousness.

The sponsor stated that the mechanism for respiratory depression was unclear. The speculated that this could occur thorugh effects of the drug on the respiratory center of the CNS or on "J-receptors" (C-fiber afferents located in the pulmonary capillary beds and activated by a number of chemical irritants and agents, including 5-HT).

The most important information pertinent to this NDA that can be taken from this study is the fact that the most likely cause of death with 311C90 administration was respiratory arrest. This is somewhat consistent with previous toxicology studies, in that labored breathing was reported in a number of the early decedent animals. It is difficult to know how relevant the predicted plasma levels experienced by these animals (100 mg/kg, i.v.; 125,000-fold greater than humans at mrdd) are in terms of potential respiratory arrest in humans, because the proposed human route of administration is oral and this rat study was done using i.v. dosing. However, based on the information

based on surface area, respiratory arrest should certainly be no problem in humans receiving the mrdd (15 mg; plasma C_{max} about 20 ng/ml).

Previous acute toxicology studies in Wistar rats by the i.v. route were consistent with this study, revealing an LLD (lowest lethal dose) of about 50-100 mg/kg i.v. bolus. For an oral dose of 311C90, an LD_{50} of about 1000-1500 mg/kg was reported.

Toxicokinetics

Toxicokinetics data were included in this review as part of each pertinent animal toxicology study, and were not included in a separate "Toxicokinetics Section." However, in order to pull together an evaluation of the toxicokinetics data for zolmitriptan, I will summarize the data from the various toxicology studies in this section.

Rat

Toxicokinetics data for zolmitriptan and metabolites from a 26-week oral toxicology study in rats are shown on page 36 of this review. Parent drug (zolmitriptan) AUCs increase proportionally with dose at both Day 1 and Day 169. However, overall AUCs increased 6-10-fold with time between Day 1 and 169. There was no difference in plasma exposure between males and females. T_{max} was 2-6 hours and $t_{1/2}$ was about 14 hours at the mid and high doses. (The half-life for a single dose in the PK studies was about 1.3 h).

Metabolites included 183C91, 1652W92, and 2161W92. Exposure to 183C91 was similar to parent drug in that AUCs increased proportionally with dose on both Days 1 and 169, but exposure levels increased about 2-3-fold with time between Days 1 and 169. Zolmitriptan:183C91 ratios were about 10:1 to 20:1 on Day 1 and 20:1 to 40:1 on Day 169. $T_{1/2}$ was 29 hours for this metabolite. Similarly, exposure levels to 1652W92 increased proportionally with dose on both Days 1 and 169, while exposures increased 2-8-fold with time between Days 1 and 169. Ratios of zolmitriptan:metabolite also increased with time. T_{max} was 2-6 hours and $T_{1/2}$ was about 21 hours for 1652W92. And finally, with 2161W92, AUCs also increased proportionally with dose on both Days 1 and 169, while exposures increased with time about 4-6-fold between these two sampling times. Ratio of AUC for zolmitriptan:2161W92 increased with time. T_{max} occurred at about 2-6 hours after dosing, and $T_{1/2}$ was about 23 h.

Dog

Toxicokinetics data for zolmitriptan and metabolites from a 1-year oral toxicology study in dogs are shown on page 63 of this review. Sampling times for this study included Days 23 and 366. Metabolites were the same as found in the rat (183C91, 1652W92 and 2161W92). Toxicokinetics for zolmitriptan show exposures (AUC) increased approximately dose-proportionally, as did AUCs for metabolites 183C91 and 2161W92. However, 1652W92 increased supra-proportionally with dose from 25 to 100 mg/kg/day, with an increase of 6- to 7-fold with the 4-fold increase in dose. There did

difference in exposures between males and females.

T_{max} for parent drug was about 0.75 h, with a $T_{1/2}$ of about 2.4 hours. The T_{max} for metabolites ranged from 0.75 to 4 h, with the average being 3.8, 2.5 and 3.4 h for 183C91, 1652W92 and 2161W92, respectively. Metabolites in descending order with respect to exposure levels were 1652W92>2161W92>183C91. 1652W92 was found at the highest plasma level, with the zolmitriptan:metabolite ratio of about 2.5 over the dose range. The ratio of 311C90:2161W92 increased slightly in a dose-dependent manner from about 2.8 at 5 mg/kg/day to about 6.5 at 100 mg/kg/day. This is consistent with formation of this metabolite approaching saturation. Exposure to 183C91 was lowest in the dog, with a zolmitriptan:metabolite average ratio of about 25:1. 183C91 is the active metabolite that in humans is found at about 50% of the exposure levels of the parent drug.

Mice

Toxicokinetics data were included in the mouse carcinogenicity study for both zolmitriptan and metabolites. In the first 6-weeks of the study, zolmitriptan exposure levels increased approximately proportionally with dose. There were no apparent differences in exposure levels between males and females. Major metabolites were the same as seen in rat and dog, and in descending order according to exposure levels were 183C91>1652W92>2161W92. $T_{1/2}$ for parent, 183C91, 1652W92 and 2161W92 were 2.6, 4.3, 2.7 and 2.7 h, respectively. AUCs for parent and all three metabolites appear to have increased in females with time, while no such accumulation appeared to occur in males.

Overall Summary and Evaluation of Toxicokinetics

Zolmitriptan plasma exposure increased with time in rat (6-10-fold) and mouse (about 2-fold) but not in dog. Elimination half-lives for zolmitriptan were longer in the rat (mid and high doses) than dog or mouse (14, 2.6 and 2.4 hours in rat, mouse and dog, respectively). Metabolites in descending order of exposure (AUC) were 2161W92>1652W92>183C91 for rat, 1652W92>2161W92>183C91 for dog, and 183C91>1652W92>2161W92 for mouse. Elimination half-lives for metabolites were longer in the rats (ranging from 21-29 hours) than in dog (ranging from 2.5-3.8 h) or mouse (2.7-4.3 h). Exposure levels to all three metabolites increased with time in the rat and mouse (females only) but not in dog.

With respect to metabolites, human exposures in descending order were 2161W92>183C91>1652W92. The only other animal species with 2161W92 at the highest exposure level was the rat. The active metabolite 183C91 was second highest in the human. In humans, 2161W92 plasma levels were similar to those of parent drug, while 183C91 and 1652W92 were present in levels about 50% of the parent. In mouse all three metabolites were found in plasma at concentrations about 5-10% of parent. In rat, 2161W92 and 1652W92 were at about 5-10% of parent, while 183C91 was at about 2-5% of parent levels. In dog, 1652W92 was at about 20-50% of parent drug levels,

CONCLUSIONS

Pharmacology

The scientific rationale for using zolmitriptan, a 5-HT_{1D} receptor agonist, in the treatment of migraine is sound. An apparent 2-fold greater potency of the drug in nonclinical pharmacology studies related to efficacy is offset by a 2-3-fold increase in potency with respect to increasing blood pressure and heart rate (conscious dog). In animal studies, there appeared to be no effect on coronary blood flow, although of some concern was a dose-related contraction of human coronary artery *in vitro*. Coronary vasospasm is one of the concerns associated with sumatriptan, an already approved migraine drug. There is at least one active metabolite, 183C91, found in man, rat, mouse and dog. This active metabolite has similar pharmacological activity to zolmitriptan, and appears to be more potent (2-6-fold). The sponsor has done an adequate job of examining the pharmacology of zolmitriptan.

ADME

ADME study results did not reveal any major cause for alarm with respect to zolmitriptan. Absorption was rapid by the oral route in all species tested, and the elimination half-life was reasonably short (1.3-3 hours for the various species studied). Oral bioavailability was good (40-75% in animals; 40% in humans). Of some concern was the fact that drug appeared to accumulate with time, but this occurred only in rats (males and females) and mice (females only), and not in dog. The sponsor also stated that this does not occur in humans with repeat dosing. Furthermore, plasma drug exposure was about 2-fold greater in female than male mice, and apparently this also holds true in humans. This may be somewhat important information in that a large proportion of migraine patients are apparently female.

As with sumatriptan, zolmitriptan appears to bind to melanin and persist in the pigmented eye for some time. With respect to metabolism, the same three major metabolites (2161W92, 183C91 and 1652W92) appear in rat, mouse, dog and humans, but at different plasma concentrations and exposure levels. 2161W92 is the major metabolite in humans, with plasma exposures similar to those of parent zolmitriptan drug, while the other two metabolites were present in levels about 50% of parent drug. In animal studies, plasma levels of metabolites were much lower compared to zolmitriptan. In rat, as in human, 2161W92 was the major metabolite. However, in rat this metabolite only constituted about 5-10% of parent plasma levels.

Finally, plasma protein binding does not appear to be a problem, there appeared to be no interaction with the MAO system, and zolmitriptan did not appear to induce liver enzymes or affect barbiturate sleep time.

Overall, the sponsor did an adequate job of examining the ADME of zolmitriptan, and there were no findings that would preclude approval of the drug for treatment of migraine.

TOXICOLOGY

General toxicology

The sponsor has completed the required acute, subchronic and chronic animal toxicology studies for an NDA for a drug to be administered in a chronic, intermittent treatment regimen (oral administration) as is the case with zolmitriptan. Results of these toxicology studies do indicate a number of findings of some concern, including convulsions and the occurrence of a number of inflammatory lesions. However, safety margins for these effects (based on AUCs) with respect to the human mrdd are adequate to support the safe administration of the drug to humans by the proposed dosing regimen. Early deaths were also reported in the subchronic and chronic toxicology studies in the rat. Further studies in the rat (i.v. administration) to try to determine the cause of death demonstrated that death may have been due to inhibition of respiration (similar results were reported in a safety pharmacology study in the cat) mediated centrally, possibly through effects of the drug on the respiratory center. While these studies were carried out i.v., and the drug is to be given p.o. in the clinic, animals receiving the lethal dose (100 mg/kg i.v.) of zolmitriptan demonstrated plasma levels about 125,000-fold greater than humans at the mrdd. Also, the LD_{50} in rats reported in the acute toxicology studies was 1000-1500 mg/kg (oral), which on a mg/m^2 basis is about 650-973-fold greater than the human mrdd. Finally, the NOEL for oral zolmitriptan in the rat studies in which early deaths occurred was about 100 mg/kg/day, which gave AUCs about 294-fold greater than in humans at the mrdd. Therefore, one might predict from these results that respiratory depression should not be a problem in humans at the mrdd.

Carcinogenicity

Rat and mouse carcinogenicity studies were carried out satisfactorily with respect to number of animals, study protocol, and choice of dose. While the sponsor did not request concurrence from the Agency with respect to choice of dose, it is my opinion that mortality data support the choice of the high dose in both studies. Based on exposure levels, in both studies the low dose would appear to be about 5- to 10-fold higher than would be appropriate for relevance to the human mrdd.

In the mouse study, there were a number of non-neoplastic lesions associated with zolmitriptan administration, but no treatment-related neoplasias were reported. In the rat study, there was a dose-related increase in thyroid follicular cell hyperplasia as well as the neoplastic lesions rat throid follicular cell adenoma and carcinoma. According to the sponsor, the occurrence of these neoplastic lesions were statistically significant ($p<0.05$) when ademomas alone or adenomas combined with carcinomas were analyzed. Carcinomas alone did not constitute a significant finding. These results regarding the adenomas should be included in the labelling.

Reproductive Toxicology

The sponsor met the requirements for reproductive toxicology testing, including a reproduction/fertility study in the rat, teratology studies in the rat and rabbit, and pre- and postnatal reproductive toxicology studies in the rat. The study protocols were consistent with the ICH guidelines for reproductive toxicology studies. The only notable findings were in the teratology studies in rats and rabbits, which revealed that oral administration of zolmitriptan may be associated with the occurrence of early fetal resorptions. While maternal toxicity (decreased body weight gain in pregnant females) confounded data interpretation at the highest dose in both species, some increase in early fetal resorptions also occurred at the mid dose in the rabbit in the absence of signs of maternal toxicity, suggesting that is may be a drug-related finding. These results should be included in the drug labelling. Furthermore, while the sponsor proposes in their draft labelling that zolmitriptan be labelled as "Category B", which indicates that there were no animal findings of concern, it is my conclusion that the drug should be labelled as "Category C". This is especially the case since a high percentage of migraine sufferers are women.

Genetic Toxicology

The sponsor has completed the full battery of genetic toxicology testing. We are in agreement that results of the mammalian gene cell mutation assay (CHO/HGPRT), mouse micronucleus test and UDS test are negative and that the results of the human lymphocyte clastogenicity test are positive. However, I disagree with the sponsor's conclusion that the results of the Ames test were also negative. It is my conclusion that the results of testing in the bacterial strains *S. typhimurium* TA1538 and TA98 are positive, based on the fact that results of the initial experiments were strongly positive and dose-related. Even though the positive results did not recur with a repeat experiment is not sufficient reason to ignore such strongly positive findings.

Miscellaneous Toxicology Studies

Results of a 14-day oral toxicology study in the rat indicated that a degradation product, 420C90, may be involved in the formation of thyroid follicular hyperplasia associated with zolmitriptan administration. Therefore, levels of this contaminant must be kept as low as possible. Results of an additional study in rats, for the purpose of determining the effects of oral zolmitriptan administration on thyroid function, demonstrated that thyroid effects were probably not due to overstimulation of the thyroid (no effect on plasma TSH). However, thyroid effects did appear to be associated with an increase in thyroxine clearance. Furthermore, this increase in thyroxine clearance did not appear to be due to induction of liver enzymes, including T_4UDPGT.

Toxicokinetics

Zolmitriptan plasma exposure (AUC) increased with time (accumulation) in rat and mouse (females), but not in dog. Exposure levels to all three metabolites also increased with time in rat and mouse (females only) but not in dog. Plasma exposure to the active metabolite, 183C91 was greatest in the mouse, while the rat was most similar to human in that metabolite 2161W92 was the major metabolite of highest exposure level in both. Overall, exposure to metabolites with respect to zolmitriptan:metabolite ratios appeared to be much greater in humans than in any of the animal species.

Overall conclusions

The sponsor has done an adequate job of completing the nonclinical workup for zolmitriptan, and study results support approval of the drug for the proposed indication, if certain conditions are met. Results of the carcinogenicity studies (thyroid follicular cell adenomas) and teratology studies (early fetal resorptions) should be included in the labelling. The drug should be labelled as "Category C" instead of the "Category B" designation that the sponsor included in their draft labelling. With respect to genetic toxicology testing, the labelling should include information stating that the drug tested positive both the human lymphocyte assay and the Ames test.

RECOMMENDATIONS

Based on my review of the nonclinical section of the NDA, I recommend that zolmitriptan be approved for the proposed indication upon compliance with the following:

1. Results of the carcinogenicity studies (thyroid follicular cell adenomas) should be included in drug labelling.

2. Results of the teratology studies (early fetal resorptions) should be included in the drug labelling and the drug should be labelled as "Category C."

3. Results of the genetic toxicology studies should be included in the drug labelling and should indicate positive findings in both the human lymphocyte clastogenicity assay and the Ames test for mutagenicity.

9-12-97

John J. Jessop, Ph.D., M.P.H.,
Pharmacologist

CC
NDA 20-768 (000)
Div file
HFD-120
/G.Fitzgerald/J.J.Jessop/L.Chen

9/16/97