In the ovaries, stromal hyperplasia occurred slightly more frequently in the drug treated rats at 104 weeks of treatment than in concurrent controls. This finding was not dose-related.

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS
TERMINAL SACRIFICE

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	06
NO.ANIMALS:	50	50	50	50	50	50
OVARIES :	50	49	50	49	48	47
- INTERST CELL HYPERPL:	1	3	-	2	-	-
- BURSAL CYST :	3	1	4	1	1	1
- FOLLICULAR CYST :	1	2	-	2	2	2
- PAROVARIAN CYST :	3	-	2	2	-	2
- CYSTIC OVIDUCT :	-	1	-	-	-	-
- ATROPHY :	13	25	9	11	16	12
- STROMAL HYPERPLASIA :	4	8	11	12	10	11
- TUBULAR HYPERPLASIA :	4	-	-	-	2	1
- P-M CONGESTION :	-	-	-	-	-	1
- ANGIECTASIS :	1	-	-	-	-	-
- ABSCESS :	-	-	-	1	-	-
UTERUS :	50	50	49	50	49	49
- CYSTIC ENDOM HYPERPL:	2	4	-	2	3	6
- DILATED GLANDS/LUMEN:	7	9	11	8	12	7
- EPITHEL HYPERPLASIA :	-	-	-	-	-	1
- ATROPHY :	1	1	-	1	1	1
- HEMORRHAGE :	-	-	-	-	1	1
- FIBROSIS :	-	-	-	-	1	-
- ABSCESS :	-	1	-	-	-	-
- SUPPURATIVE INFLAMM :	-	1	-	1	-	-
VAGINA :	49	50	50	50	45	48
- ATROPHY :	-	1	-	-	-	-
- UTERINE/VAG PROLAPSE:	-	-	-	1	1	3
SKIN/SUBCUTIS :	50	50	50	50	50	50
- EPIDERMAL CYST :	-	-	-	1	-	1
- ULCERATION :	1	2	1	-	1	-
- HYPERKERATOSIS :	-	-	-	-	1	-
- SCAB :	-	1	3	-	1	-
- CHRONIC INFLAMMATION:	2	-	1	-	1	1
- ADNEXAL ATROPHY :	8	8	10	5	2	1
- HEMATOMA :	-	-	-	-	-	1
- HEMORRHAGE :	-	-	-	-	-	1
- PODODERMATITIS :	1	-	-	-	-	-

Dose Groups 1, 2: concurrent control; Dose Groups 3, 4, 5 and 6 refer to 125, 500, 2000 and 3000 mg/kg/day dose groups, respectively.

The incidence of nasal cavity pathology was increased in male and female rats given bosentan. The findings, which included increased goblet cells, dilated submucosal glands, suppurative inflammation and squamous metaplasia, were not dose-related and were considered by the sponsor to be indicative of mild inflammation.

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: KO, INCL. DEATHS
TERMINAL SACRIFICE

SEX :							MALE
DOSE GROUP:	01	02	03	04	05	06	
NO.ANIMALS:	50	50	50	50	50	50	
NASAL CAVITY, ANTER. :	50	50	50	49	50	50	
- INCR GOBLET CELLS :	5	5	12	8	15	19	
- SUPPURATIVE INFLAMM :	5	6	2	10	7	13	
- SQUAMOUS METAPLASIA :	4	2	-	7	8	6	
- TOOTH DYSPLASIA :	-	2	1	6	2	3	
- TOOTH ROOT INFLAMM :	-	-	2	3	2	-	
- INTRANASAL EXUDATE :	13	3	2	2	10	5	
- NON-SUPPUR INFLAMM :	1	-	-	2	2	-	
- RESP EPITH HYPERPLAS:	2	-	-	4	4	2	
- DILAT SUBMUCOSAL GL :	-	1	3	9	30	5	
- FOREIGN BODY :	-	1	1	2	2	-	
- SUCUTANEOUS INFLAMM :	-	-	-	1	-	-	
NASAL CAVITY, MIDDLE :	50	50	50	49	50	50	
- INCR GOBLET CELLS :	1	-	2	-	4	1	
- DILAT SUBMUCOSAL GL :	11	3	5	21	17	16	
- EOS INCLUS OLF EPITH:	1	3	1	2	4	2	
- SUPPURATIVE INFLAMM :	3	1	3	3	3	1	
- NON-SUPPUR INFLAMM :	1	2	1	-	1	2	
- INTRALUMENAL EXUDATE:	1	1	2	-	2	1	
- ATROPHIC SUBMUCOS GL:	-	1	-	-	-	-	
- TOOTH DYSPLASIA :	-	-	3	2	-	1	
- INFLAMM NASOLAC DUCT:	-	-	1	2	-	-	
- HYPERPL SUBMUCOS GL :	-	-	-	-	-	1	
NASAL CAVITY, POST. :	50	50	50	49	50	50	
- EOSIN INCL OLF EPITH:	4	5	7	7	12	9	
- DILAT SUBMUC GLANDS :	-	5	5	17	16	7	
- INCR GOBLET CELLS :	-	-	-	-	-	1	
- NON-SUPPUR INFLAMM :	1	-	-	-	-	-	
- INTRALUMENAL EXUDATE:	-	-	-	-	2	-	
- ATROPHIC SUBMUC GL :	-	-	-	-	-	2	
- TOOTH ROOT INFLAMM :	-	-	1	-	1	-	

Dose Groups 1, 2: concurrent control; Dose Groups 3, 4, 5 and 6 refer to 125, 500, 2000 and 3000 mg/kg/day dose groups, respectively.

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0, INCL. DEATHS
TERMINAL SACRIFICE

SEX :						FEMALE
DOSE GROUP:	01	02	03	04	05	06
NO.ANIMALS:	50	50	50	50	50	50
NASAL CAVITY, ANTER. :	49	50	50	50	49	49
- INCR GOBLET CELLS :	5	4	6	11	19	22
- SUPPURATIVE INFLAMM :	-	1	1	1	2	7
- SQUAMOUS METAPLASIA :	-	-	-	5	4	4
- TOOTH DYSPLASIA :	-	-	-	-	2	1
- TOOTH ROOT INFLAMM :	-	-	-	-	-	1
- INTRANASAL EXUDATE :	-	2	2	1	-	3
- NON-SUPPUR INFLAMM :	-	-	-	2	5	2
- DILAT SUBMUCOSAL GL :	-	-	4	17	35	36
- INFLAMM NASOLAC DUCT:	1	1	-	-	-	-
- ULCERATION :	-	-	-	-	1	-
NASAL CAVITY, MIDDLE :	50	50	50	50	49	50
- INCR GOBLET CELLS :	-	3	-	1	3	3
- DILAT SUBMUCOSAL GL :	4	2	11	4	12	10
- EOS INCLUS OLF EPITH:	2	2	1	6	4	4
- SUPPURATIVE INFLAMM :	1	-	1	-	1	-
- NON-SUPPUR INFLAMM :	-	-	1	-	-	-
- INTRALUMENAL EXUDATE:	1	-	1	-	-	-
- EPIDERMAL CYST :	-	-	-	-	-	1
- TOOTH DYSPLASIA :	-	-	-	-	-	1
- TOOTH ROOT INFLAMM :	-	-	-	-	-	1
- ABSCESS :	-	1	-	-	1	-
NASAL CAVITY, POST. :	50	50	50	50	49	50
- EOSIN INCL OLF EPITH:	4	5	3	10	13	19
- DILAT SUBMUC GLANDS :	2	5	8	10	7	6
- SUPPURATIVE INFLAMM :	-	-	1	1	1	3
- NON-SUPPUR INFLAMM :	-	-	-	-	-	1
- ABSCESS :	-	1	-	-	-	1
- ATROPHIC SUBMUC GL :	-	-	-	-	2	-
- HYPERKERAT NASOPHAR :	-	-	1	-	-	-
- TOOTH ROOT INFLAMM :	1	-	3	2	1	1
- TOOTH DYSPLASIA :	-	1	1	-	-	-

Dose Groups 1, 2: concurrent control; Dose Groups 3, 4, 5 and 6 refer to 125, 500, 2000 and 3000 mg/kg/day dose groups, respectively.

Additional non-neoplastic pathology observed more frequently in male or female rats given bosentan than in concurrent controls:

Organ/ Finding	Gender	Dose (mg/kg/day)					
		0	0	125	500	2000	3000
Adrenal cortex	Male	1	0	0	1	2	5
Hypertrophy (diffuse)	Female	1	4	3	1	2	1
Forestomach	Male	2	0	2	2	3	7
Epithelial hyperplasia	Female	0	3	0	1	4	1
Kidney	Male	0	1	1	1	3	5
Cortical cysts	Female	1	0	4	1	3	3
Kidney	Male	3	1	4	1	2	1
Urethral hyperplasia	Female	5	6	5	10	9	14
Liver	Male	0	1	4	6	5	4
Dilated common bile duct	Female	1	1	1	2	3	3
Liver	Male	13	2	14	11	16	15
Eosinophilic focus	Female	6	2	1	6	2	11
Lung	Male	9	29	16	24	19	17
alveolar histiocytosis	Female	24	24	25	28	35	34
Salivary gland	Male	1	0	1	2	6	4
atrophy	Female	0	4	0	5	1	0

The incidence of thyroid follicular cell hyperplasia was not drug-related in male and female rats.

Organ/Finding	Gender	Dose (mg/kg/day)					
		0	0	125	500	2000	3000
Thyroid follicular cell hyperplasia	Male	6	5	7	6	3	6
	Female	3	1	2	2	5	2

Neoplastic findings

Thyroid follicular cell adenomas, but not combined adenomas and carcinomas, were significantly increased in male rats given 2000 and 3000 mg/kg/day compared to combined concurrent controls.[5,6] Thyroid follicular cell tumors were not significantly increased in female rats given bosentan compared to concurrent controls.

Hepatocellular adenomas, and combined adenomas and carcinomas were significantly increased in male rats given 500 and 3000 mg/kg/day compared to combined concurrent controls, but only when considered to be rare. When considered common (based on historical control data) combined adenomas and carcinomas were significant at 3000 mg/kg/day, but not at lower doses.

Hepatocellular tumors were not significantly increased in female rats given bosentan compared to concurrent controls.

Tumor Incidences

Organ/ Finding	Gender	Tumor type	Dose (mg/kg/day)						P-Trend test^	Significant trend^
			0	0	125	500	2000	3000		
Thyroid Gland Follicular Cell Tumors	Male	Adenoma (common)	6	5	9	9 0.1388[a] NS	12 0.0232[a] Signif.	16 0.0020[a] Signif.	0.0011	Significant
		Carcinoma (rare)	5	2	5	5	5	2	0.7128	NS
		Combined Adenoma and Carcinoma (common)	11	7	14	12	16 0.0158[a] NS	18 0.0178[a] NS	0.0069	NS
	Female	Adenoma (common)	2	2	0	1	4	4 0.2023[a] NS	0.0164	NS
		Carcinoma (common)	0	1	2	0	0	0	0.8930	NS
		Combined Adenoma and Carcinoma (common)	2	2	2	1	4 0.2111[a] NS	4 0.2090[a] NS	0.0538	NS
Liver, Hepatocellular Tumors	Male	Adenoma (common)	0	0	1	3 0.0227[a] NS	1 0.3017[a] NS	3 0.0308[a] NS	0.0621	NS
		Carcinoma (rare)	0	0	0	0	0	1	0.1678	NS
		Combined Adenoma and Carcinoma (common)	0	0	1	3 0.0227[a] NS	1 0.3017[a] NS	4 0.0017[a] Signif.	0.0164	NS
	Female	Adenoma (common)	0	1	1	0	1	3 0.1281[a] NS	0.0370	NS
		Carcinoma (rare)	1	0	0	0	1	0	0.5122	NS
		Combined Adenoma and Carcinoma (common)	1	1	1	0	2 0.3698[a] NS	3 0.1884[a] NS	0.0449	NS

^ vs combined concurrent control values

[a] p-value for pairwise comparison to combined concurrent control

5 Statistical analyses shown are those of the agency statistician, Roswitha Kelly.

6 A tumor is considered to be common if its historical control incidence is >1%. For a common tumor, the required trend test p-value (α) is 0.005 and the required pairwise comparison p-value is 0.01. For a rare tumor, the required trend test p-value (α) is 0.025 and the required pairwise comparison p-value is 0.05.

The sponsor provided historical control data for thyroid follicular cell tumors in Wistar rats. Two sources of data were cited: , the laboratory that performed the bosentan carcinogenicity study in Wistar rats, and published references. According to these historical control data and the criterion of CDER's statistician for differentiating rare from common tumors, thyroid follicular cell adenomas are common, and follicular cell carcinomas are rare in males but common in females.

Historical Control Data for Thyroid Follicular Cell Tumors

Source	*Adenomas*		*Carcinomas*	
Gender	*Male*	*Female*	*Male*	*Female*
(2 studies)	5% (0, 10%)	2% (0, 4%)	0 (0, 0)	2% (4%, 0)
Literature reference (1998)^ 1990-1995 (5 studies)	3.9% (1.7-6.9%)	2.8% (2.0-3.3%)	0.9% (1-1.7%)	1.5% (0-3.3%)
Literature reference (1994)^^ 1980-1990 (10 studies)	2.0% (0-10%)	0.7% (0-4.0%)	0.6% (0-2.0%)	0.9% (0-4.0%)

^Toxicological Sciences 45; 1-8 (1998)
^^Fundamental and Applied Toxicology 22; 65-72 (1994)

The sponsor provided historical control data for hepatocellular tumors in Wistar rats. Two sources of data were cited: the laboratory that performed the bosentan carcinogenicity study in Wistar rats, and published references. According to these historical control data and the criterion of CDER's statistician for differentiating rare from common tumors, hepatocellular adenomas are common, and hepatocellular carcinomas are rare.

Historical Control Data for Hepatocellular Tumors

Source	*Hepatocellular Adenomas*		*Hepatocellular Carcinomas*	
Gender	*Male*	*Female*	*Male*	*Female*
(2 studies)	3% (0, 6%)	2% (0, 4%)	0%	0%
Literature reference (1998)^ 1990-1995 (5 studies)	2.8% (0-5%)	2.2% (0-4.2%)	1.1% (0-2%)	0.2% (0-0.8%)
Literature reference (1994)^^ 1980-1990 (10 studies)	1% (0-2.5%)	2.3% (0-12%)	0.9% 0-2.5%)	0.9% (0-10%)

^Toxicological Sciences 45; 1-8 (1998)
^^Fundamental and Applied Toxicology 22; 65-72 (1994)

Brain astrocytomas were significantly increased in male rats given 3000 mg/kg/day compared to concurrent controls, but only when this tumor was considered to be rare. Brain astrocytomas were only observed in higher dose treatment groups, and were not observed in concurrent controls.

Skin keratoacanthomas were increased, but not significantly, in male rats given 500 and 3000 mg/kg/day compared to concurrent controls. Skin keratoacanthomas were not observed in concurrent controls.

Tumor Incidences

Organ	Gender	Tumor type	Dose (mg/kg/day)						P-Trend test^	Significant trend^
			0	0	125	500	2000	3000		
Brain	Male	Astrocytoma (rare tumor)	0	0	0	2 (0.0820)[a] NS	1 (0.4500)[a] NS	2 (0.0198)[a] Signif.	0.0408	NS
	Female	Astrocytoma (rare tumor)	0	0	0	0	0	1 (0.3529)[a] NS	0.1782	NS
Skin	Male	Keratoacanthoma (common tumor)	0	0	1	3 (0.0425)[a] NS	0	3 (0.0366)[a] NS	0.0978	NS
	Female		0	0	0	0	1 (0.3017)[a] NS	0	0.3099	NS

^ vs combined concurrent control values

[a] p-value for pairwise comparison to combined concurrent control

The sponson's historical control data for brain astrocytomas and skin keratoacanthomas are shown below.

Historical control data for brain astrocytomas and skin keratoacanthomas

Source	*Brain Astrocytomas*		*Skin Keratoacanthomas*	
Gender	*Male*	*Female*	*Male*	*Female*
(2 studies)	*1% (0, 2%)*	*0%*	*2% (0, 4%)*	*0%*
Literature reference (1998)^ 1990-1995 (5 studies)	*0.9% (0-2%)*	*0.7% (0-1.5%)*	*11.2% (2-22%)*	*0.7% (0-2%)*
Literature reference (1994)^^ 1980-1990 (10 studies)	*3.2% (0-8%)*	*1.3% (0-6%)*	*0.7% (0-2%)*	*0.15% (0-2%)*

^***Toxicological Sciences 45; 1-8 (1998)***

^^*Fundamental and Applied Toxicology 22; 65-72 (1994)*

- Although testes leydig cell adenomas, prostate adenomas, and seminal vesicle adenomas were more common in drug-treated than in concurrent control rats, these tumors were not significantly increased compared to concurrent controls. Hyperplasia was not observed in these organs.

Tumor Incidences

Organ	Gender	Tumor type	Dose (mg/kg/day)						P-Trend test^	Significant trend^
			0	0	125	500	2000	3000		
Testes Leydig cells	Male	Adenoma (common tumor)	1	1	3	3 (0.1446)[a] NS	3 (0.1607)[a] NS	4 (0.0854)[a] NS	0.0907	NS
		Carcinoma (rare tumor)	0	0	0	0	0	0	-	-
Prostate	Male	Adenoma (common tumor)	1	1	1	0	0	4 (0.0814)[a] NS	0.0396	NS
		Carcinoma (rare tumor)	0	0	0	0	0	0	-	-
Seminal vesicle	Male	Adenoma (rare tumor)	0	0	0	0	1	0	0.3274	NS
		Carcinoma (rare tumor)	0	0	0	1	0	0	0.5778	NS
		Combined (rare tumor)	0	0	0	1	1	0	0.3804	NS

^ vs combined concurrent control values

[a] p-value for pairwise comparison to combined concurrent control

Toxicokinetics: Exposure (AUC) to bosentan (Ro-47-0203) was dose-related in female rats at both 51 and 103 weeks of treatment and was greater in female than in male rats. Exposure was unrelated to dose in male rats given 500, 2000 and 3000 mg/kg/day.

Exposure to bosentan (AUC) in male and female rats given 125, 500, 2000 and 3000 mg/kg/day exceeded that observed in healthy male humans given 500 mg, qd for 8 days (15.03 μg.h/ml).[7]

Pharmacokinetic parameters of Ro 47-0203 in rats determined in Study Week 51 after oral administration

Study Week 51				
Dose (mg/kg/day)	Gender	C_{max} (µg / mL)	T_{max} (h)	AUC_{0-24h} (µg.h/mL)
0	M	BLQ		
	F	BLQ		
125	M	1.44 ± 0.784	24.00	25.7
	F	2.46 ± 0.932	8.00	53.6
500	M	4.55 ± 2.86	24.00	76.9
	F	8.88 ± 3.91	24.00	147
2000	M	9.06 ± 0.633	24.00	134
	F	10.6 ± 3.25	24.00	152
3000	M	5.38 ± 2.54	24.00	83.9
	F	17.9 ± 2.53	24.00	274

C_{max}: Mean ± SD, n=3; BLQ = below limit of quantification

Pharmacokinetic parameters of Ro 47-0203 in rats determined in Study Week 103 after oral administration

Study Week 103				
Dose (mg/kg/day)	Gender	C_{max} (µg / mL)	T_{max} (h)	AUC_{0-24h} (µg.h/mL)
0	M	BLQ		
	F	BLQ		
125	M	4.95 ± 1.69	24.00	63.6
	F	3.00 ± 1.11	8.00	66.1
500	M	7.93 ± 2.50	24.00	107
	F	8.82 ± 4.92	24.00	158
2000	M	6.72 ± 2.49	24.00	125
	F	13.8 ± 6.02	24.00	201
3000	M	7.86 ± 2.30	24.00	119
	F	18.2 ± 4.31	24.00	255

C_{max}: Mean ± SD, n=5; BLQ = below limit of quantification

[7] While the maximum therapeutic dose is 250 mg, bid, there is no pharmacokinetic data available for this dosing regime. Additionally, there is no pharmacokinetic data in females, and none in patients with pulmonary hypertension. The AUC in pulmonary hypertension patients likely exceeds that in healthy humans, based on Dr. Robbie's review of human pharmacokinetics of bosentan.

Exposures to bosentan metabolites (Ro 48-5033, Ro 47-8634, and Ro 64-1056) in male and female rats given 3000 mg bosentan/kg/day were low compared to parent drug exposure. This was evident at 3, 9 and 27 weeks of treatment. Exposure to metabolites in humans is also low although the absolute exposure is unknown in patients.

Pharmacokinetic parameters of Ro 47-0203 and its metabolites in rats determined after oral administration of 3000 mg/kg/day

Ro 47-0203				
Study Week	**Gender**	**C_{max} (µg / mL)**	**T_{max} (h)**	**AUC_{0-24h} (µg.h/mL)**
3	M	8.36 ± 2.23	24.00	127
	F	14.5 ± 2.80	24.00	212
9	M	4.31 ± 2.16	8.00	78.2
	F	14.6 ± 2.59	24.00	226
27	M	5.37 ± 1.09	24.00	85.4
	F	19.5 ± 5.26	24.00	332
Ro 48-5033				
Study Week	**Gender**	**C_{max} (µg / mL)**	**T_{max} (h)**	**AUC_{0-24h} (µg.h/mL)**
3	M	0.254 ± 0.030	8.00	4.99
	F	0.340 ± 0.053	24.00	7.33
9	M	0.133 ± 0.057	8.00	2.46
	F	0.558 ± 0.251	24.00	9.79
27	M	0.142 ± 0.041	24.00	2.83
	F	0.996 ± 0.498	8.00	17.1
Ro 47-8634				
Study Week	**Gender**	**C_{max} (µg / mL)**	**T_{max} (h)**	**AUC_{0-24h} (µg.h/mL)**
3	M	0.065 ± 0.018	24.00	1.05
	F	0.092 ± 0.011	24.00	1.63
9	M	0.029 ± 0.012	8.00	0.501
	F	0.116 ± 0.034	24.00	2.03
27	M	0.039 ± 0.007	24.00	0.658
	F	0.183 ± 0.039	24.00	3.39

C_{max} : Mean ± SD, n=3; plasma concentrations were determined by

Ro 64-1056				
Study Week	**Gender**	**C_{max} (ng / mL)**	**T_{max} (h)**	**AUC_{0-24h} (ng.h/mL)**
3	M	8.96 ± 1.86	8.00	171
	F	2.76 ± 0.50	8.00	62.9
9	M	5.91 ± 1.93	8.00	103
	F	5.28 ± 3.09	24.00	95.1
27	M	5.70 ± 0.88	24.00	110
	F	10.6 ± 4.8	8.00	173

C_{max} : Mean ± SD, n=3; plasma concentrations were determined by

104 Week Carcinogenicity Study in Mice

Location of Data: Volumes 1.22-1.29

Testing Facility:

Study Number: HRE/55/C

Study Dates: January 15, 1997 through January 15, 1999

GLP Compliance: Yes

~~Protocol Concurrence: The sponsor evaluated bosentan in the two-year mouse carcinogenicity study at the maximal~~ dose recommended by the EC-CAC. The sponsor did not concur with the EC-CAC's recommendation for individual animal housing. See EC-CAC minutes.

Animals: mice, 5-6 weeks of age at the start of dosing. On the day before the first day of dosing, the males weighed 25 - 35g and the females weighed 18 - 28g. The mice were housed in groups of 5 (main study) or in groups of 5 and 3 (satellites), by sex, in cages. Mice were provided powdered rodent diet and tap water, *ad libitum*.

Mode of Administration of Test Agent: Dietary admixture in powdered feed.

Dose Levels: 0, 0, 100, 450, 2000, 4500 mg/kg/day

Dietary concentrations were adjusted weekly for the first 16 weeks and every other week thereafter to maintain constant dose levels in relation to bodyweight. Dietary concentrations of test agent were determined every 13 weeks starting on week 1.

Test Article: Ro 47-0203/029 (lot number 410003A40)

Basis for Doses Evaluated: The sponsor argued that saturation of absorption occurs in mice given bosentan at doses ≥1500 mg/kg/day, but evaluated 4500 mg/kg/day as the maximum dose per EC-CAC recommendations.

Number of Animals: 50 mice/sex/group (main study animals)
25 additional mice/sex/treatment group for determination of systemic exposures

Observations/Measurements: Mice were examined daily for mortality, morbidity and clinical signs. Bodyweights were determined in all animals from all treatment groups at the start of the study, weekly for the first 16 weeks and every second week thereafter. Food intake by each cage of animals was recorded over a 6 day period in each week and group mean daily food intakes were calculated weekly for the first 16 weeks and for 1 week in 2 thereafter. Blood samples were collected from all main study animals for hematology at the end of the study or before death, where possible, for decedents. Blood samples were analysed for the hematological parameters listed below.

Red blood cell count (RBC)
Platelet count (Plate)
Total leukocyte counts (WBC)
Leukocyte differential count
- Neutrophil (Neut)
- Lymphocytes (Lymph)
- Monocytes (Mono)
- Eosinophils (Eosin)
- Basophils (Baso)

- Large unstained cells (Luc)

APPEARS THIS WAY
ON ORIGINAL

All main study animals that survived until scheduled sacrifice and main study animals that were found dead (starting at week 8) or were sacrificed *in extremis* were subjected to a complete necropsy. The following organs from the surviving 10 males and 10 females with the lowest identification numbers in each group were weighed at termination.

Adrenals	brain	Epididymides
Heart	kidney	liver + gall bladder
Lung	ovaries	pituitary
Prostate	spleen	testes
Thymus	uterus	

The following tissues were examined histologically.

adrenals	rectum
aorta	salivary gland
brain (3 sections)	sciatic nerve
caecum	seminal vesicles
colon	site of mammary gland
duodenum	skeletal muscle
epididymides	skin
eyes (incl. optic nerves)	spinal cord (3 levels)
femur (incl. marrow)	spleen
Harderian glands	stomach
head*	submandibular lymph node
heart	testes
ileum	thymus
jejunum	thyroids (incl. Parathyroids)
kidneys	tongue
liver + gall bladder	trachea
lungs (incl. mainstem bronchi)	urinary bladder
mesenteric lymph node	uterus
oesophagus	vagina
ovaries	
pancreas	
pituitary	all tumours and masses
prostate	
all gross lesions	

Two cross sections (1 level) of the nose (apical area) from animals in control group 1 and animals given 4500 mg/kg/day were examined histologically. The noses of animals from the remaining treatment groups were stored in fixative.

Plasma Exposure: Blood samples (approximately 1 ml per animal) were obtained from satellite animals (2 mice/sex/treatment group/time point) at 07.00, 15.00 and 23.00 hrs in weeks 2, 8, 26 and 52 for determination of plasma drug levels. Plasma concentrations of bosentan and its metabolites Re 48-5033, Re 47-8634, and Re 64-1056 were determined.

Interim Sacrifices: Satellite animals were sacrificed after obtaining blood samples in weeks 2, 8, 26 and 52.

Results

Mean dose levels achieved with dietary administration of bosentan were within 1% and 5% of the target doses for main study and satellite animals, respectively.

Main Study Animals

Target Dose level (mg/kg/day)	100	100	450	450	2000	2000	4500	4500
Mean achieved dose level (mg/kg/day)	99	99	446	451	2005	2016	4468	4535
Range (mg/kg/day)								

Satellite Animals

Target Dose level (mg/kg/day)	100	100	450	450	2000	2000	4500	4500
Mean achieved dose level (mg/kg/day)	105	102	470	472	2105	2052	4638	4573
Range (mg/kg/day)								

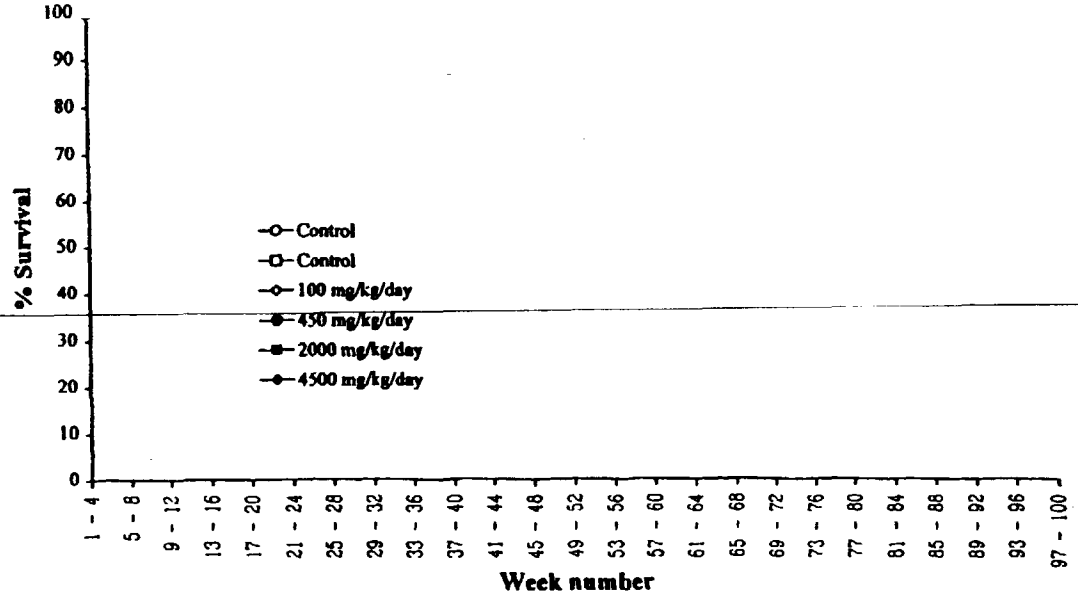
Bosentan administration did not affect survival, as shown in the following table and figures. Sufficient animals survived until scheduled sacrifice for tumor analysis. All males were sacrificed during week 100, due to low survival in male mice given 450 mg/kg/day; only 10 male mice given 450 mg/kg/day survived until week 100. There was no relationship of survival to dose given to male mice since survival in mice given 4500 mg/kg/day exceeded survival seen in mice given 450 and 2000 mg/kg/day.

Female mice were treated for the complete 104 weeks of scheduled drug treatment.

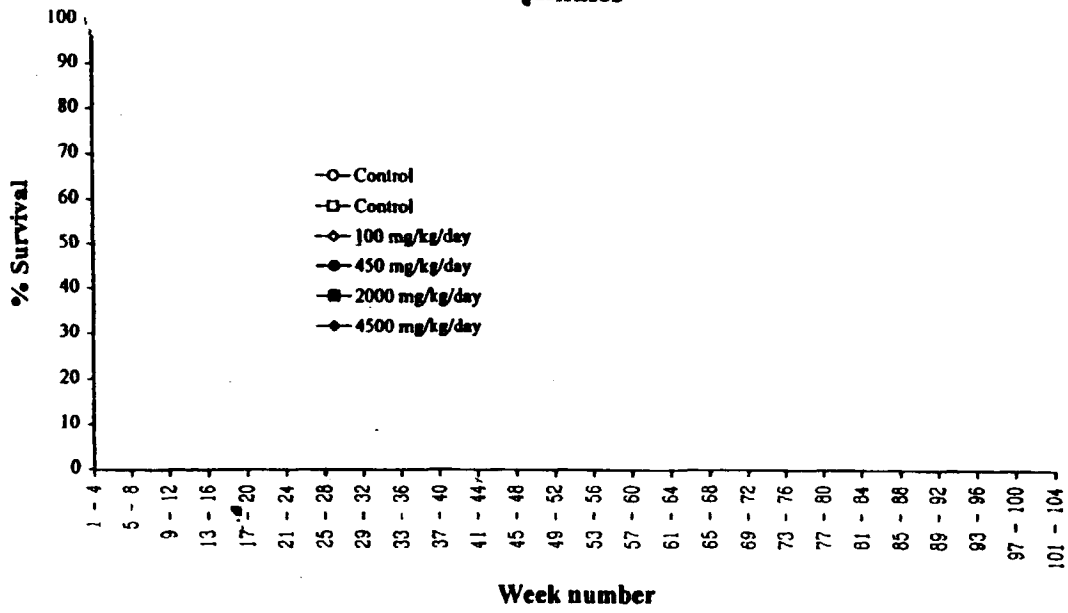
Number of mice that survived until scheduled sacrifice at week 104

Sex	Treatment week	Ro 47-0203/029 (mg/kg/day)					
		0	0	100	450	2000	4500
Male	Week 100	14	15	13	10	11	18
Female	Week 104	19	14	18	22	14	13

Survival (% of original group size) - main study males

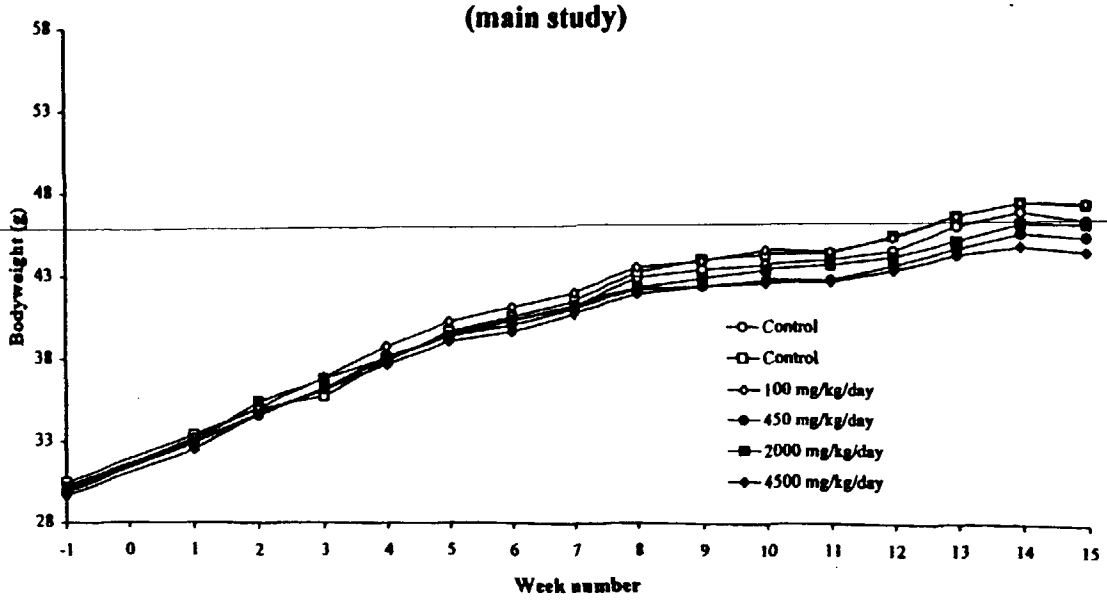


Survival (% of original group size) - main study females

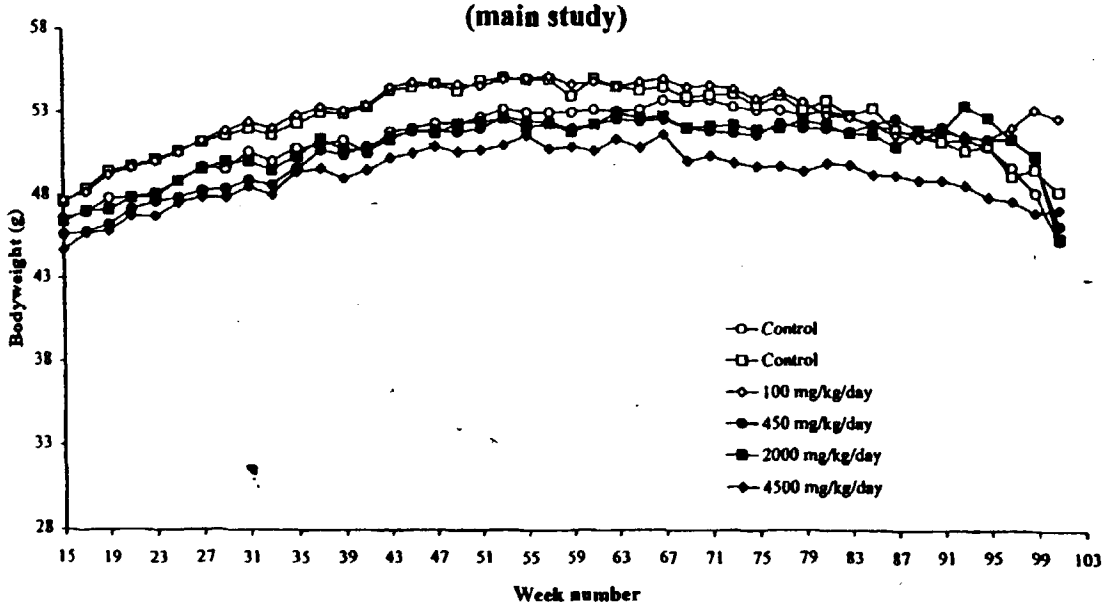


Body Weights: In male mice given bosentan at doses up to 4500 mg/kg/day, body weights were unrelated to treatment.

Group mean bodyweights (g) - weeks -1 - 15 males (main study)

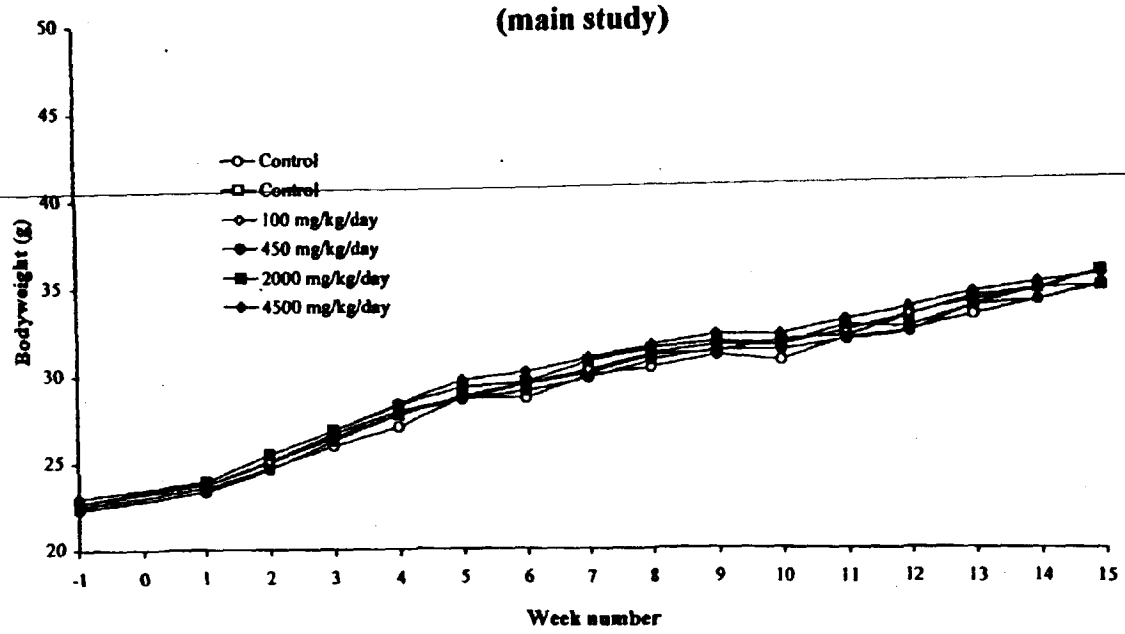


Group mean bodyweights (g) - weeks 15 - 101 males (main study)

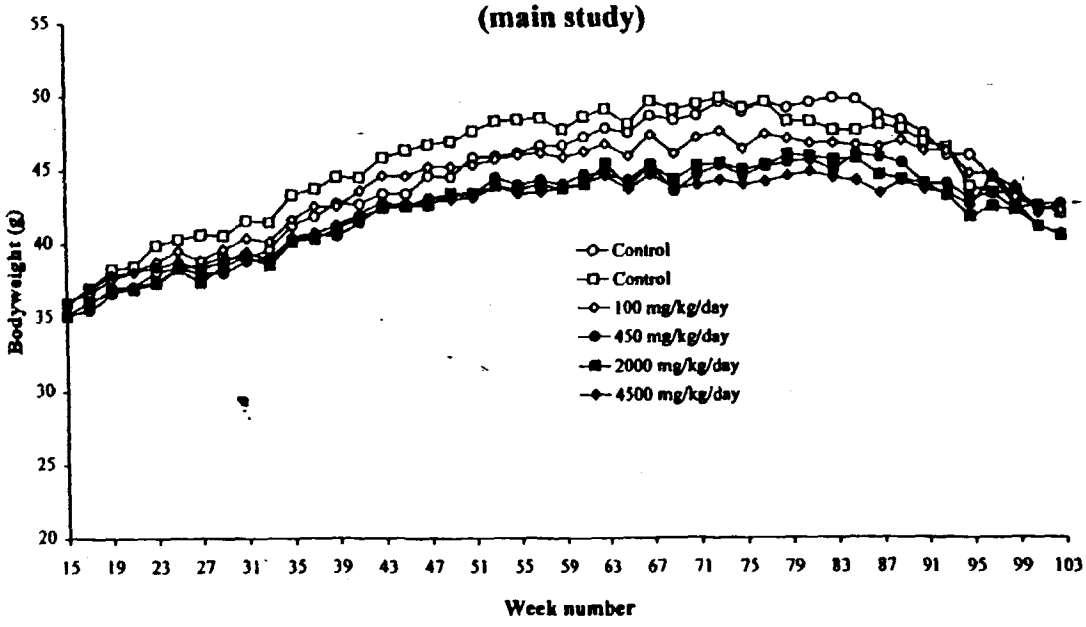


In female mice given 450, 2000 or 4500 mg/kg/day bosentan for 104 weeks, a statistically significant ($p<0.05$) reduction in bodyweight gain was present over the period Week 27 – 39. During Weeks 87 - 99, females given 2000 or 4500 mg/kg/day lost less weight than the Control Group 1, this difference was also statistically significant ($p<0.05$).

Group mean bodyweights (g) - weeks -1 - 15 females (main study)



Group mean bodyweights (g) - weeks 15 - 103 females (main study)



- *Food Consumption:* In male and female mice given 2000 and 4500 mg/kg/day, food consumption was slightly higher than in concurrent controls. This effect was more pronounced in female than in male mice.

Group mean food consumption (g/animal/day)

Group	:	1	2	3	4	5	6
Test article	:	Control			Ro 47-0203/029		
Dose (mg/kg/day)	:	0	0	100	450	2000	4500

Group sex	from: to:	98 98	100 100	Week Interval Mean# 1-16	Mean# 18-28	Mean# 30-40	Mean# 42-52	Mean# 54-64	Mean# 66-76	Mean# 78-88	Mean# 90-100	Mean# 1-100
1M	Mean	6.6	6.0	5.6	5.8	6.5	6.7	6.6	6.2	6.2	6.5	6.3
	S.D.	0.9	0.8	0.3	0.5	0.9	0.8	0.9	0.6	0.6	0.6	0.5
	N	5	4	10	10	10	10	10	9	8	8	10
2M	Mean	6.5	6.1	5.7	5.6	6.0	6.1	6.2	6.2	6.4	6.4	6.1
	S.D.	0.5	1.0	0.4	0.4	0.6	0.6	0.7	0.7	0.8	0.4	0.4
	N	8	8	10	10	10	10	10	10	10	9	10
3M	Mean	7.2	6.6	5.8	5.7	6.0	6.2	6.4	6.3	6.4	6.7	6.2
	S.D.	0.8	0.8	0.3	0.5	0.7	0.7	0.7	0.7	0.7	0.4	0.5
	N	9	7	10	10	10	10	10	10	10	9	10
4M	Mean	7.0	6.3	6.1**	6.0	6.6	6.6	6.6	6.7	6.4	6.9	6.5
	S.D.	1.0	1.0	0.4	0.1	0.6	0.7	0.6	0.4	0.5	0.7	0.4
	N	7	6	10	10	10	10	10	10	10	9	10
5M	Mean	7.0	6.8	6.0**	5.9	6.4	6.6	6.7	7.0*	6.9	7.7*	6.6
	S.D.	1.1	1.1	0.4	0.4	0.6	0.6	0.8	1.0	1.0	1.1	0.6
	N	6	5	10	10	10	10	10	10	9	9	10
6M	Mean	6.7	6.7	6.0*	6.0	6.4	6.5	6.7	6.8*	6.8	6.9*	6.5
	S.D.	0.8	0.8	0.3	0.2	0.4	0.5	0.7	0.7	0.7	0.6	0.4
	N	7	7	10	10	10	10	10	10	10	9	10

- statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001

Group mean food consumption (g/animal/day)

Group	:	1	2	3	4	5	6
Test article	:	Control		Ro 47-0203/029			
Dose (mg/kg/day)	:	0	0	100	450	2000	4500

Group sex	from: to:	98 98	100 100	102 102	104 104	Week Interval Mean# 1-16	Mean# 18-28	Mean# 30-40	Mean# 42-52	Mean# 54-64	Mean# 66-76	Mean# 78-88	Mean# 90-100	Mean# 1-104
1F	Mean	5.2	5.7	5.6	5.2	5.3	4.9	4.9	5.2	5.2	4.9	5.1	5.5	5.2
	S.D.	1.0	0.9	0.7	1.6	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.7	0.4
	N	9	9	9	9	10	10	10	10	10	10	10	10	10
2F	Mean	5.4	5.3	5.4	5.6	5.3	5.0	5.3*	5.4	5.2	5.3*	5.3	5.4	5.3
	S.D.	0.7	1.2	0.7	0.9	0.4	0.2	0.3	0.3	0.3	0.3	0.4	0.5	0.3
	N	10	8	8	8	10	10	10	10	10	10	10	10	10
3F	Mean	5.8	5.8	5.9	5.6	5.4	4.9	5.2*	5.2	5.4	5.1*	5.5	5.9	5.4
	S.D.	0.8	0.6	1.2	0.9	0.3	0.4	0.4	0.4	0.4	0.3	0.4	0.6	0.3
	N	10	10	10	9	10	10	10	10	10	10	10	10	10
4F	Mean	6.1	5.6	5.7	6.0	5.7	5.1	5.5***	5.5	5.4	5.5**	5.8**	5.9	5.6**
	S.D.	0.9	0.5	1.0	0.6	0.5	0.3	0.4	0.5	0.3	0.3	0.5	0.6	0.2
	N	7	7	7	6	10	10	10	10	10	10	10	9	10
5F	Mean	5.9	5.8	6.1	6.1	5.6	5.2**	5.6***	5.6*	5.7**	5.3**	5.6**	5.8	5.7***
	S.D.	0.6	0.7	0.8	0.6	0.5	0.3	0.4	0.3	0.4	0.4	0.5	0.6	0.4
	N	8	8	8	8	10	10	10	10	10	10	10	10	10
6F	Mean	6.3	6.2	6.4	6.4	5.5	5.3**	5.7***	5.7**	5.7**	5.7***	6.1***	6.2*	5.8***
	S.D.	0.5	1.2	1.3	1.4	0.5	0.2	0.5	0.3	0.4	0.3	0.5	0.5	0.4
	N	9	9	8	8	10	10	10	10	10	10	10	10	10

- statistically analysed *=p<0.05 **=p<0.01 ***=p<0.001

Clinical findings: There were no drug-related clinical findings.

Hematology: There were no drug-related changes in hematology parameters.

Organ weights: Liver weights and liver weight/body weight ratios were greater than concurrent control in male mice given bosentan at 2000 mg/kg/day but lower than concurrent control in male mice given 4500 mg/kg/day. Female mice given 2000 and 4500 both showed higher liver and liver/body weight ratios than concurrent control.

Parameter	Gender	Dose (mg/kg/day)					
		0	0	100	450	2000	4500
Liver wt	Male	2.37 ±0.39	2.67 ±0.66	2.96 ±0.89	2.84 ±0.87	4.04** ±1.88	2.82** ±0.74
	Female	2.10 ±0.32	1.94 ±0.39	2.16 ±0.60	2.40 ±0.67	2.62* ±0.37	3.11** ±1.02
Liver wt/body wt ratio	Male	5.21 ±0.88	5.47 ±0.99	6.12 ±1.88	6.04 ±1.94	8.74*** ±4.03	6.28* ±1.48
	Female	5.45 ±0.64	4.95 ±0.91	5.56 ±1.18	6.23 ±1.57	6.88* ±0.69	7.65** ±2.61

*Indicates significant difference from concurrent control at P <0.05
**Indicates significant difference from concurrent control at P <0.01
**Indicates significant difference from concurrent control at P <0.001

Macroscopic findings: Hepatic masses observed macroscopically at necropsy were more frequent in male mice given bosentan at 450, 2000 and 4500 mg/kg/day than in concurrent control.

Gender	Dose (mg/kg/day)					
	0	0	100	450	2000	4500
Male	5	11	15	28	22	22
Female	3	1	1	1	3	1

Non-neoplastic histopathological findings

The incidence of inflammation of the colon was greater in male mice given bosentan at 2000 mg/kg/day and in female mice given 450, 2000 and 4500 mg/kg/day than in concurrent controls. Inflammation of the colon was not observed in concurrent controls. Hyperplasia of the colon and polyps were not observed in male and female mice of any treatment group.

Inflammation of the colon

Gender	Dose (mg/kg/day)					
	0	0	100	450	2000	4500
Male	0	0	0	0	2	0
Female	0	0	0	1	1	1

The incidence of thymus lymphoid hyperplasia was greater in male mice given bosentan at 4500 mg/kg/day than in concurrent controls. The incidence of this finding was not drug-related in female mice.

Thymus lymphoid hyperplasia

Gender	Dose (mg/kg/day)					
	0	0	100	450	2000	4500
Male	1	2	4	4	4	6
Female	1	13	9	5	8	14

In males, alveolar hemorrhage in the lungs was more common at 2000 and 4500 mg/kg/day than in concurrent controls (1, 3, 1, 3, 7, 5 mice at 0, 0, 100, 450, 2000 and 3000 mg/kg/day, respectively).
There were no drug-related findings in the lungs of female mice.

The incidences of testicular tubular atrophy and mineralization were unrelated to treatment. Similarly, the average severity grade of testicular atrophy was not drug-related (2.9, 3.2, 3.3, 2.8, 3.1 and 3.1 for 0, 0, 100, 450, 2000 and 4500 mg/kg/day, respectively). There were no drug-related findings for the epididymides, prostate gland and seminal vesicles.

ORGAN/FINDING	SEX / DOSE GROUP / NO. ANIMALS	01 50	02 50	03 50	04 50	05 50	06 50
TESTES	No.Examin	50	50	50	50	50	50
- Tubular Atrophy		16	10	19	11	17	20
- Tubular Mineralizat.		4	3	3	2	4	1
- Spermatocele		-	1	3	3	1	1
- Sperm Stasis		-	1	-	-	-	-
- Hemorrhage		-	-	-	1	-	-
EPIDIDYMIDES	No.Examin	50	50	49	50	50	50
- Spermatocele		3	2	2	-	-	5
- Oligospermia		4	3	5	3	7	5
- Inflammation		-	-	-	1	-	1
- Aspermia		9	7	14	5	7	14
- Sperm Granuloma		2	1	-	-	-	-
- Cystic tubules		-	-	-	1	-	1
PROSTATE GLAND	No.Examin	50	50	49	50	50	50
- Inflammation		5	4	2	1	3	3
- Glandular Ectasia		10	17	13	5*	17	15
- Arteritis/Periarter.		-	-	-	-	-	1
- Concretions		-	1	-	-	-	-
- Edema		-	-	1	2	-	-

ORGAN/FINDING	SEX / DOSE GROUP / NO. ANIMALS	01 50	02 50	03 50	04 50	05 50	06 50
SEMINAL VESICLES	No.Examin	50	50	50	50	50	50
- Glandular Ectasia		36	36	43*	43*	39	32
- Inflammation		7	3	2	1	2	6
- Fibrosis		3	-	1	-	-	1
- Arteritis/Periarter.		-	-	-	-	-	1

Note: The sponsor's dose group designations of 01, 02, 03, 04, 05, 06 in the following tables refer to mice given bosentan at 0, 0, 100, 450, 2000 and 4500 mg/kg/day, respectively.

Neoplastic findings

In male mice, bosentan significantly increased the incidence of hepatocellular adenomas, carcinomas, and combined adenomas and carcinomas over the incidence seen in the combined concurrent controls. In female mice, the incidences of these tumors in drug treated groups were similar to concurrent control.

In male mice given bosentan at 2000 and 4500 mg/kg/day, and in female mice given bosentan at 4500 mg/kg/day, the incidence of adenomas in the colon was increased over concurrent control. In male mice, the increases were statistically significant by trend but not pairwise analyses. In female mice, the increase was not statistically significant. Adenomas of the colon were not observed in the concurrent controls and are historically rare.

Tumor incidences[8]

Organ/ Finding	Gender	Tumor type	Dose (mg/kg/day)						P-value Trend Test^	Significance
			0	0	100	450	2000	4500		
Liver, hepatocellular tumors	Male	Adenoma (common tumor)	9	10	15 0.1245[a] NS	22 0.0008[a] Signif	20 0.0042[a] Signif	23 0.0007[a] Signif	0.0024	Signif.
		Carcinoma (common tumor)	1	4	1 0.9485[a] NS	7 0.1131[a] NS	7 0.0385[a] NS	9 0.0105[a] Signif	0.0016	Signif.
		Combined (common)	10	11	16 0.1435[a] NS	26 0.0001[a] Signif	23 0.0007[a] Signif	25 0.0004[a] Signif	0.0015	Signif.
	Female	Adenoma (common tumor)	4	3	1	1	3	5	0.0757	NS
		Carcinoma (common tumor)	1	1	0	0	0	0	1.0000	NS
		Combined (common tumor)	5	4	1	1	3	5	0.1425	NS
Colon tumors [b,c]	Male	Adenoma (rare tumor)	0	0	0	0	1	2 0.1190 NS	0.0161	Signif.
		Carcinoma (rare tumor)	0	0	0	0	0	0	-	NS
	Female	Adenoma (rare tumor)	0	0	0	0	0	2 0.1935 NS	0.0518	Signif
		Carcinoma (rare tumor)	0	0	0	0	0	0	-	NS

^ vs combined concurrent control values

[a] p-value for pairwise comparison to combined concurrent control

[b] The sponsor provided two historical control studies in mice (24 month studies) from the same testing laboratory in which bosentan was evaluated. In these two studies, the overall incidence of colon adenomas was 0/160 for both male and female mice. One colon adenocarcinoma was observed in a male mouse; therefore, the overall incidence of colon adenocarcinomas was 1/160 (0.6%) for male mice (0/60 and 1/100 for each study) and 0/160 for female mice. Thus, colon adenomas and carcinomas are rare in male and female CD-1 mice.

[c] In historical control database (March 2000), the incidence of colon adenomas was 0% for both male and female mice, (0/2482 and 0/2645 for male and female mice, respectively) and is therefore considered rare. Adenocarcinomas were also rare in male and female mice (3/2482 (0.12%) in males and 0/2645 in females). Tumor incidences ranged from % to % for male mice when adenocarcinomas were observed in a study.

[8] A tumor is considered to be common if its historical control incidence is >1%. For a common tumor, the required trend test p-value (α) is 0.005 and the required pairwise comparison p-value is 0.01. For a rare tumor, the required trend test p-value (α) is 0.025 and the required pairwise comparison p-value is 0.05.

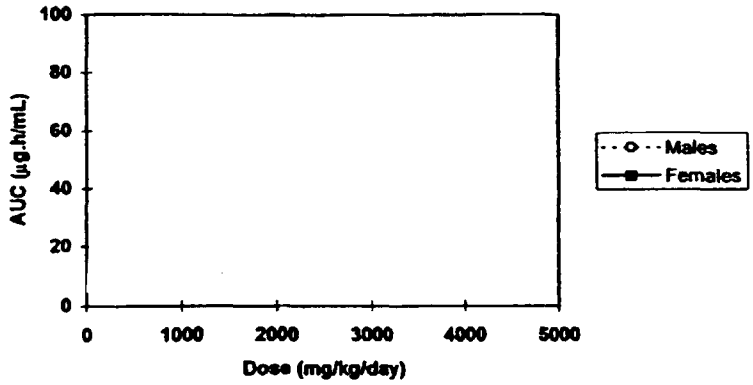
Toxicokinetics

Exposure (AUC) to bosentan (Ro 47-0203) was generally dose-related but less than dose-proportional. At doses of 450 mg/kg/day and above, AUCs exceeded that observed in healthy male humans given 500 mg, qd for 8 days (15.03 μg.h/ml).[9]

Bosentan $AUC_{0\text{-}24hr}$ (μg.hr/ml) during Study Week 52

Dose (mg/kg/day)	Gender	$AUC_{0\text{-}24hr}$ (μg.hr/ml)
100	Male	8.06
	Female	11.7
450	Male	19.4
	Female	26.7
2000	Male	22.8
	Female	41.2
4500	Male	30.4
	Female	55.4

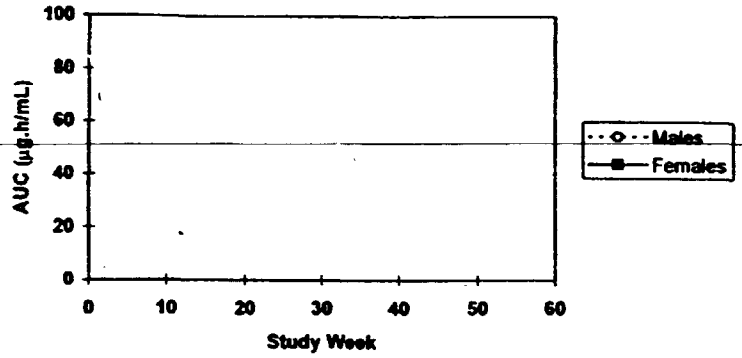
Systemic exposure to Ro 47-0203 (expressed as $AUC_{0\text{-}24h}$) versus oral dose in male and female mice: Study Week 52.



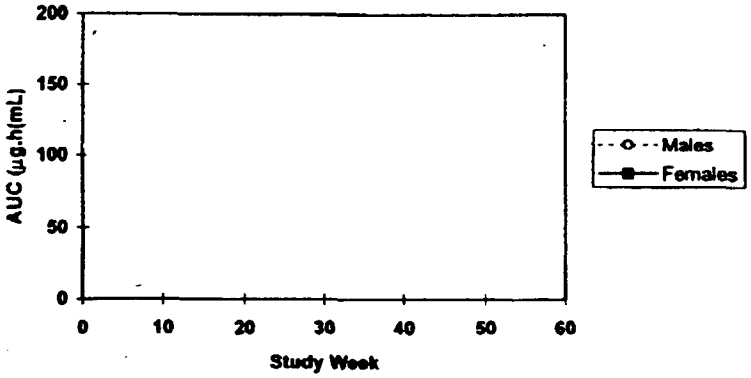
[9] While the maximum therapeutic dose is 250 mg, bid, there is no pharmacokinetic data available for this dosing regime. Additionally, there is no pharmacokinetic data in females, and none in patients with primary pulmonary hypertension. The AUC in primary pulmonary hypertension patients likely exceeds that in healthy humans, based on Dr. Robbie's review of human pharmacokinetics of bosentan.

Plasma bosentan levels (AUCs) decreased over the first year of the study (not determined after the first year) in male and female mice given 2000 and 4500 mg/kg/day. Bosentan exposure did not decrease with continued dosing in mice given 100 and 450 mg/kg/day.

Systemic exposure to Ro 47-0203 (expressed as AUC_{0-24h}) during chronic oral administration in male and female mice: Dose 2000 mg/kg/day.



Systemic exposure to Ro 47-0203 (expressed as AUC_{0-24h}) during chronic oral administration in male and female mice: Dose 4500 mg/kg/day.



Plasma concentrations of the metabolites Ro 48-5033 and Ro 47-8634 in mice were low, attaining mean levels of up to 7% and up to 0.5%, respectively, of the corresponding plasma bosentan levels. Ro 47-8634 was not detected in males, and was detected in female mice only at the highest dose of 4500 mg/kg/day. The plasma levels of metabolite Ro 64-1056 were below the limit of quantitation.

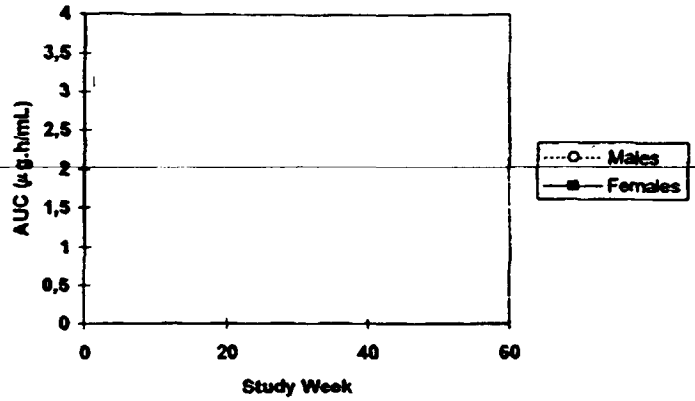
Plasma AUC $_{0\text{-}24\ hr}$ (μg.hr/ml) of Bosentan and its Metabolites during Treatment Week 52

Dose (mg/kg/day)	Gender	Bosentan	Ro 48-5033	Ro-47-8634	Ro 64-1056
100	Male	8.06	BLQ	BLQ	BLQ
	Female	11.7	0.314	BLQ	BLQ
450	Male	19.4	-	BLQ	BLQ
	Female	26.7	0.885	BLQ	BLQ
2000	Male	22.8	-	BLQ	BLQ
	Female	41.2	3.13	BLQ	BLQ
4500	Male	30.4	-	BLQ	BLQ
	Female	55.4	2.98	0.018	BLQ

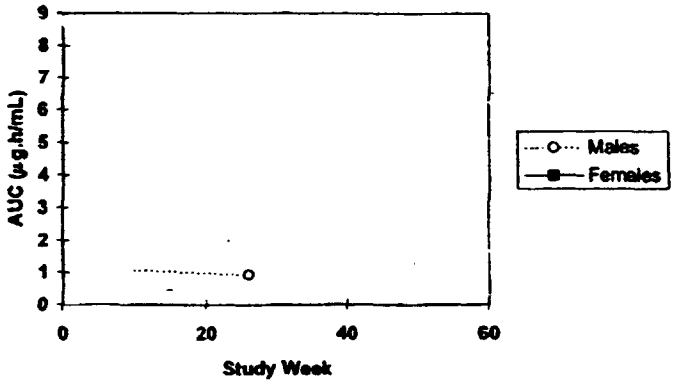
BLQ, below limit of quantitation
-, AUCs were not determined due to undetectible plasma levels at some time points.

In mice given bosentan at 4500 mg/kg/day, plasma exposure to its metabolite Ro 4-5033 decreased over the first year of study (not determined after the first year). This pattern was not evident in mice given bosentan at doses ≤ 2000 mg/kg/day.

Systemic exposure to Ro 48-5033 (expressed as AUC_{0-24h}) during chronic oral administration in male and female mice: Dose 2000 mg/kg/day.



Systemic exposure to Ro 48-5033 (expressed as AUC_{0-24h}) during chronic oral administration in male and female mice: Dose 4500 mg/kg/day.



OVERALL SUMMARY AND EVALUATION

Pharmacodynamics

Bosentan is a competitive inhibitor of endothelin receptors. *In vitro*, bosentan inhibits binding of endothelin-1 to human ET-A and ET-B receptors. Potency on ET-A receptors (Ki = 4.1-43 nM) was slightly greater than potency on ET-B receptors (Ki = 38-730 nM). Functionally, bosentan inhibits contractile effects of endothelin-1 in rat aorta and human internal mammary artery and vein. Oral administration of bosentan to rats inhibits pressor effects of big ET-1, which is the precursor to ET-1. Although bosentan decreases systemic blood pressure in rat models of hypertension, it does not lower blood pressure in normotensive rats and dogs at doses that block pressor effects of exogenous endothelin. Administration of bosentan increases plasma levels of endothelin; the consequence of this increase is not clear.

The sponsor provided preclinical evidence supporting a therapeutic role of bosentan in pulmonary hypertension. Oral administration of bosentan decreased pulmonary arterial pressure in rat models of pulmonary hypertension.[10] The predictive power of these preclinical assays for human therapeutic activity is not known.

Toxicokinetics

Plasma exposures to bosentan and its metabolites were monitored in dogs, rats and mice. Similar to humans, bosentan metabolites comprised a small percentage of drug-related product found in plasma. Although not all metabolites were found in all species evaluated, dogs exhibited all three human metabolites, and Ro 48-5033, a major human metabolite, was found in all three species.

Exposure to bosentan decreased with repeated administration in rats and dogs, similar to findings in humans. This decrease in exposure is likely due to induction of hepatic metabolism. In dogs, bosentan metabolism was 5-fold more rapid in hepatocytes from the livers of dogs given bosentan for 12 months than in hepatocytes from livers of dogs given vehicle over this same time period.

In chronic toxicology studies, dogs given bosentan orally at doses up to 25 times the human therapeutic dose exhibited higher systemic plasma exposures to unchanged parent than rats given bosentan orally at doses up to 50 times the human therapeutic dose (2-year carcinogenicity study) and mice given bosentan orally at doses up to 37 times the human therapeutic dose (2-year carcinogenicity study). All dose comparisons are based on mg/m^2 estimates. Exposure in males and females were similar in dogs, but female rats and mice showed higher exposures than male rats and mice. Animal exposures cannot be compared to exposure in pulmonary hypertensive patients since exposure in these patients has not been determined.

Species	Study Duration	Maximum Oral Dose Evaluated in Chronic Toxicology Study			Sex	Bosentan AUC (μg.h/ml)
		(mg/kg/day)	Human Equivalent Dose* (mg/kg/day)	Multiple of Maximum Recommended Human Dose^		
Dog	12 month	500	250	25	Male	466
					Female	510
Rat	24 month	3000	500	50	Male	119
					Female	255
Mouse	24 month	4500	375	37.5	Male	30
					Female	55

* The human equivalent dose corrects the animal dose for body surface area. It thus provides for dose comparisons based on a mg/M^2 estimate.

^ The maximum recommended human dose is 250 mg, bid (500 mg/day, or 10 mg/kg/day for a 50 kg person). All dose comparisons are based on mg/M^2 estimates.

[10] 100 mg/kg/day orally to rats is equivalent to 1.6 times the maximum recommended human dose of 500 mg/day (250 mg, bid), based on a mg/M^2 estimate.

Plasma protein binding of bosentan in dogs, rats, mice and rabbits was high and similar to or less than that seen in humans.

Species	Bosentan	
	% bound	% free
Human	98.1	1.9
Dog	95.9	4.1
Rabbit	96.0	4.0
Rat	98.5	1.5
Mouse	98.5	1.5

General Toxicology

Bosentan was evaluated for toxicity in mice, rats and dogs. Mice were given bosentan orally by dietary admixture for 13 weeks and 2 years at doses up to 4500 mg/kg/day. Rats were given bosentan orally by dietary admixture for 4 weeks, 6 months and 2 years at doses up to 1500 mg/kg/day, 1000 mg/kg/day, and 3000 mg/kg/day, respectively. Rats were also given bosentan orally by gavage for 4 weeks at doses up to 2000 mg/kg/day. The carcinogenicity studies in mice and rats utilized the highest bosentan doses and durations of exposure. Dogs were given bosentan orally by gavage for 4 weeks, 6 months and 12 months at doses up to 1000, 400 and 500 mg/kg/day, respectively. Sample sizes were small in the dog studies; only two dogs were given 1000 mg/kg in the 4 week study, and 4 dogs were utilized per treatment group in the 6 and 12 month studies. Repeat dose intravenous administration studies were conducted in rats (4 week duration at doses up to 40 mg/kg/day) and dogs (7 day duration at doses up to 200 mg/kg/day).

Hepatic findings

Bosentan adversely affected the liver in the 12-month oral toxicity study in dogs, with elevated mean serum alkaline phosphatase (ALP) levels seen in male and female dogs given doses of 180 and 500 mg/kg/day. In one of four male dogs given 500 mg/kg/day (25 time the maximum recommended human dose on a mg/M^2 basis), marked increases (25-30 fold) in ALP, alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and glutamate dehydrogenase (GLD) were observed, along with a 2-3 fold elevation of aspartate aminotransferase (AST). Clinical signs were not observed at the time of maximum liver enzyme elevations. Elevated transaminases were preceded by small (2-fold) increases in ALP at earlier time points. Clinical signs were noted in this high dose animal approximately 2-months after transaminases were observed to be markedly elevated.[11] Although liver transaminases were elevated on the day of clinical signs, increases were moderate, and bilirubin was not increased. Clinical signs appeared to be test agent related, since upon cessation of treatment with bosentan, this dog recovered, and when rechallenged, clinical signs reappeared; liver enzymes were not monitored when clinical signs reoccurred. Clinical signs did not reappear after bosentan administration was terminated permanently. At sacrifice 38 days after treatment termination, serum enzymes were only slightly elevated in this dog compared to baseline, and there was no evidence of hepatic necrosis or fibrosis. Consequently, liver effects appeared reversible.

The pattern of liver enzyme increases seen with bosentan suggests primary biliary obstruction with secondary hepatocyte necrosis, or cholestatic hepatotoxicity, since increases in ALP preceded increases in ALT, AST and GLD. Increases in ALP and GGT are indicative of biliary injury, and are not elevated in cases of primary hepatocyte necrosis. Consistent with this interpretation is pigmentation of bile duct canaliculi and gallbladders observed histopathologically in dogs given the highest dose. Additionally, biliary secretion appeared to be inhibited by bosentan, since biliary cholesterol was reduced by higher doses (9 and 25 times the recommended human dose), and biliary phospholipids and bile acids were reduced. Finally, non-cholesterol (dark, friable) gallstones were observed

[11] This high dose dog was found recumbent in the kennel 1.5 hours after dosing, was apathetic, sedated and the nictitating membrane was prolapsed. The dog appeared dehydrated with enophthalmia and exsiccosis, delayed capillary refilling and pale mucus membranes. Heart rate was 60 beats per minute, arrhythmic and breathing rate was 20 per minute. An ECG showed sinus bradycardia, which is normal for conscious dogs. Symptoms were absent the following morning, but test agent was discontinued for 3 days.

in one of four female dogs given 9 times the recommended human dose, suggesting the possibility of biliary obstruction. The lack of bile duct proliferation in dogs given the highest dose indicates that chronic biliary obstruction did not occur in this study.

Liver effects were also seen in rats and mice, as well as shorter-term studies in dogs.

Species	Adverse Liver Findings with Bosentan	Oral Dose			Study Duration
		mg/kg/day	Human Equivalent Dose (mg/kg/day)	Multiple of Maximum Recommended Human dose*	
Dog	Elevated Alkaline Phosphatase	400	200	20 X	6 Months
	Periacinar Hypertrophy	400	200	20 X	
	Liver Hypertrophy	60	30	3X	
	Increased ALT, Alkaline Phosphatase and liver wt	1000	500	50 X	4 Weeks
	Bile duct proliferation, single cell necrosis				
Rat	Increased liver wt	1000	166	16 X	6 Months
		125	20	2X	24 Months
Mouse	Increased liver weight	2000	166	16X	24 Months
	Hepatocellular tumors	4500	375	37.5	24 Months

*The maximum recommended human dose is 250 mg, bid (500 mg/day, or 10 mg/kg/day for a 50 kg person).

The sponsor suggests that inhibition of the canalicular bile salt export pump, with resultant accumulation of bile salts, accounts for the adverse liver findings. This is supported by elevated serum bile acids and alteration of bile composition in dogs given bosentan for 12 months.

Anemia

Bosentan affected erythroid and coagulation parameters in male and female dogs in the 12-month toxicity study. Marked transient reductions in RBCs, hemoglobin and platelets, and increases in fibrinogen and reticulocytes were observed in one of four female dogs given the highest dose (500 mg/kg/day or 25 times the maximum recommended human dose based on a mg/M^{2}estimate) coincident with clinical signs of toxicity. This female dog recovered after daily oral dosing with bosentan was temporarily discontinued. A much lower transient 14% decrease in hemoglobin and hematocrit coincident with a marked increase in reticulocytes was also seen in one of four male dogs given the highest dose. Changes in erythroid parameters observed in the 12-month study in dogs are consistent with small decreases in hemoglobin and hematocrit seen previously in 6-month oral toxicity studies in dogs and rats given ≥3 times the recommended human dose. Findings in animals are consistent with clinical findings of anemia observed in patients.

The sponsor suggests that decreases in hemoglobin and hematocrit seen in animals and humans are due to a pharmacodynamic effect of bosentan to reduce vascular permeability (since endothelin-1 increases vascular permeability), and therefore increase plasma volume, i.e., a dilutional effect. Although this hypothesis might account for bosentan's effects on hemoglobin and hematocrit, other cellular components did not appear to be decreased along with hemoglobin and hematocrit. Additionally, this hypothesis seems unlikely to account for the marked (75%) and moderate (14%) transient reductions in hemoglobin and hematocrit, and marked increases in reticulocytes observed in high dose dogs in the 12 month oral toxicology study.

Testicular tubular atrophy

In rats given bosentan orally in a 2-year carcinogenicity study, the incidence of testicular tubular atrophy (atrophy of the seminiferous tubules) was increased at all doses administered (125-3000 mg/kg/day, 2-50 times the maximum

recommended human dose).[12] While this finding was not dose-related, plasma exposures (AUCs) appeared to plateau at doses at which testicular atrophy was observed. Additionally, AUCs at the higher doses (500-3000 mg/kg/day) were only 2-3 fold higher than that seen at the lowest dose given. The no observed adverse effect level for testicular atrophy in rats given bosentan orally for 2 years was less than 125 mg/kg/day, or less than 2 times the maximum recommended human dose based on a mg/M^2 estimate.

Marked testicular tubular atrophy was also observed in one of eight rats given bosentan at 16 times the recommended human dose for 4-weeks (1000 mg/kg/day, mid dose group), while severe testicular tubular atrophy was observed in rats given 3 times the recommended human dose (200 mg/kg/day, mid dose treatment group) for 26 weeks (2 of 2 rats examined). In both the 4- and 26-week studies, testicular tubular atrophy was not observed in concurrent control or high dose groups. These findings are considered to be unrelated to bosentan treatment since they are not dose-related.

In contrast to findings in rats given bosentan for two years, the incidence of testicular tubular atrophy was not increased in mice given bosentan (up to 4500 mg/kg/day or 37 times the maximum recommended human dose on a mg/M^2 basis) orally for two years. However, AUCs in mice were at best similar to, and generally lower than those observed in rats at doses associated with testicular tubular atrophy. AUCs in mice given the highest dose were similar to those observed in rats given the lowest dose associated with testicular tubular atrophy. The incidence of testicular tubular atrophy was not increased in dogs given bosentan orally for 6 or 12 months. Although AUCs in dogs were considerably higher than those observed in rats in which testicular atrophy was observed, sample sizes in the dog studies were extremely small (4 dogs/sex/dose), and may have been insufficient to discern this effect.

Histopathologically, oligospermia was observed in the testes in one of two dogs given bosentan orally (at 1000 mg/kg/day or 50 times the maximum recommended human dose) for 4-weeks; the sponsor attributed this finding to sexual immaturity (dogs in this study were 9 months of age at sacrifice). Sperm counts, sperm motility, and fertility of male rats were not affected by bosentan given orally (at doses up to 1500 mg/kg/day or 25 times the maximum recommended human dose) for 4-6 weeks.[13]

In conclusion, bosentan increased the incidence of atrophy of the seminiferous tubules of the testes in rats when given orally for two years at doses ≥125 mg/kg/day (twice the maximum recommended human dose based on a mg/M^2 basis). A no effect level for this finding was not observed. Bosentan given orally for similar durations to mice and for shorter durations to rats and dogs (up to 6-months and one year, respectively), did not increase the incidence of testicular atrophy. Bosentan given orally to rats for 4-6 weeks at doses up to 25 times the maximum recommended human dose did not affect sperm number or motility.

Reproductive Toxicology

Bosentan was teratogenic and fetotoxic when given orally to pregnant rats during the period of organogenesis at doses as low as 60 mg/kg/day (similar to the maximum recommended human dose on a mg/m2 basis). Agenesis of the palate, craniofacial abnormalities (including shortened, misshapen mandibles, fusion of the pterygoid process with the tympanic annulus, abnormal zygomatic arch, shortened tongues, anophthalmia and microphthalmia) and blood vessel variations (abnormal origin of the right subclavian and innominate arteries) were observed in litters from bosentan-treated dams. Effects were dose-related.

Stillbirths were increased and survival of pups was markedly decreased in a dose-related way in litters from dams given 1 and 5 times, respectively, the maximum recommended human dose. The duration of gestation was slightly increased (by 1 day) at 5 times the maximum recommended human dose. There were no doses at which adverse reproductive effects were not observed.

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[12] The incidence of testicular tubular atrophy ranged from % in rats given bosentan for two years vs in concurrent controls. All severity grades appeared to be increased by bosentan.

[13] Intravenous administration of bosentan to male and female rats at doses of 10, 20 or 40 mg/kg/day (twice daily administration at 6 hour intervals) for 4-6 weeks (male) or 2 weeks (female) confirmed lack of drug effect on sperm counts, sperm viability and male and female fertility.

Adverse Developmental Finding	Lowest Dose Observed		No Observed Adverse Effect Level	
	mg/kg/day	Multiple of Maximum Recommended Human Dose*	mg/kg/day	Multiple of Maximum Recommended Human Dose*
Agenesis of the palate	120	2X	60	1X
Bent hyoid process, misshapen hyoid bone	60	1X	<60	<1X
Misshapen mandibles, abnormal zygomatic arch, shortened tongue, fusion of pterygoid process with tympanic annulus	1500	25X	300	5X
Anophthalmia/microphthalmia	300	5X	60	1X
Stillbirths^	60	1X	<60	<1X
Decreased pup survival	300	5X	60	1X
Increased gestation duration	300	5X	60	1X

* The maximum recommended human dose is 250 mg, bid (500 mg/day, or 10 mg/kg/day). Dose comparisons are based on a mg/M^2 estimates.

^ Sponsor likely overestimated stillbirths.

In utero exposure appears necessary for postnatal lethality in rats, since a litter exchange study showed litters of drug treated dams to survive poorly. In contrast, survival of litters from untreated dams that were raised by dams receiving bosentan was no different than concurrent control.

Litters of pregnant rabbits given bosentan during the period of organogenesis showed adverse effects of bosentan on fetal body weight, but only in the presence of maternal toxicity (as evidenced by maternal body weight losses during gestation and decreased food intakes). Although there was no evidence of bosentan-related teratogenicity, systemic exposure to bosentan in rabbits (AUCs) was less than in rats given teratogenic doses of the drug. Since AUCs in rabbits given bosentan at 1500 mg/kg/day are likely to be similar to those seen in patients with pulmonary hypertension receiving the maximum recommended human dose of 250 mg, bid (500 mg/day), a margin for reproductive toxicity in rabbits cannot be determined.[14]

Adverse reproductive findings observed with bosentan are likely a class effect of endothelin receptor antagonists, and are likely due to endothelin receptor blockade, since other endothelin receptor antagonists and a knockout model for endothelin shows similar effects.[15] Consequently, adverse developmental effects appear probable at bosentan doses required for therapeutic activity.

Genotoxicity

Bosentan was negative for genotoxicity in both *in vitro* and *in vivo* assay systems recommended in the ICH guidelines for evaluation of genotoxicity. Bosentan was negative for mutagenicity *in vitro* in the Ames bacterial mutagen assay, the Chinese hamster lung (V79/HPRT) assay and the rat hepatocyte unscheduled DNA repair assay. Bosentan was negative for clastogenicity *in vitro* in human peripheral lymphocytes, and *in vivo* in mice (the mouse micronucleus assay).

[14] Steady state bosentan AUC in healthy male humans receiving single daily doses of 500 mg for 8 days was 15 μg.hr/ml. (Dr. Robbie's Biopharmaceuticals review, pg 61, Report No. B-159037)

[15] Treinen KA etal., *Teratology*. 59: 51-59 (1999).
Kurihara Y etal., *Nature*. 368: 703-710 (1994).
Kurihara Y etal., *J Clin Invest*. 96: 293-300 (1995).
Lehrer SB etal., *Teratology*. 55: 42 (1997).

Carcinogenicity

Mouse Carcinogenicity Study

A 104 week carcinogenicity study was performed with bosentan administered by dietary administration to male and female mice at doses of 100, 450, 2000 and 4500 mg/kg/day. The sponsor evaluated 4500 mg/kg/day as the maximum dose per EC-CAC's recommendations. There were no significant affects of bosentan on male or female survival or male body weight gain (minor effect on female body weight gain).

There was a significant trend for hepatic adenomas, carcinomas and combined adenomas and carcinomas in male mice. Pairwise comparisons showed statistical significance for 450 mg/kg/day vs combined concurrent control for combined adenomas and carcinomas.

There was a significant trend for colon adenomas in male mice (0/50 and 0/50 for controls and 0/50; 0/50: 1/50; 2/50 for dosed groups). These neoplasms are considered rare. The contract laboratory's historical control incidence for colon adenomas in male mice was 0 of 160; a colon adenocarcinoma was observed in one of these mice. Colon adenomas and adenocarcinomas were even less common in historical control database for male mice (3/2482, all adenocarcinomas). In the present study, colon adenomas were seen only in the two highest dose male groups and the high dose female group. Adenocarcinomas of the small intestine were seen in one of two male concurrent control groups and in one of two female concurrent control groups.

There were no statistically significant neoplasms in female mice, but 2/50 female mice at the highest dose had adenomas in the colon.

Rat Carcinogenicity Study

A 104 week carcinogenicity study was performed with male and female Wistar rats given bosentan orally by dietary administration at doses of 125, 500, 2000 and 3000 mg/kg/day. The sponsor evaluated 3000 mg/kg/day as the maximum dose per EC-CAC's recommendations, using the maximum feasible dose as the dose selection criterion. Body weights of female rats given doses ≥500 mg/kg/day were significantly lower than concurrent control. There were no significant effects of bosentan on male or female survival. Toxicokinetics showed AUCs to plateau in male rats given ≥500 mg/kg/day.

In male rats, there was a significant trend for thyroid follicular cell adenomas, but not for carcinomas or combined adenomas and carcinomas. Pairwise comparisons showed thyroid follicular cell adenomas in male rats given 2000 and 3000 mg/kg/day to be significantly different than concurrent control. The Executive CAC considered the thyroid follicular adenomas to be unrelated to bosentan administration because of the lack of effect on thyroid follicular carcinomas and combined adenomas and carcinomas.

Brain astrocytomas were observed in male rats given 500, 2000 and 3000 mg/kg/day (2/50; 1/50; 2/50) and were absent from both male concurrent control groups. Although there was no significant trend, the incidence at 3000 mg/kg/day (4%) was significantly greater than control. The contract laboratory's historical control incidence for astrocytomas in male Wistar rats showed this tumor to be relatively rare. Recent literature references support this conclusion (incidences range from % with overall incidence of about %). Skin keratoacanthomas (common in this strain) were non-significantly increased in male rats given 500 and 3000 mg/kg/day compared to concurrent controls.

There were no statistically significant tumors in female rats. A brain astrocytoma was seen in one female rat at 3000 mg/kg.

CONCLUSIONS

Bosentan is teratogenic in rats at doses similar to or less than the maximum recommended human dose (on a mg/M2 basis). Teratogenicity is likely a class effect of endothelin receptor antagonists and is likely to be observed in humans given the maximum recommended human dose of 250 mg, bid.

Bosentan administration was associated with an increased incidence of tumors in rats (astrocytomas) and mice (hepatic adenomas and carcinomas, and colon adenomas).

Bosentan tested negative for genotoxicity in adequately conducted *in vitro* and *in vivo* assays.

Bosentan exhibited hepatotoxicity in dogs at doses that are high compared to the maximum recommended human dose (25 times the maximum recommended human dose on a mg/M^2 basis).

Bosentan increased the incidence of atrophy of the seminiferous tubules of the testes when given to rats for 24 months. The no observed effect level for testicular atrophy was < 2 times the maximum recommended human dose on a mg/M^2 basis.

RECOMMENDATION REGARDING APPROVABILITY

From a pharmacology perspective, bosentan is recommended for approval of the treatment of pulmonary arterial hypertension, despite the above-mentioned toxicities, because of the seriousness of the proposed indication and the lack of alternative oral therapy.

LABELING CONSIDERATIONS

(Based on label submitted on August 17, 2001)

Note: Animal:human dose ratios shown below are based on a maximum recommended human dose (MRHD) of 250 mg, bid (500 mg/day), with doses corrected for surface area (mg/m^2 dose estimates). Exposure ratios based on AUCs were not calculated since the AUC in pulmonary hypertensive patients given bosentan at 250 mg, bid is unknown.

CLINICAL PHARMACOLOGY

The proposed clinical pharmacology section should be changed to delete statements related to animal findings in pathological models since relevance of animal findings to human outcome is unknown. Additionally, statements suggesting efficacy in additional disease states, e.g., congestive heart failure, are irrevelant to the indication being considered for approval (pulmonary arterial hypertension).

The label presently reads as follows:

DRAFT

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

The pregnancy section should be changed to reflect a change in pregnancy category (from pregnancy category C to pregnancy category X), dose comparisons on a mg/m^2 basis, and low exposures in pregnant rabbits compared to pregnant rats.

We recommend that the statement be changed to read as follows:

Pregnancy Category X. *See* ***'Contraindications'*** *section.*

/S/

John E. Koerner, Ph.D:
Pharmacologist

cc. HFD-110
HFD-110/ZMcDonald
HFD-110/CResnick
HFD-110/JKoerner
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Accepted by CAR on 8/30/01

Statistical Review and Evaluation

Review of Rat Carcinogenicity Study
(ADDENDUM)

NDA#:	**21-290**
APPLICANT:	**Actelion Ltd.**
NAME OF DRUG:	**Tracleer (bosentan monohydrate)**
INDICATION:	**Pulmonary Arterial Hypertension**
STUDIES REVIEWED:	**T-00.009, two-year carcinogenicity study in rats, and T-00.008, two year study in mice; Data were Submitted Electronically.**

PHARMACOLOGY REVIEWER: John Koerner, Ph.D. (HFD-110)

STATISTICAL REVIEWER: Roswitha Kelly, M.S. (HFD-710)

1. Introduction

After the completion of the Statistical Review and Evaluation of the carcinogenicity studies for bosentan (08/29/01) there was continued concern whether the findings for astrocytoma in the brain of male rats were statistically significant. The ambiguity arose from the fact that the astrocytomas occurred in both the incidental and the fatal context. When a tumor occurs in both contexts in the same time interval the exact test is no longer accurate and the asymptotic test may be too conservative (indicating significance) when the number of tumors involved (i.e. the number of animals with the tumor) is small. Therefore, in such cases it may be difficult to pin down statistical significance when the exact test indicates lack of significance and the asymptotic test indicates statistical significance. This Addendum resolves this uncertainty.

2. Results

The following table represents the findings of astrocytoma in the brain of male rats treated with bosentan:

Table 1: Incidence of Astrocytoma in the Brain of Male Rats

TIME (WEEK)	CONTROL 1	CONTROL 2	125 MG	500 MG	2000 MG	3000 MG
92-103 *	0	0	0	0	1	0
	5	6	8	11	8	5
104-108	0	0	0	3	0	1
	38	43	36	31	35	37
81	0	0	0	0	0	1**
	47	50	46	46	46	45

* The first row of each time interval reflects the number of animals with the tumor, the second row the number of animals without the tumor.
** The fatal tumor.

As can be seen from Table 1, the fatal tumor, which occurred during week 81, did not fall into any of the intervals where the incidental tumors were found. Therefore, there is no overlap of time of the fatal and incidental tumors and the p-values from the exact permutation tests are the appropriate ones, whereas the asymptotic tests give only a conservative approximation to it. This leads to the conclusion that neither the trend nor any of the pair-wise comparisons performed reached statistical significance for astrocytoma in the brain of male rats treated with bosentan (Table 2):

Table 2: P-Values from Exact Permutation Tests for Astrocytoma Among the Male Rats

TEST	P-VALUE FROM EXACT PERMUTATION TEST	ALPHA LEVEL	CONCLUSION
TREND	0.0595	0.025	NS
C1,C2 vs. Medium	0.0820	0.05	NS
C1,C2 vs. High	0.4500	0.05	NS
C1,C2 vs. Max	0.1027	0.05	NS
C1,C2 vs. (Med, High, Max)	0.0734	0.05	NS

3. Summary

It was observed that the fatal and incidental astrocytomas of the brain among the male rats treated with bosentan occurred in non-overlapping time intervals. Therefore, the p-values associated with the exact permutation tests are the appropriate ones and indicate that in no situation did they reach statistical significance.

/S/ 10/1/01

Roswitha Kelly, MS

10/2/01

/S/

George Chi, Ph.D.

Cc: Archival NDA 21-290
HFD-110/Ms. McDonald
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HFD-710/Ms. Kelly

This addendum consists of 3 pages. 10/01/01

Statistical Review and Evaluation

Review of the Mouse Carcinogenicity Study

NDA#:	**21-290**
APPLICANT:	**Actelion Ltd.**
NAME OF DRUG:	**Tracleer (bosentan monohydrate)**
INDICATION:	**Pulmonary Arterial Hypertension**
STUDIES REVIEWED:	**T-00.009, two-year carcinogenicity study in rats, and T-00.008, two year study in mice; Data were Submitted Electronically.**

PHARMACOLOGY REVIEWER: John Koerner, Ph.D. (HFD-110)

STATISTICAL REVIEWER: Roswitha Kelly, M.S. (HFD-710)

Table of Contents

Note on Levels of Statistical Significance:

Trends in inter-current mortality are tested for statistical significance at α=0.05. Trends in tumor incidence rates are tested for statistical significance at α=0.025 and 0.005 for rare and common tumors, respectively. These levels of significance ensure an overall false positive rate of about 10 percent in the two-year two-species two-gender bioassay despite the multiplicity of testing. If pair-wise comparison of tumor incidences are performed as well, they are tested at α=0.05 and 0.01 for rare and common tumors, respectively. The definition of rare or common was based on the occurrence among concurrent controls. It is possible that some rare tumor findings loose their statistical significance if the tumors are re-classified as common based on historical evidence.

1.0 The Mouse Study

1.1 Sponsor's Results

Fifty male and female ( ) mice each received the drug as dietary admix at dose levels of 0, 0, 100, 450, 2000, and 4500 mg/kg/day. Terminal sacrifice was performed during weeks 100 (males) and 104 (females). Food and water were available ad lib. The animals were housed 5 to a cage.

The findings of satellite animals are not reported here as they were not part of the carcinogenicity evaluation of the drug.

All main study animals that were killed or found dead were examined externally and the abdominal, cranial, and thoracic cavities were macroscopically examined. All tissues of all animals were examined microscopically.

The sponsor observed no toxicologically significant effect on mortality, palpable masses, or bodyweights. However, there was a dose related increase in liver weights of both genders at all dose levels and an increase in hepatic masses in male mice treated with the drug at dose levels of 450, 2000, and 4500 mg/kg/day. The increase in the combined incidence of hepatocellular adenomas and carcinomas was statistically significant among the male mice. There was no such increase in hepatic masses or tumors among the female mice. There was no increase in the incidence of tumors at any other site in the treated male or female mice.

1.2 Reviewer's Results

The sponsor examined bodyweight gain and food consumption data for 'any obvious outlying observations' and excluded such from the statisical analyses. This approach does not appear to be statistically sound. However, the statistical findings for bodyweight gain and food consumption have probably little influence on the overall conclusions.

This reviewer agrees with the sponsor that the administration of bosentan at the levels studied did not affect the survival of either gender (see Appendix 'MICE'). There were minor differences in the number of animals surviving till terminal sacrifice, but these did not affect any conclusions.

This reviewer used the same criteria for statistical significance as the sponsor did. However, the p-values of the trend test are somewhat different for the male mice. One obvious case is hepatocellular adenoma in the liver where this reviewer observed 9, 10, 15, 22, 20, and 23 animals in the C1, C2, low, medium, high, and maximum dose groups. The corresponding numbers of the sponsor are 9, 7, 15, 19, 16, and 16. It was determined in telecon with the sponsor that the numbers presented by the sponsor are those for animals with hepatocellular adenomas only, excluding animals, which had both adenomas and carcinomas of the liver. After reconciling these differences, there remains one discrepancy, namely that the sponsor has 23 male mice with hepatocellular adenoma in the medium dose versus this reviewer having only 22. The sponsor explained that one animal (#156) with both hepatocellular adenoma and carcinoma was coded as having only liver carcinoma. Despite these discrepancies, the overall conclusions of statistical significance are consistent between this reviewer and the sponsor. Both concluded statistical significance at the α=0.005 level, the sponsor with p=0.0037, this reviewer with p=0.0024. For hepatocellular carcinoma among the male mice both the sponsor and this reviewer observed 1, 4, 1, 7, 7, and 9 animals. However, this reviewer's p-value was 0.0016 versus the sponsor's of 0.0012. Again, both results indicate statistical significance. The sponsor coded 'combined adenomas and carcinomas of the liver' as a tumor finding and observed the trend statistically significant with p=0.0021. This reviewer found one more medium dose animal with this tumor code than the sponsor (this is a typographical error in the submission according to the sponsor) and had a p=0.0015 associated with this trend test. The discrepancy of the p-values is also due to the fact that the sponsor classified all tumors of the 'combined adenomas and carcinoma of the liver' as 'incidental', whereas this reviewer maintained the incidental or fatal context of each tumor. This reviewer also combined the hepatocellular adenomas and carcinomas separately. The overall incidences of 10, 11, 16, 26, 23, and 25 were reproduced. However, the p-value was 0.0008, which is much smaller than the sponsor's p=0.0021. Again, the overall conclusion of statistical significance is consistent. However, with less dramatic data such differences could fall between statistical significance and non-significance. This reviewer also considered the trend in adenomas in the colon (0, 0, 0, 0, 1, 2) statistically significant (p=0.0161). For the female mice there were no discrepancies found when spot-checking the number of animals with tumors. However, calculated p-values were somewhat different from the sponsor's, again not affecting any conclusions. This reviewer did not observe any statistically significant trends, and when re-calculating the combined hepatocellular adenomas and carcinomas, the same incidences as the sponsor's were observed. Though not reaching statistical significance, it is noted that there were two adenomas of the colon in the highest dose group (p=0.0518). Below is a summary of this reviewer's findings, which were significant in at least one gender:

TISSUE/TUMOR	C1	C2	L	Med	H	Max	MALE	C1	C2	L	Med	H	Max	FEMALE
Liver/Hepato Adenoma	9	10	15	22*	20	23	0.0024	4	3	1	1	3	5	0.0757
Liver/Hepato Carcinoma	1	4	1	7	7	9	0.0016	1	1	0	0	0	0	1.0000
Liver/Comb. Ade+Carc (by sponsor)	10	11	16	26	23	25	0.0015	5	4	1	1	3	5	0.1425
Liver/Comb. Ade+Carc (by reviewer)	10	11	16	26	23	25	0.0008	5	4	1	1	3	5	0.1425
Colon/Adenoma	0	0	0	0	1	2	0.0161	0	0	0	0	0	2	0.0518

*Does not include one animal (# 156) which had both adenomas and carcinomas but was classified as having carcinoma of the liver only.

Statistical Significant Findings for Sub-Comparisons:

TISSUE/TUMOR	C1	C2	L	M	H	Max	MALE	C1	C2	L	M	H	Max	FEMALE
Liver/Hepatocellular Carcinoma	1		1	7	7	9	0.0013	1		0	0	0	0	1.0000
Colon/Adenoma		0	0	0	1	2	0.0239		0	0	0	0	2	0.0675
Liver/Hepatocellular Adenoma	9	10				23	0.0007	4	3				5	0.3023
Liver/Hepatocellular Carcinoma	1	4				9	0.0105	1	1				0	1.0000
Liver/Hepatocellular Ade+Carc ponsor)	10	11				25	0.0004	5	4				5	0.4646
ver/Hepatocellular Ade+Carc (reviewer)	10	11				25	0.0002	5	4				5	0.4646
Liver/Hepatocellular Adenoma	9					23	0.0035	4					5	0.4250
Liver/Hepatocellular Carcinoma	1					9	0.0070	1					0	1.0000
Liver/Hepatocellular Adenoma and Carcinoma (sponsor)	10					25	0.0026	5					5	0.5365
Liver/Hepatocellular Adenoma and Carcinoma (reviewer)	10					25	0.0017	5					5	0.5365
Liver/Hepatocellular Adenoma		10				23	0.0034		3				5	0.2937
Liver/Hepatocellular Adenoma and Carcinoma (sponsor)		11				25	0.0021		4				5	0.4444
Liver/Hepatocellular Adenoma and Carcinoma (reviewer)		11				25	0.0012		4				5	0.4444

2.0 Validity of the Female Mouse Study

As there were no statistically significant (positive) tumor findings among the female mice, the validity of this study needs to be evaluated. In order to address this issue, two questions need to be answered (Haseman, Statistical Issues in the Design, Analysis and Interpretation of Animal Carcinogenicity Studies, Environmental Health Perspectives, Vol. 58, pp. 385-392, 1984):

(i) Were enough animals exposed for a sufficient length of time to allow for late developing tumors?

(ii) Were the dose levels high enough to pose a reasonable tumor challenge in the animals?

The following rules of thumb are suggested by experts in the field: Haseman (Issues in Carcinogenicity Testing: Dose Selection, Fundamental and Applied Toxicology, Vol5, pp. 66-78, 1985) had found that on the average, approximately 50 % of the animals in the high dose group survived a two-year study. In a personal communication with Dr. Karl Lin (HFD-715), he suggested that 50 % survival of the usual 50 initial animals in the high dose group between weeks 80-90 would be considered a sufficient number and adequate exposure. Chu, Cueto, and Ward (Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays, Journal of Toxicology and Environmental Health, Vol. 8, pp. 251-280, 1981) proposed that 'To be considered adequate, an experiment that has not shown a chemical to be carcinogenic should have groups of animals with greater than 50 % survival at one year'. From these sources, it appears that the proportions of survival at weeks 52, 80-90, and at two years are of interest in determining the adequacy of exposure and number of animals at risk.

In determining the adequacy of the chosen dose levels, it is generally accepted that the high dose should be close to the MTD. Chu, Cueto, and Ward (1981) suggest:

(i) 'A dose is considered adequate if there is a detectable weight loss of up to 10 % in a dosed group relative to the controls'.

(ii) 'The administered dose is also considered an MTD if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical'.

(iii) 'In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls'.

In another paper, Bart, Chu and Tarone (Statistical Issues in Interpretation of Chronic Bioassay Tests for Carcinogenicity, Journal of the National Cancer Institute 62, pp. 957-974, 1979), stated that the mean body weight curves over the entire study period should be taken into consideration with the survival curves, when adequacy of dose levels is to be examined. In particular, 'Usually, the comparison should be limited to the early weeks of a study when no or little mortality has yet occurred in any of the groups. Here a depression of the mean weight in the treated groups is an indication that the treatment has been tested on levels at or approaching the MTD.'

Between 22 and 36 percent of the treated females lived till terminal sacrifice at week 104, showing that there was a sufficient number of animals exposed for a sufficient length of time. Survival of the maximum dosed animals compared to the controls was slightly lower (38%, 28%, and 26% for C1, C2, and maximum dose, respectively). Using the sponsor's Table 2.1 and Figures 4 and 6, average bodyweights of C1 and the maximum dosed group were about equal for the first 31 weeks. The C2 group had somewhat higher average bodyweights starting at week 15. From week 33 to week 95 the maximum dosed animals had lower average bodyweights (up to about 10% lower) than the combined

controls. However, these averages are influenced by a higher mortality in the maximum dose group during weeks 53-78. Based on these criteria, there seems to be sufficient evidence that the maximum dose was close to the MTD.

3.0 Summary

In this study male mice were treated for 98 weeks with bosentan in the feed at levels of 0, 0, 100, 450, 2000, and 4500 mg/kg/day. Survival was acceptable with 22 (medium dose) to 36 (maximum dose) percent alive at terminal sacrifice at week 99. Hepatocellular adenoma, carcinoma and combined showed statistically significant trends and pair-wise comparisons. In addition, adenoma of the colon showed a statistically significant trend with both controls and with the second control alone. The female mice received the same doses and terminal sacrifice was performed at week 104 with 22 (medium) to 38 (control 1) percent of the animals alive. There were no statistically significant trends or pairwise comparisons in tumor incidences. Survival was acceptable with about half of the maximum dose animals alive during weeks 80-90. Survival of the maximum dose was slightly lower than control 1 and about the same as control 2. With respect to average bodyweights, the maximum dose animals weighed less (up to 10% less) than the combined controls during weeks 33 to 95, indicating that this dose was probably close to the MTD.

APPENDIX MICE

Table of Content for Male/Female Mice

*Hepatocellular Adenomas and Carcinomas were also combined by this reviewer and are added at the end of each tumor table

Number of Animals
Species: Mouse
Sex: Male

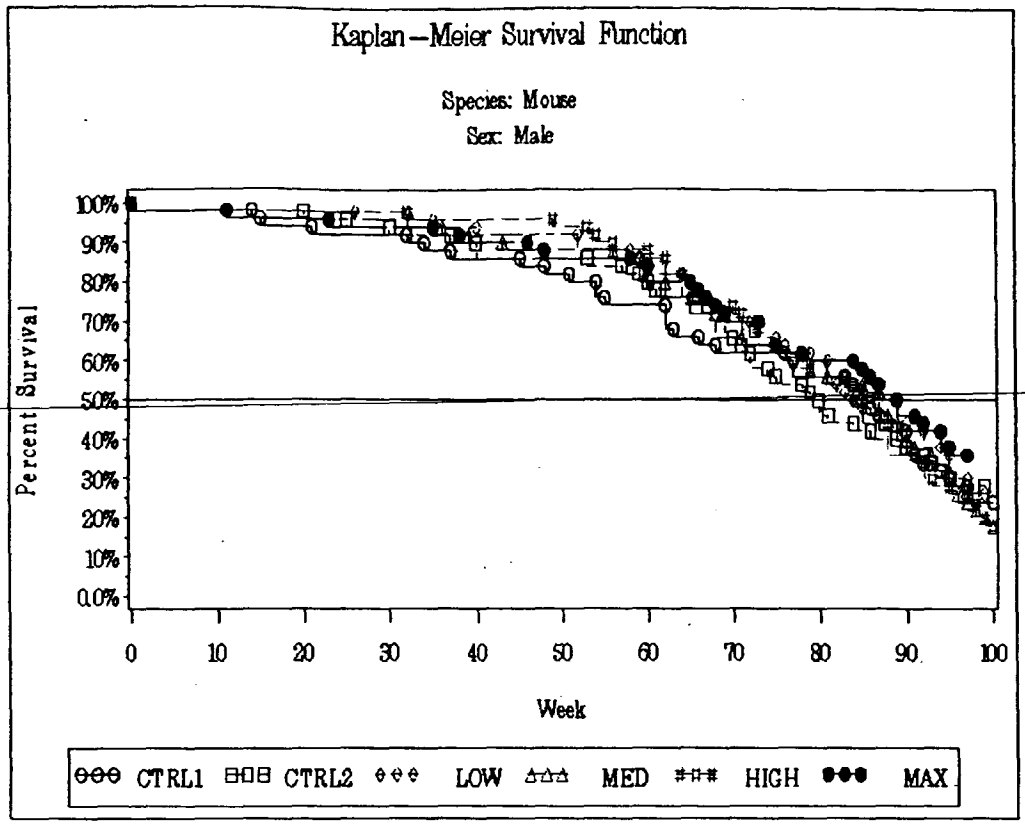
Week	Treatment Group						
	CTRL1	CTRL2	LOW	MED	HIGH	MAX	Total
	N	N	N	N	N	N	N
0-52	9	5	4	5	2	6	31
53-78	11	18	14	14	18	13	88
79-91	12	8	10	12	12	8	62
92-98	4	4	7	8	6	5	34
99-100	14	15	15	11	12	18	85
Total	50	50	50	50	50	50	300

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	1.25	0.2639
	Depart from Trend	1.06	0.9000
	Homogeneity	2.31	0.8046
Kruskal-Wallis	Dose-Mortality Trend	1.15	0.2831
	Depart from Trend	0.62	0.9609
	Homogeneity	1.77	0.8798


Kaplan–Meier Survival Function
Species: Mouse
Sex: Male
Percent Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0.0%
0
10
20
30
40
50
60
70
80
90
100
Week
CTRL1
CTRL2
LOW
MED
HIGH
MAX

Test for Dose-Tumor Positive Linear Trend

Source: Male Mouse Data

Organ Name	Organ Code	Tumor Name	Tumor Code	Natural Rate (in ctrl group)	CTRL 1	CTRL 2	LOW	MED	HIGH	MAX	Tumor type	pValue (Exact)	pValue (Asymp)
AORTA	0520	Lipoma	052001	1%	1	0	0	0	0	0	IN	1.0000	0.7696
LUNGS	0900	Alveolar/bronchiolar carc	090005	18%	12	6	11	7	11	2	MX	0.9922	0.9892
LUNGS	0900	Alveolar/bronchiolar aden	090012	15%	4	11	8	2	2	8	IN	0.5343	0.5432
STOMACH	1500	Squamous cell papilloma	150004	1%	0	1	0	2	0	0	IN	0.7323	0.8178
JEJUNUM	1602	Adenocarcinoma	160203	1%	1	0	0	0	0	0	IN	1.0000	0.7696
COLON	1702	Adenoma	170202	0%	0	0	0	0	1	2	IN	0.0161	0.0029
LIVER	1800	Hepatocellular adenoma	180010	19%	9	10	15	22	20	23	MX	0.0026	0.0024
LIVER	1800	Hepatocellular carcinoma	180016	5%	1	4	1	7	7	9	MX	0.0029	0.0016
LIVER	1800	Hemangioma	180028	0%	0	0	0	0	1	0	IN	0.3523	0.2978
LIVER	1800	Hemangiosarcoma	180031	0%	0	0	0	1	0	0	IN	0.4824	0.6860
LIVER	1800	Combined adenomas and car	180037	21%	10	11	16	26	23	25	IN	0.0015	0.0015
NCREAS	2000	Islet cell adenoma	200007	1%	0	1	0	0	0	0	IN	1.0000	0.7690
ANCREAS	2000	Hemangioma	200009	0%	0	0	1	0	0	0	IN	0.7647	0.7480
KIDNEYS	2100	Tubular cell adenoma	210009	1%	1	0	0	0	1	1	IN	0.1965	0.1444
TESTES	2500	Benign Leydig cell tumor	250003	2%	1	1	1	3	0	0	IN	0.9305	0.9228
EPIDIDYMIDES	2600	Leiomyoma	260010	0%	0	0	0	1	0	0	IN	0.5172	0.6762
SEMINAL VESICLES	2800	Adenoma	280007	1%	0	1	2	0	0	0	IN	0.9212	0.8868
THYROID GLAND	4200	C-cell adenoma	420005	0%	0	0	0	0	1	0	IN	0.3226	0.2693
ADRENAL CORTICES	4401	Hemangioma	440107	1%	0	1	0	0	0	0	IN	1.0000	0.7696
ADRENAL CORTICES	4401	Zona fasciculata adenoma	440111	1%	0	1	0	0	0	0	IN	1.0000	0.7696
ADRENAL MEDULLAS	4402	Malignant pheochromocytom	440201	1%	1	0	0	0	1	0	MX	0.5778	0.5847
ADRENAL MEDULLAS	4402	Benign pheochromocytoma	440202	2%	1	1	1	0	1	1	IN	0.4161	0.4157
HEMOLYMPHORET. SYS	4500	Malignant lymphoma	450002	12%	5	7	5	4	2	6	MX	0.6071	0.6197
HEMOLYMPHORET. SYS	4500	Histiocytic sarcoma	450003	2%	1	1	0	0	0	0	FA	1.0000	0.8501
SPLEEN	4600	Hemangioma	460005	1%	1	0	0	0	0	0	IN	1.0000	0.7637
PLEEN	4600	Hemangiosarcoma	460007	2%	1	1	0	0	0	0	IN	1.0000	0.8471

YMUS	5000	Malignant thymoma	500006	1%	1	0	1	0	0	0	MX	0.8955	0.8447
HARDERIAN GLANDS	5400	Adenoma	540003	6%	2	4	8	3	4	2	MX	0.8668	0.8653
HARDERIAN GLANDS	5400	Adenocarcinoma	540006	4%	2	2	1	0	1	1	MX	0.6633	0.6717
SKIN	5700	Fibrosarcoma	570005	6%	3	3	5	3	5	5	MX	0.2925	0.2987
SKIN	5700	Fibroma	570006	1%	1	0	0	1	1	2	IN	0.0841	0.0685
SKIN	5700	Sarcoma (not otherwise sp	570007	2%	1	1	1	2	1	1	MX	0.5515	0.5971
SKIN	5700	Sarcoma (polymorphocellul	570008	1%	1	0	1	3	1	2	MX	0.2786	0.2949
SKIN	5700	Lipoma	570009	3%	2	1	1	0	1	1	MX	0.6165	0.6164
SKIN	5700	Hemangiosarcoma	570011	1%	0	1	0	0	0	1	FA	0.3320	0.1940
SKIN	5700	Benign basal cell tumor	570012	1%	0	1	1	0	0	0	IN	0.8899	0.8361
SKIN	5700	Neural crest tumor	570014	1%	0	1	0	0	0	0	IN	1.0000	0.7680
SKIN	5700	Malignant Schwannoma	570017	0%	0	0	0	4	0	1	MX	0.4694	0.4779
SKIN	5700	Squamous cell papilloma	570020	0%	0	0	0	1	0	0	IN	0.4824	0.6860
SKIN	5700	Keratoacanthoma	570022	0%	0	0	1	0	0	0	IN	0.6588	0.7522
BONE	5900	Schwannoma	590001	1%	1	0	0	0	0	0	IN	N/A	N/A
BODY CAVITIES	6800	Sarcoma (not otherwise sp	680009	0%	0	0	0	1	0	0	FA	0.6000	0.5824
LIVER (BY REVIEWER)	**22**	**COMBINED HEPATOCELLULAR ADENOMA AND CARCINOMA**	**33**	**21%**	**10**	**11**	**16**	**26**	**23**	**25**	**MX**	**0.0009**	**0.0008**

Number of Animals
Species: Mouse
Sex: Male

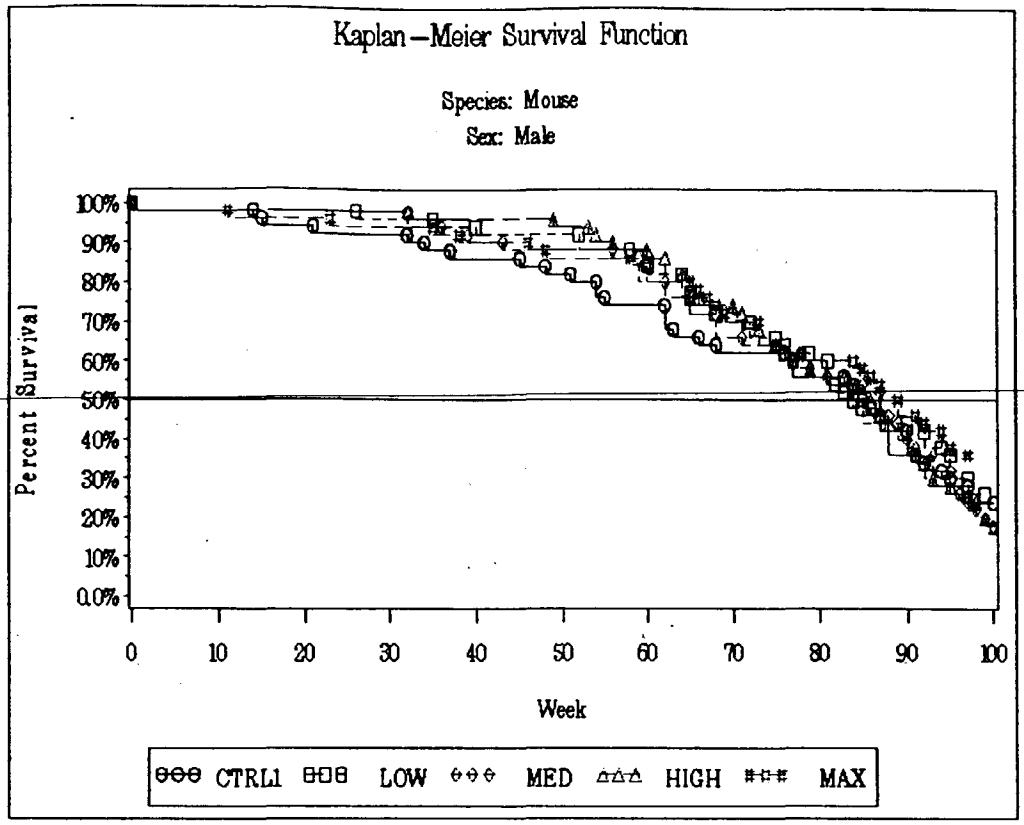
Week	Treatment Group					
	CTRL1	LOW	MED	HIGH	MAX	Total
	N	N	N	N	N	N
0-52	9	4	5	2	6	26
53-78	11	14	14	18	13	70
79-91	12	10	12	12	8	54
92-98	4	7	8	6	5	30
99-100	14	15	11	12	18	70
Total	50	50	50	50	50	250

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	1.23	0.2674
	Depart from Trend	1.10	0.7762
	Homogeneity	2.33	0.6747
Kruskal-Wallis	Dose-Mortality Trend	0.92	0.3366
	Depart from Trend	0.56	0.9061
	Homogeneity	1.48	0.8300


Kaplan–Meier Survival Function
Species: Mouse
Sex: Male
Percent Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0.0%
0
10
20
30
40
50
60
70
80
90
100
Week
CTRL1
LOW
MED
HIGH
MAX

Test for Dose-Tumor Positive Linear Trend

Source: Male Mouse Data

Organ Name	Organ Code	Tumor Name	Tumor Code	Natural Rate (in ctrl group)	CTRL1	LOW	MED	HIGH	MAX	Tumor type	pValue (Exact)	pValue (Asymp)
AORTA	0520	Lipoma	052001	2%	1	0	0	0	0	IN	1.0000	0.8064
LUNGS	0900	Alveolar/bronchiolar carc	090005	24%	12	11	7	11	2	MX	0.9974	0.9959
LUNGS	0900	Alveolar/bronchiolar aden	090012	8%	4	8	2	2	8	IN	0.2044	0.2046
STOMACH	1500	Squamous cell papilloma	150004	0%	0	0	2	0	0	IN	0.6233	0.7610
JEJUNUM	1602	Adenocarcinoma	160203	2%	1	0	0	0	0	IN	1.0000	0.8064
COLON	1702	Adenoma	170202	0%	0	0	0	1	2	IN	0.0290	0.0083
LIVER	1800	Hepatocellular adenoma	180010	18%	9	15	22	20	23	MX	0.0136	0.0131
LIVER	1800	Hepatocellular carcinoma	180016	2%	1	1	7	7	9	MX	0.0022	0.0013
LIVER	1800	Hemangioma	180028	0%	0	0	0	1	0	IN	0.4429	0.3713
LIVER	1800	Hemangiosarcoma	180031	0%	0	0	1	0	0	IN	0.5857	0.7325
LIVER	1800	Combined adenomas and car	180037	20%	10	16	26	23	25	IN	0.0110	0.0108
PANCREAS	2000	Hemangioma	200009	0%	0	1	0	0	0	IN	0.8667	0.7723
KIDNEYS	2100	Tubular cell adenoma	210009	2%	1	0	0	1	1	IN	0.2565	0.2050
TESTES	2500	Benign Leydig cell tumor	250003	2%	1	1	3	0	0	IN	0.9403	0.9336
EPIDIDYMIDES	2600	Leiomyoma	260010	0%	0	0	1	0	0	IN	0.6522	0.7342
SEMINAL VESICLES	2800	Adenoma	280007	0%	0	2	0	0	0	IN	0.8981	0.8646
THYROID GLAND	4200	C-cell adenoma	420005	0%	0	0	0	1	0	IN	0.3704	0.3105
ADRENAL MEDULLAS	4402	Malignant pheochromocytom	440201	2%	1	0	0	1	0	MX	0.6680	0.6620
ADRENAL MEDULLAS	4402	Benign pheochromocytoma	440202	2%	1	1	0	1	1	IN	0.4082	0.3889
HEMOLYMPHORET. SYS	4500	Malignant lymphoma	450002	10%	5	5	4	2	6	MX	0.4506	0.4621
HEMOLYMPHORET. SYS	4500	Histiocytic sarcoma	450003	2%	1	0	0	0	0	FA	1.0000	0.7979
SPLEEN	4600	Hemangioma	460005	2%	1	0	0	0	0	IN	1.0000	0.7888
SPLEEN	4600	Hemangiosarcoma	460007	2%	1	0	0	0	0	IN	1.0000	0.7888
THYMUS	5000	Malignant thymoma	500006	2%	1	1	0	0	0	MX	0.9626	0.8809
HARDERIAN GLANDS	5400	Adenoma	540003	4%	2	8	3	4	2	IN	0.8713	0.8696
HARDERIAN	5400	Adenocarcinoma	540006	4%	2	1	0	1	1	MX	0.5733	0.5731

LANDS												
SKIN	5700	Fibrosarcoma	570005	6%	3	5	3	5	5	MX	0.3438	0.3500
SKIN	5700	Fibroma	570006	2%	1	0	1	1	2	IN	0.1450	0.1293
SKIN	5700	Sarcoma (not otherwise sp	570007	2%	1	1	2	1	1	MX	0.5799	0.6185
SKIN	5700	Sarcoma (polymorphocellul	570008	2%	1	1	3	1	2	MX	0.4198	0.4441
SKIN	5700	Lipoma	570009	4%	2	1	0	1	1	MX	0.6351	0.6264
SKIN	5700	Hemangiosarcoma	570011	.0%	0	0	0	0	1	FA	0.2366	0.0476
SKIN	5700	Benign basal cell tumor	570012	.0%	0	1	0	0	0	IN	0.7778	0.7661
SKIN	5700	~~Malignant~~ Schwannoma	570017	.0%	0	0	4	0	1	MX	0.5796	0.5913
SKIN	5700	Squamous cell papilloma	570020	.0%	0	0	1	0	0	IN	0.5857	0.7325
SKIN	5700	Keratoacanthoma	570022	.0%	0	1	0	0	0	IN	0.8000	0.7912
BONE	5900	Schwannoma	590001	2%	1	0	0	0	0	IN	N/A	N/A
BODY CAVITIES	6800	Sarcoma (not otherwise sp	680009	.0%	0	0	1	0	0	FA	0.6667	0.6166
LIVER (BY REVIEWER)	**22**	**COMBINED HEAPATOCELLULAR ADENOMA AND CARCINOMA**	**33**	**20%**	**10**	**16**	**26**	**23**	**25**	**MX**	**0.0070**	**0.0066**

Number of Animals
Species: Mouse
Sex: Male

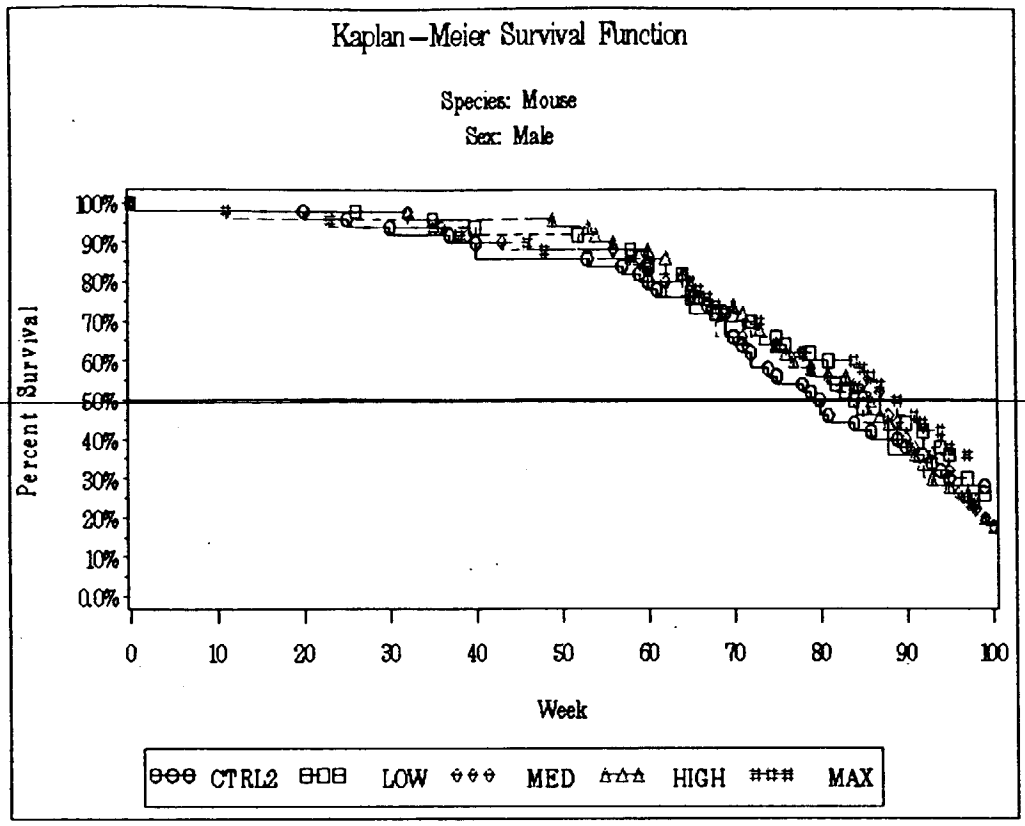
	Treatment Group					
	CTRL2	LOW	MED	HIGH	MAX	Total
	N	N	N	N	N	N
Week						
0-52	5	4	5	2	6	22
53-78	18	14	14	18	13	77
79-91	8	10	12	12	8	50
92-98	4	7	8	6	5	30
99-100	15	15	11	12	18	71
Total	50	50	50	50	50	250

Dose-Mortality Trend Tests

This test is run using Trend and Homogeneity Analyses of Proportions and Life Table Data Version 2.1, by Donald G. Thomas, National Cancer Institute

Species: Mouse
Sex: Male

Method	Time-Adjusted Trend Test	Statistic	P Value
Cox	Dose-Mortality Trend	1.25	0.2628
	Depart from Trend	1.10	0.7779
	Homogeneity	2.35	0.6716
Kruskal-Wallis	Dose-Mortality Trend	0.86	0.3540
	Depart from Trend	0.55	0.9084
	Homogeneity	1.41	0.8431


Kaplan–Meier Survival Function
Species: Mouse
Sex: Male
Percent Survival
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0.0%
0
10
20
30
40
50
60
70
80
90
100
Week
CTRL2
LOW
MED
HIGH
MAX

Test for Dose-Tumor Positive Linear Trend

Source: Male Mouse Data

Organ Name	Organ Code	Tumor Name	Tumor Code	Natural Rate (in ctrl group)	CTRL2	LOW	MED	HIGH	MAX	Tumor type	pValue (Exact)	pValue (Asymp)
LUNGS	0900	Alveolar/bronchiolar carc	090005	12%	6	11	7	11	2	MX	0.9772	0.9732
LUNGS	0900	Alveolar/bronchiolar aden	090012	22%	11	8	2	2	8	IN	0.6421	0.6484
STOMACH	1500	Squamous cell papilloma	150004	2%	1	0	2	0	0	IN	0.8377	0.8734
COLON	1702	Adenoma	170202	0%	0	0	0	1	2	IN	0.0239	0.0060
LIVER	1800	Hepatocellular adenoma	180010	20%	10	15	22	20	23	MX	0.0126	0.0120
LIVER	1800	Hepatocellular carcinoma	180016	8%	4	1	7	7	9	MX	0.0127	0.0098
LIVER	1800	Hemangioma	180028	0%	0	0	0	1	0	IN	0.4026	0.3393
LIVER	1800	Hemangiosarcoma	180031	0%	0	0	1	0	0	IN	0.5775	0.7289
LIVER	1800	Combined adenomas and car	180037	22%	11	16	26	23	25	IN	0.0097	0.0096
PANCREAS	2000	Islet cell adenoma	200007	2%	1	0	0	0	0	IN	1.0000	0.7935
PANCREAS	2000	Hemangioma	200009	0%	0	1	0	0	0	IN	0.8667	0.7723
KIDNEYS	2100	Tubular cell adenoma	210009	0%	0	0	0	1	1	IN	0.1211	0.0678
TESTES	2500	Benign Leydig cell tumor	250003	2%	1	1	3	0	0	IN	0.9394	0.9329
EPIDIDYMIDES	2600	Leiomyoma	260010	0%	0	0	1	0	0	IN	0.5921	0.7087
SEMINAL VESICLES	2800	Adenoma	280007	2%	1	2	0	0	0	IN	0.9724	0.9184
THYROID GLAND	4200	C-cell adenoma	420005	0%	0	0	0	1	0	IN	0.4000	0.3351
ADRENAL CORTICES	4401	Hemangioma	440107	2%	1	0	0	0	0	IN	1.0000	0.8035
ADRENAL CORTICES	4401	Zona fasciculata adenoma	440111	2%	1	0	0	0	0	IN	1.0000	0.8035
ADRENAL MEDULLAS	4402	Malignant pheochromocytom	440201	0%	0	0	0	1	0	FA	0.4035	0.3606
ADRENAL MEDULLAS	4402	Benign pheochromocytoma	440202	2%	1	1	0	1	1	IN	0.3532	0.3343
HEMOLYMPHORET. SYS	4500	Malignant lymphoma	450002	14%	7	5	4	2	6	MX	0.5983	0.6094
HEMOLYMPHORET. SYS	4500	Histiocytic sarcoma	450003	2%	1	0	0	0	0	FA	1.0000	0.7983
SPLEEN	4600	Hemangiosarcoma	460007	2%	1	0	0	0	0	IN	1.0000	0.8002
THYMUS	5000	Malignant thymoma	500006	0%	0	1	0	0	0	IN	0.7857	0.7840
HARDERIAN	5400	Adenoma	540003	8%	4	8	3	4	2	MX	0.9284	0.9232

GLANDS												
HARDERIAN GLANDS	5400	Adenocarcinoma	540006	4%	2	1	0	1	1	IN	0.5291	0.5319
SKIN	5700	Fibrosarcoma	570005	6%	3	5	3	5	5	MX	0.3276	0.3334
SKIN	5700	Fibroma	570006	4.0%	0	0	1	1	2	IN	0.0503	0.0372
SKIN	5700	Sarcoma (not otherwise sp	570007	2%	1	1	2	1	1	MX	0.5821	0.6207
SKIN	5700	Sarcoma (polymorphocellul	570008	4.0%	0	1	3	1	2	MX	0.3052	0.3227
SKIN	5700	Lipoma	570009	2%	1	1	0	1	1	IN	0.4783	0.4645
SKIN	5700	Hemangiosarcoma	570011	2%	1	0	0	0	1	FA	0.3868	0.2581
SKIN	5700	Benign basal cell tumor	570012	2%	1	1	0	0	0	IN	0.9662	0.8760
SKIN	5700	Neural crest tumor	570014	2%	1	0	0	0	0	IN	1.0000	0.7907
SKIN	5700	Malignant Schwannoma	570017	4.0%	0	0	4	0	1	MX	0.5919	0.6039
SKIN	5700	Squamous cell papilloma	570020	4.0%	0	0	1	0	0	IN	0.5775	0.7289
SKIN	5700	Keratoacanthoma	570022	4.0%	0	1	0	0	0	IN	0.7887	0.7881
BODY CAVITIES	6800	Sarcoma (not otherwise sp	680009	4.0%	0	0	1	0	0	FA	0.6667	0.6166
LIVER (BY REVIEWER)	**22**	**COMBINED HEPATOCELLULAR ADENOMA AND CARCINOMA**	**33**	**22%**	**11**	**16**	**26**	**23**	**25**	**MX**	**0.0060**	**0.0057**