CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-692

PHARMACOLOGY REVIEW

Memo to:

NDA 21-692 Div File

Dated: July 8, 2005

From:

Asoke Mukherjee, Ph.D, Pharmacologist

Through: Josie Yang, Ph.D, Team Leader

Re: NDA 21-692 Pharm/Tox Label Review submitted by Biovail Laboratories

An approvable letter for NDA 21-692 was issued on Oct 29, 2004. The sponsor submitted a revised label for tramadol extended release tablet (Ralivia ER) on March 7, 2005 in response to the approvable letter. The pharmacology/Toxicology data were reviewed on Dec 31, 2004 and recommendations from CACEC were made on Sept 28, 2004 for carcinogenicity studies.

The sponsor conducted a 104-week carcinogenicity study in Sprague Dawley rats at 25, 50, and 75 for male and 25, 50, 75 and 100 mg/kg in female rats. However, no treatment related incidences of tumors were observed. The CACEC recommended that the label should state "The excessive decrease in body weight gain in the treated groups in 104 week rat study might have reduced the sensitivity of rats to any potential carcinogenic effect of the drug".

Another study was conducted in p53 mice for 26 weeks at 37.5, 75 or 150 mg/kg of tramadol. CACEC concluded that the study was negative for significant tumor findings. Results for both Biovail studies should be included in the label.

Review of Pharm/Tox data dated Dec 31, 2004 suggested that no changes in the existing label for tramadol would be necessary other than inclusion of the result of two new carcinogenicity studies. Accordingly, the reviewer's recommended label is shown below along with that submitted by the sponsor.

Label proposed by the sponsor is shown below.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

A long-term carcinogenicity study was performed in rats (dosing duration of 104 weeks) at doses up to and including 75 mg/kg/day (450 mg/m^2) for male rats and 100 mg/kg/day (600 mg/m^2) for female rats. These doses are approximately 1.8-fold and 2.4-fold higher than the maximum daily human dosage of 246 mg/m^2. No evidence of carcinogenicity was observed with tramadol in male or female rats. A carcinogenicity study was also conducted in p53(+/–)-heterozygous mice (dosing duration of 26 weeks) at doses up to and including 150 mg/kg/day (450 mg/m^2) for male and female mice. This dose is approximately 1.8-fold higher than the maximum daily human dosage of 246 mg/m^2. No evidence of carcinogenicity was observed in male or female mice.

Tramadol was not mutagenic in the following assays: a bacterial reverse mutation assay using *Salmonella* and *E. coli*, a mouse lymphoma assay (in the absence of metabolic activation), and a bone marrow micronucleus test in mice. Mutagenic results occurred in the presence of metabolic activation in the mouse lymphoma assay. Overall, the weight of evidence from these tests indicates that tramadol does not pose a genotoxic risk to humans.

No effects on fertility were observed for tramadol at oral dose levels up to and including 50 mg/kg (300 mg/m^2) in male and female rats. This dosage is 1.2 times the maximum daily human dosage of 246 mg/m^2.

Pregnancy

Teratogenic Effects: Pregnancy Category B

No effects on embryofetal development were observed for tramadol at oral dose levels up to and including 50 mg/kg (300 mg/m^2) in rats and 100 mg/kg (1100 mg/m^2) in rabbits. These dosages are approximately 1.2-fold and 4.5-fold, respectively, the maximum daily human dosage of 246 mg/m^2.

Non-teratogenic Effects

Tramadol was evaluated in peri- and post-natal studies in rats. Progeny of dams receiving oral (gavage) dose levels of 50 mg/kg (300 mg/m^2 or 1.2 times the maximum daily human tramadol dosage) had decreased weights.

There are no adequate and well-controlled studies in pregnant women. Tramadol should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Neonatal seizures, neonatal withdrawal syndrome, fetal death and still birth have been reported during post-marketing reports with tramadol HCl immediate release.

LABOR AND DELIVERY

RALIVIA ER should not be used in pregnant women prior to or during labor unless the potential benefits outweigh the risks. Safe use in pregnancy has not been established. Chronic use during pregnancy may lead to physical dependence and post-partum withdrawal symptoms in the newborn (**see DRUG ABUSE AND DEPENDENCE**). Tramadol has been shown to cross the placenta. The mean ratio of serum tramadol in the umbilical veins compared to maternal veins was 0.83 for 40 women given tramadol HCl during labor.

The effect of tramadol, if any, on the later growth, development, and functional maturation of the child is unknown.

NURSING MOTHERS

RALIVIA ER is not recommended for obstetrical preoperative medication or for post-delivery analgesia in nursing mothers because its safety in infants and newborns has not been studied. Following a single IV 100 mg dose of tramadol, the cumulative excretion in breast milk within 16 hours postdose was 100µg of tramadol (0.1% of the maternal dose) and 27µg of M1.

Recommended label:

Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenic effect of tramadol was observed in p53 (+/-)-heterozygous mice for 26 weeks at oral dose up to 150 mg/kg/day (approximately 1.8-fold maximum daily human dose [MDHD] of 400 mg/kg for a 60 kg adult based on body surface conversion) and in rats for 2 years at oral doses up to 75 mg/kg/day for males and 100 mg/kg/day for females (approximately 1.8-fold and 2.4-fold MDHD, respectively). However, the excessive decrease in body weight gain observed in the rat study might have reduced their sensitivity to any potential carcinogenic effect of the drug.

Tramadol was not mutagenic in the following assays: a bacterial reverse mutation assay using *Salmonella* and *E. coli*, a mouse lymphoma assay (in the absence of metabolic activation), and a bone marrow micronucleus test in mice. Mutagenic results occurred in the presence of metabolic activation in the mouse lymphoma assay. Overall, the weight of evidence from these tests indicates that tramadol does not pose a genotoxic risk to humans.

No effects on fertility were observed for tramadol at oral dose levels up to 50 mg/kg in male and female rats (1.2-fold MDHD).

Pregnancy, Teratogenic Effects: *Pregnancy Category C*

Tramadol was not teratogenic at oral dose levels up to 50 mg/kg (1.2-fold MDHD) in rats and 100 mg/kg (4.5-fold MDHD) in rabbits during organogenesis. However, embryo-fetal lethality, reductions in fetal weight and skeletal ossification, and increased supernumerary ribs were observed at a maternal toxic dose of 140 mg/kg in mice (1.8-fold MDHD), 80 mg/kg in rats (2-fold MDHD) or 300 mg/kg in rabbits (14.5-fold MDHD).

There are no adequate and well-controlled studies in pregnant women. TRADENAME ER should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Neonatal seizures, neonatal withdrawal syndrome, fetal death and still birth have been reported during post-marketing reports with tramadol HCl immediate release.

Labor and Delivery

TRADENAME ER should not be used in pregnant women prior to or during labor unless the potential benefits outweigh the risks. Safe use in pregnancy has not been established. Chronic use during pregnancy may lead to physical dependence and post-partum withdrawal symptoms in the newborn (**see DRUG ABUSE AND DEPENDENCE**).

Tramadol has been shown to cross the placenta. The mean ratio of serum tramadol in the umbilical veins compared to maternal veins was 0.83 for 40 women given tramadol HCl during labor. The effect of TRADENAME ER, if any, on the later growth, development, and functional maturation of the child is unknown.

Nursing Mothers

Tradename ER is not recommended for obstetrical preoperative medication or for post-delivery analgesia in nursing mothers because its safety in infants and newborns has not been studied. Following a single IV 100 mg dose of tramadol, the cumulative excretion in breast milk within 16 hours postdose was 100 μg of tramadol (0.1% of the maternal dose) and 27 μg of M1 metabolite.

C.C:

NDA 21-692 Div File
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Revised on Aug 25, 2005

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/s/

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8/25/2005 11:23:01 AM
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Review and Evaluation of Carcinogenicity Studies

NDA 21-692
Date received by Center: December 31, 2003
Drug name: Ralivia ER™ (tramadol hydrochloride) extended release tablets, 100, 200, 300 mg
Indication: Management of moderate to moderately severe pain in adults
Sponsor: Biovail Laboratories, Incorporated
Review Division: Anti-inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)
Pharm/Tox reviewer: Conrad H. Chen, Ph.D.
Pharm/Tox supervisor: Josie Yang, Ph.D.
Division Director: Brian Harvey, M.D.
Project manager: Nancy Clark
Related to: IND 57,552 and IND 59,023
Drug class: Analgesic
Background: Immediate release form of tramadol hydrochloride (Ultram) was approved on March 3, 1995. Biovail Laboratories, the developer of Ralivia, originally intended to submit their product, the extended release form of tramadol hydrochloride, under ———. Therefore, they conducted a 104-week rat carcinogenicity study and a 26-week p53 mouse alternative carcinogenicity study in support of the NDA. Recently, Biovail has decided to submit their NDA under 505(b)(2) instead. The proposed labeling for Ralivia ER is similar to the labeling of currently marketed Ultram, which include two life time carcinogenicity studies in rats and mice. Biovail did not propose to include the findings from their own carcinogenicity studies in the proposed labeling for Ralivia ER.
The dose selections were based on a 26-week rat study and a 28-day mouse study and were approved by Exec CAC on June 8, 1999, February 22, 2000, and April 24, 2001.

104-Week gavage oncogenicity study with TRA P03 (tramadol hydrochloride) in rats

Test facility: ———
Study period: Oct. 21, 1999 to Sept. 21, 2001
Study completion date: May 10, 2002
Test system: Crl:CD®(SD)IGS BR rats, 60/sex/group
Dose: 0, 25, 50, 75, or 100 (females only) mg/kg/day. The high dose for females was increased to 150 mg/kg/day at Week 4 then reduced to 100 mg/kg/day at Week 10 due to overt toxicity. The dose levels were concurred by Exec CAC on June 8, 1999 and February 22, 2000.
GLP and QA compliance: Yes
Procedure: The doses were administered by oral gavage twice daily (5 to 6 hours apart) for at least 97 weeks for females and 100 weeks for males. The animals were observed twice daily for mortality and moribundity. Ophthalmic examinations were done before initiation of treatment and during Week 26, 52, 97 (females) and 100 (males). Body weight and food consumption data were collected pre-dose, weekly for Weeks 1-14, and monthly thereafter.

Blood films were made from animals terminated at unscheduled intervals and at scheduled termination. At Weeks 98 (females) and 101 (males), all surviving animals were sacrificed and necropsied. Animals that died on test or were terminated at unscheduled intervals were also necropsied. Microscopic examinations were done on tissue from each animal in the control group, group 4 and group 5 (female), and from animals that died or were terminated at an unscheduled interval. Macroscopic lesions and the lung, liver, and kidneys were also examined microscopically from each animal in group 2 and 3.

Results:

Clinical observations:

Survival was higher in all male and female treated groups compared with the respective control groups. Mortality was higher for the vehicle control animals than any of the test groups.

Mortality (number of animals died or sacrificed moribund)

Group	1	2	3	4	5
Dose (mg/kg/day)	0	25	50	75	100
Male	47/60	31/60	38/60	36/60	-
Female	48/60	37/60	30/60	32/60	41/60

At Week 97, the survival for females in each group was 20, 38, 50, 47, and 32%, respectively. At Week 100, the survival for males in each group was 22, 48, 37, and 43%, respectively. The sponsor stated that the most common cause of death was pituitary adenoma, which was considered to be unrelated to the treatment. The incidences of pituitary adenoma were 56, 52, 45, 48, and 37 in females and 39, 31, 34, and 33 in males in each group, respectively. However, the association of pituitary adenomas and deaths was not fully explained.

Clinical observations included increases in convulsions and vocalization, malocclusion, alopecia, red and rough haircoat, and sores and scabs on the paws, decrease in ataxia, and hypoactivity. The sponsor stated that some of these observations may be attributed to significantly decreased body weights resulting in increased survival. Alopecia, red and rough haircoat, sores and scabs are generally associated with older animals. Dose-related decrease of ataxia and hypoactivity was probably associated with a decrease in age-related obesity resulting form decreases in body weight for treated animals. A dose related increase in convulsions was noted in both males and females. There were no notable increases in the incidence of mass observations for treated groups. There were no treatment-related lesions noted at any of the ophthalmic examinations.

The decrease in mean body weights was dose-related for males in all treated groups and for females given 25, 50, or 75 mg/kg/day. At Week 101, mean body weights for males given 25, 50, and 75 mg/kg/day were 77, 68, and 64% of the control, respectively. At Week 98, mean body weights for females were 73, 67, 67, and 71% of the control, respectively. Mean body weight gains were generally decreased for all male and female treated groups beginning at the first week of the study to Week 62 and 66, respectively. Overall body weight gains for all treated male groups (Weeks 1 to 101) and treated female groups (Weeks 1 to 98) were significantly decreased compared with controls.

Summary of Body Weight Gains:

Sex	Group, (Dose, mg/kg/day)	1 (0)	2, (25)	3 (50)	4 (75)	5 (100)
Male	N (Week 1-101)	13	29	22	25	-
	Mean (%)	719 (100%)	511* (71%)	433* (60%)	394* (55%)	-
	S.D.	106.2	95.7	46.3	80.2	-
Female	N (Week 1-98)	12	23	30	28	19
	Mean	439 (100%)	284* (65%)	248* (56%)	249* (57%)	269* (61%)
	S.D.	99.6	48.7	39.6	47.5	58.1

* Group mean is significantly different from the mean of the control group (Group 1) at $p \leq 0.05$

Mean food consumption was generally decreased throughout the study for male and female treated groups, corresponding with decreases in mean body weights and body weight gains.

Non-neoplastic findings:

There were no test article-related changes for organ weights or macroscopic or microscopic findings. Increases or decreases in several microscopic findings were attributed to decreased body weight or increased survival, or both in the treated groups. Infiltrates of pulmonary macrophages and cage sores were observed more frequently in treated groups. Infiltrates of pigmented macrophages in the mediastinal lymph nodes were observed more frequently in treated female groups. Various changes as proteinaceous casts of the kidneys, hemorrhage of the adrenal cortex, hypercellularity of the bone marrow, extramedullary hematopoiesis of the spleen, and edema and erosions of the glandular stomach were present more frequently in some treated animals than in control group. These findings were considered as common spontaneous lesions and the variation in incidence of these findings was considered incidental.

Some other findings were decreased in treated animals than in control. These findings included cystic degeneration of adrenal cortex, chronic inflammation and vacuolation of liver, and chronic progressive nephropathy and mineralization of renal pelves, etc. The decreased incidences of these common findings were considered as related to decreased body weights and increased survival in the treated groups.

Neoplastic findings:

There was no significant increase in any of the neoplastic findings.

The incidence of thyroid C-cell adenomas was higher for females given 100 mg/kg/day than for the controls, but the increase was not statistically significant. Pituitary adenomas, mammary fibroadenomas, and mammary carcinomas were observed less frequently for females given 100 mg/kg/day.

Statistical Methods:

Based on the study pathologist's review, the lesions were assigned "incidental", "fatal", or "palpable" status. The "incidental" tumors were analyzed by the logistic regression techniques (Dines and Lagakos, 1983). The "fatal" and "palpable" tumors were analyzed by the Cox-Tarone binary regression techniques. When the same tumor type was assigned to the death of some animals ("fatal") and not in some others ("incidental"), an IRAC-type analysis (Peto et al, 1980) was performed. Although not mentioned in the submission, it appears that pair-wise comparisons were conducted in the analysis.

The incidence of thyroid C-cell adenomas (I) was shown below:

Group	1	2	3	4	5
Dose, mg/kg/day	0	25	50	75	100/150/100

Male	16/60	4/33	6/41	11/60	
P-value				0.1105 -	
Female	6/59	6/38	3/31	9/60	13/60
P-value				0.4988 +	0.0749 +

I = Incidental; - = Decreased Direction; + = Increased Direction;

The results of the analysis of other neoplastic lesions were shown in the following tables.

TEXT TABLE 2: RESULTS OF ANALYSIS OF NEOPLASTIC LESIONS- MALES

	Group 1	Group 2	Group 3	Group 4	
HEMATO NEOPLASIA- MALIGNANT LYMPHOMA (I)					
	0/60	3/60	11/60	2/60	
p-value	.2892 +	.1555 +		.1190 +	
HEMATO NEOPLASIA- MALIGNANT SARCOMA, HISTIOCYTIC (I+F)					
	2/60	1/60	5/60	1/60	
p-value	.4768 -		.4400 +		
PITUITARY- BENIGN ADENOMA (I+F)					
	39/60	31/38	34/47	33/60	
p-value				.0702 -	
PARATHYROID- BENIGN ADENOMA (I)					
	2/58	0/31	0/38	0/55	
p-value				.0505 -	(E)
SKIN- BENIGN KERATOACANTHOMA (P)					
	4/60	4/37	2/40	2/60	
p-value				.2114 -	
THYROID- BENIGN ADENOMA, C-CELL (I)					
	16/60	4/33	6/41	11/60	
p-value				.1105 -	
THYROID- MALIGNANT CARCINOMA, C-CELL (I)					
	1/60	0/33	0/41	1/60	
p-value					
THYROID- ADENOMA/CARCINOMA, C-CELL (I)					
	17/60	4/33	6/41	12/60	
p-value				.1102 -	
THYROID- BENIGN ADENOMA, FOLLICULAR CELL (I)					
	9/60	5/33	1/41	2/60	
p-value				.0155 - *	
SKIN/SUBCUTIS (MULTIPLE ORGANS)- BENIGN FIBROMA (P)					
	1/60	3/37	4/40	5/60	
p-value				.1841 +	
SKIN/SUBCUTIS (MULTIPLE ORGANS)- MALIGNANT FIBROSARCOMA (P)					
	2/60	2/37	0/40	2/60	
p-value					
SKIN/SUBCUTIS (MULTIPLE ORGANS)- FIBROMA/FIBROSARCOMA (P)					
	3/60	5/37	4/40	7/60	
p-value				.3013 +	

Note: I = Incidental; F = Fatal; P = Palpable; + = Increased Direction; - = Decreased Direction; * = Only one animal had fatal designation; E = Exact Probability.

TEXT Table 3: RESULTS OF ANALYSIS OF NEOPLASTIC LESIONS- FEMALES

	Group 1	Group 2	Group 3	Group 4	GROUP 5
ADRENAL, CORTEX- BENIGN ADENOMA (I)					
	1/60	0/43	0/39	5/60	0/60
p-value				.0801 +	
HEMATO NEOPLASIA- MALIGNANT LYMPHOMA (I+F)					
	1/60	2/60	5/60	1/60	0/60
P-value	.1781 +		.3601 +		
MAMMARY- MALIGNANT CARCINOMA (P)					
	11/60	8/53	5/39	7/60	4/59
P-value				.1148 -	.0448 - *
MAMMARY- BENIGN FIBROADENOMA (P)					
	23/60	27/53	19/39	11/60	11/59
P-value				.0019 - **	.0217 - *
MAMMARY- BENIGN ADENOMA (P)					
	2/60	6/53	1/39	4/60	1/59
P-value				.4878 +	
MAMMARY- FIBROADENOMA/ADENOMA/CARCINOMA (P)					
	29/60	36/53	22/39	18/60	14/59
P-value				.0040 - **	.0037 - **
PITUITARY- BENIGN ADENOMA (I+F)					
	56/60	52/54	45/50	48/59	37/59
P-value				.0051 - **	.0153 - *
SKIN/SUBCUTIS- BENIGN FIBROMA (P)					
	3/60	1/41	3/33	3/60	1/60
P-value					.3142 -
SKIN/SUBCUTIS- MALIGNANT FIBROSARCOMA (P)					
	1/60	2/41	0/33	0/60	0/60
P-value					
SKIN/SUBCUTIS- FIBROMA/FIBROSARCOMA (P)					
	4/60	3/41	3/33	3/60	1/60
P-value					.1567 -
THYROID- BENIGN ADENOMA, C-CELL (I)					
	6/59	6/38	3/31	9/60	13/60
P-value				.4988 +	.0749 +
MULTIPLE ORGANS- BENIGN POLYP (I)					
	41/60	5/49	5/40	6/60	4/60
P-value				.3853 +	

Note: I = Incidental; F = Fatal; P = Palpable; + = Increased Direction; - = Decreased Direction; ! = Only one animal had fatal designation; * = Significant at p≤0.05; ** = Significant at p≤0.01.

TEXT Table 3: RESULTS OF ANALYSIS OF NEOPLASTIC LESIONS- FEMALES (CONTD.)

	Group 1	Group 2	Group 3	Group 4	GROUP 5
UTERUS- MALIGNANT SARCOMA, ENDOMETRIAL STROMAL (I)					
	0/60	0/49	0/40	1/60	0/60
P-value					
MULTIPLE ORGANS- POLYP/SARCOMA (I)					
	41(!)/60	5/49	5/40	7/60	4/60
P-value				.2941 +	
MULTIPLE ORGANS- BENIGN LEIOMYOMA (I)					
	0/60	1/37	0/30	1/60	1/59
P-value					
MULTIPLE ORGANS- MALIGNANT LEIOMYOSARCOMA (I)					
	0/60	0/49	1/40	0/60	1/60
P-value					
MULTIPLE ORGANS- LEIOMYOMA/LEIOMYOSARCOMA (I)					
	0/60	1/49	1/40	1/60	2/60
P-value					.3931 + (E)

Note: I = Incidental; F = Fatal; P = Palpable; + = Increased Direction; - = Decreased Direction; ! = Only one animal had fatal designation; E = Exact Probability.

Evaluation and Comment:

The purpose of this study was to assess the oncogenic potential of tramadol HCl (TRA P03) when administered twice daily by oral gavage to male and female Sprague-Dawley

rats at 0, 25, 50, 75, or 100 (female only) mg/kg/day. Due to decreased survival in the control groups, males were terminated after 100 weeks of treatment and females were terminated after 97 weeks of treatment.
Based on the results of this study, tramadol HCl did not produce any evidence of oncogenic potential.

26-Week gavage carcinogenicity study with tramadol hydrochloride in p53 (+/-) C57BL/6 mice
Test facility:
Study period: July 12, 2001 to January 17, 2002
Study completion date: October 25, 2002
Test system: Male and female C57BL/6 — -Trp53^{tm1} (heterozygote) mice, 20/sex/group, received on 26 June, 2001 from at the initiation of dosing, the mice were 7 to 8 weeks of age with body weights from 18.8 to 28.2 g for males and from 14.6 to 21.1 g for females.
Dose: 0, 37.5, 75, or 150 mg/kg/day tramadol HCl or positive control article (400 mg/kg/day p-cresidine in corn oil) at a dose volume of 10 mL/kg by gavage once daily for 26 weeks; the dose levels were concurred by Exec CAC at a meeting on April 24, 2001.
GLP and QA compliance: Yes
Procedure: Mouse was observed twice daily for mortality and moribundity. Once daily, cageside observations were conducted and findings were recorded. Once weekly, grossly visible or palpable mass was recorded. Body weight and food consumption were measured weekly. The absorption of test article was confirmed by collecting plasma samples at the terminal sacrifice for toxicokinetic analysis. Blood samples were collected for hematology tests 3 to 4 days prior to study termination from the orbital sinus. Animal found dead or sacrificed in extremis were grossly examined. After 26 weeks of treatment, all surviving mice were sacrificed. Tissues from each animal in the control and high-dose groups and animal that died or at an unscheduled interval were examined microscopically.
Results: In the control and tramadol treated groups, nine mice (three males and one female in 150 mg/kg/day (group 4), four 75 mg/kg/day females (group 3) and one control female) either died or were sacrificed in extremis. Three males and two females in p-cresidine group also died or were sacrificed in extremis during the study. The cause of death for three group 3 females and one control female was identified as malignant lymphoma. The cause for other death was not determined. The sponsor stated that there were no treatment-related effects on mortality.
The mortalities in each group are tabulated below:

Group, (n=20)	1	2	3	4	5
Dose, mg/kg/day	0	37.5	75	150	400 (p-cresidine)
Male	0	0	0	3	3
Female	1	0	4	1	2

There were no treatment-related clinical observations. The incidence of rough hair coat increased in the p-cresidine-treated males. No treatment-related changes in body weight were found in tramadol-treated groups. Body weights and body weight gains in p-cresidine-treated group were consistently decreased. There were sporadic decreases in food consumption in male groups but there were no significant effects on body weight

gains. Food consumption in female groups was not affected by the treatment. In p-cresidine group, the food consumption was 19 % less than the control group and correlated with decreased body weights and body weight gains.
Exposure to tramadol in the circulating plasma was dose-related. In general, increases in C_{max} and AUC_{0-t} were greater than dose-proportional for both genders. The metabolite, O-desmethyltramadol, increased in a dose-related manner (but less than dose-proportional). The metabolite to parent ratios, however, generally decreased with the increase in dose level, indicating possible saturation of this metabolic pathway.
There were no significant changes in organ weights that were considered as treatment-related. For instance, the mean liver and kidney weights were significantly decreased in some treated groups but there were no microscopic correlates. The brain-to-body weight ratio and heart-to-body weight ratio of group 4 animals were significantly increased but there were no microscopic correlates.
None of the macroscopic alterations were treatment-related. Among the animals of unscheduled deaths, the thymic masses in one control and two group 3 females correlated with the microscopic findings of malignant lymphoma. Upon microscopic examinations, the malignant lymphomas were found in one control female and three group 3 females. One case of thymic lymphoma was also found in one control female at terminal sacrifice. In summary, lymphomas were found in two control females and three group 3 females in this study. Lymphoma was not found in other treated groups (including group 4) among terminal sacrifices and unscheduled deaths and there was no dose-effect relationship. No other tramadol-related histomorphologic findings occurred after 26 weeks of treatment. However, malignant lymphoma is a common tumor in p53 (+/-) mice.
The positive control group (p-cresidine group) exhibited histomorphologic lesions in the urinary bladder and salivary gland. In the urinary bladder, slight to moderately severe transitional cell hyperplasia was found in all animals. The transitional cell hyperplasia was diffuse in nature and usually seen with areas of squamous cell metaplasia and occasionally with focal areas of dysplasia. Eight males and one female had transitional cell carcinoma of the urinary bladder. Because of the low tumor incidence of urinary bladder (1/20) in female group, the results from positive control appeared a little shaky. Two mice exhibited macroscopically large masses in the salivary gland that correlated with the microscopic diagnosis of undifferentiated sarcoma.

The incidences of microscopic observations were shown in the following two tables:

Table 10
Incidence of Microscopic Observations – Terminal Sacrifice

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE---------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
*** TOP OF LIST ***											
BRAIN (BR)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
CORD, CERVICAL (CE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
CORD, THORACIC (TC)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
CORD, LUMBAR (LC)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
PITUITARY (PI)	NUMBER EXAMINED:	17	0	0	15	0	18	0	0	19	0
	NOT REMARKABLE:	17	0	0	15	0	18	0	0	19	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	19	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	4	0	0	3	0	0	0	0	0	0
--PIGMENT		14	0	0	13	0	17	0	0	15	0
--CONGESTION		0	0	0	0	0	17	0	0	19	0
--HYPERPLASIA, SUBCAPSULAR CELL		1	0	0	4	0	18	0	0	18	0
--UNILATERALLY EXAMINED		1	0	0	0	0	1	0	0	1	0
--FOCAL HYPERTROPHY		1	0	0	0	0	0	0	0	0	0
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	19	0	0	15	0	19	0	0	18	0
	NOT REMARKABLE:	17	0	0	15	0	16	0	0	18	0
--UNILATERALLY EXAMINED		2	0	0	0	0	3	0	0	1	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS – TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE---------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
THYROID (TY)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	18	0
	NOT REMARKABLE:	18	0	0	15	0	14	0	0	16	0
--UNILATERALLY EXAMINED		2	0	0	2	0	5	0	0	4	0
--HYPERPLASIA, "C" CELL		0	0	0	0	0	0	0	0	1	0
PARATHYROID (PT)	NUMBER EXAMINED:	18	0	0	11	0	13	0	0	12	0
	NOT REMARKABLE:	18	0	0	11	0	13	0	0	12	0
ESOPHAGUS (ES)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	18	0
	NOT REMARKABLE:	13	0	0	15	0	14	0	0	14	0
--CHRONIC INFLAMMATION, TUNICA MUSCULARIS		7	0	0	2	0	5	0	0	5	0
TRACHEA (TR)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
LUNG (LU)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	7	0	0	10	0	5	0	0	4	0
--INFLAMMATION, CHRONIC		8	0	0	1	0	9	0	0	6	0
--HEMORRHAGE		1	0	0	2	0	0	0	0	1	0
--PERIBRONCHIAL/PERIVASCULAR, INFILTRATION, LYMPHOID		5	0	0	4	0	10	0	0	11	0
--ALVEOLAR MACRODRAGES		11	0	0	4	0	9	0	0	5	0
HEART (HT)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	19	0	0	16	0	19	0	0	19	0
--FOCI OF MINERALIZATION		1	0	0	0	0	0	0	0	0	0
--CARDIOMYOPATHY, DEGENERATIVE		0	0	0	1	0	0	0	0	0	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
SPLEEN (SP)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--PIGMENT		20	0	0	17	0	19	0	0	19	0
--CONGESTION		0	0	0	1	0	0	0	0	0	0
--EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		0	0	0	2	0	0	0	0	0	0
LIVER (LI)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	5	0	0	3	0	1	0	0	2	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	0	0	0
--FOCI OF CHRONIC INFLAMMATION		9	0	0	9	0	10	0	0	12	0
--FOCI OF CHRONIC ACTIVE INFLAMMATION		3	0	0	2	0	3	0	0	2	0
--FOCI OF EXTRAMEDULLARY HEMATOPOIESIS		1	0	0	0	0	2	0	0	3	0
--LYMPHOID ACCUMULATION, PERIVASCULAR		9	0	0	6	0	16	0	0	8	0
--NECROSIS, INDIVIDUAL CELL		0	0	0	1	0	0	0	0	0	0
--CAPSULITIS		0	0	0	0	0	0	0	0	1	0
GALLBLADDER (GB)	NUMBER EXAMINED:	20	0	0	16	0	19	0	0	15	0
	NOT REMARKABLE:	9	0	0	8	0	10	0	0	14	0
--INFLAMMATION, CHRONIC ACTIVE		11	0	0	8	0	9	0	0	5	0
KIDNEY (KD)	NUMBER EXAMINED:	20	0	0	17	3	19	0	0	19	1
	NOT REMARKABLE:	9	0	0	11	0	7	0	0	10	0
--TUBULE, MINERALIZATION		2	0	0	0	3	1	0	0	0	0
--TUBULE, DILATATION		0	0	0	0	2	0	0	0	0	0
--TUBULE, REGENERATION		9	0	0	8	3	8	0	0	1	1
--CHRONIC INFLAMMATION, PELVIS		8	0	0	7	1	6	0	0	3	0
--PELVIS, DILATATION		1	0	0	2	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		1	0	0	1	2	4	0	0	1	0
--PROTEINACEOUS CASTS		0	0	0	1	0	3	0	0	6	0

*** CONTINUED ON NEXT PAGE ***

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
*** FROM PREVIOUS PAGE ***											
KIDNEY (KD)	NUMBER EXAMINED:	20	0	0	17	3	19	0	0	19	1
	NOT REMARKABLE:	9	0	0	11	0	7	0	0	10	0
--ATROPHY, CORTEX		1	0	0	2	0	0	0	0	0	0
--PAPILLA, NECROSIS		0	0	0	0	2	0	0	0	0	0
STOMACH, NONGL (SG)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
STOMACH, GL (ST)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	19	0	0	17	0	18	0	0	18	0
--DILATATION, MUCOSAL GLAND		1	0	0	0	0	1	0	0	1	0
DUODENUM (DU)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
JEJUNUM (JE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
ILEUM (IL)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
PANCREAS (PA)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	19	0	0	13	0	17	0	0	19	0
--FOCAL CHRONIC INFLAMMATION		1	0	0	3	0	2	0	0	0	0
--FOCAL ACINAR HYPERPLASIA		0	0	0	1	0	0	0	0	0	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN B53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
CECUM (CE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
COLON (CO)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
RECTUM (RE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
LN, MESENTERIC (MS)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
SALIV GL, MANDIB (SG)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	10	0	0	7	0	18	0	0	18	0
--FOCAL MONONUCLEAR CELLS, PERIDUCTAL		10	0	0	10	0	1	0	0	1	0
THYMUS (TH)	NUMBER EXAMINED:	20	0	0	17	0	18	0	0	18	0
	NOT REMARKABLE:	20	0	0	17	0	17	0	0	18	0
--H-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	0	0	0
AORTA, THORACIC (AO)	NUMBER EXAMINED:	20	0	0	17	0	17	0	0	19	0
	NOT REMARKABLE:	19	0	0	17	0	15	0	0	19	0
--FOCAL INTIMAL THICKENING		1	0	0	0	0	2	0	0	0	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN B53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=T;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	20	20	20	17	17	19	20	16	19	18
EYE (EY)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	12	0	0	11	0	12	0	0	9	0
--RETROBULBAR/INTRA-OCULAR INFLAMMATION, UNILATERAL		8	0	0	6	0	4	0	0	10	0
--BULBI PTHYSIS		1	0	0	0	0	0	0	0	0	0
--RETROBULBAR HEMORRHAGE		0	0	0	0	0	4	0	0	1	0
HARDERIAN GLAND (HG)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	6	0	0	10	0	9	0	0	10	0
--HYPERPLASIA		2	0	0	0	0	2	0	0	2	0
--CHRONIC INFLAMMATION, FOCAL		13	0	0	6	0	8	0	0	9	0
--CHRONIC ACTIVE INFLAMMATION, FOCAL		0	0	0	1	0	1	0	0	0	0
--UNILATERALLY EXAMINED		0	0	0	0	0	1	0	0	0	0
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
TESTIS (TE)	NUMBER EXAMINED:	20	0	0	17	0	0	0	0	0	0
	NOT REMARKABLE:	4	0	0	6	0	0	0	0	0	0
--UNILATERALLY EXAMINED		1	0	0	0	0	0	0	0	0	0
--DEGENERATION, SEMINIFEROUS TUBULE		15	0	0	11	0	0	0	0	0	0
EPIDIDYMIS (EP)	NUMBER EXAMINED:	20	0	0	17	0	0	0	0	0	0
	NOT REMARKABLE:	19	0	0	16	0	0	0	0	0	0
--UNILATERALLY EXAMINED		1	0	0	0	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	1	0	0	0	0	0	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-26 DEATH=T;FIND=ALL;SUBSET=ALL	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	20	20	20	17	17	19	20	16	19	18
PROSTATE (PR)	NUMBER EXAMINED:	20	0	0	17	0	0	0	0	0	0
	NOT REMARKABLE:	17	0	0	15	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		3	0	0	2	0	0	0	0	0	0
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	20	0	0	17	0	0	0	0	0	0
	NOT REMARKABLE:	20	0	0	17	0	0	0	0	0	0
URINARY BLADDER (UB)	NUMBER EXAMINED:	20	0	0	16	17	19	0	0	19	18
	NOT REMARKABLE:	14	0	0	16	0	19	0	0	19	0
--INTRALUMINAL PROTEINACEOUS MATERIAL		6	0	0	0	0	0	0	0	0	0
--HYPERPLASIA, TRANSITIONAL CELL		0	0	0	0	17	0	0	0	0	18
--FOCAL DYSPLASIA, TRANSITIONAL CELL		0	0	0	0	4	0	0	0	0	3
--M-TRANSITIONAL CELL CARCINOMA		0	0	0	0	6	0	0	0	0	1
--SQUAMOUS CELL METAPLASIA, FOCAL		0	0	0	0	4	0	0	0	0	17
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	1	0	0	0	0	4
--ARTERITIS/PERIARTERITIS		0	0	0	0	14	0	0	0	0	16
--FIBRINOID NECROSIS OF ARTERIAL WALL		0	0	0	0	0	0	0	0	0	2
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	14	0	0	15	0
--FOLLICLE, CYST		0	0	0	0	0	4	0	0	2	0
--BURSA, CYST		0	0	0	0	0	1	0	0	0	0
--UNILATERALLY EXAMINED		0	0	0	0	0	0	0	0	2	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	5	0	0	1	0
--DILATATION		0	0	0	0	0	1	0	0	0	0
--DILATED ENDOMETRIAL GLANDS		0	0	0	0	0	14	0	0	18	0

*** CONTINUED ON NEXT PAGE ***

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-26 DEATH=T;FIND=ALL;SUBSET=ALL	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	20	20	20	17	17	19	20	16	19	18
*** FROM PREVIOUS PAGE ***											
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	5	0	0	1	0
--CYST, ENDOMETRIAL		0	0	0	0	0	1	0	0	3	0
--INFLAMMATION, CHRONIC ACTIVE		0	0	0	0	0	0	0	0	1	0
CERVIX (CV)	NUMBER EXAMINED:	0	0	0	0	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	19	0	0	19	0
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	0	19	0	0	19	0
	NOT REMARKABLE:	0	0	0	0	0	19	0	0	19	0
MAMMARY, FEMALE (MF)	NUMBER EXAMINED:	0	0	0	0	0	18	0	0	18	0
	NOT REMARKABLE:	0	0	0	0	0	18	0	0	18	0
SKIN (SK)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	16	0	19	0	0	19	0
--DUCTECTASIS, PREPUTIAL GLAND		0	0	0	1	0	0	0	0	0	0
MARROW, FEMUR (FE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0
BONE, FEMUR (FE)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	19	0	0	19	0

TABLE 10
INCIDENCE OF MICROSCOPIC OBSERVATIONS - TERMINAL SACRIFICE

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-28 DEATH=T;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	20	20	20	17	17	19	20	16	19	18
HEMATO NEOPLASIA (MH)	NUMBER EXAMINED:	20	0	0	17	0	19	0	0	19	0
	NOT REMARKABLE:	20	0	0	17	0	18	0	0	19	0
--M-LYMPHOMA		0	0	0	0	0	1	0	0	0	0
SKIN, OTHER (SS)	NUMBER EXAMINED:	6	0	0	4	1	6	0	0	2	1
	NOT REMARKABLE:	0	0	0	1	0	3	0	0	1	0
--INFLAMMATION, CHRONIC ACTIVE		4	0	0	1	1	1	0	0	1	1
--NECROSIS		3	0	0	0	0	1	0	0	0	1
--ULCER		4	0	0	0	1	1	0	0	0	1
--FIBROSIS		1	0	0	1	0	0	0	0	1	0
--NECROTIC CELLULAR DEBRIS, EPIDERMAL SURFACE		3	0	0	1	0	1	0	0	0	0
--ACANTHOSIS		2	0	0	1	0	1	0	0	0	1
--HEMORRHAGE		2	0	0	0	0	0	0	0	1	0
--INFLAMMATION, CHRONIC		2	0	0	2	0	2	0	0	0	0
LN, OTHER (LN)	NUMBER EXAMINED:	0	0	0	1	0	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
--MACROPHAGES, PIGMENTED		0	0	0	1	0	0	0	0	0	0
*** END OF LIST ***											

Table 11
Incidence of Microscopic Observations -- Unscheduled Deaths

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-28 DEATH=UNSCHED;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	0	0	0	3	3	1	0	4	1	2
** TOP OF LIST **											
BRAIN (BR)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
CORD, CERVICAL (CS)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
CORD, THORACIC (TC)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
CORD, LUMBAR (LC)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
PITUITARY (PI)	NUMBER EXAMINED:	0	0	0	3	3	2	0	3	1	2
	NOT REMARKABLE:	0	0	0	3	1	1	0	3	1	2
--CONGESTION		0	0	0	0	2	0	0	0	0	0
ADRENAL, CORTEX (AC)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	0	2
	NOT REMARKABLE:	0	0	0	0	2	0	0	0	0	0
--PIGMENT		0	0	0	1	0	1	0	1	0	0
--CONGESTION		0	0	0	3	0	1	0	4	0	1
--HYPERPLASIA, SUBCAPSULAR CELL		0	0	0	0	0	0	0	0	0	0
--UNILATERALLY EXAMINED		0	0	0	0	1	0	0	0	0	0
ADRENAL, MEDULLA (AM)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	0	2
	NOT REMARKABLE:	0	0	0	3	2	1	0	4	0	2
--UNILATERALLY EXAMINED		0	0	0	0	1	0	0	0	0	0

TABLE I1
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-28 DEATH=UNSCHED;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	0	0	0	3	3	1	0	4	1	2
THYROID (TY)	NUMBER EXAMINED:	0	0	0	3	2	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	2	1	0	2	1	0
--UNILATERALLY EXAMINED		0	0	0	1	0	0	0	2	0	2
PARATHYROID (PT)	NUMBER EXAMINED:	0	0	0	1	1	0	0	2	1	1
	NOT REMARKABLE:	0	0	0	1	1	0	0	2	1	1
ESOPHAGUS (ES)	NUMBER EXAMINED:	0	0	0	3	2	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	2	1	0	1	1	2
--CHRONIC INFLAMMATION, TUNICA MUSCULARIS		0	0	0	1	0	0	0	3	0	0
TRACHEA (TR)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	2	1	0	4	1	2
--M-UNDIFFERENTIATED SARCOMA		0	0	0	0	1	0	0	0	0	0
LUNG (LU)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	1	1	0	0	1	0	2
--INFLAMMATION, CHRONIC		0	0	0	0	0	0	0	2	0	0
--CONGESTION		0	0	0	2	1	0	0	1	1	0
--ALVEOLAR MACROPHAGES		0	0	0	0	0	1	0	2	0	0
--LEUKOCYTOSIS		0	0	0	0	1	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	3	0	0
HEART (HT)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	2	0	0	3	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	1	0	0

TABLE I1
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-28 DEATH=UNSCHED;FIND=ALL;SUBSET=ALL		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	0	0	0	3	3	1	0	4	1	2
SPLEEN (SP)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	0	0	0	0	1	0	0
--PIGMENT		0	0	0	3	3	1	0	1	1	2
--DEPLETION, LYMPHOID		0	0	0	0	2	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	2	0	0
LIVER (LI)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	1	0	0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	2	0	0
--CONGESTION		0	0	0	0	0	0	0	1	0	0
--FOCI OF CHRONIC INFLAMMATION		0	0	0	1	0	1	0	1	1	0
--FOCI OF CHRONIC ACTIVE INFLAMMATION		0	0	0	1	0	0	0	0	0	0
--FOCI OF EXTRAMEDULLARY HEMATOPOIESIS		0	0	0	0	0	0	0	0	0	1
--LYMPHOID ACCUMULATION, PERIVASCULAR		0	0	0	1	0	0	0	1	0	1
--VACUOLIZATION		0	0	0	0	1	1	0	0	0	0
--HEPATOCELLULAR DEGENERATION, CENTRILOBULAR		0	0	0	0	1	0	0	0	0	0
--PIGMENT, SINUSOIDAL CELL		0	0	0	0	1	0	0	0	0	1
GALLBLADDER (GB)	NUMBER EXAMINED:	0	0	0	2	2	1	0	3	1	1
	NOT REMARKABLE:	0	0	0	2	2	1	0	1	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	2	0	0
KIDNEY (KD)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	1	0	1	0	3	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
--TUBULE, MINERALIZATION		0	0	0	0	1	0	0	0	0	0
--TUBULE, DILATATION		0	0	0	0	0	0	0	0	0	1
--TUBULE, VACUOLIZATION		0	0	0	0	1	0	0	0	0	0
--TUBULE, REGENERATION		0	0	0	2	2	0	0	0	0	0

** CONTINUED ON NEXT PAGE **

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	0	0	0	3	3	1	0	4	1	2
** FROM PREVIOUS PAGE **											
KIDNEY (KD)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	1	0	1	0	3	1	1
--PELVIS, DILATATION		0	0	0	1	0	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	1	1	0	0	0	0	0
--ATROPHY, CORTEX		0	0	0	0	1	0	0	0	0	0
--PAPILLA, NECROSIS		0	0	0	0	1	0	0	0	0	0
--SUPPURATIVE PYELONEPHRITIS		0	0	0	0	0	0	0	0	0	1
STOMACH, NONGL (SG)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
STOMACH, GL (ST)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	1
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	1
DUODENUM (DU)	NUMBER EXAMINED:	0	0	0	3	2	1	0	3	1	2
	NOT REMARKABLE:	0	0	0	3	2	1	0	2	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
JEJUNUM (JE)	NUMBER EXAMINED:	0	0	0	3	2	1	0	3	1	2
	NOT REMARKABLE:	0	0	0	3	2	1	0	2	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
ILEUM (IL)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	3	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

		-- NUMBER-OF-ANIMALS-AFFECTED --									
	SEX:	----------MALE---------					---------FEMALE--------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION	NUMBER:	0	0	0	3	3	1	0	4	1	2
PANCREAS (PA)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	3	1	0	4	1	2
--FOCAL ACINAR HYPERPLASIA		0	0	0	1	0	0	0	0	0	0
CECUM (CE)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
COLON (CO)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
RECTUM (RE)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
LN, MESENTERIC (MS)	NUMBER EXAMINED:	0	0	0	2	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	3	1	0	3	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
SALIV GL, MANDIB (SG)	NUMBER EXAMINED:	0	0	0	3	3	1	0	3	1	2
	NOT REMARKABLE:	0	0	0	3	2	1	0	3	1	1
--M-UNDIFFERENTIATED SARCOMA		0	0	0	0	1	0	0	0	0	1
THYMUS (TH)	NUMBER EXAMINED:	0	0	0	3	2	1	0	4	1	0
	NOT REMARKABLE:	0	0	0	3	1	0	0	1	1	0
--DEPLETION, LYMPHOID		0	0	0	0	1	0	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	1	0	3	0	0

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	0	0	0	3	3	1	0	4	1	2
AORTA, THORACIC (AO)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
EYE (EY)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	3	0	0	3	1	2
--RETROBULBAR HEMORRHAGE		0	0	0	1	0	1	0	0	0	0
--LENS, DEGENERATION		0	0	0	0	0	0	0	1	0	0
HARDERIAN GLAND (HG)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	3	1	0	4	1	1
--CHRONIC INFLAMMATION, FOCAL		0	0	0	1	0	0	0	0	0	1
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	0	0	0	1	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
TESTIS (TE)	NUMBER EXAMINED:	0	0	0	3	3	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	3	1	0	0	0	0	0
--DEGENERATION, SEMINIFEROUS TUBULE		0	0	0	0	2	0	0	0	0	0
EPIDIDYMIS (EP)	NUMBER EXAMINED:	0	0	0	3	3	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	3	3	0	0	0	0	0
PROSTATE (PR)	NUMBER EXAMINED:	0	0	0	3	3	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	3	2	0	0	0	0	0
--INFLAMMATION, CHRONIC		0	0	0	0	1	0	0	0	0	0

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS

TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-28
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION		-- NUMBER OF ANIMALS AFFECTED --									
	SEX:	----------MALE----------					----------FEMALE----------				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	0	0	0	3	3	1	0	4	1	2
SEMINAL VESICLE (SV)	NUMBER EXAMINED:	0	0	0	3	3	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	3	3	0	0	0	0	0
URINARY BLADDER (UB)	NUMBER EXAMINED:	0	0	0	2	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	2	0	1	0	3	1	0
--HYPERPLASIA, TRANSITIONAL CELL		0	0	0	0	3	0	0	0	0	2
--M-TRANSITIONAL CELL CARCINOMA		0	0	0	0	2	0	0	0	0	0
--ARTERITIS/PERIARTERITIS		0	0	0	0	1	0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
OVARY (OV)	NUMBER EXAMINED:	0	0	0	0	0	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	0	0	1	0	2	1	1
--FOLLICLE, CYST		0	0	0	0	0	0	0	1	0	0
--UNILATERALLY EXAMINED		0	0	0	0	0	0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
UTERUS (UT)	NUMBER EXAMINED:	0	0	0	0	0	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	0	0	1	0	3	1	2
--DILATED ENDOMETRIAL GLANDS		0	0	0	0	0	0	0	1	0	0
CERVIX (CV)	NUMBER EXAMINED:	0	0	0	0	0	1	0	4	1	1
	NOT REMARKABLE:	0	0	0	0	0	1	0	3	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
VAGINA (VA)	NUMBER EXAMINED:	0	0	0	0	0	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	0	0	1	0	3	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS
TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-26
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	0	0	0	3	3	1	0	4	1	2
MAMMARY, FEMALE (MF)	NUMBER EXAMINED:	0	0	0	0	0	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	0	0	1	0	4	1	2
SKIN (SK)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	3	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
MARROW, FEMUR (FM)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	3	1	1
--HYPERPLASIA, MYELOID		0	0	0	0	0	0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	0	0	0	0	1	0	0
BONE, FEMUR (FE)	NUMBER EXAMINED:	0	0	0	3	3	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	3	1	0	4	1	2
HEMATO NEOPLASIA (HN)	NUMBER EXAMINED:	0	0	0	3	0	1	0	4	1	2
	NOT REMARKABLE:	0	0	0	3	0	1	0	1	1	2
--M-LYMPHOMA		0	0	0	0	0	0	0	3	0	0

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS - UNSCHEDULED DEATHS
TWENTY-SIX WEEK GAVAGE CARCINOGENICITY STUDY WITH TRAMADOL HYDROCHLORIDE IN P53(+/-)C57BL/6 MICE

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;WEEKS=1-26
DEATH=UNSCHED;FIND=ALL;SUBSET=ALL

ORGAN AND FINDING DESCRIPTION	SEX:	-- NUMBER-OF-ANIMALS-AFFECTED -- MALE					FEMALE				
	GROUP:	-1-	-2-	-3-	-4-	-5-	-1-	-2-	-3-	-4-	-5-
	NUMBER:	0	0	0	3	3	1	0	4	1	2
SKIN, OTHER (SS)	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0
LN, OTHER (LN)	NUMBER EXAMINED:	0	0	0	0	0	0	0	0	0	0
	NOT REMARKABLE:	0	0	0	0	0	0	0	0	0	0

*** END OF LIST ***

Evaluation and Comment:

The purpose of this study was to evaluate the carcinogenic potential of tramadol hydrochloride when administered daily by oral gavage to p53 (+/-) heterozygous mice for 26 weeks. The dose levels of this study were concurred by Exec CAC at a meeting on April 24, 2001. As recommended by the Exec CAC, the sponsor has determined the drug plasma levels at the termination of the study to confirm the drug exposure. The Exec CAC also stated at the meeting that because of the limited experience with transgenic mouse models, the types of tumors that may need to be combined may not be adequately described in the recommendations by McConnell et. al.
The results showed that at dose levels up to 150 mg/kg/day tramadol hydrochloride did not produce any drug-related non-neoplastic or neoplastic changes in the animals. As expected, the p-cresidine group (positive control) produced neoplastic and non-neoplastic lesions in the urinary bladder of the p-53 mice.

Overall Summary:

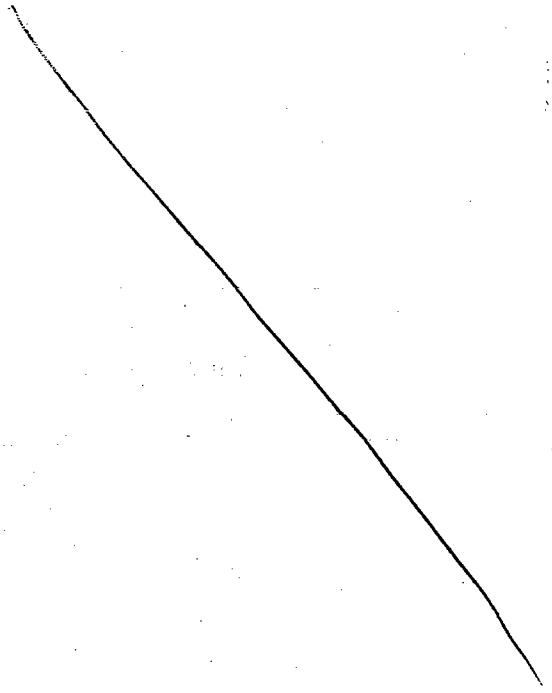
Tramadol was not mutagenic in the following assays: Ames *Salmonella* microsomal activation test, CHO/HPRT mammalian cell assay, mouse lymphoma assay (in the absence of metabolic activation), dominant lethal mutation tests in mice, chromosome aberration test in Chinese hamsters, and bone marrow micronucleus tests in mice and Chinese hamsters. Weakly mutagenic results occurred in the presence of metabolic activation in the mouse lymphoma assay and micronucleus test in rats. Overall, the weight of evidence from these tests indicates that tramadol does not pose a genotoxic risk

to humans. Since tramadol may be considered as an equivocal genotoxic compound, the utility of p53 mice model as an alternative carcinogenicity study model may be appropriate.

NDA 21-692 is currently submitted under 505(b)(2). In the proposed carcinogenicity labeling, the section similar to the labeling for Ultram (NDA 20-281) is adopted. The sponsor of NDA 21-692 does not plan to include the results from two studies reviewed here in the labeling. It is recommended that the results from both 104-week rat ( ) and 26-week p53 (+/-) mouse ( ) studies be included in the labeling if NDA 21-692 is approved.

Conrad H. Chen, Ph.D.
Pharmacology Reviewer

Concurrence by: Josie Yang, Ph.D.
Pharmacology Team Leader



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CAC minutes:

Executive CAC
Date of Meeting: September 28, 2004

Committee: Jeri El Hage, Ph.D., HFD-510, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Barry Rosloff, Ph.D., HFD-120, Alternate Member
Josie Yang, Ph.D., HFD-550, Team Leader
Conrad H. Chen, Ph.D., HFD-550, Presenting Reviewer

Author of Draft: Conrad H. Chen

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-692
Drug Name: Ralivia™, Tramadol Hydrochloride Extended Release Tablets (100, 200, and 300 mg)
Sponsor: Biovail Laboratories, Incorporated

General Background:
Immediate release form of tramadol hydrochloride was approved in 1995. Biovail Laboratories, the developer of Ralivia, [redacted]. Therefore, they conducted a 104-week rat carcinogenicity study and a 26-week p53 mouse alternative carcinogenicity study in support of the NDA. Recently, Biovail has decided to submit their NDA under 505(b)(2) instead. The proposed labeling for Ralivia ER is similar to the labeling of currently marketed tramadol product, which includes life time carcinogenicity studies in rats and mice. The dose levels (up to 30 mg/kg/day) in these studies were considered below the MTD. The dose levels used by Biovail in the new carcinogenicity studies were 25, 50, 75, 100/150/100 mg/kg/day in 104-week rat study and 37.5, 75, and 150 mg/kg/day in the 26-week p53 mouse study.
Biovail did not propose to include the findings from their own carcinogenicity studies in the proposed labeling for Ralivia ER.
The dose selections were based on a 26-week rat study and a 28-day mouse study and were approved by the Executive CAC on June 8, 1999, February 22, 2000, and April 24, 2001.

Rat Carcinogenicity Study:
In the rat study, there was no significant treatment-related increase in neoplastic findings. The incidence of thyroid C-cell adenomas was higher for females given 100 mg/kg/day than for the controls, but the increase was not statistically significant. Pituitary adenomas, mammary fibroadenomas, and mammary carcinomas were observed less frequently than

control for females given 100 mg/kg/day, probably secondary to the marked decreases in body weight gain at that dose.

P53 Mouse Carcinogenicity Study:
In the mouse study, none of the macroscopic alterations were treatment-related. Among the unscheduled deaths, the thymic masses in one control and two medium dose females correlated with the microscopic findings of malignant lymphoma. Upon microscopic examination, malignant lymphomas were found in one control female and three medium dose females. One case of thymic lymphoma was also found in one control female at terminal sacrifice. In summary, lymphomas were found in two control females and three medium dose females in this study. Lymphoma was not found in other treated groups (including high dose) among terminal sacrifices and unscheduled deaths and there was no dose-effect relationship. Malignant lymphoma is a common tumor in p53 (+/-) mice. No other tramadol-related histomorphologic findings occurred after 26 weeks of treatment.

Executive CAC Recommendations and Conclusions:

Rat:

The Committee agreed that the study was acceptable, noting that the doses had prior FDA concurrence and concluded that there were no treatment-related tumor increases. However, sensitivity to any potential carcinogenic effect of the drug may have been reduced by the relatively large decreases in bodyweight gains seen.

The labeling for the currently marketed tramadol product says that carcinogenicity testing was done below the MTD. The Committee noted that Biovail study could be used to verify whether MTD had been reached in the previous rat study with Ultram if the same strain of rats were used; that is, the excessive decrease in body weight gain (and possible tumor suppression) in the Biovail study could have suggested that the original study was done at an adequate dose. However, the previous carcinogenicity study was conducted in a different strain of rats (Wister rats) than the Biovail study which was conducted in Sprague-Dawley rats. Therefore, in essence, the doses in the two studies cannot be compared meaningfully.
Both studies were negative for tumor findings, and the drug probably has been adequately tested.
The results from the Biovail study can be included in the labeling provided that the following statement is added: The excessive decrease in body weight gain in the treated groups in 104-week rat study might have reduced the sensitivity of rats to any potential carcinogenic effect of the drug.

P53 Mouse:

The Committee agreed that the study was adequate, noting that the doses had prior FDA concurrence.

The Committee concluded that the study was negative for significant tumor findings and results from the p53 mouse study should be included in the labeling.

Jeri El Hage, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-550
/Team leader, Josie Yang, HFD-550
/Reviewer, Conrad Chen, HFD-550
/CSO/PM, Nancy Clark, HFD-550
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Conrad Chen
8/22/2005 03:22:58 PM
PHARMACOLOGIST
Recommended revision of carcinogenicity labeling.

Josie Yang
8/29/2005 11:00:12 AM
PHARMACOLOGIST
The format of proposed labeling was not in compliance with current Center's practices.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-692
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	December 31, 2003
DRUG NAME:	Ralivia (tramadol hydrochloride) Extended Release Tablets
INDICATION:	Moderate to moderately severe pain
SPONSOR:	Biovail Laboratories, Incorporated
DOCUMENTS REVIEWED:	Electronic submission
REVIEW DIVISION:	Division of Anti-inflammatory, Analgesic, and Ophthalmic Drug Products (HFD-550)
PHARM/TOX REVIEWER:	Conrad H. Chen, Ph.D.
PHARM/TOX SUPERVISOR:	Josie Yang, Ph.D.
DIVISION DIRECTOR:	Brian Harvey, M.D.
PROJECT MANAGER:	Nancy Clark

Date of review submission to Division File System (DFS): October 20, 2004

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability
Approval of NDA is recommended.

B. Recommendation for nonclinical studies
None

C. Recommendations on labeling
Findings from 104-week carcinogenicity study in rats and 26-week carcinogenicity study in p53 mice should be included in the labeling (see Suggested Labeling on page 27 of review). The labeling for Pregnancy, Fertility, and Mutagenesis as proposed by the sponsor are acceptable.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings
The repeat-dose toxicity findings were mainly CNS related (convulsions, tremors, excessive salivation, etc.) In the genotoxicity studies, tramadol was positive in the mouse lymphoma assay in the presence of S9 activation but was negative in the absence of S9. It was also negative in the Ames test (±S9) and the mouse micronucleus test. Tramadol was not teratogenic but appeared to be embryotoxic and fetotoxic in the reproductive studies. The results from 104-week rat study and 26-week p53 mouse study showed that tramadol was not carcinogenic.

B. Pharmacologic activity
The analgesic effects of tramadol and its active metabolite O-desmethyltramadol (M1) are mediated by their bindings to μ-opioid receptors. Weak inhibitions of serotonin and norepinephrine uptakes by tramadol and M1 are also demonstrated.

C. Nonclinical safety issues relevant to clinical use
Convulsions and tremors were observed in animal toxicity studies.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

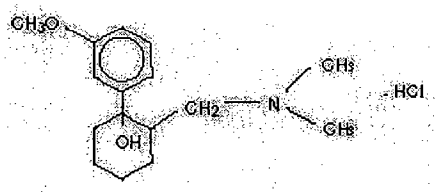
NDA number: 21-692
Review number: No.1
Sequence number/date/type of submission: 000/December 31, 2003/Commercial
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Biovail Laboratories, Incorporated
Manufacturer for drug substance:

Reviewer name: Conrad H. Chen, Ph.D.
Division name: Anti-inflammatory, Analgesic, And Ophthalmic Drug Products
HFD #: 550
Review completion date: October 9, 2004

Drug:

Trade name: Ralivia™
Generic name: Tramadol Hydrochloride Extended Release Tablets (100, 200, and 300 mg tablets
Code name: TRA P03
Chemical name: (±) cis-2-[(dimethylamino) methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride.

CAS registry number: 22204-88-2
Molecular formula/molecular weight: $C_{16}H_{25}NO_2 \cdot HCl/299.8$
Structure:



Relevant INDs/NDAs/DMFs: IND 57,552, IND 59,023

Drug class: Centrally acting analgesic

Indication: Management of moderate to moderately severe pain in adults

Clinical formulation: 100, 200, and 300 mg tablets

Route of administration: Oral

Proposed use: For patients with moderate to moderately severe chronic pain —

Start at a dose of 100 mg QD and titrated up if required by 100 mg increments every 5 days as necessary for pain relief and depending on tolerability, not to exceed [redacted] /day.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary: Tramadol is a centrally acting analgesic with an active metabolite, O-desmethyltramadol (M1). The immediate release form of tramadol is currently marketed (Ultram, NDA 20281, approved on March 3, 1995). Since there is a considerable understanding of its pharmacological activity, no new pharmacology studies were conducted. This NDA is a 505(b)(2) submission. The sponsor is partially relying on the FDA's previous finding of safety and effectiveness of Ultram for the approval of their NDA.

Tramadol has been shown to produce an analgesic effect in several different animal pain models. The potency of tramadol was similar to that of codeine and ranged from 2 to 10-fold less potent than morphine. While the mechanism of action is not wholly understood, the analgesic effects of tramadol appear to be mediated via binding to μ-opioid receptors and a weak inhibition of serotonin uptake. Tramadol also produces a weak inhibition of norepinephrine uptake, but how much this effect contributes to its analgesic activity is not clear.

2.6.2.2 Primary pharmacodynamics:

The analgesic effects of tramadol appear to be mediated via binding to μ-opioid receptors.

2.6.2.3 Secondary pharmacodynamic:

Tramadol produces weak inhibitions of serotonin uptake and epinephrine uptake.

2.6.2.4 Safety pharmacology: Safety pharmacology studies from the literature showed that tramadol minimally impaired performance in a mouse rotarod test. In a kindled rat model of epilepsy, tramadol, its enantiomers, and O-desmethyl metabolite produced anticonvulsant effects at IP doses below 30 mg/kg; however, at a dose of 30 mg/kg, tramadol and its enantiomers induced seizures in the majority of animals tested. This dose had no effect in nonkindled control animals. Tramadol inhibited mouse colonic propulsive motility, with the activity residing with the (+)-enantiomer. The results of cardiovascular studies showed that tramadol may possess a mild myocardial depressant activity. Tramadol produced no renal effects in nephritic rats.

Abuse liability: The potential for physical or psychological dependence appeared to be lower than morphine. Repeated administration of tramadol produced some physical dependence in naïve monkeys as discontinuation produced mild withdrawal symptoms qualitatively similar to those of morphine. The reinforcing and drug-seeking effects of tramadol studied in monkeys were less than those observed for pentazocine or codeine.

Other: Studies have demonstrated that less tolerance develops to the effects of tramadol compared to other narcotic analgesics, and the cross-tolerance between tramadol and morphine were shown to be minimal.

2.6.2.5 Pharmacodynamic drug interactions: No information is submitted.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

In animal studies, tramadol HCl was rapidly absorbed and rapidly metabolized. Based on the AUC values, the exposure in rodents and dogs is lower than that observed in humans after a single oral dose of the extended-release formulation of tramadol HCl. Animal studies have demonstrated that the tissue distribution of tramadol is primarily to the liver. Tissue distribution studies to determine the levels of tramadol and M1 in the brain demonstrated that, in mice, tramadol plasma levels were approximately 35% lower than M1 plasma levels. The levels of M1 in brain and plasma were similar, whereas brain levels of tramadol were approximately 2.5-fold higher than plasma levels of tramadol. Similar results were observed in rats, although the tramadol levels observed in brain were approximately 3- to 5-fold higher than levels in plasma.

Tramadol HCl is rapidly metabolized and, in animal and human studies, at least 30 metabolites of tramadol have been characterized and identified. Six pathways were proposed for the formation of metabolites: O-demethylation, N-demethylation, cyclohexyl oxidation, oxidative N-dealkylation, dehydration, and conjugation. In general, the metabolism of tramadol is similar in rats, dogs, and humans. The major metabolites in all three species were O-desmethyltramadol (M1), N-desmethyltramadol (M2), N,N-didesmethyltramadol (M3), N,O-didesmethyltramadol (M5), and the glucuronide of M5. In rats and humans, the glucuronide of M1 was also a major metabolite. Humans excreted more unchanged tramadol (>10%) as compared to rats and dogs (2%). In humans, tramadol and several of its metabolites undergo sulfation, a pathway that does not appear to be observed in rats and dogs.

Urinary excretion studies demonstrated that, although the proportions varied across species, metabolite M1 and its conjugate were the major metabolites measured, except in humans where M2 was found at approximately similar levels.

2.6.4.2 Methods of Analysis: For the following items: Methods of analysis, Absorption, Distribution, Metabolism, Excretion, Drug interaction, etc., see the summary above. The sponsor relies on the pharmacokinetic information from literature and the previous findings of FDA for tramadol.

2.6.4.3 Absorption: see above

2.6.4.4 Distribution: see above

2.6.4.5 Metabolism: see above

2.6.4.6 Excretion: see above

2.6.4.7 Pharmacokinetic drug interactions: see above

2.6.4.8 Other Pharmacokinetic Studies: see above

2.6.4.9 Discussion and Conclusions: See the summary above.

2.6.4.10 Tables and figures to include comparative TK summary

Several toxicokinetic studies were conducted in conjunction with repeat-dose toxicity studies. The AUC values for the high dose in each study are presented in Table 5.3-3 and are compared to AUC values in humans after a single dose of the extended-release formulation of tramadol HCl.

Table 5.3-3: AUC Values for Tramadol in Mice, Rats, Dogs, and Humans

Species	Dose	Duration	AUC_{0-t} (ng hr/mL)	Report No.
Mouse	150 mg/kg/day	28 Days	Male: 4155 Female: 4826	AA14YE2G32.04BTL
Mouse	150 mg/kg/day	26 Weeks	Male: 3175 Female: 3173	6802-159
Rat	75 mg/kg/day	26 Weeks	Male: 2463 Female: 10977	6802-120
Dog	60 mg/kg/day	39 Weeks	Male: 536 Female: 335	6802-123
Human	100 mg 200 mg 400 mg	Single dose	2778[a] 6356 15213	B01-569PK-TRAP03

[a] Combined values for males and females

The exposure of mice and rats in the high-dose groups in the toxicity studies was generally comparable to the exposure in humans after a single 100-mg dose of the extended-release formulation of tramadol HCl; however, exposure in rodents was less than that observed in humans after a single 200- or 400-mg dose. Exposure in dogs was substantially less than that observed in humans.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary:

The toxic effects of tramadol hydrochloride were evaluated in repeat-dose toxicity studies, as well as in studies designed to determine carcinogenic potential. The mutagenic potential of tramadol was assessed in a battery of *in vitro* and *in vivo* genetic toxicity tests. Oral reproductive toxicity was determined in fertility, embryofetal development, and perinatal/postnatal development reproductive studies. A summary of the nonclinical toxicology study conducted by Biovail is presented in Table 5.4-1.

Table 5.4-1: Summary of Nonclinical Safety Program

Category/Test Type/Article	Route	Species	Report No.
Repeat-Dose Studies			
5-Day/Range-finding/Tramadol HCl	PO	Mouse	AA14YE.2G32.04.BTL
28-Day/Toxicity/Toxicokinetics/Tramadol HCl	PO	Mouse	AA14YE.2G32.04.BTL
4-Week/Toxicity/Tramadol HCl	PO	Rat	6802-111
13-Week/Toxicity/Tramadol HCl	PO	Rat	6802-117
26-Week/Toxicity/Toxicokinetics/Tramadol HCl	PO	Rat	6802-120
4-Week/Toxicity/Tramadol HCl	PO	Dog	6802-114
13-Week/Toxicity/Toxicokinetics/Tramadol HCl immediate-release/Tramadol HCl extended-release	PO	Dog	6802-158
39-Week/Toxicity/Toxicokinetics/Tramadol HCl	PO	Dog	6802-123
Genotoxicity			
Bacterial reverse mutation/Tramadol HCl	*In vitro*	*S. typhimurium* *E. coli*	G98AW50.502
Mammalian cell gene mutation/Tramadol HCl	*In vitro*	L5178Y/TK$^{+/-}$ cells	G98AW50.704
Micronucleus test/Tramadol HCl	PO	Mouse	G98AW50.123
Carcinogenicity			
26-Week/Tramadol HCl	PO	Mouse (p53(+/-))	6802-159
104-Week/Tramadol HCl	PO	Rat	6802-126
Reproduction Studies			
Fertility/Embryofetal development/Range-finding/Tramadol HCl	PO	Rat	2102-007P
Fertility/Embryofetal development/Tramadol HCl	PO	Rat	2102-007
Developmental toxicity/Range-finding/Tramadol HCl	PO	Rabbit	2102-008P
Developmental toxicity/Tramadol HCl	PO	Rabbit	2102-008
Perinatal-postnatal development/Range-finding/Tramadol HCl	PO	Rat	2102-009P
Perinatal-postnatal development/Tramadol HCl	PO	Rat	2102-009

2.6.6.2 Single-dose toxicity: No single-dose toxicity studies were conducted.

2.6.6.3 Repeat-dose toxicity:

Repeat-dose toxicity studies with tramadol HCl were conducted in mice and rats administered by gavage and in dogs administered by gelatin capsule.

One study in dogs compared the effects of an immediate-release formulation and extended-release tablets; the tablets were put in gelatin capsules for administration. The results of the repeat-dose toxicity studies demonstrated that treatment with tramadol HCl was well-tolerated at doses up to 150 mg/kg/day in mice (28 days; Report No. AA14YE.2G32.04.BTL), 25 mg/kg/day in rats (26 weeks; Report No. 6802-120), and 20 mg/kg/day in dogs (39 weeks; Report No. 6802-123). In general, clinical signs in mice were limited to lethargy, although high doses produced tonic seizures, and no treatment-related clinical signs were observed in rats at the doses administered. No treatment-related clinical signs were observed in dogs at doses up to 60 mg/kg/day for 4 weeks (Report No. 6802-114), but longer treatment (39 weeks) produced signs of neurotoxicity. A 13-week bridging study (Report No. 6802-158) was conducted in dogs to compare the toxicity of the immediate-release formulation (Lot No. 3TRMDN09034) with that of extended-release tablets (200 or 300 mg/day). Dogs treated with the extended-release formulation received tablets from Lot No. 000303 from Days 1 through 70 and Lot No. 99H060 from Days 71 through 92. The only treatment-related finding was a decrease in

body weight gains for dogs treated with the immediate-release formulation; this finding was not observed in dogs treated with the extended-release formulation.
None of the toxicology studies demonstrated any treatment-related effects on hematology, clinical chemistry, or urinalysis parameters and there were no treatment-related macroscopic or microscopic findings or effects on organ weights.

Study title: 26-week gavage toxicity study in rats

Key study findings:
Study no.: 6802-120
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 10-29-98
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 25, 50, or 75 mg/kg/day, divided into 2 daily doses for 26 weeks
Species/strain: Crl:CD(SD)IGS BR rats
Number/sex/group or time point (main study): 20/sex/group
Route, formulation, volume, and infusion rate: oral gavage
Satellite groups used for toxicokinetics or recovery:
Age: 33 to 39 days old
Weight (nonrodents only):
Unique study design or methodology (if any):

Observation times and results: One control female and two females in 50 mg/kg/day group died on the test. The rest of the animals survived to the scheduled necropsy. Lower body weights, body weight gains, and food consumption were found in the treated groups. At the completion of the study (Week 27), mean body weights for males and females given 25, 50, or 75 mg/kg/day were 91.6, 84.7, and 80.1%, and 91.9, 88.1, and 90.4%, respectively, of those of the control animals. Overall body weight gains (Weeks 1 to 27) for males and females given 25, 50, or 75 mg/kg/day, were 89.0, 80.4, and 73.7%, and 86.5, 80.9, and 84.2%, respectively, when compared to with those of the respective control animals. The differences in body weights and body weight gains shown above were all statistically significant.
At 25 mg/kg/day, no apparent changes in clinical observation were found. No specific drug-related macroscopic or microscopic changes were found in the treated groups. The differences in organ weight data (absolute weights, organ-to-body percentages, and organ-to-organ weight ratios) for the treated groups were considered as related to the decreases in the terminal body weights. The sponsor considered 25 mg/kg/day as the NOAEL in 26-week rat study based on the clinical findings.

Study title: Thirty nine-week capsule toxicity study in dogs

Key study findings:
Study no.: 6802-123
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 10-16-98
GLP compliance: Yes
QA report: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 20, 40 or 80/60 mg/kg/day
Doses were selected based on results from a 4-week toxicity study where 15- 30, and 60 mg/kg/day were used. The 80 mg/kg/day dose was reduced to 60 mg/kg/day on Day 8 because of toxicity.
Species/strain: beagle dogs
Number/sex/group or time point (main study): 4/sex/group
Route, formulation, volume, and infusion rate: oral, in gelatin capsules for 39 weeks
Satellite groups used for toxicokinetics or recovery:
Age: 5 months old
Weight (nonrodents only): 5.6 to 8.0 kg
Unique study design or methodology (if any):

Observation times and results: Two males administered 80/60 mg/kg/day were terminated at unscheduled intervals on Day 80 and 114. Neurological observations including convulsions, tremors, ataxia, hyperactive behavior, and sensitive to touch were noted for these animals. All other animals survived to the scheduled necropsy. The same neurological findings were also found in several others in 80, a few in 40 and one in 20 mg/kg/day groups.
Test material-related lower mean body weights and food consumption were noted for males given 80/60 and for females given 20, 40, or 80/60 mg/kg/day. Mean body weight losses during the first week of treatment for males and females given 80 mg/kg/day were 10.5% and 7.8%, respectively. During the Weeks of 1 and 2, while the control males and females gained 0.3 and 0.1 kg, the 80/60 mg/kg/day males and females lost 0.8 and 0.5 kg, respectively. At the termination of the study, the mean body weights for males and females in the treated groups were 105, 97.2, 90.7% and 94.5, 90.1, and 92.3% of controls, respectively.
Administration of tramadol had no obvious effects on electrocardiographic findings, ophthalmic observations, or clinical pathology test results.
No drug-related effects were seen on absolute or relative organ weights in any treated groups. There were no drug-related effects on macroscopic or microscopic pathology findings at terminal sacrifice. Two males in high dose groups that were terminated early had testicular atrophy and aspermia.
Tramadol was rapidly absorbed and rapidly eliminated in dogs. The increase in tramadol C_{max} and AUC were not consistently proportional to the increase in the dose. Based on the results of this study, the NOAEL was considered as 20 mg/kg/day.

6.6.6.4 Genetic toxicology: Tramadol HCl was negative in an *in vitro* bacterial reverse mutation assay using *S. typhimurium* and *E. coli* tester strains in the absence and presence of metabolic activation (Report No. G98AW50.502). The results of the *in vitro* mutation assay with tramadol HCl in mouse lymphoma L5178Y cells were negative in the absence of metabolic activation and positive in the presence of metabolic activation (Report No. G98AW50.704). At oral doses up to 150 mg/kg, tramadol HCl was negative in an *in vivo* mouse micronucleus test (Report No. G98AW50.123).

Study title: Bacterial reverse mutation assay

Key findings: Negative
Study no.: G98AW50.502
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: 6-11-98
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: [redacted]

Methods
Strains/species/cell line: *S. typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *E. coli* tester strain WP2uvrA in the presence and absence of Aroclor-induced rat liver S9.

Doses used in definitive study: 100-5,000 µg/plate, neither precipitate nor appreciable toxicity was observed.

Basis of dose selection: establishment of dose range preliminary toxicity

Negative controls: water

Positive controls: 2-aminoantracene, 2-nitrofluorene, sodium azide, 9-amino acridine, and methyl methanesulfonate

Incubation and sampling times: 48 to 72 hours

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

The following criteria must be met for the mutagenicity assay to be considered valid. All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene. All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60. To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL. The mean of each positive control must exhibit at least a three-fold increase in the number of revertants over the mean value of the respective vehicle control. A minimum of three non-toxic dose levels are required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) A reduction in the background lawn.

Study outcome:

The results of mutagenesis assay were presented in the following table.

Salmonella/E. coli Mutagenicity Assay
Summary of Results

Table 16

Test Article Id : TRA P03
Study Number : G98AW50.502 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	14 ± 1	115 ± 6	9 ± 2	5 ± 3	13 ± 2
100	12 ± 3	118 ± 12	10 ± 1	3 ± 1	17 ± 6
333	12 ± 3	112 ± 8	5 ± 3	5 ± 2	16 ± 3
1000	13 ± 2	122 ± 16	11 ± 3	4 ± 1	15 ± 2
3333	14 ± 3	132 ± 9	6 ± 2	4 ± 2	14 ± 2
5000	10 ± 1	117 ± 10	12 ± 5	6 ± 0	10 ± 2
Pos	248 ± 177	537 ± 30	471 ± 52	514 ± 85	173 ± 13

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100	TA1535	TA1537	WP2 uvrA
0.0	21 ± 1	129 ± 8	10 ± 2	6 ± 2	19 ± 2
100	20 ± 3	122 ± 16	9 ± 2	4 ± 0	19 ± 5
333	19 ± 4	121 ± 4	7 ± 3	7 ± 4	15 ± 2
1000	17 ± 3	123 ± 5	9 ± 4	6 ± 1	17 ± 3
3333	21 ± 2	141 ± 15	11 ± 3	6 ± 4	17 ± 2
5000	17 ± 5	116 ± 4	7 ± 1	3 ± 1	14 ± 3
Pos	871 ± 38	859 ± 54	92 ± 7	140 ± 31	491 ± 20

0.0 = Vehicle plating aliquot of 50 µL
Pos = Positive Control concentrations as specified in Materials and Methods section.

Under the condition of this study, tramadol HCl was concluded to be negative in the bacterial reverse mutation assay.

Study title: *In vitro* mammalian cell gene mutation test

Key findings: Under the conditions of this study, tramadol HCl was concluded to be negative without S9 activation and positive with S9 activation in the L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay.

Study no.: G98AW50.704

Volume #, and page #:
Conducting laboratory and location: ———
Date of study initiation: 6-12-98
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: —>

Methods

Strains/species/cell line: L5178Y/TK$^{+/-}$ mouse lymphoma cells, clone 3.7.2C, obtained

Doses used in definitive study: 25 to 600 µg/mL

Basis of dose selection: Preliminary toxicity assay was used to establish the dose range.

Negative controls: water

Positive controls: In the presence of S9: 7, 12 Dimethylbenz (a) anthracene
In the absence of S9: methyl methanesulfonate

Incubation and sampling times: 24 and 48 hours

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The following criteria must be met for the mutagenesis assay to be considered valid:
Negative Controls:
The spontaneous mutant frequency of the solvent control cultures must be within 20 to 100 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%.
Positive Controls:
At least one concentration of each positive control must exhibit mutant frequencies of $\geq$100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies (Moore *et al.*, 1985; Aaron *et al.*, 1994).
Test Article-Treated Cultures:

A minimum of four analyzable concentrations with mutant frequency data will be required.

Study outcome:

Under the conditions of this study, tramadol HCl was concluded to be negative without S9 activation and positive with S9 activation in the L5178Y/TK$^{+/-}$ mouse lymphoma mutagenesis assay.
The results of mutagenesis assay are presented in the following tables.

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH TRA P03 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

Test Article Concentration (µg/mL)		TFT Colonies Counts				Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1	25	40	25	30	±7	111	123	112	115	±5	52		
Solvent	2	30	32	33	32	±1	149	137	156	147	±8	43		
Mean Solvent Mutant Frequency= 48														
100	A	55	35	39	43	±9	155	148	153	152	±3	57	9	100
100	B	30	38	38	35	±4	142	104	133	126	±16	56	8	84
250	A	83	83	78	81	±2	120	98	78	99	±17	165	117	54
250	B	81	96	101	93	±8	97	85	112	98	±11	189	142	53
500	A	116	105	103	108	±6	89	78	86	84	±5	256	209	30
500	B	97	105	98	100	±4	111	104	91	102	±8	196	149	35
600	A	90	86	76	84	±6	98	91	92	94	±3	179	132	23
600	B	92	84	101	92	±7	64	91	96	84	±14	221	173	20
750	A	72	66	79	72	±5	112	92	92	99	±9	147	99	11
750	B	74	73	68	72	±3	94	73	90	86	±9	167	120	15
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)														
2.5		146	145	149	147	±2	151	159	166	159	±6	185	137	95
4		178	156	166	167	±9	98	112	103	104	±6	319	272	49

Solvent = water

A and B or 1 and 2 are duplicate cultures

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH TRA P03
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
Independent Repeat Assay

Test Article Concentration (µg/mL)		TFT Colonies Counts			Mean	VC Colonies Counts				Mean	Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
Solvent	1				+	+	+	151	151	±0			
Solvent	2	89	73	68	77 ±9	181	181	208	190	±13	81		
Mean Solvent Mutant Frequency= 81													
200	A	87	83	73	81 ±6	158	138	152	149	±8	108	28	91
200	B	73	80	100	84 ±11	172	157	139	156	±13	108	27	98
300	A	74	77	68	73 ±4	191	171	164	175	±11	83	3	98
300	B	68	71	85	75 ±7	197	153	153	168	±21	89	8	89
400	A				+	140	138	152	143	±6			64
400	B	55	55	53	54 ±1	168	169	175	171	±3	64	-17	66
500	A	28	25	27	27 ±1	155	158	150	154	±3	35	-46	39
500	B	20	28	31	26 ±5	184	170	166	173	±8	30	-50	50
600	A	33	24	31	29 ±4	210	212	171	198	±19	30	-51	37
600	B	24	25	33	27 ±4	155	143	164	154	±9	35	-45	32
Positive Control - Methyl Methanesulfonate (µg/mL)													
2.5		126	140	155	140 ±12	124	125	113	121	±5	233	152	56
5		155	153	164	157 ±5	96	81	84	87	±6	362	281	34

Solvent = water

A and B or 1 and 2 are duplicate cultures

+ - Culture lost to contamination

[a] - Mutant frequency (per 10^6 surviving cells)=(Average # TFT colonies / average # VC colonies) x 200

[b] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] - % total growth = (% suspension growth x % cloning growth) / 100

Study title: Mouse erythrocyte micronucleus test

Key findings:
Study no.: G98AW50.123
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: 6-11-98
GLP compliance: yes
QA reports: yes (x) no ( )

Drug, lot #, and % purity: ____,

Methods

Strains/species/cell line: ICR mice, 5/sex/group

Doses used in definitive study: 38, 75, or 150 mg/kg by single oral gavage

Basis of dose selection: pilot toxicity test

Negative controls: water

Positive controls: cyclophosphamide (CP)

Incubation and sampling times: 24 and 48 hours

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):
The mean incidence of micronucleated polychromatic erythrocytes must not exceed 5/1000 polychromatic erythrocytes (0.5%) in the vehicle control. The incidence of micronucleated polychromatic erythrocytes in the positive control group must be significantly increased relative to the vehicle control group ($p \leq 0.05$, Kastenbaum-Bowman Tables).

Study outcome:
The incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes scored and the portion of polychromatic erythrocytes per total erythrocytes are summarized and presented for each treatment group by sacrifice time in Table 6.

Table 6

Summary of Bone Marrow Micronucleus Study Using TRA P03

Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- sd)	Change from Control (%)	Micronucleated Polychromatic Erythrocytes Number per 1000 PCEs (Mean +/- sd)	Number per PCEs Scored
Water							
20 mL/kg	M	24	5	0.48 ± 0.06	---	0.4 ± 0.22	4 / 10000
	F	24	5	0.50 ± 0.04	---	0.5 ± 0.33	5 / 10000
TRA P03							
38 mg/kg	M	24	5	0.42 ± 0.07	-13	0.4 ± 0.42	4 / 10000
	F	24	5	0.47 ± 0.08	-6	0.7 ± 0.27	7 / 10000
75 mg/kg	M	24	5	0.51 ± 0.04	6	0.2 ± 0.27	2 / 10000
	F	24	5	0.45 ± 0.04	-10	0.2 ± 0.27	2 / 10000
150 mg/kg	M	24	5	0.41 ± 0.08	-15	0.3 ± 0.27	3 / 10000
	F	24	5	0.48 ± 0.09	-4	0.2 ± 0.27	2 / 10000
CP, 50 mg/kg	M	24	5	0.37 ± 0.04	-23	32.0 ± 9.54	*320 / 10000
	F	24	5	0.38 ± 0.04	-24	28.7 ± 7.27	*287 / 10000
Water							
20 mL/kg	M	48	5	0.55 ± 0.05	---	0.2 ± 0.27	2 / 10000
	F	48	5	0.43 ± 0.05	---	0.7 ± 0.45	7 / 10000
TRA P03							
150 mg/kg	M	48	5	0.49 ± 0.06	-11	0.4 ± 0.42	4 / 10000
	F	48	5	0.47 ± 0.04	9	0.1 ± 0.22	1 / 10000

[1]*, p<0.05 (Kastenbaum-Bowman Tables)

Slight reductions of 4 to 15% in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the test article-treated groups relative to their respective vehicle controls. Reductions were observed in male dose groups 24 hours after treatment with 38 mg/kg and 24 and 48 hours after treatment with 150 mg/kg. Decrease in ratio of polychromatic erythrocytes to total erythrocytes was apparent in female mice 24 hours after treatment with 38, 75, and 150 mg/kg. The number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in test article-treated groups was not statistically increased relative to their respective vehicle control in either male or female mice, regardless of dose level or bone marrow collection time ($p>0.05$). cyclophosphamide (CP) induced a significant increase in micronucleated polychromatic erythrocytes in both male and female mice ($p<0.05$).

All criteria for a valid test were met. Under the conditions of the assay described in this report, TRA P03 (tramadol hydrochloride) did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow and was concluded to be negative in the micronucleus test using male and female ICR mice.

2.6.6.5 Carcinogenicity: Please see the separate "Review and Evaluation of Carcinogenicity Studies" for detail.

Study title:

104-week gavage oncogenicity study with TRA P03 (tramadol hydrochloride) in rats
26-week gavage carcinogenicity study with tramadol hydrochloride in p53 (+/-) C57BL/6 mice

Key study findings: Two carcinogenicity bioassays, one using a traditional rat model and one using a transgenic mouse model, demonstrated that tramadol HCl had no carcinogenic potential.

Adequacy of the carcinogenicity study and appropriateness of the test model:
The dose selections for these two carcinogenic studies have been concurred by Exec CAC.
Evaluation of tumor findings:

104-week rat study: There was no significant increase in any of the neoplastic findings. The incidence of thyroid C-cell adenomas was higher for females given 100 mg/kg/day than for the controls, but the increase was not statistically significant. Pituitary adenomas, mammary fibroadenomas, and mammary carcinomas were observed less frequently for females given 100 mg/kg/day.

26-week mouse study: None of the macroscopic alterations were treatment-related. Among the animals of unscheduled deaths, the thymic masses in one control and two group 3 females correlated with the microscopic findings of malignant lymphoma. Upon microscopic examinations, the malignant lymphomas were found in one control female and three group 3 females. One case of thymic lymphoma was also found in one control female at terminal sacrifice. In summary, lymphomas were found in two control females and three group 3 females in this study. Lymphoma was not found in other treated groups (including group 4) among terminal sacrifices and unscheduled deaths and there was no dose-effect relationship. No other tramadol-related histomorphologic findings occurred after 26 weeks of treatment. However, malignant lymphoma is a common tumor in p53 (+/-) mice.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Combined fertility and developmental toxicity study in rats

Key study findings: No treatment-related effects on fertility, embryo/fetotoxicity, and teratogenicity were observed under the condition of this study.
Study no.: 2102-007
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation: January 1999
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Table 6

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Treatment	Sex	Time (hr)	Number of Mice	PCE/Total Erythrocytes (Mean +/- sd)	Change From Control (%)	Micronucleated Polychromatic Number per 1000 PCEs (Mean +/- sd)	Erythrocytes Number per PCEs Scored[1]
Water							
20 mL/kg	M	24	5	0.48 ± 0.06	---	0.4 ± 0.22	4 / 10000
	F	24	5	0.50 ± 0.04	---	0.5 ± 0.35	5 / 10000
TRA P03							
38 mg/kg	M	24	5	0.42 ± 0.07	-13	0.4 ± 0.42	4 / 10000
	F	24	5	0.47 ± 0.08	-6	0.7 ± 0.27	7 / 10000
75 mg/kg	M	24	5	0.51 ± 0.04	6	0.2 ± 0.27	2 / 10000
	F	24	5	0.45 ± 0.04	-10	0.2 ± 0.27	2 / 10000
150 mg/kg	M	24	5	0.41 ± 0.08	-15	0.3 ± 0.27	3 / 10000
	F	24	5	0.48 ± 0.09	-4	0.2 ± 0.27	2 / 10000
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	F	24	5	0.38 ± 0.04	-24	28.7 ± 7.27	*287 / 10000
Water							
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	F	48	5	0.43 ± 0.05	---	0.7 ± 0.45	7 / 10000
TRA P03							
150 mg/kg	M	48	5	0.49 ± 0.06	-11	0.4 ± 0.42	4 / 10000
	F	48	5	0.47 ± 0.04	9	0.1 ± 0.22	1 / 10000

[1]*, p=0.05 (Kastenbaum-Bowman Tables)

Slight reductions of 4 to 15% in the ratio of polychromatic erythrocytes to total erythrocytes were observed in some of the test article-treated groups relative to their respective vehicle controls. Reductions were observed in male dose groups 24 hours after treatment with 38 mg/kg and 24 and 48 hours after treatment with 150 mg/kg. Decrease in ratio of polychromatic erythrocytes to total erythrocytes was apparent in female mice 24 hours after treatment with 38, 75, and 150 mg/kg. The number of micronucleated polychromatic erythrocytes per 1000 polychromatic erythrocytes in test article-treated groups was not statistically increased relative to their respective vehicle control in either male or female mice, regardless of dose level or bone marrow collection time ($p>0.05$). cyclophosphamide (CP) induced a significant increase in micronucleated polychromatic erythrocytes in both male and female mice ($p<0.05$).

All criteria for a valid test were met. Under the conditions of the assay described in this report, TRA P03 (tramadol hydrochloride) did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow and was concluded to be negative in the micronucleus test using male and female ICR mice.

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2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Combined fertility and developmental toxicity study in rats

Key study findings: No treatment-related effects on fertility, embryo/fetotoxicity, and teratogenicity were observed under the condition of this study.
Study no.: 2102-007
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: January 1999
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 10, 25 and 50 mg/kg/day by gavage; 28 days before cohabitation in males, 15 days before cohabitation in females through day 17 of gestation (DG 17)
Species/strain: Crl:CDBR VAF/Plus (Sprague-Dawley)
Number/sex/group: 25/sex/group
Route, formulation, volume, and infusion rate: by oral gavage once daily, 10 ml/kg
Satellite groups used for toxicokinetics:
Study design: Female rats were sacrificed by carbon dioxide asphyxiation on DG 20, Caesarean-sectioned and a gross necropsy was performed, and fetal examinations were conducted. The dose selection was based on a preliminary dose-range finding study.
Parameters and endpoints evaluated:

Results

Mortality: There were no mortalities during the study.

Clinical signs: Treatment-related clinical signs in males consisted of localized alopecia in the 50 mg/kg/day group and dilated pupils in the 25 and 50 mg/kg/day groups. In females, treatment-related clinical signs consisted of excess salivation in the 50 mg/kg/day group during gestation and localized alopecia in all groups during the precohabitation and gestation periods.

Body weight: In males, body weight gains were significantly reduced in all treated groups for the entire precohabitation period; body weight gains for the entire study period were significantly reduced in the 25 and 50 mg/kg/day groups (84.7% and 77.6% of control, respectively). Feed consumption was significantly reduced in the 25 and 50 mg/kg/day groups during the precohabitation period but was generally comparable to controls, or increased, after cohabitation. Absolute feed consumption values were significantly reduced in the 25 and 50 mg/kg/day groups for the entire study.
In females, significant body weight loss occurred in the 25 and 50 mg/kg/day groups on Days 1 to 8 of the study and significant reductions in body weight gain occurred on Days 8 to 15. As a result, body weight gains were significantly reduced in the 25 mg/kg/day group and significant body weight loss occurred in the 50 mg/kg/day group for the entire precohabitation period. Body weight gains for the entire gestation period were significantly reduced in the 50 mg/kg/day group (89.9% of control). Based on the changes in body weight and body weight gain, it appears that maternal MTD has been attained in this study.

PROTOCOL 2102-007: COMBINED (GAVAGE) FERTILITY AND DEVELOPMENTAL TOXICITY STUDY OF TRA P03 IN RATS
(SPONSOR'S STUDY NUMBER: B98-PC055-TRA P03)

TABLE C4 (PAGE 1): BODY WEIGHT CHANGES - PRECOHABITATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	25	50
RATS TESTED	N	25	25	25	25
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+6.4 ± 6.6	+3.6 ± 5.2	-1.0 ± 6.9**	-3.4 ± 4.6**
DAYS 8 - 15b	MEAN±S.D.	+9.6 ± 6.6	+9.0 ± 4.7	+5.2 ± 6.4*	+3.4 ± 6.2**
DAYS 1 - 15b	MEAN±S.D.	+16.0 ± 8.7	+12.6 ± 6.3	+4.2 ± 7.8**	-0.1 ± 7.5**

DAYS = DAYS OF STUDY
a. Dosage occurred on day 1 of study through day 17 of presumed gestation.
b. Last value recorded before cohabitation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

PROTOCOL 2102-007: COMBINED (GAVAGE) FERTILITY AND DEVELOPMENTAL TOXICITY STUDY OF TRA P03 IN RATS
(SPONSOR'S STUDY NUMBER: B98-PC055-TRA P03)

TABLE C5 (PAGE 1): MATERNAL BODY WEIGHTS - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	25	50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	24	25
INCLUDED IN ANALYSES	N	23b	23c	24	25
MATERNAL BODY WEIGHT (G)					
DAY 0	MEAN±S.D.	277.6 ± 15.8	272.3 ± 13.6	263.8 ± 11.8**	261.3 ± 13.7**
DAY 7	MEAN±S.D.	310.8 ± 19.0	301.6 ± 15.3	291.0 ± 13.6**	285.6 ± 15.0**
DAY 14	MEAN±S.D.	343.3 ± 22.3	331.2 ± 15.4*	320.8 ± 14.1**	317.8 ± 16.1**
DAY 18	MEAN±S.D.	391.0 ± 29.1	375.1 ± 18.2	365.2 ± 17.6**	363.6 ± 19.8**
DAY 20	MEAN±S.D.	423.8 ± 33.0	412.6 ± 20.9	399.6 ± 16.2* [23]d	392.7 ± 21.4**

DAY = DAY OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 17 of gestation.
b. Excludes values for dam 505, which had a litter consisting of one conceptus.
c. Excludes values for dam 542; the mating date was presumed incorrectly identified.
d. Excludes a value that was not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

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PROTOCOL 2102-007: COMBINED (GAVAGE) FERTILITY AND DEVELOPMENTAL TOXICITY STUDY OF TRA P03 IN RATS
(SPONSOR'S STUDY NUMBER: B98-PC055-TRA P03)

TABLE C6 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 10	III 25	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	24	25
INCLUDED IN ANALYSES	N	23b	23c	24	25
MATERNAL BODY WEIGHT CHANGE (G)					
DAYS 0 - 7	MEAN±S.D.	+33.2 ± 7.9	+29.3 ± 5.6	+27.2 ± 6.9**	+24.3 ± 7.0**
DAYS 7 - 14	MEAN±S.D.	+32.4 ± 6.2	+29.6 ± 3.6	+29.8 ± 5.9	+32.2 ± 8.5
DAYS 14 - 18	MEAN±S.D.	+47.7 ± 11.0	+43.9 ± 7.6	+44.4 ± 8.1	+45.9 ± 8.3
DAYS 0 - 18	MEAN±S.D.	+113.3 ± 18.6	+102.9 ± 10.5*	+101.4 ± 12.0*	+102.3 ± 14.1
DAYS 18 - 20	MEAN±S.D.	+32.8 ± 6.4	+37.4 ± 8.2*	+36.0 ± 6.1 [23]d	+29.0 ± 7.6
DAYS 0 - 20	MEAN±S.D.	+146.2 ± 22.0	+140.3 ± 16.2	+136.6 ± 11.7 [23]d	+131.4 ± 14.8*

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 17 of gestation.
b. Excludes values for dam 505, which had a litter consisting of one conceptus.
c. Excludes values for dam 542; the mating date was presumed incorrectly identified.
d. Excludes a value that was not recorded.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Food consumption: Absolute and relative feed consumption values were significantly decreased in all treated groups for the entire precohabitation and gestation periods.

The absolute consumption values during Days 0 to 20 of gestation were 100, 91.9, 86.5, and 86.1% for 0, 10, 25, and 50 mg/kg/day groups, respectively.

PROTOCOL 2102-007: COMBINED (GAVAGE) FERTILITY AND DEVELOPMENTAL TOXICITY STUDY OF TRA P03 IN RATS
(SPONSOR'S STUDY NUMBER: B98-PC055-TRA P03)

TABLE C10 (PAGE 1): MATERNAL RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - GESTATION - SUMMARY - Fo GENERATION FEMALE RATS

DOSAGE GROUP DOSAGE (MG/KG/DAY)a		I 0 (VEHICLE)	II 10	III 25	IV 50
RATS TESTED	N	25	25	25	25
PREGNANT	N	24	24	24	25
INCLUDED IN ANALYSES	N	23b	23c	24	25
MATERNAL FEED CONSUMPTION (G/KG/DAY)					
DAYS 0 - 7	MEAN±S.D.	83.6 ± 7.8	77.9 ± 4.9**	75.6 ± 6.3**	75.2 ± 5.3**
DAYS 7 - 14	MEAN±S.D.	80.6 ± 5.3	75.6 ± 3.2**	75.0 ± 4.8**	76.0 ± 5.8**
DAYS 14 - 18	MEAN±S.D.	75.5 ± 5.6	71.8 ± 4.2*	71.0 ± 5.1**	70.7 ± 4.0**
DAYS 0 - 18	MEAN±S.D.	80.2 ± 5.3	75.5 ± 3.2**	74.2 ± 4.6**	74.3 ± 4.6**
DAYS 18 - 20	MEAN±S.D.	67.4 ± 4.7	67.6 ± 4.3	67.4 ± 4.8 [23]d	63.9 ± 5.6*
DAYS 0 - 20	MEAN±S.D.	78.5 ± 4.6	74.4 ± 2.8**	72.5 ± 3.7** [23]d	73.0 ± 4.1**

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on day 1 of study through day 17 of gestation.
b. Excludes values for dam 505, which had a litter consisting of one conceptus.
c. Excludes values for dam 542; the mating date was presumed incorrectly identified.
d. Excludes a value that was associated with spilled feed.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

The relative food consumption values during Days 0 to 20 of gestation were 100, 94.8, 92.7, and 93% for 0, 10, 25, and 50 mg/kg/day groups, respectively.

Toxicokinetics: Not performed

Necropsy: There were no treatment-related effects on absolute weights of epididymides, testes, seminal vesicles, or prostate. Caudal epididymal sperm motility, count, and density were unaffected by treatment. There were no necropsy observations that were considered to be related to treatment other than the localized alopecia.

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Treatment had no effect on the reproductive performance of males and females. Caesarean-sectioning and litter parameters were unaffected by administration of the highest dose level of 50 mg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, and percent live male fetuses were comparable among the groups. All gross external, soft tissue, or skeletal malformations and variations in the fetuses were considered unrelated to treatment.
Female reproductive performance was unaffected by dosages of the test article as high as 50 mg/kg/day. There were no statistically significant or biologically important differences in the number of days in cohabitation, number of rats that mated, the fertility index, rats with confirmed mating dates, and rats pregnant per rats in cohabitation.
The percentage of dams with any resorptions was significantly increased ($p<0.05$ to $p<0.01$) in the 10, 25, and 50 mg/kg/day dosage groups. The sponsor considered this apparent increase in resorptions as unrelated to the test article because: 1) the changes were not dosage-dependent; and 2) the values are within the ranges observed historically at the Testing Facility. All placentas appeared normal. **However, it appears that the embryotoxic effects of tramadol can not be ruled out. Embryotoxic and fetotoxic effects of tramadol have been reported in the labeling for Ultram.**
Fetal alterations were defined as: 1) malformations (irreversible changes that occur at low incidences in this species and strain); 2) variations (common findings in this species and strain, and reversible delays or accelerations in development).
In the 0 (vehicle), 10, 25, and 50 mg/kg/day dosage groups, litters with fetuses with any alteration were 11 (45.8%), 10 (41.7%), 7 (29.2%) and 9 (36.0%), respectively. The numbers of fetuses with any alteration observed were 13 (4.0%), 12 (3.6%), 17 (4.9%) and 10 (2.8%), and the percentages of fetuses per litter with any alterations were 7.6%, 3.5%, 4.7% and 2.7% in the four dosage groups, respectively. All fetal alterations were considered unrelated to the test article because the incidences: 1) were not dosage-dependent; and/or 2) were within the ranges observed historically at the Fasting Facility according to the sponsor.
Based on the results of this study, the NOAEL for parental toxicity was considered to be less than 10 mg/kg/day and the reproductive and embryofetal development NOAEL was considered to be greater than 50 mg/kg/day.

Embryofetal development

Study title: Oral developmental toxicity study in rabbits

Key study findings:
Study no.: 2102-008

Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: November 1998
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 10, 50, and 100 mg/kg/day on days 6 through 18 of gestation
Species/strain: [redacted] White [redacted]
Number/sex/group: 20 females/group
Route, formulation, volume, and infusion rate: 10 ml/kg once daily by stomach tube
Satellite groups used for toxicokinetics:
Study design: On gestation day 29, all surviving rabbits were sacrificed, Caesarean-sectioned, and a gross necropsy was performed. The dose selection was based on a preliminary dose-range finding study.
Parameters and endpoints evaluated:

Results

Mortality (dams): There were no unscheduled deaths.

Clinical signs (dams): There was no treatment-related abortion, premature delivery, and clinical signs or necropsy observations.

Body weight (dams):
Body weight gains were reduced or significantly reduced ($p \leq 0.05$ to $p \leq 0.01$) in the 50 and 100 mg/kg/day dosage groups for the entire dosage period (calculated as DGs 6 to 19), the entire gestation period after the initiation of dosing (DGs 6 to 29), and the entire gestation period (DGs 0 to 29). Significant reductions ($p \leq 0.01$) in body weight gain or significant ($p \leq 0.01$) body weight loss occurred in the 50 mg/kg/day dosage group on DGs 12 to 15, and in the 100 mg/kg/day dosage group on DGs 7 to 8, 9 to 12 and 12 to 15. During the postdosage period (DGs 19 to 29), body weight gain was significantly increased ($p \leq 0.05$) in the 100 mg/kg/day dosage group, a rebound phenomenon that commonly occurs in these types of studies. Absolute body weights were significantly reduced ($p \leq 0.05$) in the 100 mg/kg/day dosage group on DG 18.
Significant ($p \leq 0.05$) body weight loss occurred in the 50 mg/kg/day dosage group on DGs 0 to 6, but was not considered related to the test article because dosing was not initiated until DS 6.
Body weights and body weight gains were unaffected by the 10 mg/kg/day dosage of the test article. A significant increase (≤ 0.01) in body weight gain on DGs 8 to 9 in the 10 mg/kg/day dosage group was considered transient and unrelated to the test article.
Body weight gains were significantly reduced for the entire gestation period for animals in the 50 and 100 mg/kg/day groups (72% and 77% of control values, respectively).

PROTOCOL 2202-066: ORAL (STOMACH TUBE) DEVELOPMENTAL TOXICITY STUDY OF TRA P01 IN RABBITS
(SPONSOR'S STUDY NUMBER: ESE-DC659-TRA P01)

TABLE 5 (PAGE 1): MATERNAL BODY WEIGHT CHANGES - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	50	100
RABBITS TESTED	N	20	20	20	20
PREGNANT	N	20	20	19	19
MATERNAL BODY WEIGHT CHANGE (KG)					
DAYS 0 - 6	MEAN±S.D.	+0.04 ± 0.09	+0.07 ± 0.07	-0.03 ± 0.12*	+0.04 ± 0.10
DAYS 6 - 7	MEAN±S.D.	+0.01 ± 0.04	+0.03 ± 0.04	+0.03 ± 0.06	+0.03 ± 0.05
DAYS 7 - 8	MEAN±S.D.	+0.01 ± 0.04	+0.02 ± 0.03	-0.01 ± 0.04	-0.03 ± 0.04**
DAYS 8 - 9	MEAN±S.D.	+0.00 ± 0.03	+0.04 ± 0.04**	+0.00 ± 0.03	+0.00 ± 0.04
DAYS 6 - 9	MEAN±S.D.	+0.03 ± 0.06	+0.08 ± 0.07	+0.02 ± 0.09	-0.01 ± 0.04
DAYS 9 - 12	MEAN±S.D.	+0.04 ± 0.04	+0.03 ± 0.06	+0.01 ± 0.06	+0.01 ± 0.06**
DAYS 12 - 15	MEAN±S.D.	+0.12 ± 0.06	+0.08 ± 0.05	+0.04 ± 0.05** [18]b	+0.04 ± 0.06**
DAYS 15 - 19	MEAN±S.D.	+0.05 ± 0.07	+0.04 ± 0.07	+0.09 ± 0.09 [18]b	+0.02 ± 0.09
DAYS 19 - 24	MEAN±S.D.	+0.14 ± 0.10	+0.12 ± 0.13	+0.10 ± 0.11 [18]b	+0.20 ± 0.09
DAYS 24 - 29	MEAN±S.D.	+0.12 ± 0.06	+0.07 ± 0.07 [19]b	+0.10 ± 0.09 [17]b	+0.15 ± 0.06 [18]b
DAYS 6 - 19	MEAN±S.D.	+0.23 ± 0.17	+0.24 ± 0.12	+0.16 ± 0.17 [18]b	+0.02 ± 0.15**
DAYS 19 - 29	MEAN±S.D.	+0.26 ± 0.15	+0.22 ± 0.08 [19]b	+0.33 ± 0.10 [17]b	+0.36 ± 0.13* [18]b
DAYS 6 - 29	MEAN±S.D.	+0.49 ± 0.20	+0.47 ± 0.11 [19]b	+0.40 ± 0.17 [17]b	+0.37 ± 0.18* [18]b
DAYS 0 - 29	MEAN±S.D.	+0.53 ± 0.18	+0.54 ± 0.12 [19]b	+0.38 ± 0.19** [17]b	+0.41 ± 0.17* [18]b

DAYS = DAYS OF GESTATION
[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 6 through 18 of gestation.
b. Excludes values for does that were moribund sacrificed, aborted or delivered.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

Food consumption (dams): Absolute and relative food consumption values were also significantly decreased for the entire gestation period in 50 and 100 mg/kg/day groups.

Toxicokinetics: Not performed

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.):
No caesarean-sectioning or litter parameters were affected by treatment. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the groups. No doe had a litter consisting of only resorbed conceptuses and there were no dead fetuses.

Offspring (malformations, variations, etc.):
No gross external, soft tissue or skeletal fetal alterations (malformations or variations) were caused by dosages of the test article as high as 100 mg/kg/day. There were no dosage-dependent or significant differences in the litter or fetal incidences of any gross external, soft tissue or skeletal alterations.
Litters with fetuses with alterations were 5 (25%), 9 (47.4%), 10 (58.8%), and 10 (55.6%) in the 0, 10, 50, and 100 mg/kg/day groups, respectively. The numbers of fetuses with any alteration observed were 7 (4.1%), 12 (8.3%), 15 (11.3%), and 18 (8.7%) and the percentages of fetuses with any alteration per litter were 4.4, 9.6, 11.6, and 8.4% in these same respective dosage groups. None of the gross external, soft tissue, or skeletal

alterations (malformations or variations) were considered to be treatment-related. Based on the changes in body weight gains, it appears that the maternal MTD has been reached in this study.
Based on the results of this study, the maternal NOAEL was considered to be 10 mg/kg/day and the embryofetal development NOAEL was considered to be greater than 100 mg/kg/day.

Prenatal and postnatal development

Study title: Developmental and perinatal/postnatal reproduction toxicity study in rats, including a postnatal behavioral/functional evaluation

Key study findings:
Based on the results of this study, it was concluded that the NOAEL for maternal toxicity was less than 5 mg/kg/day and that the developmental NOAEL was 25 mg/kg/day, based on the decreased pup body weights observed in the 50 mg/kg/day group. The NOAEL for viability and growth of the F_1 generation was 25 mg/kg/day based on the reduced postweaning body weights observed in the 50 mg/kg/day. The Sexual maturation, development, and mating performance of the F_1 generation were not affected at doses as high as 50 mg/kg/day.

Study no.: 2102-009
Volume #, and page #:
Conducting laboratory and location: [redacted]
Date of study initiation: November 1998
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 5, 25, and 50 mg/kg/day from day 7 of gestation (DG 7) through day 20 of lactation (DL 20)
Species/strain: [redacted]
Number/sex/group: 25 females/group
Route, formulation, volume, and infusion rate: 10 ml/kg once daily by oral gavage
Satellite groups used for toxicokinetics:
Study design, parameters and endpoints evaluated:
The parameters determined for F_0 generation females included clinical observations, abortions, premature delivery, body weight, food consumption, duration of gestation, litter size, pup viability at birth, and maternal behavior. F_0 generation females were sacrificed and necropsied on DL 21.
The parameters determined for F_1 generation pups included body weight, viability, clinical observations, and feed consumption. Beginning on postpartum Day 24 (DP 24), one male and one female rat from each litter were evaluated in a passive avoidance test for learning, short-term retention, and long-term retention. Female rats were evaluated for the age of vaginal patency beginning on DP 28;

male rats were evaluated for the age of preputial separation beginning on DP 39. Beginning on DP 70, one male and one female rat from each litter were evaluated in a water-filled M-maze for overt coordination, swimming ability, learning, and memory. The F_1 generation rats were mated at approximately 90 days of age. Macroscopic examination was conducted on all F_1 generation pups that died and all pups culled on DL21. Male rats selected for continued evaluation were sacrificed after completion of the 21-day cohabitation period, subjected to gross necropsy, and organ weights were obtained for testes and epididymides. The female rats selected for continued evaluation were sacrificed on DG 20, caesarean-sectioning and litter parameters were determined, and a gross necropsy was performed.
The parameters determined for F_2 fetuses included body weight, sex, and gross external alterations.

Results

F_0 in-life:
There were no F_0 mortalities during the study. Treatment-related clinical signs were observed for rats in the 25 and 50 mg/kg/day groups and consisted of dilated pupils, localized alopecia, and mydriasis during gestation and lactation. There were no gross lesions observed at necropsy.
Maternal body weight gains for the entire gestation period and for the dosage period were slightly reduced in the 5 mg/kg/day group (96% and 97% of control group values, respectively). Maternal body weight gains were significantly reduced in the 25 mg/kg/day group (86% and 83% of control values, respectively) and in the 50 mg/kg/day group (82% and 77% of control values, respectively) during the same time periods. Maternal body weight gains for the entire lactation period were increased in the 5 mg/kg/day group and significantly increased in the 25 and 50 mg/kg/day groups.
Absolute and relative feed consumption values were significantly decreased in the 25 and 50 mg/kg/day groups for the entire gestation period and the dosage period. Absolute and relative feed consumption values were unaffected during the lactation period.

F_0 necropsy:
No natural delivery parameters were affected by administration of tramadol HCl. The number of dams delivering litters, duration of gestation, implantation sites per delivered litter, the number of dams with stillborn pups, the number of dams with no liveborn pups, the gestation index, and the number of dams with all pups dying were comparable among the groups.

F_1 physical development:
Pup body weights were significantly reduced in the 50 mg/kg/day group on DL 1, 4, 7, 14, and 21. The numbers of pups delivered, the viability index, the number of surviving pups, the percentage of male pups, and the live litter size on each weighing day were comparable among the groups.

The numbers of pups found dead or presumed cannibalized were significantly increased on DL 5 to 7 and 15 to 21 in the 5 and 50 mg/kg/day groups, respectively. As a result, the lactation index on DL 21 was significantly reduced in the 5 and 50 mg/kg/day groups. The sponsor considered these increases as unrelated to the test article because both apparent increases could be attributed to pup mortality in only one litter in each group. **However, the toxic effects on the postpartum survivals cannot be ruled out.**
All F1 clinical observations were considered unrelated to administration of tramadol HCl to the F0 dams because the incidences were not dosage-dependent and/or occurred in only one or two pups. There were no treatment-related mortalities and all pups appeared normal at necropsy on DL 21.

PROTOCOL 2102-007: ORAL (GAVAGE) DEVELOPMENTAL AND PERINATAL/POSTNATAL REPRODUCTION TOXICITY STUDY OF TRA P03 IN RATS, INCLUDING A POSTNATAL BEHAVIORAL/FUNCTIONAL EVALUATION (SPONSOR'S STUDY NUMBER: BSS-PC056-TRA P03)

TABLE E11 (PAGE 1): LITTER OBSERVATIONS (NATURALLY DELIVERED PUPS) - SUMMARY - F1 GENERATION LITTERS

MATERNAL DOSAGE GROUP		I	II	III	IV
MATERNAL DOSAGE (MG/KG/DAY)		0 (VEHICLE)	5	25	50
DELIVERED LITTERS WITH ONE OR MORE LIVEBORN PUPS	N	24	23	25	24
PUPS DELIVERED (TOTAL)	N	336	315	351	356
	MEAN+S.D.	14.0 ± 1.6	13.9 ± 3.0	14.0 ± 3.4	14.8 ± 1.9
LIVEBORN	MEAN+S.D.	13.9 ± 1.6	13.6 ± 3.6	13.9 ± 3.3	14.6 ± 2.0
	N(%)	334(99.4)	312(97.8)	347(98.9)	350(98.3)
STILLBORN	MEAN+S.D.	0.1 ± 0.3	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.7
	N(%)	2(0.6)	4(1.2)	4(1.1)	6(1.7)
UNKNOWN VITAL STATUS	N	0	3	0	0
PUPS FOUND DEAD OR PRESUMED CANNIBALIZED					
DAY 1	N/N(%)	0/334(0.0)	4/312(1.3)	3/347(0.9)	6/350(1.7)
DAYS 2- 4	N/N(%)	6/334(1.8)	1/308(0.3)	6/344(1.7)	8/344(2.3)
DAYS 5- 7	N/N(%)	1/328(0.3)	9/307(2.9)**	4/338(1.2)	0/336(0.0)
DAYS 8-14	N/N(%)	0/327(0.0)	1/298(0.3)	1/334(0.3)	2/336(0.6)
DAYS 15-21	N/N(%)	0/327(0.0)	0/297(0.0)	0/333(0.0)	4/317(1.3)**a
VIABILITY INDEX b	%	98.2	98.4	97.4	96.0
	N/N	328/334	307/312	338/347	336/350
LACTATION INDEX c	%	99.7	95.7**	98.5	98.1*a
	N/N	327/328	297/307	333/338	313/319

DAY(S) = DAY(S) POSTPARTUM
a. Excludes values for litter 19377, which had an extra pup found on day 20 postpartum; it was presumed this pup was from litter 19378 (Group IV).
b. Number of live pups on day 4 postpartum/number of liveborn pups on day 1 postpartum.
c. Number of live pups on day 21 postpartum/number of live pups on day 4 postpartum.
* Significantly different from the vehicle control group value ($p \leq 0.05$).
** Significantly different from the vehicle control group value ($p \leq 0.01$).

F1 males from the 50 mg/kg/day group had significantly reduced body weight gains for the entire postweaning period; body weight gains in F1 females were significantly reduced from postweaning Days 1 through 57. Absolute feed consumption values were reduced and relative feed consumption values were increased for F1 males and females from the 50 mg/kg/day group.

F1 behavioral evaluation:
Sexual maturation of the F1 generation rats was unaffected by administration of tramadol to F0 dams. There were no biologically important or statistically significant differences in the values for learning, short-term retention, long-term retention, or response inhibition in the F1 generation males or females as evaluated by performance in a passive avoidance paradigm and a watermaze swim task.

F1 reproduction:
Mating performance of the F1 generation was unaffected. There were no statistically significant or biologically important differences in the number of days in cohabitation,

number of rats that mated, fertility index, rats with confirmed mating dates, and number of pregnant rats. Caesarean-sectioning and litter parameters were also unaffected

F_2 findings:
There were no treatment-related gross fetal alterations or malformations in F_2 generation fetuses.

2.6.6.7 Local tolerance: No information is submitted.

2.6.6.8 Special toxicology studies: No information is submitted.

2.6.6.9 Discussion and Conclusions:
The toxic effects of tramadol hydrochloride were evaluated in repeat-dose toxicity studies, as well as in studies designed to determine carcinogenic potential. The mutagenic potential of tramadol was assessed in a battery of *in vitro* and *in vivo* genetic toxicity tests. Oral reproductive toxicity was determined in fertility, embryofetal development, and perinatal/postnatal development reproductive studies.

2.6.6.10 Tables and Figures: See individual section in above for information.

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
Tramadol is a centrally acting analgesic with an active metabolite O-desmethyltramadol (M1). The immediate release form of tramadol is currently marketed (Ultram, NDA 20281, approved on March 3, 1995). Since there is a considerable understanding of its pharmacological activity, no new pharmacology studies were conducted. This NDA is a 505(b)(2) submission. The sponsor is partially relying on the FDA's previous finding of safety and effectiveness of Ultram for the approval of this NDA.
The sponsor has conducted several nonclinical toxicity studies for tramadol hydrochloride. The toxic effects of tramadol hydrochloride were evaluated in repeat-dose toxicity studies (e.g. 26-week rat and 39-week dog), as well as in studies designed to determine carcinogenic potential (104-week rat and 26-week p53 mouse). In general, the CNS- related toxicities (convulsions, tremors, excess salivation, etc.) were observed in those studies. No carcinogenic effects were observed at doses up to 75-100 mg/kg/day in rats and up to 150 mg/kg/day in p53 mice.
The mutagenic potential of tramadol was assessed in a battery of *in vitro* and *in vivo* genetic toxicity tests. At oral doses up to 150 mg/kg, tramadol HCl was negative in an *in vivo* mouse micronucleus test. Tramadol HCl was negative in an *in vitro* bacterial reverse mutation assay using *S. typhimurium* and *E. coli* tester strains in the absence and presence of metabolic activation. The results of the *in vitro* mutation assay with tramadol HCl in mouse lymphoma L5178Y cells were negative in the absence of metabolic activation and positive in the presence of metabolic activation. These results were similar to that from the studies previously conducted for Ultram.

Oral reproductive toxicity was determined in fertility, embryofetal development, and perinatal/postnatal development reproductive studies in rats and rabbits. Tramadol had no toxic effect on fertility in rats at doses up to 100 mg/kg/day. No teratogenic effects were observed in rats (at doses up to 50 mg/kg/day) and in rabbits (at doses up to 100 mg/kg/day). However, fetal resorptions increased and fetotoxic effects (increases in fetal resorptions) were suspected. The sponsor considered these effects not caused by drug because it was not dose-related and it was within the historical control values. In a perinatal/postnatal developmental study in rats, tramadol at 5 and 50 mg/kg/day produced a decrease in postnatal survivals, although the findings could be caused by a single litter effect. Embryotoxic and fetotoxic effects of tramadol were reported in previous animal reproductive studies conducted for Ultram.
Unresolved toxicology issues (if any): None

Recommendations: The approval of NDA 21-692 is recommended from the pharmacology perspective.

Suggested labeling:
The findings from the new genotoxicity studies and reproductive toxicity studies conducted by/for Biovail are similar to the findings described in the labeling for Ultram. Therefore, the proposed labeling for Ralivia, which is similar to that of Ultram, is acceptable.
The findings from the 104-week rat carcinogenicity study and the 26-week p53 mouse carcinogenicity study should be included in the labeling. The suggested labeling is:

A slight, but statistically significant, increase in two common murine tumors, pulmonary and hepatic, was observed in a mouse carcinogenicity study, particularly in aged mice. Mice were dosed orally up to 30 mg/kg (0.36 times the maximum daily human dosage [MDHD] of ——/day) for approximately two years, although the study was not done with the Maximum Tolerated Dose. This finding is not believed to suggest risk in humans. No such finding occurred in a rat carcinogenicity study (dosing orally up to 30 mg/kg or 0.73 times MDHD). In recent studies in rats for 104 weeks (75 mg/kg/day, 1.8 times MDHD) and in p53 mice for 26 weeks (150 mg/kg/day, 1.8 times MDHD) there were no carcinogenic effects. However, the excessive decrease in body weight gain in the treated groups in recent 104-week rat study might have reduced the sensitivity of rats to any potential carcinogenic effect of the drug.

Signatures (optional):

Reviewer Signature Conrad H. Chen, Ph.D.

Supervisor Signature Concurrence Yes ___ No ___
Josie Yang, Ph.D.

APPENDIX/ATTACHMENTS

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/s/

Conrad Chen
10/20/04 10:30:11 AM
PHARMACOLOGIST
The approval is recommended from the pharmacology perspective.

Josie Yang
10/21/04 12:06:11 PM
PHARMACOLOGIST

Executive CAC
Date of Meeting: September 28, 2004

Committee: Jeri El Hage, Ph.D., HFD-510, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Barry Rosloff, Ph.D., HFD-120, Alternate Member
Josie Yang, Ph.D., HFD-550, Team Leader
Conrad H. Chen, Ph.D., HFD-550, Presenting Reviewer

Author of Draft: Conrad H. Chen

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA # 21-692
Drug Name: Ralivia™, Tramadol Hydrochloride Extended Release Tablets (100, 200, and 300 mg)
Sponsor: Biovail Laboratories, Incorporated

General Background:
Immediate release form of tramadol hydrochloride was approved in 1995. Biovail Laboratories, the developer of Ralivia, Therefore, they conducted a 104-week rat carcinogenicity study and a 26-week p53 mouse alternative carcinogenicity study in support of the NDA. Recently, Biovail has decided to submit their NDA under 505(b)(2) instead. The proposed labeling for Ralivia ER is similar to the labeling of currently marketed tramadol product, which includes life time carcinogenicity studies in rats and mice. The dose levels (up to 30 mg/kg/day) in these studies were considered below the MTD. The dose levels used by Biovail in the new carcinogenicity studies were 25, 50, 75, 100/150/100 mg/kg/day in 104-week rat study and 37.5, 75, and 150 mg/kg/day in the 26-week p53 mouse study.
Biovail did not propose to include the findings from their own carcinogenicity studies in the proposed labeling for Ralivia ER.
The dose selections were based on a 26-week rat study and a 28-day mouse study and were approved by the Executive CAC on June 8, 1999, February 22, 2000, and April 24, 2001.

Rat Carcinogenicity Study:
In the rat study, there was no significant treatment-related increase in neoplastic findings. The incidence of thyroid C-cell adenomas was higher for females given 100 mg/kg/day than for the controls, but the increase was not statistically significant. Pituitary adenomas, mammary fibroadenomas, and mammary carcinomas were observed less frequently than control for females given 100 mg/kg/day, probably secondary to the marked decreases in body weight gain at that dose.

P53 Mouse Carcinogenicity Study:
In the mouse study, none of the macroscopic alterations were treatment-related. Among the unscheduled deaths, the thymic masses in one control and two medium dose females correlated with the microscopic findings of malignant lymphoma. Upon microscopic examination, malignant lymphomas were found in one control female and three medium dose females. One case of thymic lymphoma was also found in one control female at terminal sacrifice. In summary, lymphomas were found in two control females and three medium dose females in this study. Lymphoma was not found in other treated groups (including high dose) among terminal sacrifices and unscheduled deaths and there was no dose-effect relationship. Malignant lymphoma is a common tumor in p53 (+/-) mice. No other tramadol-related histomorphologic findings occurred after 26 weeks of treatment.

Executive CAC Recommendations and Conclusions:

Rat:

The Committee agreed that the study was acceptable, noting that the doses had prior FDA concurrence and concluded that there were no treatment-related tumor increases. However, sensitivity to any potential carcinogenic effect of the drug may have been reduced by the relatively large decreases in bodyweight gains seen.

The labeling for the currently marketed tramadol product says that carcinogenicity testing was done below the MTD. The Committee noted that Biovail study could be used to verify whether MTD had been reached in the previous rat study with Ultram if the same strain of rats were used; that is, the excessive decrease in body weight gain (and possible tumor suppression) in the Biovail study could have suggested that the original study was done at an adequate dose. However, the previous carcinogenicity study was conducted in a different strain of rats (Wister rats) than the Biovail study which was conducted in Sprague-Dawley rats. Therefore, in essence, the doses in the two studies cannot be compared meaningfully.
Both studies were negative for tumor findings, and the drug probably has been adequately tested.
The results from the Biovail study can be included in the labeling provided that the following statement is added: The excessive decrease in body weight gain in the treated groups in 104-week rat study might have reduced the sensitivity of rats to any potential carcinogenic effect of the drug.

P53 Mouse:

The Committee agreed that the study was adequate, noting that the doses had prior FDA concurrence.

The Committee concluded that the study was negative for significant tumor findings and results from the p53 mouse study should be included in the labeling.

Jeri El Hage, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-550
/Team leader, Josie Yang, HFD-550
/Reviewer, Conrad Chen, HFD-550
/CSO/PM, Nancy Clark, HFD-550
/ASeifried, HFD-024

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Jeri El Hage
10/6/04 08:25:57 AM