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IND # [redacted] Dec. 19, 1994

Sponsor: SmithKline Beecham Pharmaceuticals
King of Prussia, PA 19495 (215) 832-3707

Submission Date: 11/8/1994
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REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
INFORMATION AMENDMENT

Drug: BRL49653C [(±)-5-{(4-(2-(methyl-2-pyridinylamino) ethoxy)phenyl)methyl}-2,4-thiazolidinedione maleate]

Class: Thiazolidinedione antidiabetic agent

Related to: IND# [redacted]
IND# [redacted]

This amendment contains 8 preclinical studies conducted in rats and dogs. At least one of the studies was designed to test the sponsor's hypothesis that myocardial hypertrophy which was induced by the test substance would be secondary to an increase in plasma volume.

1. BRL 49653: EFFECT OF 14 DAYS TREATMENT ON CIRCULATING ERYTHROCYTE MASS IN FEMALE SPRAGUE DAWLEY RATS(PF-1001-BRL-049653/1)

A. METHODS: Batch #HGC-E-01C of BRL 49653C used in this study. A total of 31 female Sprague Dawley rats were implanted with arterial and venous cannulae. One week after surgery, 10 rats were treated by gavage with 5 ml/kg methylcellulose(control group) and 9 rats were given BRL 49653, 80 mg/kg, orally for 14 days. Hematocrit, total blood volume, red cell mass and plasma volume were either calculated or determined by conventional methods.

B. RESULTS: As shown in Table 1, treatment the rats with BRL, 80 mg/kg, for 14 days decreased hematocrit by 12% and increased total blood volume and plasma volume by 27% and 33%, respectively.

C. COMMENTS AND CONCLUSIONS: The volume of vehicle, 5 ml/kg, was somewhat large, but might not affect the quality of the data. A 30% increase in plasma volume as a result of the drug action appeared to have little effect on myocardial function and no histological changes have been documented.

2. BRL 49653: REPEAT ORAL DOSE STUDIES ON PACKED RED CELL VOLUME, PLASMA VOLUME AND HEART WEIGHT IN THE FEMALE SPRAGUE-DAWLEY RAT(PG-1021/BRL-049653/1)

A. METHODS: BRL 49653, Batch No. HGC-E-01C. A total of 40 female Sprague-Dawley rats were divided into 5 groups. Eight rats in each group were given BRL49653 in doses of 0, 3.5, 10, 35, or 80 mg/kg once daily by oral gavage for 14 days. Packed red cell volume, total plasma volume, and cardiac protein content were determined.

B. RESULTS: BRL had little effect on body weight gain at low doses, but there was a significant increase in BW gain at the highest dose (80 mg/kg) group. Packed red cell volume was significantly increased at 10 mg/kg or higher doses. There was a dose-dependent increase in plasma volume up to 30-40% in the high dose group. Cardiac wet or dry weight was also increased up to 20% and, in the high dose group, myocardial protein content was elevated by 30%(Table 2).

C. COMMENTS: Blood volume was increased by 30%, which might explain the body weight gain by 4%.

3. EFFECTS OF BRL 49653 ON SYSTEMIC HEMODYNAMICS AND RENAL FUNCTION IN THE FEMALE SPRAGUE-DAWLEY RAT(PP-1001/BRL-049653/1)

A. METHODS: BRL 49653, batch HGC-E 01C. Female SD rats were given BRL at a dose of 80 mg/kg once daily by oral gavage for 7 days. Cardiac output was monitored by a pulsed Doppler flow probe. Measurements of BP, HR and renal clearance were carried out by conventional methods. Total peripheral resistance(TPR) was calculated as BP(MAP)/CO, stroke volume(SV) as CO/HR, left ventricular minute work(LVMW) as MAP x CO, and left ventricular stroke work(LVSW) as LVMW/HR.

B. RESULTS: BRL, 80 mg/kg, had no effect on body weight after the 7 day-treatment, although a similar treatment for 14 days increased total blood volume significantly(Table 1). It reduced mean atrial pressure without an increase in HR. Hematocrit was reduced as in previous studies. Urine flow was reduced from 15.7 ± 1.4 to 10.0 ± 1.2 μl/min.100 g. as renal blood flow was reduced by 35%(Table 3).

C. COMMENTS: It appears that the hypotensive action of BRL was depended upon the drug-induced reduction in TPR. The mechanism of this action is not clear. The underlying mechanism of cardiac hypertrophy appears to be a drug-induced increase in myocardial tissue weight and protein, which may allow for an increase in stroke volume and cardiac output.

4. BRL49653: ACUTE AND REPEAT ORAL HIGH-DOSE STUDIES ON FLUID AND ELECTROLYTE BALANCE, AND ON VARIOUS PLASMA HORMONES, IN THE FEMALE SPRAGUE-DAWLEY RATS(REPORT NO. PF-1004/BRL-049653/1)

A. METHODS: At least 23 adult female SD rats were used. The rats were given orally either 1% methylcellulose (control group) or BRL 49653, 80 mg/kg, for 7 days. Following this active drug treatment period there was a recovery period for a week with vehicle only. Twenty-four hour urine volume, osmolality, creatinine clearance, major ion excretion, vasopressin, IGF-1 and aldosterone were measured.

B. RESULTS: The significant changes were noted in body weight gain(5.4 ± 2.6 vs. 21.3±2.5 g), food intake(141.8±3.1 vs. 158.9±2.9 g), water gain (67.84±5.53 vs. 85.57±5.30 g), sodium balance (2974±264 vs. 4734±253 μmol), chloride balance(-524 ±283 vs. 642±271 μmol), plasma aldosterone (258±107 vs. 575±102 pg/ml), heart weight(0.85±0.03 vs. 0.95±0.03 g). Most of the changes were reversible upon the withdrawal of the test substance.

C. COMMENTS: This report has no direct impact on the main issue of the drug-induced cardiac toxicity. However, it appears that BRL acted on renal tubules to retain salts and water by releasing aldosterone, which will increase blood volume. Heart became enlarged due to this change and vascular resistance was reduced to compensate for enlarged heart.

5. BRL 49653: A REPEAT ORAL DOSE STUDY ON PACKED RED CELL VOLUME AND HEART WEIGHT IN NON-DIABETIC AND STREPTOZOTOCIN-DIABETIC FEMALE SD RATS(PF-1003/BRL-049653/1)

A. METHODS: Diabetes was induced in 20 conscious SD female rats by intravenous administration of streptozotocin (60 mg/kg body weight). BRL 49653 was administered once daily by oral gavage at a dose of 80 mg/kg for 14 days. Packed RBC, and other parameters were determined.

B. RESULTS: BRL did not increase body weight (234±12 vs. 266±16 g) in non-diabetic animals, which is not consistent with an increase in body weight gain reported by the sponsor previously. This negative effect of BRL was true in streptozotocin-treated rats. However, BRL reduced plasma glucose(11.1±1.2 vs. 9.7±0.8 mmol/l) and plasma insulin(86.3±52.6 vs. 31.9±14.5 nmol/l) in non-diabetic rats. Streptozotocin-diabetic rats were characterized by high plasma glucose levels (37.3±3.0) with a low level of plasma

insulin 4.8±2.4 nmol/1), which was not reversed by BRL treatment. BRL reduced packed red cell volume(37.4±1.0 vs. 33.3±1.65) with an increase in heart wet weight(791±53 vs. 968±53 mg). Streptozotocin had no effect on these parameters.

C. COMMENTS: BRL produced a significant reduction in packed RBC volume and a significant increase in heart weight in non-diabetic rats but had no effect on either parameter in streptozotocin-diabetic animals. The sponsor argued that these indicate that BRL have no direct and independent action on the heart that results in an increase in heart weight. The reviewer has no problem in this interpretation since the primary action of BRL appears to be on renal tubule and vasculature.

6. BRL 49653: EFFECT OF 14 DAYS TREATMENT ON HEMATOCRIT, PLASMA VOLUME AND HEART WEIGHT IN FEMALE BRATTLEBORO RATS WITH DIABETES INSIPIDUS(PF-1002/BRL-049653/1)

A. METHODS: Twenty-five female Brattleboro D/I rats were conditioned for 7 days. Following the acclimation period the rats were given orally either placebo or BRL 49653, 80 mg/kg, for 2 weeks. Packed RBC volume and total plasma volume were measured by conventional assay procedures.

B. RESULTS: A significant reduction in hematocrit(38.2 ± 0.5 vs 35.6 ± 0.7) and an increase in heart weight(301 ± 4 vs 370 ± 6 mg/100 g body weight) was noted.

7. A STUDY TO INVESTIGATE THE PHARMACOKINETICS OF BRL 49653 IN RATS FOLLOWING A SINGLE INTRAVENOUS DOSE OF 0.4 MG/KG BRL 49653C AND A SINGLE ORAL DOSE OF 0.4 MG/KG BRL49653C(BF-1024/BRL-049653/1)

A. METHODS:

Three male SD rats were given once intravenously BRL 49653C at a dose of 0.4 mg/kg via the femoral vein. After a washout period of 3 days, the three rats and two additional rats were given BRL 49653c(0.4 mg/kg) once orally. Blood samples(approximately 0.15 ml) were obtained from the jugular vein at pre-dose and at 15, 30, 35, 45 minutes and 1, 1.25, 1.5, 2.5, 4.5, 6.5, 8.5, 10.5 and 24.5 hours after the initiation of BRL administration. PK parameters such as C_{max}, T_{max}, and AUC were determined by conventional methods. $T_{½}$ was calculated as $\ln(2)/\alpha$, here α indicates the apparent terminal elimination rate constant. The absolute bioavailability, F, was calculated as follows:

$F = AUC_{po}/AUC_{iv} \times Dose_{iv}/Dose_{po} \times T_{½po}/T_{½iv}$

B. RESULTS: are summarized in Table 4.

8. STUDY TO INVESTIGATE THE PHARMACOKINETICS OF BRL49653 IN DOGS FOLLOWING A SINGLE INTRAVENOUS DOSE OF 0.4 MG/KG AND A SINGLE ORAL DOSE OF 0.4 MG/KG(BF-1023/BRL049653/1)

A. METHODS: Three male Beagle dogs were given intravenously BRL(0.4 mg/kg) via cephalic vein. After a washout period of 7 days, the same dogs were given orally the same dose once. A series of blood samples (1.5 ml) were obtained from a cephalic vein at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 hours post-dose. Pharmacokinetic parameters were either determined or calculated as described under item 7.

B. RESULTS: are summarized in Table 5.

9. OVERALL COMMENTS

1. The data that support myocardial hypertrophy-induced by BRL49653C appears to be related to plasma expansion, which was mainly due to salt and water retention in female rats. However, the mechanism(s) that cause salt and water retention is not clear. Although the sponsor pointed out that the drug reduced total peripheral resistance(TPR), its salt and fluid retaining property is this division's concern since they are known to be detrimental to the cardiovascular system.

NAI

/S/ [redacted]
Herman M. Rhee, Ph. D.

/S/ [redacted]
1/5/95

cc: Original IND, HFD-510
A. Jordan/H. Rhee

[redacted]
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Table 1.

PARAMETER	CONTROL GROUP	BRL 49653 GROUP
Number of rats	10	9
Haematocrit (Initial blood sample)	39.6 ± 0.8	34.9 ± 0.7** ↓ 11.6%
Total blood volume (ml)	12.3 ± 0.7	15.6 ± 0.9* ↑ 26.8%
Plasma volume (ml)	7.5 ± 0.4	10.0 ± 0.6* ↑ 33.3%
RBC mass (ml)	4.8 ± 0.3	5.6 ± 0.3
Plasma erythropoietin concentration (mU/ml)	40.8 ± 2.4	48.7 ± 3.0

All data values above are expressed as the group mean value ± standard error of the mean (sem).
Asterisks indicate a statistically significant difference between the drug treatment and control groups: * $p<0.01$; ** $p<0.001$

Table : 2

Effect of repeat oral administration of BRL 49653 on heart weight and heart protein content in the female Sprague-Dawley rat

Dose of BRL 49653 (mg/kg body wt)	Heart Parameter				
	Heart Wet Weight (mg)	Heart Dry Weight (mg)	Dry wt/Wet wt (%)	Protein content (mg/heart)	Protein content (% wet wt)
0	904 ± 63 0.	199 ± 11	22 ± 0.5	38.6 ± 4.8	21.4 ± 2.0
3.5	939 ± 65 3.9	206 ± 16 3.5	22 ± 0.6	45.3 ± 7.6 14.7	24.1 ± 3.2
10.0	*989 ± 75 9.4	*217 ± 14 9.0	22 ± 0.5	42.3 ± 4.2 12.2	21.4 ± 1.7
35.0	**1038 ± 83 14.8	**227 ± 15 14.1	22 ± 0.4	**47.9 ± 4.7 24.1	23.2 ± 3.4
80.0	***1083 ± 71 19.8	***235 ± 15 18.0	22 ± 0.4	***51.4 ± 5.6 33.2	**23.7 ± 1.5

38.6

BRL 49653, or control solution, was administered once daily, by oral gavage for 14 days. On the 15th day, after an overnight fast, rats were anaesthetised with Na pentobarbitone. Following removal of a blood sample from the dorsal aorta, the thoracic cavity was opened and the heart removed. Results are mean ± SD (n=8). P; by Student's 't' test, *<0.05, **<0.01, ***<0.001.

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TABLE 3

Renal effects of BRL 49653 in female Sprague-Dawley rat

N = 5	Control	BRL 49653
Body weight, g	248 ± 8	229 ± 9
MAP, mm Hg	112 ± 3.0	100 ± 2.2*
Hematocrit, %	38 ± 0.7	34 ± 1.2*
Posm, mOsm/kg H_2O	294 ± 1.1	294 ± 0.8
Urine flow, µl/min•100 g	15.7 ± 1.4	10.0 ± 1.2*
Uosm, mOsm/kg H_2O	821 ± 57	971 ± 68
GFR, µl/min•100 g	1062 ± 69	973 ± 56
RBF, µl/min•100 g	5122 ± 88	3801 ± 194*
RVR (MAP/RBF)	21.9 ± 0.7	26.6 ± 1.4*
FF (GFR/RPF), %	33.2 ± 2.1	39.0 ± 1.4*
FE of sodium, %	1.8 ± 0.3	1.1 ± 0.1*
FE of potassium, %	45.1 ± 5.7	37.8 ± 2.6
C H_2O, µl/min•100 g	-27.0 ± 1.6	-21.9 ± 1.1
Cosm, µl/min•100 g	42.8 ± 1.3	31.9 ± 1.9*

The values under BRL 49653 are the means ± SEM of results obtained on me day after a single oral dose. P, plasma; U, urine, GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; FE, fractional excretion; C, clearance. *, significantly different from control, $P<0.05$.

TABLE 4

Parameter [units]	Intravenous [n=5]	Oral [n=4]
Cmax [ug/mL]	1.88 (1.58 - 2.27)	1.60 (1.17 - 1.92)
Tmax [hours]	0.50 (0.48 - 0.75)	0.49 (0.48 - 1.00)
AUC(0-inf) [ug.h/mL]	5.15 (3.26 - 6.57)	5.71 (5.04 - 6.55)
T1/2 [hours]	2.09 (1.71 - 2.51)	2.18 (1.76 - 2.64)
CL* [mL/h]	38.2 (29.5 - 58.3)	33.3 (28.5 - 38.1)
CL* [(ml/h)/kg]	81.0 (61.9 - 120.2)	70.4 (61.2 - 78.1)
Vss* [mL]	108.0 (85.5 - 135.7)	115.2 (94.9 - 156.3)
Vss* [mL/kg]	229.2 (177.8 - 279.8)	242.9 (205.9 - 320.9)

* - for the oral phase this parameter is divided by the fraction of dose absorbed. ** - median (range)

TABLE 5

Parameter [units]	Intravenous [n=3]	Oral [n=3]
Cmax [ug/mL]	3.04 (2.38-4.05)	0.90 (0.69-1.11)
Tmax * [hours]	0.50 (0.50-0.58)	2.00 (0.52-2.00)
AUC(0-inf) [ug.h/mL]	3.46 (2.28-4.97)	2.20 (1.40-3.29)
T1/2 [hours]	0.74 (0.62-0.88)	1.06 (0.87-1.29)
CL [mL/h]	1921 (1196-2404)	ND
CL [(mL/h)/kg]	127 (77.7-177)	ND
Vss [mL]	1807 (1423-2111)	ND
Vss [mL/kg]	119 (92.4-155)	ND

* - median (range)

ND - not determined

H. Rhee

IND # [redacted] October 20, 1993

OCT 22 1993

Sponsor: SmithKline Beecham Pharmaceuticals
King of Prussia, PA 19495 (215) 832-3707

Submission Date: 9/22/1993 Assigned Date: 10/4/1993

Drug: BRL49653C [(±)-5-{(4-(2-(methyl-2-pyridinylamino) ethoxy)phenyl)methyl}-2,4-thiazolidinedione maleate]

Class: Thiazolidinedione antidiabetic agent

Related to: IND [redacted]
IND# [redacted]

COMMENTS AND RECOMMENDATIONS

1) In the study, entitled "BRL 49653: Single oral dose studies on heart rate and blood pressure in conscious normotensive male Sprague-Dawley rat", you concluded that BRL reduced heart rate in the conscious, restrained rats(page 296). However, from the data in tables 1(pg 302) and 3(pg 304). it appears that batch 3 increased HR while batch 6 reduced it, although batch 6 produced tachycardia. Pleae explain.

2) In the study, entitled " A 4-week oral repeat dose study in dogs followed by a 4-week off-dose period", relative heart weights were increased by 20 and 28% respectively in intermediate and high dose females and by 32% in high dose males. After 4 week off-dose, relative heart weights remained increased by 19% and 12% in males and females, respectively. How and why does BRL 49653 increase cardiac weight without significant effects on cardiovascular functions such as BP and HR?

3) The results from the dose range-finding studies and the doses selected for the rat and mouse carcinogenicity studies should be submitted for our review prior to initiation of the carcinogenicity studies.

/S/ [redacted]

Herman M. Rhee, Ph. D.

cc: Original IND, HFD-510
A. Jordan/H. Rhee

/S/ [redacted]

10/22

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IND # [redacted] October 20, 1993

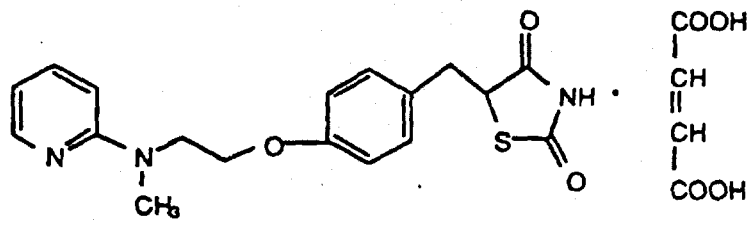
Sponsor: SmithKline Beecham Pharmaceuticals
King of Prussia, PA 19495 (215) 832-3707

Submission Date: 9/22/1993 Assigned Date: 10/4/1993

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
ORIGINAL SUMMARY

Drug: BRL49653C [(±)-5-{(4-(2-(methyl-2-pyridinylamino) ethoxy)phenyl)methyl}-2,4-thiazolidinedione maleate]

Chemical Structure:



BRL 49653C

Class: Thiazolidinedione antidiabetic agent

Related to: IND [redacted]
IND# [redacted]

Dosage form: 0.1 mg, 1.0 mg, and 5.0 mg capsules

Route of administration: Oral

Clinical Investigational Plans:

Several clinical studies are planned. Initial clinical trial is a single blind, randomized, dose-rising study in healthy 12 male volunteers, which is designed to determine the safety, tolerability and pharmacokinetics of BRL49653C {0.2, 0.5, 1.0, 2.0, and 5.0 mg}. A 10 day repeat dose study of safety, pharmacokinetics and preliminary pharmacodynamics in 24 obese insulin resistant or mildly diabetic subjects. In 24 normal volunteers, an open-label, crossover study of the effect of food on the pharmacokinetics of the hard gel capsule formulation will be studied. The combined administration of BRL49653C and an oral sulfonylurea will

be also studied in patients with NIDDM. The final study is a 6 week repeat dose study to evaluate the efficacy, safety and tolerability of 2-3 doses of BRL 49653C or placebo in 140 patients with NIDDM.

A. PHARMACOLOGY

1. BRL49653: Antihyperglycemic activity and effects on blood hemoglobin after administration for 8 days to C57B1/6 ob/ob mice. A comparison with CS-045(PG-1005/BRL-09653/2)

Groups of 6-8 mice were given either BRL49653 (Batch R1) or CS-045 (Batch GBD1) for 8 days by dietary admixture. The doses of BRL49653 ranged from 0.3 mol (0.108 mg) to 10,000 mol(3.6 g) per kg of diet. BRL 49653 produced a dose-dependent reduction in fasting blood glucose and improved the disposal of an oral glucose load. At high doses (>300 mol/kg diet) BRL caused a significant reduction in blood hemoglobin content and it appeared that BRL is about 100 times more potent than CS-045.

2. BRL 49653: antihyperglycemic activity and effects on pancreatic and serum insulin concentrations and on serum lipid concentrations after repeat administration to C57B1/KsJdb/db diabetic mice. A comparison with pioglitazone(AD4833,PG-1011/BRL-049653/2)

Groups of 5 female C57Bl/KsJ db/db diabetic mice were given either BRL49653 (Batch R5) or pioglitaone (Batch GBD2) for 21 days by dietary admixture. BRL 49653 was used at 3 doses levels: 10 mol(3.6 mg), 100 mol and 1000 mol/kg of diet. Pioglitazone was studied at doses of 300 (107mg), 3,000 and 30,000 mol per kg of diet. BRL 49653 produced a dose-dependent reduction in non-fasted blood glucose concentrations. I_{50} dose for antihyperglycemia was about 30 mol/kg of diet and BRL is about 30 times more potent than pioglitazone as an antihyperglycemic agent. BRL produced dose dependent falls in serum insulin concentration and concomitant increases in pancreatic insulin content, which suggest multiple action sites of this drug.

3. BRL 49653: Effect of repeat oral administration on plasma lipid and amylin concentrations in lean (Fa/?) and obese(fa/fa) male Zucker rats(PG-1015/BRL-049653/2)

Groups of 10 male Zucker fatty fa/fa rats and their lean male liter mate(Fa/?) were used. The animals were dosed daily by oral gavage for 21-28 days with BRL 49653 (3 mol/kg, body weight). Plasma amylin was detected by RIA,

using ^{125}I-rat amylin(10,000 cpm). Plasma cholesterol, TG and fatty acids in fa/fa rats were increased compared to those in lean(Fa/?) litermates. After 14 days of administration to fa/fa rats BRL reduced in total plasma cholesterol, TG and fatty acids, while it had little or no effect in lean rats. BRL also reduced plasma anylin in fa/fa rats.

4. BRL 49653: Single and repeat oral dose studies on blood glucose concentrations in the streptozotocin-diabetic rat(PG-1003/BRL-049653/1)

Diabetes was induced in conscious male Sprague-Dawley rats by intravenous(tail vein) injection of streptozotocin (80 mg/kg). In the rats, a single oral dose of BRL (3 or 30 mg/kg) had no effect on blood glucose levels. Repeated administration of the same doses for 7 days produced similar results, suggesting that the presence of endogenous insulin is a prerequisite for the action of this drug. This finding is also true for the mice, of which diabetes was induced by alloxan monohydrate (100 mg/kg).

5. BRL 49653: Effect of repeat oral administration on whole body, hepatic and peripheral tissue insulin sensitivity in the male Zucker fatty fa/fa rat(Pg-1014/BRL-049653/2)

Groups of 7-10 male Zucker fa/fa rats were given daily BRL 3 mol/kg by oral gavage for 21-31 days. Measurement of whole body and peripheral tissue insulin sensitivity was performed under hyperinsulinemic-euglycemic clamp conditions. At a steady state insulin level of 2 mU/ml and a steady state glucose concentration of 5 mM, the rate of insulin of glucose required to maintain euglycemic was significantly greater in BRL -treated rats. Hepatic glucose output was suppressed in both groups of rats in this condition.

6. BRL 49653: Effect of repeat oral administration to C57B1/6 ob/ob mice on insulin binding, glucose transport and cell surface glucose transporters(GLUT-4) in isolated white adipocytes(Pg-1009/BRL-049653/2)

Groups of 12 or 18 male C57Bl/6 ob/ob mice were administered by dietary admixture at a dose of 30 mol/kg for 15 to 24 days. Photoaffinity labelling of glucose transporters, immunoprecipitation of GLUT-4, electrophoresis and quantification of GLUT-4 transporters by Western blotting were performed in addition to standard glucose transport and insulin binding. Repeat oral administration of BRL (30 mol/kg of diet) produced a two-fold increase in insulin-binding to adipocytes isolated from white adipose tissue. Affinity of the insulin receptor for insulin is unchanged by the drug. Increased insulin-binding caused a 2.5-fold increase in total adipocyte content of GLUT-4 glucose transporter. BRL did not alter Km to insulin of adipocyte-glucose transport, nor is

there any effect on basal rates of glucose transport. It appears that BRL increased insulin responsiveness due to its action on translocation of GLUT-4 from intracellular storage sites to the cell surface.

7. BRL 49653: Effect on amylin-inhibition of insulin-stimulated glycogen synthesis and lactate output by the rat isolated soleus muscle(GP-1017/BRL-049653/2)

In the soleus muscle of male Wistar rats amylin produced a dose-dependent inhibition of radiolabelled glucose incorporation. Amylin also reduced lactate formation, of which effect was not affected by BRL, suggesting that the drug does not directly interact with the mechanisms whereby amylin opposes insulin action in the skeletal muscle in vitro.

8. BRL 49653: Single oral dose studies on heart rate and blood pressure in the conscious normotensive male Sprague-Dawley rat(PG-1002/BRL-049653/1)

BRL 49653, Batch no. R3 and R6, was used for this experiment. Groups of male Sprague-Dawley rats were given BRL 30 mg/kg by gavage. BP and HR were measured conventional methods at various time points. BRL, Batch 3, increased HR 30 min after drug administration in restrained and conscious rats(Table 1). BRL, batch 6 tended to decrease the parameter(Table 2), although inconsistent findings were noted with the same batch 6(Table 3). It appears that BRL has little or no effect on BP. In conscious male SHR BRL, batch R3, had no significant effect on neither mean arterial BP nor HR after 10 mol(3.6 mg)/kg p. o. daily.

B. TOXICOLOGY REPORTS

1. BRL 49653C - An intravenous acute toxicity study in rats(TF-1008/BRL-049653/1)

Eleven Sprague-Dawley rats per sex were injected intravenously single doses of 64 or 121 mg/kg of BRL(batch no. BMC-32518-162). The high dose killed one male 20 min after dosing. Toxic signs comprised ataxia, shallow respiration, convulsion and dilated pupils followed by gasping and a red nasal discharge prior to death. Adverse signs at 64 mg/kg were limited to transient, slight ataxia in one female. There were no macroscopic abnormality related to treatment.

2. An oral acute toxicity study in rats (TF-1003/BRL-049653/1)

Six Sprague-Dawley rats of each sex were administered a single dose of 2000 mg/kg by gavage. There was no mortality and treatment related signs were

transient post-dose salivation in one female. No remarkable pathologic findings were noted.

3. A comparison of BRL 48482 and BRL 49653 in a 4-week oral repeat dose study in the female rat(TF-1016/BRL-049653/1)

Groups of female SD rats were given orally BRL 48482(0.4, 4. and 40 mg/kg/day) or BRL 49653(0.36, 3.6 and 36 mg/kg/day) for-28 days. Swelling in the dorso-thoracic region were noted from week 2 in intermediate and high dose animals treated with BRL 48482, and in one that received BRL 49653 high dose.
On days 15 and 28, RBC parameters were slightly reduced in high dose BRL 48482 animals and high dose BRL 49653 animals. Heart weight was increased at the intermediate and high doses of both compounds, and liver weight slightly increased at the high dose of BRL 48482(Table 4). No toxicological findings were noted at 0.36 mg/kg BRL 49653 whereas bone marrow hypocellularity was detected at the equimolar dose of BRL 48482(0.4 mg/kg/day).

4. BRL 49653C: A maximum tolerated dose study in the dog by the oral route(TF-1005/BRL-049653/1)

One male and one female beagle dogs were given orally BRL(batch No. BMC-32518-128) 10 mg/kg on day 1, and the dose was doubled every day until the reaction to treatment and/or the effects on RBC parameters were considered to be dose-limiting. At the weekend the animals remained off-dose and the previous dose was resumed again on the Monday. When the MTD was reached the animals were left off-dose for 20 days and monitored for signs of recovery.

No mortality was observed. Marked body weight loss, approximately 10%, occurred in the female after receiving 320 mg/kg, which was recovered within 3 days off-dose. A slight non-progressive decrease in hemoglobin and RBC count was noted in both animals from day 3 after 20 mg/kg. Pale areas were noted around the cortico meduallary junction in the kidneys of the female.

5. BRL 49653c: A range-finding repeat dose study in dogs by the oral route(TF-1010/BRL-049653/2)

Two male and two female Beagle dogs were given orally either 20 mg/kg or 80 mg/kg of BRL(batch No. BMC-32518-128) for 28 days. At 80 mg/kg/day, sporadic emesis was noted between 1 and 4 hours after dosing. No treatment related changes in body weight, ECG and ophthalmoscopy were noted. No significant alterations in hematology, blood chemistry, urinalysis and/or macro-/microscopic observations were noted.

Toxicokinetic data indicated that there was systemic exposure to the parent compound, with both C_{max} and AUC(0-24) of BRL 49653 increased with increasing dose. There were also no obvious sex differences in exposure.

6. A 4-week oral repeat dose study in rats followed by a 4-week off-dose period(TF-1011/BRL-049653/2)

15-20 SD rats/gp/sex were given orally either 0(control), 0.4, 2.0 or 80.0 mg/kg/day of BRL(Batch No. BMC-32518-128) for 28 days.

a. Mortality : None.

b. Clinical Observations:

At the dose of 80 mg/kg/day, a firm swelling in the mid scapular region, first noted in some animals from day 22, occurred in 12/20 females, which persisted to the end of the study.

c. Body weights:

During the first week of treatment, marked increases in bodyweight gain of 39% and 167% were noted in high dose males and females respectively. In this group body weight gain during the 4-week off-dose period was reduced by 20% and 49% respectively.

d. Food consumption and utilization

Food consumption in high dose males was increased throughout treatment, with an overall increase of 13% relative to controls.

e. Ophthalmoscopy: No treatment related findings.

f. Hematology

At the high dose, hemoglobin, packed cell volume and red cell counts were lower than those of the controls on days 15 and 28(males) and 16 and 29(females) as shown in table 5. One male from each of the low, intermediate and high dose groups(animals 22, 37 and 52), showed a shorter activated partial thromboplastin time than the controls on day 29.

g. Toxicokinetics

BRL was detected in plasma from all treated animals, indicating that the drug was absorbed and systemically available at all doses tested. Drug plasma

concentration, C_{max} and AUC(0-24) increased with increasing dose between the low and intermediate doses, and less than proportionately between the intermediate and high doses. C_{max} was observed between 0.5 and 2.0 hours after dosing in all animals and the range of T_{max} was similar on days 1 and 24. Elimination half-lives was ranging from 1.6 to 4.5 hours at all dose levels. The results are summarized (TTK 1-4).

h. There was an increase in brown adipose tissue, particularly white adipose tissue in male. In females, only the weight of brown adipose tissue was elevated, including the increase in heart weight (Table 6).

i. Macroscopic and microscopic observation

Enlarged brown adipose tissue was seen in 11/13 females and in 1/13 males for the high dose group at the end of the treatment. In the adipose tissue, a minimal to moderate increase in cytoplasmic vacuolation was noted in 4/13 low dose, 10/13 intermediate dose and all high dose males. Both the incidence and the severity of this finding were dose related.

7. A 4-week oral repeat dose study in dogs followed by a 4-week off-dose period(TF-1012/BRL-049653/1)

4 Beagle dogs/sex/gp were given orally either 0(control), 0.4, 5.0 or 60.0 mg/kg/day of BRL, Batch BMC-32518-162, for 28 days.

a. Mortality: None.

b. Clinical observations:

Loose feces were occasionally noted in intermediate dose males and females and slightly more frequently in high dose males. Isolated emesis was also noted in several animals in the high dose group of both sex.

c. Body weight:

There was no overall treatment related efffect on group mean body weights. But, during the first 3 weeks of treatment bodyweight loss of approximately 13% was noted in one high dose male.

d. Food consumption: No significant reduction in food consumption was noted during the treatment period.

e. Ophthalmoscopy: No treatment related findings.

f. Electrocardiogram: In HD males at 1 hour post-dose, a significant increase in HR was noted. No treatment related effects on cardiac rhythm or ECG waveform were observed.

g. Hematology:

A significant, and progressive decrease in hemoglobin was noted(Table 7), whereas there was an increase in plasma volume a week after drug treatment in the high dose group. In general, males were affected to a greater degree than females, that is, on day 22, mean RBC count was decreased by approximately 27% and 20%, respectively in the HD group.

h. Blood chemistry

All animals in the intermediate and high dose groups had increased alanine aminotransferase(ALT). The change tended to be progressive in a number of animals, particularly at the high dose, though it appeared to be reversible upon drug-withdrawal.

i. Urinalysis: No changes.

j. Toxicokinetics

Plasma concentrations of BRL increased with dose and both median C_{max} and AUC(0-24) increased approximately proportionately with increasing dose. C_{max} was observed between 0.5 and 2.0 hours after dosing in all animals and T_{max} was similar on days 1 and 24. It was not possible to determine AUC(0-inf) for all animals as a result of a poorly defined terminal phase. Drug elimination was rapid with terminal phase half-lives ranging from 0.5-5.3 hours at all dose levels, with no notable difference between days 1 and 24 or between sex. The results are summarized(TTK 6-9).

k. Organ weights

Relative heart weights were increased by 20 and 28% respectively in intermediate and high dose females and by 32% in high dose males. After 4 week off-dose, relative heart weights remained increased by 19% and 12% in males an females respectively (Table 8).

l. Macroscopic observations: No treatment related findings.

m. Microscopic observations:

Basophilic tubules in the kidney cortex, minimal to mild in degree, were seen in all high dose animals including those killed after the 28 day recovery period. In the spleen, a minimal increase in hemopoiesis was noted in all high dose animals and in occasional animals in all other groups, including two control makes.

n. Conclusion

In the rats, dose related increases in brown fat weight, with 2.8 and 7.9 fold increases in HD males and females respectively, were associated with firm swelling in the mid scapular region in a total of 12/20 high dose females during the treatment period. At the HD, hemoglobin, packed cell volume and RBC count were slightly lower than controls in males and females. There was an increase in weight and cytoplasmic vacuolation of the brown adipose tissue at the intermediate and high doses and in males at the low dose of 0.4 mg/kg/day.

In the dogs, the main treatment-related effects of BRL at 0.4, 5.0 and 60 mg/kg were limited to the high dose. Reduced RBC parameters, increases in ALT and increases in the absolute and relative heart weight were noted at the high dose. Particularly, the increase in heart weight was significant even at the intermediate dose in female dogs, which might indicate compromises of cardiovascular function as a result of the drug treatment.

8. Bacterial mutation assay(TF-1017/BRL-049653/1)

Utilizing 5 types of S. typhimurium and positive control system with/out S-9 mix, effects of 0. 312.5, 625, 1250, 2500, and 5000 g/plate of BRL were tested by counting the mean number of revertant colonies per treatment group. Substantial increases in revertant colony numbers were observed with the concurrent positive control compounds, suggesting the sensitivity of the assay system. BRL did not the number of revertant colonies, which indicate this drug is not mutagenic under the testing conditions. But, this drug is found to be weakly mutagenic in mouse lymphoma L5178Y cells in the presence of S9 mix only(See table TTK-5).

9. Study to evaluate the potential of BRL 49653C to induce unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure(TF-1007/BRL-049653/1)

Groups of 3 male SD rats were given twice 2000 mg/kg or 1000 mg/kg BRL 49653 on 2 occasions approximately 12 hours apart. Hepatocytes were harvested from the animals and the cells were labelled with 10 Ci/ml [^{3}H]-thymidine for autoradiography. The positive control with 2-acetamidofluorene increased grain count. Treatment with BRL at doses up to 2000 mg/kg produced mean number of grain(NG) values in the range -0.3 to 0.2, of which count is not significant. It

appears that BRL showed little or no genotoxic activity in in-vivo micronucleus test in either sex of CD-1 mice after two oral administrations of 700 mg/kg.

10. A preliminary investigation of the absorption, excretion and plasma concentrations of radioactive material in male dogs following a single oral dose of [C] BRL 49653C at a target dose level of 60 mg free base/kg(BF-1005/BRL-04953/1)

Two male Beagle dogs were given orally [C]BRL(Batch 31872-49), of which dose level was 60 mg free base/kg. Urine and plasma samples were subjected to scintillation counting for data calculation. Excretion of radioactive drug in the urine and feces over the study period amounted to 27 and 62% of the total dose, respectively. Urinary excretion occurred largely during the first 24 h after dosing, whereas fecal excretion was slow. It appears that the drug excreted in urine was equivalent to the amount of drug that was absorbed.

11. An in-vitro study to determine the rate of interconversion of the enantiomers of BRL 49653 in mouse, rat, dog and human plasma(BF-1008/BRL-049653/1)

BRL 49653(Racemate), SB 206846{S(-)} and SB 210232 {R(+)} were each spiked at two different concentrations (100 ng/ml and 1 g/ml) into samples of mouse, rat, dog and human control plasma. The mixtures were incubated at 37°C for 6 h, when aliquots of each plasma sample were extracted and analyzed for the two chiral compounds by chiral HPLC. The results are summarized(Table 9).

C. ATTATCHMENT

18 tables(1 through 9, and TTK 1-9)

Table 1

BEST POSSIBLE

Effect of BRL 49653 (Batch R3) on heart rate in the 18hr fasted, male Sprague-Dawley rat

Time after dosing (min)	Heart Rate (beats/min)		Increase in heart rate relative to control (beats/min)
	Control	BRL 49653 (30mg/kg)	
-30	427 ± 50	438 ± 51	10
0	434 ± 43	435 ± 22	0
30	398 ± 30	439 ± 27	40*
60	384 ± 37	452 ± 29	67***
90	396 ± 32	432 ± 28	35

Heart rate was measured in restrained, conscious 18hr fasted Sprague-Dawley rats by ECG recording. After determination of baseline heart-rate, BRL 49653 (30 mg/kg body wt) or control vehicle (2 ml/kg body wt) was administered orally, by gavage. Heart rate was measured over a 90 min post-dosing period. Results are mean heart rates (beats/min) ± SD (n=6 rats per group). Statistical comparisons were made between treatment groups by Student's 't' test (two-tailed) for non-paired samples. *p<0.05; ***p<0.001 vs control.

Table 2

Effect of BRL 49653 (Batch R6) on heart rate in the 18hr fasted, male Sprague-Dawley rat

Time after dosing (min)	Heart Rate (beats/min)				
	Control	BRL 49653 (3mg/kg)	Increase relative to control	BRL 49653 (30mg/kg)	Increase relative to control
-30	404 ± 31	400 ± 28	-4	386 ± 20	-18
0	384 ± 38	384 ± 32	0	384 ± 34	0
30	388 ± 27	392 ± 31	4	374 ± 29	-14
60	391 ± 34	400 ± 46	9	369 ± 18	-23
90	389 ± 24	394 ± 31	5	371 ± 24	-18

Heart rate was measured in restrained, conscious 18hr fasted Sprague-Dawley rats by ECG recording. After determination of baseline heart-rate, BRL 496 control vehicle (2 ml/kg body wt) was administered orally, by gavage. Heart rate was measured at 30 min intervals over a 90 min post-dosing period. Results are mean heart rates (beats/min) ± SD (n=6 rats per group). Statistical comparisons were made by Student's 't' test (two-tailed) for non-paired samples.

BEST POSSIBLE

Effect of BRL 49653 (Batch R6) on heart rate in the 18hr fasted, male Sprague-Dawley rat

Time after dosing (min)	Heart Rate (beats/min)				
	Control	BRL 49653 (3mg/kg)	Increase relative to control	BRL 49653 (30mg/kg)	Increase relative to control
-30	414 ± 44	395 ± 20	-10	390 ± 18	-11
0	399 ± 26	390 ± 27	0	386 ± 19	0
30	378 ± 27	398 ± 30	29*	397 ± 20	32*
60	367 ± 35	380 ± 25	22	380 ± 22	26
90	348 ± 18	375 ± 30	36*	384 ± 28	49***

Heart rate was measured in restrained, conscious 18hr fasted Sprague-Dawley rats by ECG recording. After determination of baseline heart rate, BRL 49653 or control vehicle (2 ml/kg body wt) was administered orally, by gavage. Heart rate was measured at 30 min intervals over a 90 min post-dosing period. Results are mean heart rates (beats/min) ± SD (n=6 rats per group). Statistical comparisons were made by Student's 't' test (two-tailed) for non-paired samples. *p<0.05; ***p<0.001 vs control.

Table 4

PARAMETER		GROUP 1 CONTROL	GROUP 2 X48482/001 0.400 MG/KG	GROUP 3 X48482/001 4.00 MG/KG	GROUP 4 X48482/001 40.0 MG/KG	GROUP 5 X49653/001 0.360 MG/KG	GROUP 6 X49653/001 3.60 MG/KG	GROUP 7 X49653/001 36.0 MG/KG
FEMALES								
BODYWT	MEAN	242.6	237.4 NS	250.4 NS	256.4 NS	240.5 NS	259.0 NS	242.8 NS
	%DIFF	+0	-2	+3	+6	-1	+7	+0
(g)	S.D.	16.04	19.14	22.09	19.42	34.41	28.02	16.49
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
HEART	MEAN	0.9340	0.9579 NS	1.180***	1.328***	0.9360 NS	1.098*	1.175***
	%DIFF	+0	+3	+26	+42	+0	+18	+26
(g)	S.D.	0.0721	0.0499	0.1275	0.1723	0.1415	0.1639	0.1437
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
HEART	MEAN	0.3851	0.4048 NS	0.4708***	0.5166***	0.3894 NS	0.4226*	0.4837***
	%DIFF	+0	+5	+22	+34	+1	+10	+26
(%)	S.D.	0.0191	0.0232	0.0195	0.0402	0.0230	0.0251	0.0474
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
LIVER	MEAN	6.786	6.587 NS	7.244 NS	7.965**	6.926 NS	7.212 NS	6.835 NS
	%DIFF	+0	-3	+7	+17	+2	+6	+1
(g)	S.D.	0.6273	0.4703	0.7908	0.8627	1.031	0.9347	0.4508
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
LIVER	MEAN	2.795	2.780 NS	2.890 NS	3.103***	2.880 NS	2.780 NS	2.819 NS
	%DIFF	+0	-1	+3	+11	+3	-1	+1
(%)	S.D.	0.1536	0.1363	0.0928	0.1749	0.1071	0.1212	0.1495
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
SPLEEN	MEAN	0.6251	0.5519 NS	0.6175 NS	0.6133 NS	0.5621 NS	0.6211 NS	0.5546 NS
	%DIFF	+0	-12	-1	-2	-10	-1	-11
(g)	S.D.	0.0871	0.1079	0.0874	0.0980	0.1123	0.1067	0.0498
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
SPLEEN	MEAN	0.2571	0.2310 NS	0.2464 NS	0.2389 NS	0.2329 NS	0.2396 NS	0.2294(*)
	%DIFF	+0	-10	-4	-7	-9	-7	-11
(%)	S.D.	0.0268	0.0291	0.0262	0.0310	0.0197	0.0285	0.0252
	(N)	(8)	(8)	(8)	(8)	(8)	(8)	(8)

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2. 3. 4. 5. 6. 7
% differences calculated on unrounded data
Significance levels for t-test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 () P > 0.05 for overall F test NT not test

			X49653/001 0 MG/KG	X49653/001 0.400 MG/KG	X49653/001 2.00 MG/KG	X49653/001 80.0 MG/KG
MEASUREMENT CODE	SEX: MALES DAYS					
HB	15	MEAN	148.3	149.7 NS	146.2 NS	135.9***
		SD	5.3	3.7	6.6	7.6
g/l		N	10	10	10	9
PCV	15	MEAN	0.428	0.434 NS	0.422 NS	0.397***
		SD	0.016	0.014	0.019	0.023
l/l		N	10	10	10	9
RBC	15	MEAN	7.53	7.75 NS	7.46 NS	6.99**
		SD	0.24	0.45	0.34	0.40
10^12/l		N	10	10	10	9
MCH	15	MEAN	19.71	19.36 NS	19.62 NS	19.46 NS
		SD	0.46	1.02	0.47	0.37
pg		N	10	10	10	9
MCV	15	MEAN	57.0	56.2 NS	56.6 NS	57.0 NS
		SD	1.6	2.7	1.6	1.4
fl		N	10	10	10	9
MCHC	15	MEAN	346.1	345.2 NS	346.6 NS	342.2(*)
		SD	2.6	5.1	2.5	3.6
g/l		N	10	10	10	9
RETICS	15	MEAN	3.42	4.23 NS	4.50(*)	3.97 NS
		SD	0.82	0.75	1.59	1.20
%		N	10	10	10	10
NUC.RBC	15	MEAN	0.0	0.0 NT	0.0 NT	0.0 NT
		SD	0.0	0.0	0.0	0.0
/100WBC		N	10	10	10	10

SD = Standard deviation N = Number of animals

t-Test:- GROUPS 2, 3, 4 COMPARED WITH GROUP 1
Significance levels for t-Test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 ()

Table 6

PARAMETER	MALES		GROUP 1	GROUP 2 0.400 MG/KG	GROUP 3 2.00 MG/KG	GROUP 4 80.0 MG/KG
BODYWT		MEAN	390.8	399.5 NS	415.4 NS	416.7 NS
		%DIFF	.0	.2	.6	.7
(g)		S.D.	30.22	24.72	39.01	28.03
		(N)	(10)	(10)	(10)	(10)
BOTH ADRENAL		MEAN	0.0619	0.0548 NS	0.0546 NS	0.0574 NS
		%DIFF	.0	-11	-12	-7
(g)		S.D.	0.0091	0.0056	0.0109	0.0074
		(N)	(10)	(10)	(9)	(10)
BOTH ADRENAL		MEAN	0.0160	0.0137(*)	0.0132(*)	0.0138 NS
		%DIFF	.0	-14	-17	-13
(%)		S.D.	0.0030	0.0014	0.0027	0.0022
		(N)	(10)	(10)	(9)	(10)
BRAIN		MEAN	2.133	2.206 NS	2.141 NS	2.139 NS
		%DIFF	.0	.3	.0	.0
(g)		S.D.	0.0770	0.0982	0.1324	0.1527
		(N)	(10)	(10)	(10)	(10)
BRAIN		MEAN	0.5488	0.5544 NS	0.5186 NS	0.5147 NS
		%DIFF	.0	.1	-6	-6
(%)		S.D.	0.0480	0.0478	0.0467	0.0429
		(N)	(10)	(10)	(10)	(10)
BROWN ADIPOSE		MEAN	0.3982	0.4256 NS	0.6135 NS	1.218***
		%DIFF	.0	.7	.54	.206
(g)		S.D.	0.0842	0.1243	0.1713	0.7716
		(N)	(10)	(10)	(10)	(10)
BROWN ADIPOSE		MEAN	0.1019	0.1066 NS	0.1470 NS	0.2874***
		%DIFF	.0	.5	.44	.182
(%)		S.D.	0.0194	0.0310	0.0381	0.1652
		(N)	(10)	(10)	(10)	(10)

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 () P

PARAMETER			MG/KG	MG/KG	MG/KG
MALES					
SPLEEN	MEAN	0.8533	0.7728 NS	0.8285 NS	0.8204 NS
	%DIFF	.0	-9	-3	-4
(g)	S.D.	0.1068	0.1172	0.1464	0.1212
	(N)	(10)	(10)	(10)	(10)
SPLEEN	MEAN	0.2185	0.1934(*)	0.1991 NS	0.1973 NS
	%DIFF	.0	-11	-9	-10
(%)	S.D.	0.0228	0.0262	0.0267	0.0300
	(N)	(10)	(10)	(10)	(10)
BOTH TESTES	MEAN	4.669	4.773 NS	4.653 NS	4.348*
	%DIFF	.0	.2	.0	-7
(g)	S.D.	0.3544	0.3736	0.2073	0.2842
	(N)	(10)	(10)	(10)	(10)
BOTH TESTES	MEAN	1.201	1.198 NS	1.128 NS	1.049**
	%DIFF	.0	.0	-6	-13
(%)	S.D.	0.1304	0.1036	0.1048	0.1087
	(N)	(10)	(10)	(10)	(10)
THYMUS	MEAN	0.4595	0.5251 NS	0.4845 NS	0.5289 NS
	%DIFF	.0	.14	.5	.15
(g)	S.D.	0.0786	0.1104	0.1768	0.1437
	(N)	(10)	(10)	(10)	(10)
THYMUS	MEAN	0.1173	0.1310 NS	0.1156 NS	0.1257 NS
	%DIFF	.0	.12	-1	.7
(%)	S.D.	0.0165	0.0248	0.0389	0.0282
	(N)	(10)	(10)	(10)	(10)
WHITE ADIPOSE	MEAN	4.725	5.054 NS	6.856**	6.956***
	%DIFF	.0	.7	.45	.47
(g)	S.D.	1.721	1.288	1.500	0.8512
	(N)	(10)	(10)	(10)	(10)
WHITE ADIPOSE	MEAN	1.194	1.264 NS	1.635**	1.673***
	%DIFF	.0	.6	.37	.40
(%)	S.D.	0.3532	0.3143	0.2373	0.2021
	(N)	(10)	(10)	(10)	(10)

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2. 3. 4
% differences calculated on unrounded data
Significance levels for t-test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 () P

Table 6(Con

PARAMETER		GROUP 1	GROUP 2 0.400 MG/KG	GROUP 3 2.00 MG/KG	GROUP 4 80.0 MG/KG
FEMALES					
BODYWT	MEAN	259.2	255.6 NS	256.7 NS	274.3 NS
	%DIFF	.0	-1	-1	.6
(g)	S.D.	18.32	10.76	20.39	19.15
	(N)	(10)	(10)	(10)	(10)
BOTH ADRENAL	MEAN	0.0694	0.0701 NS	0.0730 NS	0.0739 NS
	%DIFF	.0	.1	.5	.6
(g)	S.D.	0.0115	0.0102	0.0108	0.0104
	(N)	(10)	(10)	(10)	(10)
BOTH ADRENAL	MEAN	0.0268	0.0274 NS	0.0286 NS	0.0271 NS
	%DIFF	.0	.2	.7	.1
(%)	S.D.	0.0042	0.0039	0.0047	0.0046
	(N)	(10)	(10)	(10)	(10)
BRAIN	MEAN	2.039	2.058 NS	2.068 NS	2.020 NS
	%DIFF	.0	.1	.1	-1
(g)	S.D.	0.1231	0.0735	0.0852	0.1344
	(N)	(10)	(10)	(10)	(10)
BRAIN	MEAN	0.7896	0.8066 NS	0.8093 NS	0.7395 NS
	%DIFF	.0	.2	.2	-6
(%)	S.D.	0.0669	0.0483	0.0602	0.0704
	(N)	(10)	(10)	(10)	(10)
BROWN ADIPOSE	MEAN	0.2485	0.3002 NS	0.4193 NS	2.105***
	%DIFF	.0	.21	.69	.747
(g)	S.D.	0.0470	0.0738	0.1526	0.9439
	(N)	(10)	(10)	(10)	(10)
BROWN ADIPOSE	MEAN	0.0[illegible]	0.1177 NS	0.1619 NS	0.7610***
	%DIFF	.0	.23	.69	.693
(%)	S.D.	0.0172	0.0292	0.0532	0.3311
	(N)	(10)	(10)	(10)	(10)

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2. 3. 4
% differences calculated on unrounded data
Significance levels for t-test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 () P

SEX: MALES			0.400 MG/KG	5.00 MG/KG	60.0 MG/KG
EASUREMENT CODE	DAYS				
WB	-7	MEAN	144.0	143.0	140.3	137.6
'1		SD	3.6	9.0	6.8	7.2
		N	5	3	3	5
	-3	MEAN	142.6	143.7	145.7	137.8
		SD	5.5	4.5	6.5	4.1
		N	5	3	3	5
	3	MEAN	137.8	137.7	135.0	125.6*
		SD	4.3	4.0	10.1	7.2
		N	5	3	3	5
	8	MEAN	140.0	147.0	136.0	123.6**
		SD	5.6	3.6	6.1	11.4
		N	5	3	3	5
	15	MEAN	145.2	152.7	140.7	119.4***
		SD	5.0	6.4	1.5	12.8
		N	5	3	3	5
	22	MEAN	152.2	150.7	146.0	107.4***
		SD	3.6	8.1	9.5	9.8
		N	5	3	3	5
	29	MEAN	140.0	144.3	139.3	98.2***
		SD	6.4	3.5	6.1	4.3
		N	5	3	3	5
	45	MEAN	154.0			134.0
		SD	7.1			7.1
		N	2			2
	57	MEAN	152.5			146.0
		SD	2.1			25.5
		N	2			2

D = Standard deviation N = Number of animals

-Test:- GROUPS 2, 3, 4 COMPARED WITH GROUP 1
ignificance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F
NT not tested All other values not significant

Table 8

PARAMETER	MALES		GROUP 1	GROUP 2 0.400 MG/KG	GROUP 3 5.00 MG/KG	GROUP 4 60.0 MG/KG
SPLEEN		MEAN	72.80	78.43 NS	60.17 NS	44.40 NS
(g)		%DIFF	+0	+8	-17	-39
		S.D.	24.25	7.107	19.03	9.946
		(N)	(3)	(3)	(3)	(3)
SPLEEN		MEAN	0.5979	0.6375 NS	0.4958 NS	0.3639 NS
(%)		%DIFF	+0	+7	-17	-39
		S.D.	0.2173	0.1056	0.1310	0.0969
		(N)	(3)	(3)	(3)	(3)
HEART		MEAN	84.27	86.60 NS	90.27 NS	111.7**
(g)		%DIFF	+0	+3	+7	+33
		S.D.	1.563	15.04	7.859	9.280
		(N)	(3)	(3)	(3)	(3)
HEART		MEAN	0.6872	0.6957 NS	0.7493 NS	0.9097**
(%)		%DIFF	+0	+1	+9	+32
		S.D.	0.0107	0.0871	0.0309	0.0841
		(N)	(3)	(3)	(3)	(3)
THYMUS		MEAN	11.77	15.68 NS	21.28*	12.92 NS
(g)		%DIFF	+0	+33	+81	+10
		S.D.	2.807	3.565	4.632	2.770
		(N)	(3)	(3)	(3)	(3)
THYMUS		MEAN	0.0955	0.1273 NS	0.1762**	0.1045 NS
(%)		%DIFF	+0	+33	+84	+9
		S.D.	0.0196	0.0335	0.0327	0.0174
		(N)	(3)	(3)	(3)	(3)
LUNG		MEAN	114.7	113.6 NS	114.4 NS	124.8 NS
(g)		%DIFF	+0	-1	+0	+9
		S.D.	4.521	16.58	13.83	9.47
		(N)	(3)	(3)	(3)	(3)
LUNG		MEAN	0.9349	0.9212 NS	0.9487 NS	1.014 NS
(%)		%DIFF	+0	-1	+1	+8
		S.D.	0.0273	0.1679	0.0708	0.0214
		(N)	(3)	(3)	(3)	(3)

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-test:
NS P > 0.05 * P < 0.05 ** P < 0.01 *** P < 0.001 () P > 0.05 for over

Table 9

Final ratios (SB 206846/SB 210232) after incubation at 37 °C for 6 h.

Plasma	BRL 49653 spiked		SB 206846 spiked		SB 210232 spiked	
	1 µg/mL	100 ng/mL	1 µg/mL	100 ng/mL	1 µg/mL	100 ng/mL
Mouse	61.6/38.4	63.6/36.4	62.3/37.7	62.9/37.1	62.5/37.5	62.6/37.4
Rat	69.9/30.1	75.0/25.0	71.0/29.0	74.2/25.8	70.1/29.9	72.1/27.9
Dog	54.9/45.1	58.7/41.3	55.2/44.8	57.5/42.5	54.5/45.5	55.5/44.5
Human	24.0/76.0	25.6/74.4	27.1/72.9	26.6/73.4	21.1/78.9	23.3/76.7

Interconversion half-lives (h) from modelling the enantiomer ratio-time data.

Plasma	BRL 49653 spiked		SB 206846 spiked		SB 210232 spiked	
	1 µg/mL	100 ng/mL	1 µg/mL	100 ng/mL	1 µg/mL	100 ng/mL
Mouse	0.38	0.37	0.74	0.89	0.61	0.61
Rat	0.58	0.46	1.27	1.69	0.73	0.76
Dog	0.85	0.95	0.93	1.17	0.86	0.96
Human	2.02	2.10	1.53	2.03	0.94	0.79

TTK-1

Individual Maximum Observed Plasma BRL 49653 Concentrations (Cmax) [mg/mL] on Days 1 and 24

0.4 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
33	2.1	1.8	103	3.4	3.1
34	3.7	3.2	104	2.3	2.7
35	5.9	2.3	105	3.9	3.0
Median Value	3.7	2.3	Median Value	3.4	3.0

2.0 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
48	7.7	10.2	118	14.0	13.7
49	10.3	9.9	119	15.8	16.4
50	7.9	10.5	120	14.6	15.1
Median Value	7.9	10.2	Median Value	14.6	15.1

80 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
68	204	122	138	267	208
69	159	137	139	246	179
70	149	104	140	231	232
Median Value	159	122	Median Value	246	208

TTK-2

Area Under the Plasma BRL 49653 Concentration Time Curve [mg.h/mL], AUC(0-24h) on Days 1 and 24

0.4 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
33	5.5	5.3*	103	8.8	15.8
34	11.2*	12.1	104	10.8	17.3*
35	7.9	5.3*	105	7.5	17.0
Median Value	7.9	5.3	Median Value	8.8	17.0

2.0 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
48	28.4*	25.9	118	68.9	93.3
49	28.1*	33.1	119	51.8	63.4
50	36.2*	38.9	120	71.6	63.4*
Median Value	28.4	33.1	Median Value	68.9	63.4

80 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
68	1259	472	138	1462*	1394
69	766	448	139	2039	1338
70	888	526	140	1434*	1467
Median Value	888	472	Median Value	1462*	1394

* AUC(0-24h) due to no result being obtained at 24 hours.

TTK-3

Area Under the Plasma BRL 49653 Concentration Time Curve [mg.h/mL]
AUC(0-inf) on Days 1 and 24

0.4 mg/kg/day

Animal No.	Males		Animal No.	Females	
-	Day 1	Day 24		Day 1	Day 24
33	5.6 (1.5)	5.4 (2.5)	103	9.0 (2.3)	15.9 (0.4)
34	11.9 (6.1)	12.2 (0.6)	104	10.8 (0.4)	22.8 (24.1)
35	8.2 (3.7)	5.4 (3.2)	105	7.6 (1.6)	N.D.
Median Value	8.2	5.4	Median Value	9.0	19.4

2.0 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
48	29.0 (1.9)	N.D.	118	69.3 (0.6)	94.4 (1.2)
49	N.D.	33.1 (0.2)	119	51.8 (0.1)	63.5 (0.2)
50	38.4 (5.7)	39.1 (0.6)	120	72.0 (0.6)	74.1 (14)
Median Value	33.7	36.1	Median Value	69.3	74.1

80 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
68	1260 (0.1)	473 (0.1)	138	N.D.	1409 (1.1)
69	766 (0.1)	448 (0.0)	139	2047 (0.4)	1346 (0.6)
70	888 (0.0)	527 (0.2)	140	N.D.	1484 (1.1)
Median Value	888	473	Median Value	2047*	1409

Bracketed values are the portion extrapolated to infinity expressed as a percentage.
N.D. not determined
* single value

TTK-4

Terminal Phase Half-Life (hours) of BRL 49653 on Days 1 and 24

0.4 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
33	1.6 (5)	1.8 (5)	103	1.8 (6)	3.0 (5)
34	2.4 (6)	3.2 (7)	104	3.0 (6)	4.5 (4)
35	2.2 (5)	1.9 (6)	105	1.7 (5)	N.D.
Median Value	2.2	1.9	Median Value	1.8	3.8

2.0 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
48	1.6 (4)	N.D.	118	3.2 (5)	3.7 (6)
49	N.D.	2.7 (5)	119	2.4 (7)	2.7 (6)
50	2.3 (4)	3.3 (5)	120	3.2 (6)	3.5 (6)
Median Value	2.0	3.0	Median Value	3.2	3.5

80 mg/kg/day

Animal No.	Males		Animal No.	Females	
	Day 1	Day 24		Day 1	Day 24
68	2.5 (5)	2.2 (4)	138	N.D.	3.6 (5)
69	2.1 (5)	2.1 (5)	139	2.8 (4)	3.2 (5)
70	1.9 (4)	2.7 (5)	140	N.D.	3.7 (5)
Median Value	2.1	2.2	Median Value	2.8*	3.6

Bracketed values are the number of concentration-time pairs used in the determination of the terminal phase rate-constant.
N.D. not determined
* single value

Evidence of mutagenic activity was obtained in both experiments conducted in the presence of S9 mix. A summary of the key results is given below (mean values from replicate cultures).

First Experiment in the Presence of S9 Mix (Assay 2)

Treatment	Dose Level ($\mu g.ml^{-1}$)	Relative Total Growth %	Mutant Fraction $x10^{-6}$	Fold Increase Over Control
Dimethylsulphoxide	(100 μl added)	100	49	-
3-Methylcholanthrene	2.5	39	673	13.9
BRL 49653C	12.5	112	53	1.1
	25	111	47	1.0
	50	101	61	1.3
	100	86	62	1.3
	200	56	90	1.9
	400	Too toxic to assess		

Second Experiment in the Presence of S9 Mix (Assay 4)

Treatment	Dose Level ($\mu g.ml^{-1}$)	Relative Total Growth %	Mutant Fraction $x10^{-6}$	Fold Increase Over Control
Dimethylsulphoxide	(100 μl added)	100	50	-
3-Methylcholanthrene	2.5	33	707	14.2
BRL 49653C	50	90	49	1.0
	100	77	73	1.5
	150	72	90	1.8
	[illegible]	[illegible]	98	2.0
	[illegible]	Too toxic to assess		

TTK-6

Maximum Observed Plasma BRL 49653 Concentrations (Cmax)
[mg/ml] on Days 1 and 24

0.4 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
6	0.48	0.51	22	0.31	0.18
7	0.28	0.46	23	0.37	0.37
8	0.35	0.36	24	0.72	0.61
Median Value	0.35	0.46	Median Value	0.37	0.37

5.0 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
9	2.05	1.63	25	5.88	6.87
10	4.17	4.16	26	2.69	5.94
11	3.95	6.42	27	10.23	10.05
Median Value	3.95	4.16	Median Value	5.88	6.87

60 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
12	37.47	31.88	28	50.44	56.44
13	23.12	77.55	29	40.70	62.02
14	25.11	58.53	30	64.82	60.65
Median Value	25.11	58.53	Median Value	50.44	60.65

TTK-7

Area Under the Plasma BRL 49653 Concentration Time Curve, (AUC(0-24h)[mg.h/mL]) on Days 1 and 24

0.4 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
6	0.61	0.55	22	0.46	0.31
7	0.39	0.54	23	0.68	0.80
8	0.66	0.72	24	0.64	0.62
Median Value	0.61	0.55	Median Value	0.64	0.62

5.0 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
9	2.70	2.77	25	7.26	10.26
10	8.99	6.31	26	5.44	7.35
11	6.61	8.10	27	11.37	11.06
Median Value	6.61	6.31	Median Value	7.26	10.26

60 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
12	54.99	97.15	28	121.59	121.84
13	75.77	156.41	29	82.46	82.09
14	84.07	132.96	30	119.01	140.48
Median Value	75.77	132.96	Median Value	119.01	121.84

TTK-8

Area Under the BRL 49653 Concentration Time Curve (AUC(0-inf)[mg.h/mL]) on Days 1 and 24

0.4 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
6	0.62 (0.6)	0.56 (0.2)	22	0.47 (1.6)	N.D.
7	0.40 (1.5)	N.D.	23	0.69 (1.2)	0.84 (4.0)
8	0.67 (2.1)	0.74 (1.7)	24	0.65 (1.2)	0.62 (0.7)
Median Value	0.62	0.65	Median Value	0.65	0.73

5.0 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
9	2.70 (0.3)	2.77 (0.2)	25	7.15 (0.3)	10.27 (0.1)
0	N.D.	N.D.	26	N.D.	7.39 (0.6)
11	6.64 (0.5)	8.11 (0.1)	27	11.38 (0.1)	N.D.
Median Value	4.67	5.44	Median Value	9.27	8.83

60 mg/kg/day

	Males			Females	
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
12	N.D.	98.82 (1.7)	28	121.61 (0.0)	N.D.
13	N.D.	N.D.	29	82.48 (0.0)	N.D.
14	84.08 (0.0)	132.99 (0.0)	30	N.D.	N.D.
Median Value	84.08*	115.91	Median Value	102.05	N.D.

Bracketed values are the portion extrapolated to infinity expressed as a percentage.
N.D. - not determined
* single value

TTK-9

Terminal Phase Half-Life [hours] of BRL 49653
on Days 1 and 24

0.4 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
6	0.5 (3)	0.7 (4)	22	0.5 (3)	N.D.
7	0.6 (3)	N.D.	23	0.7 (3)	1.0 (3)
8	0.9 (3)	0.8 (3)	24	0.6 (3)	0.5 (4)
Median Value	0.6	0.8	Median Value	0.6	0.8

5.0 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
9	1.4 (3)	1.1 (5)	25	1.5 (3)	1.2 (3)
10	N.D.	N.D.	26	N.D.	2.2 (3)
11	1.4 (4)	1.2 (3)	27	1.1 (4)	N.D.
Median Value	1.4	1.2	Median Value	1.3	1.7

60 mg/kg/day

Males			Females		
Dog No.	Day 1	Day 24	Dog No.	Day 1	Day 24
12	N.D.	5.3 (4)	28	1.9 (3)	N.D.
13	N.D.	N.D.	29	2.9 (3)	N.D.
14	2.0 (4)	2.3 (3)	30	N.D.	N.D.
Median Value	2.0*	3.8	Median Value	2.4	N.D.

Bracketed values are the number of concentration-time pairs used in the determination of the terminal phase rate-constant.
N.D. - not determined
* - single

LABELING REVIEW: (To be communicated to the sponsor)

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis: Two-year carcinogencity studies were conducted in Charles River CD-1 mice at doses of 0.4, 1.5 and 6 mg/kg/day in the diet(**top dose equivalent to 12 times human AUC at the maximum recommended human dose of 8 mg/day**). Two-year carcinogencity studies in Sprague-Dawley rats at oral gavage doses of 0.05, 0.3 and 2 mg/kg/day (**top dose equivalent to approximately 10 and 20 times of human AUC for male and female rats, respectively**).

Rosiglitazone was not carcinogenic in the mouse. There was an increase in incidence of adipose hyperplasia in the mouse at doses >1.5 mg/kg/day (approximately 2 times human AUC). In rats, there was a significant increase in the incidence of benign adipose tissue tumors (lipomas) at doses >0.3 mg/kg/day (approximately 2 times human AUC). These proliferative changes in both species are considered due to the persistent pharmacological overstimulation of adipose tissue. [redacted]

Mutagenesis: Rosiglitazone was not mutagenic or clastogenic in the *in vitro* bacterial assays for gene mutation, the *in vitro* chromosome aberration test in human lymphocytes, the *in vivo* mouse micronucleus test and the *in vivo/in vitro* rat UDS assay. There was a small (about 2-fold) increase in mutation in the *in vitro* mouse lymphoma assay in the presence of metabolic activation [redacted]

Impairment of Fertility: Rosiglitazone had no effects on mating or fertility of male rats given up to 40 mg/kg/day(**Please provide AUC ratio**). Rosiglitazone altered estrous cyclicity (>2 mg/kg/day) and reduced fertility (40 mg/kg/day) of female rats in association with lower plasma levels of progesterone and estradiol with no such effects at 0.2 mg/kg/day (**AUC ratio=4, compared to human AUC value**). In monkeys, rosiglitazone (0.6 and 4.6 mg/kg/day (**AUC ratio = 2.74 and 14.71, compared to human AUC values**) diminished the follicular phase rise in serum estradiol with consequential reduction in the luteinizing hormone surge, lower luteal phase progesterone levels, and amenorrhea. The mechanism for these effects appears to be direct inhibition of ovarian steroidogenesis.

Animal Toxicology

Heart weights were increased in mice(10 mg/kg/day), rats(5 mg/kg/day), and dogs(2 mg/kg/day) with rosiglitazone treatments. The doses represent multiples of human exposure of 18, 21, and 1.2, respectively, based on AUC comparison. Morphometric measurement indicated that there was hypertrophy in cardiac ventricular tissues, which may be due to increased heart work as a result of the drug action. In a 26-week dog study hepatic enzymes were not elevated at a dose of rosiglitazone 2 mg/kg/day. But, they were elevated 10 times of normal levels at 20 mg/kg/day, which represents a multiple of human exposure of 12 based on AUC comparisons.

Pregnancy

Pregnancy Category C
There was no effect on implantation or the embryo with rosiglitazone treatment during early pregnancy in rats, but treatment during mid-late gestation was associated with fetal death and growth retardation in both rats and rabbits. Teratogenicity was not observed. Rosiglitazone caused placental pathology in rats (>3 mg/kg/day) but not in rabbits at 100 mg/kg/day(**Please provide AUC ratio**). Treatment of rats during gestation through lactation reduced litter size, neonatal viability and postnatal growth with growth retardation reversible after puberty. For effects on the placenta, embryo/fetus and offspring, the no-effect dose was 0.2 mg/kg/day (**AUC ratio=4, compared to human AUC value**) in rats and 15 mg/kg/day (**AUC ratio=4.2**) in rabbits.

Nursing Mothers

Drug related material was detected in milk from lactating rats. It is not known whether the drug is excreted in human milk. Because many drugs are excreted in human milk, the drug should not be administered to a nursing woman.

Statistical Review and Evaluation
Carcinogenicity

(An Addendum Report)

Date: APR 10 1999

IND No:	21071
Applicant:	SmithKline Beecham Pharmaceuticals
Drug Name:	Avandia ™ (rosiglitazone maleate) Tablets
Document Review:	Volume 1.5.034 – 1.5.041
Data Source:	four diskettes - mice G95501 rat G95500
Pharmacologist:	Dr. Herman Rhee (HFD-510)
Statistical Reviewer:	Moh-Jee Ng, (HFD-715)

1. Introduction

In this NDA submission, two animal carcinogenicity studies (one in mice and in rats) were included. A statistical review was conducted by Division of Biometrics II and a statistical review and evaluation report was issued on March 22, 1999.

Dr. Rhee of HFD-510, who is the reviewing pharmacologist of this NDA, has requested Division of Biometrics II to perform a pairwise comparisons on the hemangiosarcoma tumor incidence rates of male mice.

2. Results of the Additional Analysis

The incidence rates of hemangiosarcoma tumor of male mice are as follow:

Control 1	Control 2	Low	Medium	High
0	0	4	0	0

There is no significant positive dose-positive relationship in hemangiosarcoma tumor.

A rule for adjusting the effect of multiple testings proposed by Haseman (1983) is used to adjust for the effect of multiple testings in pairwise comparisons. The rule states that in order to keep the overall false-positive rate at the nominal level of approximately 0.1, tumor types with spontaneous tumor rates of 1% or less (rare tumors) should be tested at 0.05 significant level, otherwise (common tumors) a 0.01 significance level should be used .

This reviewer compared the hemangiosarcoma tumor incidence rates of the combined control groups versus the low dose using the Fisher exact test. Since hemangiosarcoma tumor types with spontaneous tumor rates of 1% or less, it should be tested at 0.05 significant level. The difference is a statistically significant with p=0.012.

/S/ [redacted]

Moh-Jee Ng
Mathematical Statistician

Concur: /S/ [redacted]

4/9/99

Karl Lin, Ph.D.
Expert Mathematical Statistician
(Applications in Pharmacology & Toxicology)

cc:
HFD-510/Division File
HFD-510/Dr. Rhee
HFD-715/Division File, Chron
HFD-715/ENevius, TSahlroot, KLin, MNg

APPEARS THIS WAY ON ORIGINAL

Statistical Review and Evaluation
Carcinogenicity

Date: MAR 22 1999

NDA No:	21071
Applicant:	SmithKline Beecham Pharmaceuticals
Drug Name:	Avandia ™ (rosiglitazone maleate) Tablets
Document Review:	Volume 1.5.034 – 1.5.041
Data Source:	four diskettes - mice G95501 rats G95500
Pharmacologist:	Dr. Herman Rhee (HFD-510)
Statistical Reviewer:	Moh-Jee Ng (HFD-715)

1. Introduction

Two animal carcinogenicity studies (one in mice and one in rats) were included in this NDA submission. The goal of these two studies was to evaluate the carcinogenic potential of Avandia ™ given by oral administration in the diet to mice and rats for two years. Dr. Herman Rhee of HFD-510, who is the reviewing pharmacologist of this NDA, requested the Division of Biostatistics II to perform a statistical review and evaluation of these studies.

2. The Mouse Study

2.1 Design

The sponsor reported results of two experiments, one in male mice and one in female mice. In each of these experiments there were two control groups and three treated groups known as low, medium, and high. The mice in control groups 1 and 2 were fed ground diet alone. Three treated groups (Groups 3, 4 and 5) received Avandia ™ at dose levels of 0.4, 1.5 or 6.0 mg/kg/day. Each group had a size of 60 mice. All surviving male mice given high dose group were killed in week 95 because of the large number of deaths in this group. All surviving males from the remaining groups and all surviving female mice were killed in weeks 101 and 105, respectively.

All mice were weighed once weekly during weeks 1–28 and once every four weeks thereafter. The food consumption of all mice was recorded once weekly during weeks 1 to 12 and once every 4 weeks thereafter.

2.2 Sponsor's Analyses

All statistically analyses were carried out separately for males and females. Mortality was analyzed using log rank methods. The survival times were compared to test whether there was a trend of decreasing survival time with increasing dose. Kaplan-Meier survival curves were plotted to visually compare the survival distributions of the five treated