CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 021071

PHARMACOLOGY REVIEW(S)

NDA 21-071 April 26, 1999

Sponsor: SmithKline Beecham Pharmaceuticals
1250 South Collegeville Road
Collegeville, PA 19426-0989

MAY 11 1999

Submission Date: Nov, 24, 1998 Received: Nov. 25, 1999

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original Summary

1. Drug: Rosiglitazone Maleate Tablets, Avandia™(BRL49653C)
2. Related: IND [redacted]
   IND
   IND
3. Pharmacological Class: Antidiabetic agent(Thiazolidinedione)

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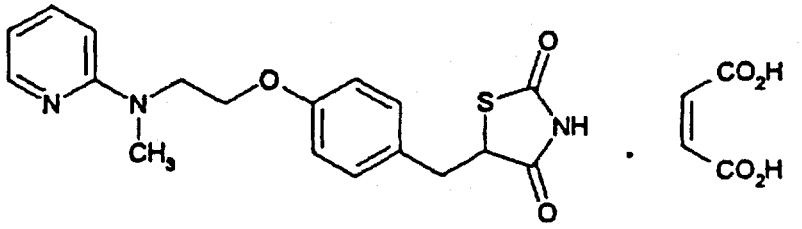
2. Related: IND [redacted]
   IND
   IND
3. Pharmacological Class: Antidiabetic agent(Thiazolidinedione)
4. Indicated Use: Type II diabetes monotherapy or in combination with metformin.
5. Clinical Dosage: Human AUC(1.61 µg.h/ml) was noted following 10 days repeat dosing at 5 mg/day(Study 002). Subsequently the clinical AUC was documented to be 3 µg.h/ml when clinical dose was raised to 8 mg/day.

6. Structure:



7. Chemical Name: 2,4-Thiazolidinedione, 5-[[4-[2-(methyl-2-pyridinylamino) ethoxy]phenyl]methyl]-(Z)-2-butenedioate (1:1)

8. Foreign Studies: Yes - Please see individual studies.

A. INTRODUCTION

Rosiglitazone is a thiazolidinedione derivative that has antihyperglycemic action which might be a useful antidiabetic agent. The mechanism of action has not been clearly

established, although its stimulatory effect on peroxisomal proliferator activated receptor gamma (PPARγ) is well known. The primary molecular target for the action of rosiglitazone might be in the nuclear receptors, but how its activation of the receptors improves glycemic control remains to be elucidated. In humans, PPARγ receptors are found in key target tissues for insulin action such as adipose tissue, skeletal muscle, and liver. It binds to animal and human PPARγ receptors as well as adipocytes with a high affinity, where it may alter carbohydrate metabolism. In this NDA review, rosiglitazone preclincal pharmacology, safety toxicology, reproductive toxicology, genotoxicity, and carcinogenicity are reviewed.Studies previously reviewed under the IND are summarized in this review.

B. PHARMACOLOGY AND PHARMACODYNAMIC STUDIES

Most of pharmacology studies were performed at SKB, the Frythe, Hertfordshire, U.K. Please also see previous IND reviews dated on 10/20/1993, 11/8/1994, 12/9/1994, 5/17/1995, 6/8/1995, 8/8/1995, 6/26/1996, 7/9/1996, 7/11/1996, 7/17/1996, 9/18/1966, and 11/8/1996. In the toxicological studies in different animals, the high doses usually produced cardiac weight increases, fluid accumulation in the chest cavity, reductions in hematocrit, and atrial thrombosis in the mice, although the low and mid-dose effects near human exposure levels were not clear. Therefore, in the agency review dated on 9/18/1996, the sponsor was requested to provide the exposure ratios(AUC) of the animals to human for the lowest doses at which cardiac hypertrophy, hydrothorax, and/or anemia were occurred.

1). Effects of Rosiglitazone on 2-Deoxyglucose Uptake in Differentiated 3T3-L1 Adipocytes. A Comparison with Troglitazone and Pioglitazone(Project#PG-1018/BRL-049653/3)

Methods: Rosiglitazone(Batch R7), pioglitazone(Batch GBD-2) and troglitazone(Batch GBD1) were synthesized by SmithKline Beecham. 3T3-L1 adipocytes (American Type Culture Collection, Cat#CCL92-1) were seeded at a density of 10, 000 cells per well plate and were exposed to drugs for a total of 48 hours. Uptake of 2-deoxyglucose in all cells was determined by ^{3}H-glucose(25μM/ 0.5 μCi per well).

Results: There were drug dose-dependent increases in 2-deoxyglucose uptake as shown below. The EC_{50} of rosiglitazone was lowest in comparison to the two other thiazoladinediones.

Table#1. EC_{50} Values of Rosiglitazone in 2-Deoxyglucose Uptake in 3T3-L1 Adipocytes

Concentration of Drug	2-Deoxyglucose Uptake(pmol/well/10 minutes)		
	Rosiglitazone	Pioglitazone	Troglitazone
Control	26.5 ± 4.8	26.5 ± 4.8	22.3 ± 2.0
1 nM	15.5 ± 4.0	18.2 ± 9.4	21.0 ± 2.8
10 nM	24.1 ± 5.7	26.3 ± 2.6	21.5 ± 2.6
100 nM	63.9 ± 13.1	34.6± 9.9	27.4 ± 1.8
1 μM	66.6 ± 17.5	40.3 ± 1.3	39.7 ± 8.7
10 μM	62.6 ± 4.2	54.3 ± 4.8	71.0 ± 4.1
EC_{50} (M)	50 nM	3 μM	2 μM

2). Effects of Rosiglitazone on Insulin Receptor Phosphotyrosine Phosphatase(PTP) Activity in Liver and Muscle in Diabetic Mice(PF-1007/BRL-049653/2)

Methods: Female C57/BL/KsJ db/db mice and their lean matched-litter controls (+/?) were treated with rosiglitazone (3 μmol/kg in diet) for 21 days. The liver or muscle was freeze-clamped and it was pulverized under liquid N_2 in a mortar. The tissues were homogenized and subsequently centrifuged at 100,000g for 60 min. The supernatant and the pellet were assayed for PTPas activity.

Results: The hepatic cells of both control and diabetic db/db mice had high PTPase activities in particulate fractions. The diabetic mice had low PTPase activity, which was not altered by the treatment of rosiglitazone as shown below.

Table #2. Effects of Rosiglitazone on Insulin Receptor PTP Activity in Diabetic Mice.

Fraction	Mice	Control	Rosiglitazone
Cytosolic	+/?	45.40 ±1.1	45.09± 0.71
	Db/db	18.84 ±1.5*	21.03 ±2.2*
Particulate	+/?	331.90± 6.7	325.40± 5.51
	Db/db	212.13 ±12.2*	208.50 ±13.1*

@Results are pmol Pi released/mg protein/min, and are expressed as mean + SEM of the three preparations. * indicate $P<0.001$, compared to lean control.

3). Antihyperglycemic Activities of Rosiglitazone, Troglitazone and Pioglitazone in the KK-Ay Mouse(BRL049653/100RXF/1). This study was conducted by [redacted]

Methods: Twelve male KK-Ay mice/group were given by dietary admixture either vehicle(RMI diet), rosiglitazone(1.07, 3.57, and 10.7 mg/kg), pioglitazone(10.7, 35.7, and 107 mg/kg), or troglitazone(132, 441, and 1324 mg/kg) for 10 days. Serum glucose, triglyceride, and insulin were determined before and during the study.

Results:

Body Weight and Food Consumption: The three drugs did not reduce body weight. Actually there was a significant increase in this parameter after pioglitazone treatment in parallel with the increase of food consumption. The effects of the drugs on serum glucose, triglyceride, neutral esters fat acid(NEFA) and insulin levels are summarized in a table below. Rosiglitazone decreased blood glucose and insulin levels in drug dose-dependently. Pioglitazone and troglitazone also reduced the parameters.

Table#3. Comparison of Antihyperglycemic Activities of Glitazones in KK-Ay Mouse

Drug	Dose (mg/kg)	N#	Glucose (mg/dl)	NEFA (mEq/l)	TG (mg/dl)	Insulin (ng/ml)
Control	--	12	163.3	1.078	135.8	10.92
Rosigli-	1.07	10	152.2	1.017	105.5	11.89
tazone	3.57	11	150.5	0.904	103.3	3.34
	10.7	10	113.4	0.840	90.4*	3.34**
Pioglita-	10.7	11	150.5	0.855	102.7	8.27
zone	35.6	12	104.8*	0.904	110.5	4.68
	107	12	92.4**	0.873	113.9	1.93**
Troglita-	132	12	134.6	0.918	125.0	8.15
zone	441	12	140.3	0.915	118.7	5.33
	1324	12	111.3	0.879	106.6	3.45**

* and ** indicate P <0.05 and <0.001, respectively. N indicates the number of determinations.

4). Effects of Rosiglitazone and its Metabolites on Vascular Tone in Rat Isolated Aorta(BRL-049653/RSD-100N28/1). This study was performed at SKB, the Frythe, Hertfordshire, U.K.

Methods: In order to compare the vascular effects of rosiglitazone and its metabolites rat aortic tissue was used. SD rat's aortic strips (2mm in length) were incubated under 1 g resting tension for 1 hr before use. The maximum tension was achieved by 1 μM phenylephrine and per cent relaxation of the tissues was analyzed in the presence of rosiglitazone and its metabolites including troglitazone. EC_{50} values were calculated for each drugs. Tested metabolites were: SB-237216(N-demethylated form), SB-244675(3-OH form), SB-243914(3-OH, N-demethylated form), SB-275286(5-OH form), SB-280789(5-OH, N-demethylated form) and SB-271258(phenoxyacetic acid-form).

Results: The vehicle control including 84 μl DMSO produced 3.6% relaxation of the maximal contraction. At a concentration causing 50% relaxation of the maximal tone the order of potency was troglitazone> rosiglitazone> SB-244675>SB-275286>SB-237216>SB-243914>SB-280789 as shown below. The data indicate that rosiglitazone had less vascular effect than troglitazone. Rosiglitazone's metabolites were largely inactive in aortic smooth muscle contractility.

Table #4. EC_{50} Values and % Maximum Relaxation in SD Rat Aortic Strip

Compound	EC_{50} (95% confidence limits)	% maximum relaxation
Troglitazone	$5.92X10^{-5}$M(4.47-7.85)	97.5±2.26
Rosiglitazone	$1.29X10^{-4}$M(1.19-1.40)	93.2±5.59
SB-237216*	$4.08X10^{-4}$M(3.53-4.72)	89.2±5.64
SB-243914*	$5.00X10^{-4}$M(4.31-5.80)	76.1±4.20
SB-280789*	$6.50X10^{-4}$M(5.85-7.22)	71.8±4.66
SB-275286*	$3.85X10^{-4}$M(3.44-4.32)	96.2±2.26
SB-244675*	$3.78X10^{-4}$M(3.11-4.34)	83.8±2.69
SB-271258*	Not attainable	15.6±4.40
Vehicle	Not attainable	3.60±1.86

*Please see page 12 for structures of the metabolites.

5). Covalent binding of Rosiglitazone to Human Serum Albumin in Vitro(TF-1036/BRL-049653/1)

Methods: To determine the extensiveness of covalent(irreversible) binding of rosiglitazone to human serum albumin, [redacted]C-rosiglitazone was incubated with albumin at 37°C for 6 and 24 hours. The concentrations of rosiglitazone were 0.25 and 2.5 μg/ml, which were about one and ten times the maximum clinical plasma levels.

Results: Trichloroacetic acid precipitable radioactivity (covalent binding) of rosiglitazone was proportional to the initial drug concentration, which was not increased with incubation time. The results are summarized below in Table 5.

Table 5. Rosiglitazone Binding to Human Albumin in Phosphate Buffered Saline

Drug Concentration (μg/ml)	Incubation Time (hours)	Albumin Binding (nm/g protein)	Albumin Binding (% radioactivity)*
0.25	6	0.779	6.68
	24	0.599	4.97
2.5	6	5.400	4.58
	24	7.450	6.76

*Calculated as final dpm/g protein as a % of initial radioactivity after dialysis, precipitation and extraction process as in Methods.

6) Binding Affinity to Peroxisomal Proliferator Activated Receptor Gamma (PPARγ), PPARγ agonist potency and Antidiabetic Potency of Rosiglitazone and its Metabolites

Methods: Peroxisomal proliferator activated receptor gamma (PPARγ) is considered as a potential receptor for thiazolidinedione. To understand the binding affinity of rosiglitazone and its metabolites to the PPARγ, the metabolites were synthesized and tested for their concentration that inhibits 50% of the maximum binding(IC_{50}). The concentrations that required to activate 50% of the maximum activity of PPARγ as agonists were also compared with in vivo antidiabetic potency.

Results: M10 was the most active in the binding to PPARγ next to rosiglitazone while the binding affinity of M9 and M11 was poor. It appeared that there were some correlations in the binding affinity and the agonist activity, although there were some exceptions. The finding indicates that the drug action might not be solely mediated through the receptor binding since in vivo antidiabetic potency and agonist activity were not in parallel with the parameter. The detailed results are summarized below in Table 6.

Table 6. PPARγ Binding and Agonist Potency of Rosiglitazone and its Metabolites

Compound@	PPARγ* Binding(IC_{50})	Agonist* Activity(EC_{50})	In vivo antidiabetic Potency(%)	Study Report Numbers
Rosiglitazone	44	118	100	PG-1005
237216(M12)	9750	2185	1	RSD-1009ZS/1
243914(M9)	>10,000	4254	Inactive	RSD-100BFZ/1
274675(M11)	>10,000	2510	1	RSD-00DPX/2
271258(M1)	226	135	10	RSD-100N27/4
275286(M13)	2471	1398	1	RSD-00S7W/1
280789(M7)	2471	1398	1	RSD-00S7W/1
332650(M10)	5.4	8170	Not Determined	RSD-00TNV/1

@Each number indicates rosiglitazone's metabolites as shown on page 10 and * the unit is in nM.

7) SUMMARY OF ROSIGLITAZONE PHARMACOLOGY

Rosiglitazone reduced blood glucose in C57B1/6 mice and Zucker (fa/fa) rats including streptozotocin-induced diabetic rats. It also reduced cholesterol in some animals and also decreased amylin, which might antagonize insulin action. But, it had no effect on insulin receptor phosphotyrosine phosphatase activity in liver and muscle in diabetic mice. In KK-Ay mice, it appeared that rosiglitazone had the most potent antihyperglycemic activity, which was followed by pioglitazone and troglitazone, respectively. Rosiglitazone increased GLUT-4 transporter and deoxyglucose uptake, which is consistent with its expected antihyperglycemic action.

C. PHARMACOKINETICS AND ROSIGLITAZONE METABOLISM

1). Pharmacokinetic Data of Rosiglitazone in the Mouse following Dietary Administration for 14 Days(RBL-102I)

Methods: Nine CD-1 male mice per group were given either control diet or diet containing rosiglitazone at doses of 2, 5, and 10 mg/kg/day for 14 days. Two blood samples were taken from each animal in all drug-treated dose groups, at various times during the 24-hour light/dark cycle, commencing approximately at the time

the lights were switched off on day 14. Plasma samples were analyzed by [redacted] with [redacted] detection.

Results: Maximum observed plasma concentrations at all dose levels were achieved at approximately 4 hours after the start of the dark phase. Overall, achieved dose levels were within 4% of the nominal dose. Increasing dose levels of the drug over the range 2 to 10 mg/kg/day were associated with approximately proportional increases in both Cmax and AUC(0-24) as shown below.

Table 7. Summary of pharmacokinetic parameters for BRL 49653 in mice after dietary administration of rosiglitazone for 14 days

Dose	Cmax [ug/mL]	Tmax* [h]	AUC(0-24) [ug·h/mL]
2 mg/kg/day	0.53	4.03	6.77
5 mg/kg/day	1.24	4.14	18.7
10 mg/kg/day	2.84	4.26	44.1

Rosiglitazone pharmacokinetic data such as blood clearance(CL_b), volume of distribution at steady state condition(V_{ss}) and absolute bioavailability in rat, dog and human are compared in a table form below.

Parameter	Rat	Dog	Man
CL_b ([mL/h]/kg)	140	220	60
Vss (mL/kg)	230	120	190
T1/2 (h)	2	1	4
F (%)	100	60	≥ 95
Plasma protein binding (%)	99.8	99.0	99.8

CL_b = Blood clearance V_{ss} = Volume of distribution at steady state
F = Absolute bioavailability

2). In vitro Metabolism of Rosiglitazone in Human Cytochrome P450 Enzymes(RSD-100CPZ/1)

Methods: [redacted]C-Rosiglitazone (Batch# KL5439-112A2, SA 120μCi/mg) at a final concentration of 10 μM, was incubated with human liver microsomes (n = 47) and microsomes from insect cells transfected with human cytochrome P450 cDNA, CYP2C8 and CYP2C9-Arg 144. The human microsomes were obtained from [redacted]

[redacted] The products of rosiglitazone metabolism were investigated using [redacted] and [redacted]. The effect of retinoic acid, on the metabolism in human liver microsomes was investigated to indicate the involvement of CYP2C8. The inhibitory potential of the drug on taxol 6α-hydroxylase (CYP2C8) using human liver microsomes (n = 3) was also investigated. The structure of rosiglitazone and its three metabolites are illustrated below.

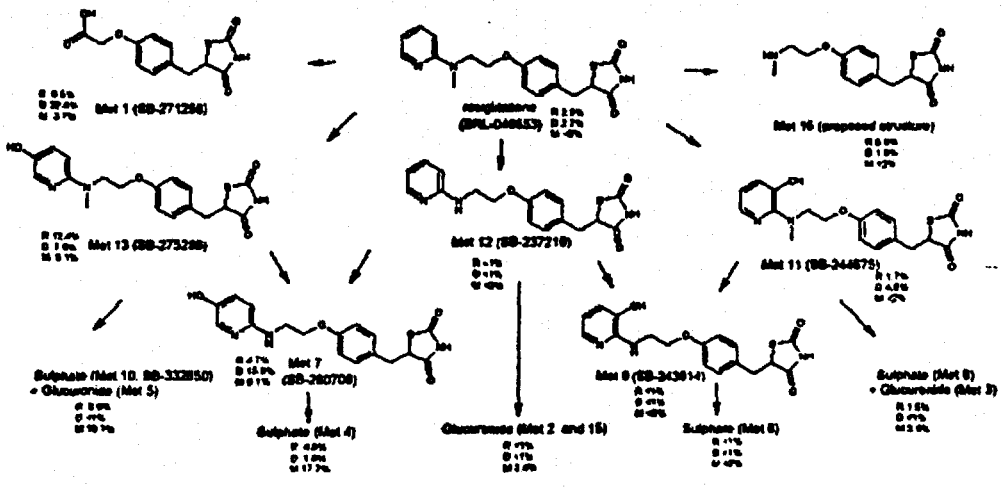


Table 8. Putative Metabolites of Rosiglitazone

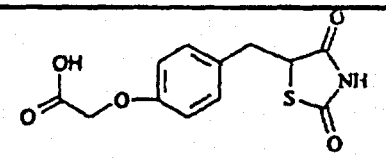
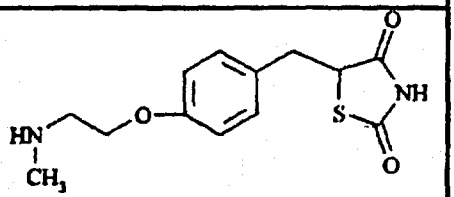
Results: Rates of rosiglitazone 3-hydroxylation and N-demethylation varied over 35-fold in the human livers investigated. The formation of 3-hydroxy and N-desmethyl rosiglitazone were inhibited by retinoic acid (> 50%), they were found to correlate with taxol 6α-hydroxylation ($p < 0.001$) in a bank of human livers and both metabolites were produced by CYP2C9-Arg 144 and more significantly CYP2C8 supersomes. Rosiglitazone also had a moderate inhibitory effect on taxol 6α-hydroxylase activity, with a mean IC_{50} of 18 μM.

Conclusions: CYP2C8 is primarily responsible for the hydroxylation and N-demethylation of rosiglitazone in human liver; with minor contributions from CYP2C9. The drug is a moderate inhibitor of CYP2C8 activity in human liver microsomes.

3). Hepatic Metabolism of Rosiglitazone in Rat, dog and Human Liver Slices(RSD-100KNN/1)

Methods: [redacted]C- Rosiglitazone (maleate salt: batch# HGC-E-01C, SA 4440 KBq/mg)) was incubated at approximately 10 and 100 μM over a pre-determined time-course with rat, dog, and human precision cut liver slices. In addition, appropriate no-drug and no-cell control incubations were also performed. Samples were analyzed by [redacted] to provide preliminary qualitative information on the biotransformation of rosiglitazone (BRL-049653) in rat, dog and man.

Results: Preliminary qualitative assessment of the metabolism of rosiglitazone by rat, dog and human liver slices indicated that it was primarily metabolized to a number of metabolites, that were mainly the products of N-demethylation, pyridine ring hydroxylation, O-glucuronidation and O-sulfation. All the major components in the rat and dog liver slice incubations were also observed *in vivo*, indicating that the slices represented a good predictive model for rosiglitazone metabolism in man as shown below.

Molecular Mass	Rat	Dog	Human	Metabolite ID
281	√	√	x	 Phenoxyacetic acid derivative
280	√	√	√	 N-despyridinyl
519	x	√	√	N-desmethyl glucuronide
519	√	x	√	N-desmethyl glucuronide
549	x	√	√	O-glucuronide
439	√	√	√	N-desmethyl O-sulphate
439	√	√	x	N-desmethyl O-sulphate
359	x	√	x	N-desmethyl 5-hydroxy (SB-243914)
549	√	√	√	O-glucuronide
359	x	√	x	N-desmethyl hydroxy
453	x	√	√	O-sulphate
453	√	√	√	O-sulphate
549	√	√	√	O-glucuronide
373	√	√	x	5-hydroxy (SB-244675)
343	√	√	√	N-desmethyl
373	√	x	√	Hydroxy or N-oxide
357	√	√	√	BRL-049653

x - Peak not observed by [redacted].

Conclusion: Rosiglitazone was primarily metabolized by N-demethylation, pyridine ring hydroxylation, glucuronidate ion and sulfation in rat, dog and human liver slices. All metabolites present in the human liver slice incubations were also detected in either the rat or dog incubations. There appeared to be no unique human metabolites. The metabolism in the liver slices reflected the metabolism *in vivo* for each species.

D. TOXICOLOGY STUDIES

1). A 13-Week Dietary Range-Finding Study in Mice(TF-1037/BRL-049653/1)

Methods: Eight Crl:CD-1 mice/sex/group were given rosiglitazone(batch HGC-E-01C) at doses of 0, 0.4, 2, 10, and 20 mg/kg/day by dietary admixture for 13 weeks.

Results:

Mortality and Clinical Observation: There was no mortality. No remarkable clinical signs were noted except firm, but palpable swellings in the scapular areas were noted in 14/16 animals in the high dose group and 6/16 animals dosed at 10 mg/kg/day.

Body Weight and Food Consumption: There were no clear treatment related effects on body weight since there was no drug dose dependent change in this parameter in either sex. There were no treatment-related effects on food or water consumption.

Blood Chemistry: In the high dose group there were slight(8-9%) decreases in mean hemoglobin and red cell count. Mean reticulocyte count was also decreased (by 38%) in these animals, which appears drug dose related. Total protein was moderately decreased (12%) in high dose male group without remarkable histopathological changes.

Organ Weight: In females of 10 and 20 mg/kg/day groups, there were significant increases in inter-scapular brown adipose tissue(up to 89%). Relative ovary weights were also 97% higher at the high dose. In males, renal weights were increased(10%) at 2 mg/kg/day and heart weights were increased by 15% at 10 mg/kg/day groups, which suggests LOAEL might be near 2 mg/kg/day. The brown adipose tissue weight was also increased by 73% in the high dose group.

2). One-Year Study of Dietary Rosiglitazone in Mice(Protocol# 195653/RSD-100HKX/1)

Methods: Sixteen CD-1 mice/sex/group were given either vehicle(regular maintenance diet#1) or rosiglitazone by dietary administration at doses of 0, 3, or 6 mg/kg/day for a year.

Results:

Mortality and Clinical Signs: A total of fourteen mice died or were killed during the treatment period. Five males died after blood sampling operation, and one female was killed as a result of eye bleeding. A further five treated animals and 3 control mice died or were killed, of which macroscopic observations revealed natural deaths of this age and strain. Thus, no deaths are related to treatment. There were no clinical signs that were considered to be related to drug treatment.

Body Weight and Food Consumption: By Week 52,the group mean body weights of males given 3 and 6 mg/kg/day were 7 and 13% higher than the mean control. The parameter in the high dose group females were increased by 6% over the control animals. There were no consistent treatment-related effects on food intake.

Hematology: Hemoglobin concentration in all treated mice was reduced by 13% from the control value. Mean absolute reticulocyte counts were up to 432% lower than control group means in one female group in all weeks. In mice receiving 6 mg/kg/day, mean platelet count was lower than the controls by up to 20% in males and in females at most of all blood samples. Other differences from control were small or were not seen consistently over the 1-year period, and are not considered to be related to drug treatment.

Organ Weight: The group mean myocardial volume was 36% greater than the control in females of the high dose group and the pituitary gland volume was 63% less than the control as shown below. There was a strong correlation(Correlation Coefficient=0.89) between cardiac volume and cardiac wet weight at necropsy as revealed by Magnetic Resonance Imaging(MRI).

Table 8. Effects of Rosiglitazone on Cardiac Changes in Female Mice by MRI

	Female Control	Females on 6 mg/kg/day
Heart volume(µl)	240 ± 53	327 ± 37*
Heart Wet Weight(mg)	150 ± 29	185 ± 16*
Pituitary Volume(µl)	1.66 ± 0.40	0.62 ± 0.47*
Pituitary Wet Weight(mg)	2.28 ± 0.56	2.12 ± 0.44

Each value represents group mean ± SD of 9 to 11 determinations. * $P<0.05$.

Macroscopic Observations: There was a high incidence of enlarged scapular fat pads and increased amounts of brown and white adipose tissue in the drug-treated mice. The changes were considered to be a result of treatment with the drug.

Comments : The mid-dose did not produce significant findings. Thus, NOEL may be 3 mg/kg/day. It appears that the top dose might be near the LOAEL, which were still two low to draw meaningful toxicological conclusion.

3). A 26-Week Oral Repeat Dose Study in Rats Followed by a 12-Week Off-Dose Period(TF-1023/BRL-049653/1).

Methods: Twelve Crl:CD rats/sex/group were given either vehicle(methylcellulose) or rosiglitazone (batch# HGC-E-01C) orally at doses of 0, 0.2, 1.0 or 40 mg/kg/day for 26 weeks. The doses were selected based on the findings from a 28-day repeat dose study, using 0, 0.4, 2.0, or 80 mg/kg/day. In the high dose group there were low erythrocyte parameters and high heart weight, which were considered as evidence of drug-related toxicity.

Results:

Mortality: One control female, one intermediate dose male, and two males and five females in the high dose group, occurred during the course of treatment. One high dose male also died during the off-dose period. The major cause of deaths in the high dose group appeared to be fluid accumulation in the thoracic cavity.

Clinical Signs: In the high dose group of both sexes there were firm palpable swellings at various sites were noted in all animals, which was first observed in Week 4. There was no such swelling at either of the 0.2 or 1.0 mg/kg/day groups. There were no other clear treatment-related findings.

Body Weights and Food Consumption: At 40 mg/kg/day, overall body weight gain in males was higher than controls by 22%. In females, overall weight gain was 19% greater than controls. The intermediate doses did not clearly reduce body weight. Food consumption in animals dosed at 40 mg/kg/day was increased throughout treatment, with an overall increase of 10% in males and 7% in females. The parameter was not affected by treatment in the low and intermediate doses.

Hematology: Rosiglitazone dosed at 40 mg/kg/day induced a progressive, reduction in hemoglobin, packed cell volume and red blood cell counts in both sexes. This change was exemplified by a reduction in group mean RBC at weeks 5 and 26 of 7% and 13% in males, and 13% and 25% in females. Mean platelet counts were reduced in males and females in the high dose group and in females given 1.0 mg/kg/day. No toxicologically significant findings were noted at 0.2 mg/kg/day.

In females of the high group, mean total protein (albumin and globulin) was higher than control in weeks 9 and 14, by 5 to 14%, respectively. In blood samples taken from the high dose females which were killed due to hydrothorax, an animal (#168) had a reduced alanine aminotransferase(ALT) and total globulins. In some animals there was an increased cholesterol by 40-60% of the control values.

Toxicokinetic Analysis: There was a small difference in the pharmacokinetics between sexes, females having 50-100% higher mean values of AUC at all three doses. At least 67% of female animals had higher Cmax and AUC values than those observed in male animals as shown below.

Table 9. SUMMARY OF PARMACOKINETIC DATA IN RATS AT WEEK 22

Dose (mg/kg/day)	Sex	Cmax (µg/ml)	Tmax (hours)	$AUC_{(0-t)}$ (µg.h./ml)	Clinical Ratio#	T½ (hours)
0.2	M	1.08*	0.5*	4.63*	1.5	3.5*
	F	1.26	1.2	8.61	2.9	- 5.5
1.0	M	4.46	0.5	15.78	5.3	2.8
	F	4.88	0.6	22.38	7.5	3.6
40.0	M	83.42	1.2	330.91	110	2.6
	F	107.00	1.2	652.69	217	3.3

N=3, *Represent mean or median of data obtained from 2 animals. #Calculated based on human exposure(3 µg.h/ml) following 10 days dosing at 8 mg/day.

Organ Weights: At the end of the treatment rosiglitazone(40 mg/kg/day) increased heart weights of 45% in males and 39% in females compared to control. The parameter was restored partially during off-dose period in both sexes. Dose-related increases in scapular adipose tissue weight were also noted. In the high dose, the increases were 417% and 685% in males and females, respectively. Effects of rosiglitazone on liver and heart weights are summarized below, although epididymides, prostate and seminal vesicle weights were also altered in high dose group.

In order to determine the drug effect on cardiac hypertrophy, morphometric measurements were carried out at the end of the study(T93566/Protocol#T94640/RSD-100NPK/1). Areas of left ventricular wall(LVW), left ventricular lumen(LVL), inter ventricular septum(VS), right ventricular lumen(RVL) and right ventricular wall(RVW) were measured as shown below. The increase in the ventricular areas might be secondary to the increased cardiac work as a result of the drug action.

Table 10. Mean Right and Left Ventricular Wall and Lumen Areas(mm^2) in Rats

Dose(mg /kg/day)	LVW	LVL	VS	RVL	RVW	Total Wall	Heart Wt(g)
Control	49.48	8.29	27.05	9.31	15.51	91.74	1.83
0.2	49.16	10.34	26.98	10.42	14.91	91.75	1.75
1.0	51.85	9.99	28.51	10.22	15.89	100.82	1.84
40.0	69.84*	13.61*	37.76*	18.82*	22.81*	130.41*	2.96*

All values except organ weight are expressed in mm^2 which were obtained from 10 to 12 determinations. LVW, LVL, VS, RVL and RVW indicate areas for left ventricular wall, left ventricular lumen, inter ventricular septum, right ventricular lumen and right ventricular wall, respectively. *P<0.01.

Table 11. Effects of Rosiglitazone on Cardiac and Hepatic Weights (gram) in Rats

Tissues	Sex	Control	0.2 mg/kg/day	1.0 mg/kg/day	40 mg/kg/day
Heart	M	1.831	1.750	1.810	2.956*
	F	1.133	1.097	1.204	1.750*
Liver	M	19.78	19.25	17.72	22.86*
	F	10.98	10.82	10.96	13.98*

All values are expressed in g from 10 to 12 animals. *<0.05.

Pathology: Treatment related changes occurred in brown and white adipose tissue, sternal medullary fat, the heart, and pituitary gland at 1.0 and 40 mg/kg/day. At necropsy, macroscopically enlarged hearts in 8/20 high dose animals and an enlarged pituitary gland in a single intermediate dose female. There were no associated histopathological findings with either organ.

4). A 26-Week Oral Repeat Dose Study in Dogs Followed by a 12-Week Off-Dose Period(TF-1022/BRL-04953/1)

Methods: Pure bred beagle dogs(4-6 dogs/sex/group) were given either vehicle(starch) or rosiglitazone (batch#HGC-E-01C) once daily by capsule at doses of 0.2, 2.0 or 20 mg/kg/day for 26 weeks. An additional two males and two females dogs assigned to each of the control and high dose groups(recovery animals) were dosed for the same period and then maintained off-dose for 12 weeks. The post mortem examinations at the end of the treatment period were performed in weeks 27 and 39 respectively.

Results:

Mortality and Clinical Signs: There were no deaths. An increased incidence of loose feces was noted among high dose males.

Body Weight and Food Consumption: At the end of treatment, group mean bodyweights were reduced by 7 to 8% in high dose animals, which was significant($P<0.01$). During the recovery period the parameter was partially recovered. There were no effects in the low dose group, although the intermediate group had a slight reduction in bodyweight gain. There were no treatment-related findings in food consumption.

Hematology: In dogs dosed with high dose, there were progressive reductions in hemoglobin, packed cell volume, and RBC counts from week 3 to 5 of the study. For example, group mean RBC counts reduced to 33% in males and 25% in females from the control values. Plasma volume increased in 5/6 males and 5/6 females, which was generally progressive from week 5 in 3 males and in 3 females. The intermediate dose group showed a marginal alteration in the parameters. No toxicologically significant hematological changes were noted in dogs of the low dose group.

Blood Chemistry: There were marked increases in alanine aminotransferase(ALT) in all high dose animals. In particular, males in the group had elevated ALT level up to 12-times of baseline values at 17 weeks. Alkaline Phosphatase(AKP), aspartate aminotransferase(AST) and lactate dehydrogenase were also elevated in males of the high dose group toward the end of the study as shown below. Albumin and total bilirubin(1.88 vs. 2.45 μmol/l at Week 19) were increased in males of the high dose group.

Table 12. Effective of Rosiglitazone on Hepatic Enzyme Levels in 26-Week Dog Studies

Test	Weeks	Control		0.2mg/kg/day		2.0 mg/kg/day		20 mg/kg/day	
		M	F	M	F	M	F	M	F
AKP	5	143.2	134.7	122.0	108.8	110.5	117.3	111.2	92.7*
	19	104.0	124.2	95.5	116.5	218.8	96.0	379.7*	135.2
	25	103.0	120.0	92.8	107.3	280.0	105.5	507.2*	160.2
ALT	5	42.8	35.5	42.8	38.5	68.0	59.0	95.3*	61.3
	12	50.0	36.2	43.0	40.0	189.5	74.3	321.3*	117.2*
	17	39.3	35.3	46.5	43.8	138.3	84.0	487.5*	163.5
	19	44.7	35.5	49.8	41.8	255.5	66.5	400.2*	160.8*
	25	40.2	36.2	50.3	47.0	331.5	113.0	386.5*	199.0*
AST	17	26.3	26.5	27.8	25.3	32.5	24.5	48.0*	29.3
	25	28.8	28.8	32.8	28.0	36.3	22.8	46.2*	32.7
LDH	25	46.2	49.5	44.3	47.0	55.5	54.8	80.7*	59.8

All values represent means of 8 to 12 determinations and the units are in IU/l. AKP, ALT and LDH stand for alkaline phosphatase, alanine aminotransferase and lactate dehydrogenase, respectively. *<0.05.

Toxicokinetics: There was no significant difference in PK data between sexes, although the AUC values were somewhat higher in males than in females in rats. The PK data were also similar between two time periods at 4 and 24 weeks in dogs as shown below.

Table 13. Pharmacokinetic Data of Rosiglitazone in 26-Week Dog Studies

Dose (mg/kg)	SEX	PK Data at Week 4				PK Data at Week 24			
		Cmax(µg/ml)	Tmax (hrs)	AUC_{0-t} (µg.hr/ml)	T½ (hrs)	Cmax (µg/ml)	Tmax (hrs)	AUC_{0-t} (µg.hr/ml)	T½ (hrs)
0.2	M	0.14	2.0	0.28	1.52*	0.19	1.0	0.54	1.75
	F	0.18	2.0	0.37	ND	0.29	1.0	0.48	1.30
2	M	2.92	0.5	4.38	1.26	2.18	1.0	4.14	1.52*
	F	2.22	1.0	3.55	0.82	3.72	0.5	7.31	1.08
20	M	22.83	1.0	40.48	1.57	27.15	1.0	69.60	2.36
	F	22.06	2.0	47.63	1.26	20.44	1.0	56.59	2.71

N=3, *Represent mean or median of data obtained from 2 animals. M and F stand for males and females. In the high dose group at week 24, clinical ratios for males and females were 23 and 19 respectively (clinical exposure = 3 µg.h./ml at a dose of 8 mg/day).

Organ Weights: Rosiglitazone increased significantly heart and liver weights in the high dose group(Please see table below). Relative heart weights were increased by 44% and 38% and liver weights by 52% and 27% in males and females, respectively. After twelve weeks off-dose, liver weights were comparable to controls while relative heart weights remained increased in some animals. The intermediate dose also increased the heart weights by 34% in males and 12% in females. Remarkable changes in high dose organ weights increased relative kidney weights in males(21%) and absolute adrenal weights in both sexes(approx. 20%). There were no organ weight changes at the low dose.

Table 14. Effects of Rosiglitazone on Heart Weights in Dogs after 26-Week Treatment

Tissues	Sex	Control	0.2 mg/kg/day	2.0 mg/kg/day	20 mg/kg/day
Heart (g)	M	105.4	108.8	141.1*	144.5*
	F	100.9	106.1	111.8	127.4*
Liver (g)	M	346.3	364.5	415.0	501.8*
	F	347.3	330.3	335.3	390.8

Macroscopic Observations: Changes in the heart consisting of fluid in the pericardial sac were noted in 3/8 animals from the high dose group. White areas on the left papillary muscles were observed in a male dog from the group after the off dose period, which was not seen in animals from other groups.

Microscopic Observations: Treatment related changes occurred in heart, sternum, liver, spleen and thymus. The changes were generally dose-related in incidence and /or severity.The changes were characterized by left ventricular myocardial hypertrophy, increased sternal medullary fat deposition, and increased thymic atrophy. In the liver, increased hemosiderin was noted. Other treatment-related renal basophilic tubules in animals of both sexes at the high dose group were observable, which might be reversible upon 12-week off-dose period.

5). One Year Oral Toxicity Study in Dogs(BRL-049653/RSD-100HJW/2; Protocol# G96626). This study was performed by [redacted]

Methods: Pure bred beagle dogs(4 dogs/sex/group) were given either vehicle(starch) or rosiglitazone (batch#HGC-E-01C) once daily by capsule at doses of 0.05, 0.5 or 5 mg/kg/day for one year.

Results:

Mortality and Clinical Signs: There were no deaths and there were no clinical signs that were considered to be related to the treatment.

Body Weight and Food Consumption: After 52 weeks of treatment a small (4%) mean body weight loss was recorded for males receiving the high dose. There were no significant changes in the parameter in all groups. Food consumption over the 52-week period was not affected by the treatment.

Ophthalmoscopy and Electrocardiography: There were no ocular lesions which might have affected by the treatment. Electrical waveform or heart rate were not affected by the treatment.

Hematology: Group mean hemoglobin levels at Weeks 4 and 12 were as marginally increased for males (12.7%, 15.3%) and females (24.3%, 5.4%) receiving the high dose. It appears that the findings are not consistent with other hematological findings since marginally reduced red cell indices were noted for males in the high dose group as in the case of 26-week studies.

Biochemistry: Statistically significant increases in mean ALT values for males occurred at 0.5 (60.9%) or 5.0(82.6%) mg/kg/day group at Week 4. Subsequently 460% in males and 360% in females of the high dose group increased the ALT values.

Organ weights: Since the sponsor selected low doses, the only finding of note was a small increase in-group mean heart weight in high dose group(25.6% in females and 16% in males).

Macroscopic post mortem Findings and Histopathology: There were no macroscopic post mortem findings that were considered to be related to treatment. A slight increase in incidence and severity of pigmented macrophages was seen in male dogs receiving the high dose

COMMENT: In dogs, a dose of rosiglitazone(20 mg/kg/day) might be near the MTD as demonstrated in 26-week studies. Thus, the sponsor could not reveal any toxicologically meaningful findings in one-year study with the maximum dose of 5 mg/kg/day except that 5 mg/kg/day might be a minimally toxic dose.

Table 15. Comparison of Histopathological Effects of Rosiglitazone in Mice, Rats, and Dogs

Spec-ies	Sex	Lesion	LED (mg/kg/day)	AUC at LED(µg.h/ml)	Exposure* Ratio(animal:man)
Mou-se	F	Cardiac Hypertrophy	10	53.5	17.8
	F	Erythrocyte Parameters	20	112.0	37.3
Rat	M	Cardiac Hypertropy	5	62.5	20.8
	M	Erythrocyte Parameters	1	16.2	5.4
Dog	M/F	Cardiac Hypertrophy	2	3.6	1.2
	M/F	Erythrocyte Parameters	2	3.6	1.2

LED stands for Lowest Effective Dose and * calculated based on human AUC 3 µg.h/ml following clinical dose at 8 mg/day.

E. SPECIAL TOXICITY STUDIES

1). Effects of 28 Day Oral Administration of Rosiglitazone on Cardiac Function and Morphology(RSD-100S4M/1) in Dogs.

Methods: Female Beagle dogs(8/group) were given either vehicle(blend gelatin capsules) or rosiglitazone (60 mg/kg)capsules orally once daily for 28 days. Serial transthoracic

echocardiographic examinations were performed to evaluate cardiac function and morphology at Day 1, 7, 14 and 28. A terminal invasive evaluation was conducted to monitor ventricular functions and contractile reserve.

Results: In life summary is presented below.

Table 16. Effects of 28-Day Oral Administration of Rosiglitazone on Cardiac Function in Dogs.

Systemic Parameters	Baseline Values at Day 1		% Change at Day 28	
	Control	Rosiglitazone	Control	Rosiglitazone
R-R interval(msec)	654.4	686.3	11	-19*
RV wall thickness(cm)	0.31	0.27	8	19
LVPWTd(cm)	0.49	0.39	17	55*
LV Mass(g)	43.2	34.2	7	64
LV cardiac output(ml/min)	1867	1524	4	78*
LV ejection fraction(%)	0.59	0.55*	-2	11

Each value represents mean of 6 to 8 determinations. LV and LVPWTd stand for left ventricle and left ventricular posterior wall thickness during diastole. *P<0.05.

<u>2). Effects of 4-Week Rosiglitazone Treatment on Canine Hepatic Enzyme Activities</u>

Methods: Female beagle dogs (3 /group) were given either vehicle(gelatin capsules) or rosiglitazone(Batch# HGC-E-14C) at a dose of 60 mg/kg/day for 28 days. Another set of 6 female dogs were also studied similarly using troglitazone at a dose of 80 mg/kg/day for 28 day.

Results: The main highlights of the study were summarized below.

Table 17. Hematocrit and Liver Enzyme after 4-Week Rosiglitazone Treatment in Dogs

Treatment	Hct*	Hct@	ALT(IU/mL)*	ALT(IU/ml) @	Relative Liver Wt(%)
Control	0.453	0.447	30.3	32.6	3.10
Rosiglitazone 60 mg/kg	0.477	0.367	23.0	48.3	35.7
Control	0.491	0.487	22.8	29.5	
Troglitazone 80 mg/kg	0.471	0.452	27.5	61.8	

*Hematocrit was measured at Day 4 for rosiglitazone test while at Day 1 for troglitazone test. @ was determined at Day 29 for rosiglitazone test while at Day 27 for troglitazone test. All values represent means of 3 to 6 animals.

3). Effects of Rosiglitazone and Troglitazone on Rat Hepatocytes In Vitro(RSD-100S4S/1)

Methods: Hepatocytes were prepared by the two-stage collagenase perfusion, using male SD rats and cultured on collagen type I plastic ware at a density of 3x104 cells/well for 96 well plates. The cultured hepatocytes were subjected to 5 major tests in the absence or presence of troglitazone(Lot# SM96258-060A1), rosiglitazone(Lot# HGC-E-03C) or their metabolites including acetaminophen(Lot# 02825HR). The five agents were 1) Neutral red(50 µg/ml) uptake for energy dependent functions; 2) Lactate dehydrogensase release for plasma membrane permeability; 3)Fluorescein diacetate retention(50 ng/ml) for plasma, microsomal and lysosomal membrane permeability; 4) Alamar Blue reduction for mitochonrial redox function; and 5)Coomassie blue staining for total cell protein.

Results: The concentrations required to cause a 25% change from the control(EC_{50}) for troglitazone(Lot# SM96258-060A1), rosiglitazone(Lot# HGC-E-03C) and acetaminophen(Lot# 02825HR) are summarized below.

Table 18. EC_{25} Values (μM) for Troglitazone, Rosiglitazone and Acetaminophen in Hepatocytes

Compound	Neutral Red	LDH	Fluorescein Diacetate	Alamar blue	Coomassie blue
Rosiglitazone (4 hour)	60	GLS	GLS	GLS	GLS
Rosiglitazone (24 hour)	62	GLS	GLS	GLS	GLS
Troglitazone (4 hour)	20	40	40	70	50
Troglitazone (24 hour)	30	22	45	40	34
Acetaminophen (4 hour)	300	30,000	33,000	29,000	31,000

The drugs were added to the hepatocytes either 4 or 24 hours' post-isolation incubation. GLS stands for greater than limit of solubility of drugs.

4). SUMMARY OF TOXICOLOGICAL STUDIES:

Toxicology studies in the mouse, rat and dog are summarized in a table below. This table also shows a comparison of the extent of drug exposure in relation to human. In many studies, it was difficult to determine an accurate no effective level of rosiglitazone since neither the low nor the intermediate dose produced significant toxicological effects. Even the high doses were not sufficiently high enough to induce frank toxicity in some studies. An exception would be 26 week-treatment of rats at 40 mg/kg/day, which killed 2 male and 5 female rats at the high dose and the 1 year dog study where the high doses elicited significant elevations of ALT ans AST. To this reviewer, it is difficult to evaluate the toxicological action of rosiglitazone in drug-dose dependent manner. It is quite clear that a long-term exposure to the drug produced significant toxicity even at the fractions of the high dose in most of all toxicological studies. In particular, the exposure-duration dependent elevation of alkaline phosphatase(AKP) and alanine aminotransferase(ALT) was clearly demonstrated in male dogs of 26-week studies(Study#: TF-1022). The 500 to 1000% increases in the liver enzymes in dogs were confirmed in one year dog studies(Study#RSD-100HJW/2). The elevations in hepatic enzymes, which were drug-dose and exposure-duration dependent, do not allow a simple prediction of potential consequence after a life-long exposure of rosiglitazone, although the metabolic profile in canine might be similar to humans. There were increased cardiac weights as well as reductions in hematological parameters in most of high dose groups along with increased incidence of pulmonary thrombosis, which might be the typical class actions of

thiazolidinediones. The main problem is an inability to extrapolate the preclinical data to clinical setting because of wide dosing intervals used in most toxicological studies.

Table 19. Rosiglitazone Dose in Mice, Rats, and Dogs in Major Toxicological Studies

SKB Study#	Species /Strain	Treatment Dura-tion	Dose(mg/kg/day)			Clinical AUC ratio	Toxicity Comments
			Low	Mid	High		
TF-1047	Mice /CD-1	4W*	0.4	2/10	20.0		No Effect
TF-1037	Mice /CD-1	13W	0.4	2/10	20.0	69(F)	No Death ↓RBC(38%)
RSD-100HKY /1	Mice /CD-1	52W	3		6		No death ↑Heart Wt(23%)
TF-1011	Rats/SD	4W	0.4	2.0	80.0		No death
TF-1023	Rat/CD	26W	0.2	1.0	40.0	110(M) 217(F)	Death(58%) ↓RBC(25%)
RSD-100S4M	Female Dog/ Beagle	4W			60.0		↑LVPWT (55%)
TF1022	Dog/ Beagle	26W	0.2	2.0	20.0	23(M)/ 19(F)	↑AKP(5x), ALT(10x) in male
RSD100 HJW/2	Dog/ Beagle	1Y	0.05	0.5	5.0		No death ↑ALT

*W indicates treatment duration in week. M or F stand for clinical AUC ratio in male or female. LVPWT, AKP, and ALT stand for left ventricular posterior wall thickness, alkaline phosphatase, and alanine aminotransferase, respectively. ↑ and ↓ indicate an increase or a decrease of relevant parameters.

F. REPROTOXICITY STUDY

Please also see reviews dated on 10/20/1993, 6/8/1995, 7/11/1996 and 7/17/1996.

1) Oral Study for Effects on Pre- and Postnatal Development in Rats(RSD-100M82/2)

Methods: Sixty male and 130 female SD rats were used in the study. The females rats (F0) were cohabitated with male rats(F0). Postcoitus Day 0 was designated when insemination was confirmed. The mated female rats(24/group) were given either vehicle or rosiglitazone (Lot# HGC-E-03C) orally at doses of 0.2, 1.0 and 3.0 mg/kg/day from day 6 postcoitus through day 20 postpartum. The high dose(3mg/kg/day) gave 3.6 times human multiple, based on body surface area.

Results:

Mortality and Clinical Signs: There were no unscheduled deaths and no drug-related clinical signs were observed.

F0 female Body Weight: Females at 3 mg/kg/day gained 44% more weight than controls during days 6 to 10 postcoitus and 26% during day 10 to 14 postcoitus. There was no difference during lactation. There were no adverse effect on the parameter in the low and intermediate dose groups.

F0 Necropsy Observations: There were no drug-related observations.

F0 Natural Deliveries: The mean duration of gestation was 21.3 to 21.5 days, which was not affected by drug treatment. Average delivery time per pup (elapsed time of deliveries observed divided by number of pups observed) was also not significantly different from the control group.

F1 Offspring Number, Viability, and Examinations: The results are summarized below. In the high dose group, the live birth index was reduced significantly, although the low and mid-dose did not reduce the parameter. Likewisely, per cent viability up to 21 postpartum was reduced significantly, although the top dose was 3.6 times of human multiple.

F01 Offspring Body Weight: At or shortly after the time of birth (Day 0 or 1pp), male and female offspring body weight was less than controls by up to 10% at 1.0 mg/kg/day and by up to 15% at the high dose. By the time of weaning (Day 21 pp), there were no differences among the groups.

F1 Offspring Physical Development: A decrease in offspring body weight during days 28 pp to 42 pp was noted in the high dose group. Vaginal opening and balano preputial

separation in males were delayed in the group. There were no statistical differences in the parameter at the two low doses.

Table 20. Female(F0) Natural Delivery and Offspring (F1) Number and Viability in Rats

Dose(mg/ kg/day)	Live Pups#	Implants #	Dead Pup#	Live Birth Index(%)	Viability(%) at Day		
					0-4pp	4-7pp	7-21pp
Control	14.2	15.9	0.4	96.6	99.4	99.6	98.5
0.2	15.6	16.8	0.3	98.1	98.5	100.0	98.9
1.0	14.5	15.5	0.2	98.1	98.4	99.3	97.4
3.0	12.0	16.2	1.6	87.5*	85.7*	95.4*	97.2

Life birth index(%) = 100 x (live pups born)/(live and death pups born). Percent viability at Day 0-4pp = 100 x (pups alive on Day 4 postpartum)/(pups alive on Day 0 postpartum).

F1 Offspring Reflex Behavior: There were isolated differences for both higher and lower groups from controls, which was not clearly dose-dependent.

F1 Offspring Learning and Retention: A passive avoidance test indicated that animals in all groups acquired avoidance behavior, which was not different from the control and the treated groups.

F1 Offspring Motor Activity: All animals showed high levels of activity during the nocturnal period and in response to an amphetamine challenge. It appeared that there was no effect of maternal (F0) drug treatment on offspring (F1) motor activity.

F1 Offspring reproductive Performance: There were no significant effects on offspring estrous cyclicity, mating incidence (90 to 100%) or pregnancy incidence (89 to 100%). Average pup delivery time was different between the control (7.3 min/pup), 1.0 mg/kg/day(9.9 min/pup) and 3.0 mg/kg/day (12.6 min/pup) groups. Litters size (live F2 pups/litter) was 16% less than controls for the high dose group.

The data indicate that rosiglitazone had significant effects on pre- and postnatal development and survival in rat at relatively low human multiple.

<u>2). Placental Transfer of Radioactive Rosiglitazone(RSD-100KXP/1)</u>

Methods: Fifteen time-mated female pregnant SD rats were given a single oral administration of [redacted]C-rosiglitazone(Batch# AL54415-153A, SA 122.5 μCi/mg) at a dose of 0.2 mg free base/kg on day 18 of gestation. Three rats were killed at each of 1, 2, 6, 24 and 72 hours post dose and concentrations of total radioactivity were determined in fetal

tissue, amniotic fluid, fetal placenta, maternal liver, kidney, mammary tissue, ovaries, uterus, and plasma.

Results: Radioactivity was detected in all fetal and maternal tissues investigated. For most of the tissues, the highest mean concentration of drug-related radioactivity was noted at 2 hr after dosing except mammary and ovary tissues. Beyond 2 hr, the concentrations in all tissues were seen to decline time-dependently as shown below. Fetal tissues and placenta also accumulated the radioactivity 12 and 24% of plasma level of drug.

Table 21. Radioactive Rosiglitazone in Pregnant Rat Tissues after 0.2 mg/kg Injection

Tissue Samples	Rosiglitazone Concentration (μg free base /g) in Tissues*				
	1 hr	2hr	6 hr	24 hr	72 hr
Amniotic fluid	0.129	0.142	0.087	0.016	0.018
Fetal tissue	0.114	0.122	0.075	0.017	0.007
Kidney	0.271	0.278	0.191	0.027	0.002
Liver	0.681	0.778	0.632	0.094	0.004
Mammary tissue	0.178	0.167	0.111	0.048	0.003
Ovaries	0.245	0.243	0.135	0.020	0.001
Fetal placenta	0.227	0.230	0.177	0.052	0.016
Uterus	0.273	0.303	0.202	0.040	0.014
Plasma	0.945	0.945	0.612	0.073	0.002

*Each value represents mean of three independent determinations.

3). Secretion of Radioactive rosiglitazone in the milk of Lactating Rats(RSD-100KXR/1)

Methods: : Fifteen lactating SD rats were given a single oral administration of [redacted]C-rosiglitazone(Batch# AL54415-153A, SA 122.5 μCi/mg) at a dose of 0.2 mg free base/kg. Milk and plasma samples were collected from three rats at each of the following time points: 1, 2, 6, 24, and 72 hr after dosing.

Results: The table below shows that secretion of drug-related radioactivity into milk was detected at all time points investigated and the mean concentration being highest at 1 hr

after dosing. The concentrations of drug-related radioactivity in milk were lower than those in plasma, resulting in milk to plasma ratios of 0.1 to 0.3.

Table 22. Drug Levels in Milk and Plasma of Lactating Rats after 0.2 mg/kg Injection

Sample	Rosiglitazone Concentration (µg free base equivalent/g)				
	1 hr	2 hr	6 hr	24 hr	72 hr
Plasma	1.101	0.652	0.539	0.018	0.001
Milk	0.121	0.109	0.110	0.006	0.001*
Ratio@	0.111	0.169	0.205	0.297	NA

Each value represents mean of at least 3 determinations. @ indicates ratio of radioactivity in milk to the plasma and * indicates calculated values from data less than 30 dpm above background counts.

4). Embryo-Fetal Development in Rabbits(TF-1042/BRL-049653/1)

Methods: Batch HGC-E-01C. Twenty-two premated female New Zealand White rabbits were given rosiglitazone orally at doses of 2, 15 or 100 mg/kg/day. The animals were dosed from days 6 to 20 postcoitus (pc) inclusive and were necropsied on day 28 pc.

Results: There were no treatment-related clinical signs. Three females aborted, one control animal on day 15 pc and two high dose satellite animals on days 22 and 28 pc. Towards the end of gestation there was a slight difference in the weight gain profile of high dose animals compared with controls, reflecting the reduced litter weight at this dose. Food intake was reduced at the high dose throughout the treatment period. Water intake was unaffected.

At the high dose, there was a statistically significant reduction in foetal weight resulting in a reduced litter weight compared with controls. A higher incidence of some minor skeletal anomalies/variants was seen at the high dose compared with controls, the majority of which were considered to be indicative of fetal immaturity. At the high dose, there were reduction of appoximately 20% in hemaglobin.

SUMMARY AND CONCLUSION:

There was no remarkable 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. 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=11.94 µg.h/mL) in rats and 15 mg/kg/day (AUC=12.5µg.h/mL) in rabbits.

G. GENOTOXICITY STUDY

Please also see the reviews dated on 10/20/1993 and 5/17/1995.

1). Rosiglitazone: Mouse Lymphoma Mutation Assay(TF-1006/)

Methods: Rosiglitazone(12.5 to 400 µg/ml) was dissolved in DMSO before use. The incidence of forward mutations at the thymidine kinase locus of mouse lymphoma L5178Y cells was assessed by counting the numbers of trifluorothymidine-resistant cells able to form colonies in semi-solid medium. The tests were performed both in the presence and absence of a post-mitochondrial supernatant fluid preparation (S9 mix) from the livers of Aroclor 1254-induced adult male rats. The positive control substances used were ethyl methanesulfonate(EMS: 250 µg/ml) and 3-methylcholanthrene(3-MC: 2.5µg/ml).

Results: Rosiglitazone, at a concentration of 150 µg/ml, reduced the relative suspension growth to 65% and 75% in the absence and presence of S9 mix, respectively. A concentration of 400 µg/ml was too toxic since it killed the cells. In the absence of S9 mix, the duplicated cultures gave increases of 2.3 –fold over the vehicle control mean at 100 µg/ml as shown below. In the presence of S9 mix there was evidence of an increase at the highest concentration of 200 µg/ml. The duplicate cultures gave a mean 1.85-fold increase over the vehicle controls, of which difference is significant by both Dunnett's test and by linear regression for positive trend. The sponsor repeated the tests in the absence and presence of S9 mix. In the absence of S9 mix, rosiglitazone appeared not to be mutagenic, although power of statistics indicated that it was mutagenic in the presence of S9 fraction. The positive agents clearly increased the mutant fraction as expected.

Drug	Dose (µg/ml)	Viable Count (VC)	Clon. Eff. (%)	Suspension Growth	Total Cell Growth	Total Growth (5)	Mutant Count (MC)	Mutant Fraction x 10^{-6*}
DMSO	100	144	72	18.7	13.4	100	28	39
EMS	250	122	61	15.3	9.3	69	218	361
3-MC	2.5	130	65	18.8	12.2	90	33	51
Rosi-	12.5	135	67	16.8	11.5	84	46	68
Glita-	25	121	61	17.2	10.5	78	37	63
Zone	50	130	65	12.0	7.9	58	38	60
	100	116	58	12.4	7.0	53	53	91
	200	138	70	11.9	8.3	62	44	64
	400	NP	-	-	-	-	NP	-

*Calculated by 200x mutant counts(MC) /viable counts(VC). EMS, 3-MC, Con. Eff., and NP stand for ethyl methanesulfonate, 3-methylcholanthrene, cloning efficiency and not plated due to toxicity, respectively.

Conclusion: Rosiglitazone is mutagenic in mouse lymphoma L51178Y cells in the presence of S9 mix, although it was equivocal in the absence of S9 mix.

2). Rosiglitazone: Amess Test (TF-1015)

Methods: The strains of S. typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) and E. coli(WP2 uvrA) were used. 0.1 ml of test compound was added to cultures at 5 concentrations separated by a two-fold interval. A top dose level of 5000 µg/plate was used followed by dose levels at 2500, 1250, 625 and 312.5 µg/plate. 0.1ml of positive control agents(9-aminoacridine at 80 µg/plate with TA 1537, N-Ethyl-N-nitro-N-nitrosoguanidine with WPw urvA, TA100 and TA1535, 2-nitrofluorene, aminoanthracene at 0.5 to 20 µg/plate) were also included.

Results: The revertant colony counts for rosiglitazone obtained in the preliminary toxicity test indicated that the drug was not toxic towards the tester strains. Substantial increases in revertant colony numbers were observed with the concurrent positive control compounds, which demonstrated the sensitivity and validity of assay system. It is concluded that rosiglitazone was not mutagenic in the tested bacterial system.

3). SUMMARY AND CONCLUSION OF GENOTOXIC STUDIES:

The overall genotoxicity potential of rosiglitazone appears to be equivocal since the tests of chromosomal aberration, unscheduled DNA, in vivo mouse micronucleus were all negative, while the incidence of forward mutations at the thymidine kinase locus of mouse lymphoma L5178Y cells was increased by rosiglitazone in triplicate assays in the presence of S-9 mix.

H. CARCINOGENICITY STUDY

1). 2-Year Dietary Carcinogenicity Study in Mice(RSD-100HJN/1)

Methods: Sixty albino CD-1 mice/sex/group were given either control diet or diet containing rosiglitazone at doses of 0.4, 1.5 and 6 mg/kg/day for 2 years.

Selection of Dose and its Justification: The doses were selected based on 13-week mouse study with rosiglitazone at doses of 0.4, 2, 10 and 20 mg/kg/day. The 13-week study indicated that the high dose produced cardiac enlargement (approx. 16%) in males only with a reduction in erythrocyte parameters (by 8%). The AUC ratio was calculated to be approximately 40 at 10 mg/kg/day, based on the maximum anticipated clinical dose of 4 mg/day (Amendment Serial #019 dated 1/6/1995). But, the sponsor reduced the high dose to 6 mg/kg/day, while clinical dose was raised from 4 to 8 mg/kg. The ratio would likely be below 10. Clinical AUC ratio could not be calculated due to unavailability of mouse AUC values.

Furthermore, they did not perform pharmacokinetic and metabolic studies in 2-year carcinogenity study in mice. But, the choice of 6 mg/kg/day as a suitable high dose might be supported, at least in male mice, by the following findings in the carcinogenicity study. There was a trend to reduce survival time with increasing dose in male mice (not in females). Survival was reduced in the high dose males, 70% mortality at week 95 compared with 55% in the controls (Please see table 23), which forced them to terminate the high dose group males at Week 95 instead of Week 104. The high dose mortality was associated with an increase in the incidence of left atrial thrombosis as evidenced by high mortality (23/47 vs. 6/41 controls; please see table 24). In the high dose females, there was a small increase in the incidence of left atrial enlargement (5/60 vs. 1/120 controls) and thrombosis (9/60 vs. 6/120 controls), of which data are not suitable for the justification of dose for female cell.

Results:

Mortality and Clinical Signs: There was a significant($P<0.05$) trend of increasing mortality with increasing drug dose. Early deaths among the high dose males resulted in the sacrifice at Week 95 before other male groups. The high mortality in the high dose group had to do with left atrial thrombosis as shown subsequently. There was an increase in the number of palpable masses in the scapular region of males at all doses and the females given the intermediate and the high doses.

Table 23. Mortality of Mice in 2-Year Carcinogenicity Studies

Sex	Time Period (Weeks)	Rosiglitazone Dose(mg/kg/day) in Mice			
		0	0.4	1.5	6
Males	1-52	8(13%)	7(12%)	7(12%)	5(8%)
	53-95/101*	33(55%)	28(47)	37(62%)	42(70%)
	Total	41(68%)	35(58%)	44(73%)	47(78%)
Females	1-52	4(7%)	5(8*)	3(8%)	5(8%)
	53-105#	28(47%)	26(43*)	31(52%)	30(50%)
	Total	32(53%)	31(52%)	34(57%)	35(58%)

*High dose male were killed at week 95 and all other male killed in week 101. #All female groups were killed at week 105. Initial mice were 60 in all groups.

Body Weight and food Consumption: There were no reductions in the parameters in any groups in both sexes. Actually there were slight(statistically not significant) increases in body weight gain and food consumption in the high dose females.

Macroscopic Observations: Left atrial thrombosis was increased in males of the high dose group as shown below. There was also an increase in the incidence of left atrial enlargement in mice in that group, which was evident in the males.

Table 24. Incidence of Left Atrial Thrombosis in Mice in 2-Year Carcinogenicity Studies

Mice Sex	Rosiglitazone Dose(mg/kg/day) in Mice			
	0	0.4	1.5	6
Males	6/41	0/35	3/44	23/47
Females	0/32	0/31	0/34	4/35

Microscopic Observations: Neoplastic Findings: Hemangiosarcoma was seen in the liver of 4/60 males that received the low dose, but did not occur in mice from other dose groups. Although the incidence was significant (P=0.013) in a pairwise comparison, there was no dose-related trend as shown below.

Table 25. Incidence of Neoplastic Lesions in 2-Year Carcinogenecity Studies in Mice

Tumor	Dose in Males(mg/kg/day)				Dose in Females(mg/kg/day)			
	0	0.4	1.5	6.0	0	0.4	1.5	6.0
Adenoma	5	7	4	-	1	-	-	-
Carcinoma	7	7	5	1	1	-	1	-
Hemangio sarcoma	-	4	-	-	-	-	-	-
Lipoma	1	-	-	-	-	-	-	-

The total number of animals in each group was 60, from which the tissues were examined for tumors.

Non-Neoplastic Findings: There was an increased incidence of left atrial thrombosis in male mice in the high dose group as reported above. There were also increases in the incidences of adipocyte hyperplasia of the scapular fat pad and marrow of the sternum and femur of mice that received the high dose. A marginal increase in the incidence of mixed inflammatory cell infiltrate in the heart was seen in males at the dose as shown below.

Table 26. Cardiac and Hepatic Infiltration in 2-Year Carcinogenicity Study in Mice

Inflamed Tissue	Sex	Rosiglitazone Dose (mg/kg/day) 2-Year Mice Studies			
		0	0.4	1.5	6.0
Cardiac Infiltrate	Males	2	3	1	9
	Females	1	1	4	0
Hepatocellular Vacuolation	Males	0	9	9	5
	Females	0	1	1	0

Comments and Conclusion: The sponsor initially indicated that the clinical ratio would be 25 in mice and rats(Amendment#020 dated Jan. 24, 1995). The dose might be MTD since the thrombus in associated with cardiomegaly in the males might be the limit of the dose in male, although the picture in females was not clear. There were significant high incidence of male mortality in the high dose group. Drug treatment was associated with hyperplasia of the adipocytes of the scapular fat pad of animals in the high dose

group, which was correlated with the increased incidence of palpable masses in that areas. This is consistent with the pharmacology of thiazolidinediones which facilitate the differentiation of preadipocytes and the conversion of free fatty acids to fat. It appears that dietary administration of rosiglitazone to mice at doses of 0.4, 1.5 or 6 mg/kg/day for 95 weeks did not produce a clear increase in the incidence of malignancy or multiplicity of tumors in male mice. The high dose for females was not likely MTD. Thus, one cell in the mouse carcinogenicity studies appeared to be invalid.

2). 2-Year Carcinogenicity Studies in Rats after Oral(Gavage) Administration(RSD-100HJV/2)

Methods: Crl:CD(SD)BR rats(60/sex/group) were given either vehicle (1% methylcellulose) or rosiglitazone(batch# HGC-E03C) orally(gavage) at doses of 0.05, 0.3, or 2 mg/kg/day for 2 years. Toxicokinetic measurements were performed in Weeks 3 and 54, using additional animals(24 rats/sex/group).

Selection of Dose and Dose Justification in Rats: The patterns of circulating metabolites of rosiglitazone in rat, dog and man were comparable (See table 8), although the amounts of the metabolic products recovered in urine or feces in the three species' were quite different. This in combination with the genotoxicity findings makes the criteria of AUC ratio be unsuitable for the justification of the dose selection. Since the clinical dose was increased from 5 to 8 mg/day, the ratio (12 for males, 19 for females) had to be too low.

Animal survival was reduced in high dose males, 77% mortality compared with 58% in the controls, although the opposite picture was clear in case of female rats (Please see table 27). The reduced survival was associated with a higher incidence of cardiovascular related mortality such as hydrothorax, cardiomyopathy and hypertrophy in the high dose male (12/46 vs. 1/70 in controls). In case of female rats, there was a slight increase in the incidence of atrial hypertrophy (10/60 vs. 0/120 in controls). Therefore, the high dose might be the MTD for both sexes since the cardiovascular toxicity was depended upon the drug-concentration and -exposure duration.

Mortality: There was a significant ($P<0.05$) higher mortality among males and a significant($p<0.05$) decrease in mortality in females receiving 2 mg/kg/day, compared to the controls as shown below. Incidence of mortality indicates that there were time-dependent gradual increases in deaths in all groups.

Table 27. Summary of Mortality Data in 2-Year Carcinogenicity Study in Rats

Dose	Rosiglitazone Dose(mg/kg/day) in 2-Year Carcinogenicity Study in Rats							
	Control		0.05		0.3		2.0	
Sex	M	F	M	F	M	F	M	F
Size*	60	60	60	60	60	60	60	60
W1-52@	2	3	2	5	3	2	4	2
W53-104	33	41	27	39	27	43	42	33
W1-104	35	44	29	44	30	45	46	35

* Initial group size was 60 in all groups in both sexes and @ indicate deaths during the week one through week 104.

Clinical Signs: There was an increased incidence of masses noted in the interscapular region, which was more pronounced in females than in males. There were no dose-related clinical signs noted in any group immediately following oral administration of the test substance.

Bodyweight and Food consumption:

Bodyweight gain for treated groups of males was slightly higher up to 14% than the concurrent controls over the 104 weeks of treatment. The parameter for females during the 104 weeks of treatment was similar to the control group. Food consumption over the 104 weeks of treatment was similar in all groups and not affected by treatment.

Toxicokinetics: Plasma drug concentrations increased with increasing drug-dose. Maximum plasma concentrations were reached between 0.5 and 4.0 hours after dosing. In general, females had higher AUC values than males and clinical exposure ratio reflected the effects as shown below. The ratio in males was less than 25.

Table 29. Summary of Toxicokinetic Data in 2-Year Carcinogenicity Studies in Rats

Dose(mg/kg/day)	Sex	Cmax(µg/ml)		AUC_{0-t}(µg.h/ml)		Tmax(hours)		Clinical Ratio*
		Wk 13	Wk 54	Wk 13	Wk 54	Wk 13	Wk 54	
0.05	M	0.165	0.336	0.707	1.84	0.5	4.0	0.61
0.3	M	1.10	1.03	5.49	5.20	1.0	0.5	1.78
2.0	M	10.0	6.70	30.4	35.7	0.5	2.0	11.88
0.05	F	0.210	0.207	1.05	1.68	1.0	2.0	0.56
0.3	F	1.30	1.51	6.36	10.7	0.5	0.5	3.56
2.0	F	9.70	11.2	48.4	58.1	0.5	0.5	19.34

* calculated from AUC values at Week 54 based on human AUC 3(µg.h/ml) following 10 days dose at 8 mg/day.

Cardiac Weights: Group mean heart weight following 104 weeks of treatment was increased in animals treated with the high dose and in males treated with the intermediate dose when compared to controls as shown below.

Table 30. Effects of Rosiglitazone in Cardiac Weights in 2-Year Carcinogenicity in Rats

Sex	Rosiglitazone Dose(mg/kg/day) in 2-Year Carcinogenicity Study in Rats				
	Control 1	Control 2	0.05	0.30	2.00
Males	2.10	2.27	2.21	2.31*	2.53*
Females	1.63	1.52	1.51	1.62	1.95*

Each value expressed in gram and *<0.05.

Macroscopic Pathology: Prominent effects of rosiglitazone are summarized below. Other minor findings were: Incidences of scab and/or alopecia in females were higher than the controls. Adipose tissue(both white and brown) and scapular brown fat pad(hibernating gland) are generally increased in high dose group in both sexes.

Table 31. Rosiglitazone Effects on Pathology in 2-Year Carcinogenicity Study in Rats

Tissues Examined	Dose(mg/kg/day) in 2-Year Carcinogenicity Study in Rats					
	Control		0.3		2.0	
	M	F	M	F	M	F
Thoracic Fluid	4/70*				19/46	
Lung Congestion	4/70	6/87			22/46	11/36
Flaccid Testes	7/60		12/60		21/60	

*indicates incidences at terminal or in decedent out of total animal examined.

Microscopic Pathology: Neoplastic findings

There was a significant increased incidence of lipoma in males at 0.3 mg/kg/day (P=0.001) and in females at the dose(P=0.003) by pair wise comparison as shown below. The lipoma is different from liposarcoma or focal hyperplasia because it was defined as discrete demarcated subcutaneous mass and tumor cells forming lobules. Focal white adipocyte hyperplasia was seen in all treated male groups and in females at in the high dose group. Focal brown adipocyte hyperplasia was also seen in the scapular fat pads in males at the high dose and in females at the intermediate and high dose groups as shown below. Incidences of several common tumors (thyroid C-cell adenoma, fibroma, and pancreatic islet cell adenoma) were reduced at the high dose. There was a single instance of a urinary bladder tumor(transitional cell carcinoma) in the female high dose group.

Table 32. Neoplastic Effects of Rosiglitazone in 2-Year Carcinogenicity Study in Rats

Tissues Incidence	Sex	Dose(mg/kg/day) in 2-Year Carcinogenicity Study in Rats				
		Control 1	Control 2	0.05	0.30	2.00
Subcutaneous Lipomas	M	3/60	4/60	5/59	13/58	6/60
	F	1/60	2/60	3/60	1/59	9/60
Hyperplasia, White	M	0/60	0/60	2/59	4/58	4/60
	F	0/60	0/60	0/60	0/59	3/60
Hyperplasia, Brown	M	0/60	0/60	0/60	1/59	15/60
	F	0/60	0/60	0/59	0/58	3/60

*White and brown indicate focal subcutaneous white and scapular brown adipocyte hyperplasia, respectively.

Non-neoplastic Findings:

In decedent males treated with the high dose, there was an increase in the incidence of fatal cardiopathy (hypertrophy, cardiomyopathy and hydrothorax) as shown below, which might contribute to mortality. There was also an increase in the incidence of and severity of atrial myocyte hypertrophy, with cardiomegaly, and atrial thrombi at the high dose. At the high dose, acute centrilobular hypatocyte necrosis was seen in several decedent males, which was associated with cardiomyopathy and atrial hypertrophy.

Table 33. Incidence of Non-neoplastic Lesions in 2-Year Carcinogenicity Studies in Rats

Tissues Incidence	Sex	Dose(mg/kg/day) in 2-Year Carcinogenicity Study in Rats				
		Control 1	Control 2	0.05	0.30	2.00
Cardiopathy	M	1/33	0/37	0/29	0/30	12/46
	F	0/43	0/44	0/44	0/45	0/35
Atrial Hypertrophy	M	7/60	4/60	6/60	9/60	30/60
	F	0/60	0/60	2/60	2/60	10/60
Atrial Thrombi	M	0/60	0/60	1/60	0/60	5/60
	F	0/60	0/60	0/60	0/60	1/60

3). Comment and Conclusion:

In this study designed to assess the carcinogenicity of rosiglitazone in the rat, the high dose (2 mg/kg/day) was associated with significantly higher(58% in control vs. 78%) mortality in males. The mortality in females was reduced from the control, 73% to 58% in the high dose group. The estimation of clinical exposure ratio(AUC ratio) in males and females was 12 and 19, respectively, which indicate the high dose might be near the MTD since the metabolic profile of this drug appears to be similar in rats and human.

The only tumor showing a statistically significant increase in the treated rats was subcutaneous lipoma in males in males at 0.3 mg/kg/day and females at 2.0 mg/kg/day. The treatment-related increase in lipomas and adipocyte hyperplasia may be considered to be consistent with the drug action, which facilitate the differentiation of pre-adipocytes into adipocytes.

An increased incidence of enlargement of the heart was seen at necropsy in both decedent and terminal rats on the 0.3 and 2.0 mg/kg/day dose groups, which was correlated with increased incidence of atrial hypertrophy. Additionally twelve decedents dosed at 2.0 mg/kg/day showed cardiac effects characterized by macroscopic cardiomegaly and fluid in the thoracic cavity and left atrial thrombi formation, which appeared to be one of important contributing factors of high mortality in the males dosed at the high dose. This incidence and severity of atrial myofiber hypertrophy with cardiomegaly in couple with left atrial thrombi might suggest the long-term toxic potential of this drug in cardiopulmonary system. The severe cardiopulmonary action of this drug appears to be related to the drug exposure duration rather than the dose because there were no clear such effect in the 4-week(doses: 0.4, 2 and 80 mg/kg/day 0 or 6-month (doses: 0.2, 1 and 40 mg/kg/day) toxicological studies. In the 2-year carcinogenicity studies, the systemic exposure of the drug was similar during Weeks 13 and 54 at the dose of 2 mg/kg/day, based on AUC values at the time. But, at terminal examination, cardiopulmonary complications in connection with the formation of thrombi in the left atria was confirmed in 2-year carcinogenicity studies in male mice and rats. This suggests that a chronic exposure of human or animal subjects to the drug could lead to potential cardiac and/or pulmonary complications such as cardiomyopathy, hypertrophy and hydrothorax. Despite the difficulties in drug-dose justification and in interpreting the data are clear, the results support that rosiglitatzone is not likely to be carcinogenic.

I. OVERALL SUMMARY AND CONCLUSIONS

Pharmacology and pharmacodynamic studies indicate that rosiglitazone produced antidiabetic effects by activating peroxisomal proliferator activated receptor gamma (PPARγ). The precise underlying mechanism of the beneficial action of rosiglitazone remains to be elucidated. Toxicological data indicate that the drug produced various toxicities such as left atrial thrombosis in mice, hydrothorax in rats, cardiac hypertrophy and elevation of hepatic enzymes at high dose in dogs.

In mice, toxicology studies for 4, 13 and 52 weeks indicate there was no drug-induced mortality, although there was a reduction in RBC counts (38%) and an increase in heart weight (23%) in high dose groups (Please see Table 19). In the high dose group of the 1-year studies, 14 deaths were noted, which were not drug-induced mortality since 5 mice died during blood sampling, a mouse died of bleeding from eye, and 8 deaths due to natural course. In 26-Week rat toxicity studies, the top dose (40 mg/kg/day) was toxic since the dose killed 58% of the animals(Table 19). The toxicity was also demonstrated by ventricular hypertrophy (Table 10). But, the low and intermediate doses had no significant adverse effects, which suggests they were below LOAEL. An inspection of toxicokinetic data of the studies at Week-22 indicates that the top dose gave AUC ratio over 100, while the AUC ratio in the intermediate dose was under 10(See Table 9). The

dosing intervals were too large to establish the dose-dependent toxicities of rosiglitazone (Please see table 19).

Similar problems are noted in toxicology studies in dogs. The top dose (20 mg/kg/day) in 26-Week studies produced an increase in alanine aminotransferase by 1,000% at week 17 in males (Table 12). The intermediate dose did not significantly increase the parameter as well as alkaline phosphatase. The top dose gave AUC ratio only 23 and 19 in male and female dogs. For one-Year dog studies the sponsor-selected rosiglitazone at doses of 0.05, 0.5 and 5.0 mg/kg/day, which produced toxicologically negligible results (Table 19). That is, low and intermediate doses in most of all experiments in mice, rats and dogs did not produce toxicologically meaningful data. However, a toxicological profile indicating primarily heart and liver target endpoints was established at very high doses of the testing article. It is not possible, however, to determine how close to clinical exposures to predict long-term human toxicological potential of the drug.

The fundamental problems that caused by exceptionally wide dosing interval were multiple. A dose-response relationship in any toxicological action of rosiglitazone could not be established since there was only one point of values derived from the high dose. It is not scientifically logical to extrapolate the current toxicological findings to clinical settings since the margin of safety could not be estimated. At the high dose, rosiglitazone produced various toxicities such as left atrial thrombosis, hydrothorax, cardiohypertrophy and elevations of hepatic enzymes in the high dose group. In this reviewer's opinion it is not possible to anticipate potential human toxicities, based on limited data derived from one high dose in almost of all investigations. Furthermore, it is even more difficult to predict long-term human toxicological potential of the drug with unidentified preclinical toxicities at clinical doses.

For instance, toxicities of rosiglitazone depend upon the exposure duration of the drug according to animal experiments. In 26-Week dog studies, AKP was increased by 77%, 365% and 492% at weeks 5, 19 and 25, respectively in the high dose group male dogs (Please see Table 12). Similarly, ALT was also increased by 222%, 623% and 1240% at Week 5, 12, and 17, respectively. Toxicological profiles of this drug for long-term exposure has not been well documented. Similar concern is in carcinogencity studies, which were performed in mice at doses of 0.4, 1.5 and 6 mg/kg/day. The top dose was approximately 10 times of human multiple, which killed 49% of males (See Table 24). A chronic exposure of rosiglitazone even with a low dose could produce toxic complications with chronic exposure.

The left atrial thrombosis in mouse carcinogencity studies might be consistent with the pulmonary congestion, which was manifest due to during hydrothorax. The top dose might be near MTD for males, but the dose was not MTD for females because of low drug-induced mortality. In rat carcinogencity studies, the top dose was 2 mg/kg/day, which gave 12 and 19 of human multiples in females and males, respectively. The mortality of male rats was increased by the top dose of rosiglitazone, while it reduced it in females. Sex dependent action of rosiglitazone is not clear, although the AUC ratio

and metabolic profile were comparable between sexes. In the studies, there were no remarkable findings except significant increases in lipoma and in incidence of adipose hyperplasia in the mouse. It appears that the doses for both mouse and rat carcinogenicity were too low to evaluate its carcinogenicity potential. The doses used for reproductive studies in rats also appear to be too low.

The facts that low and intermediate doses did not produce toxicologically relevant data casue the reviewer concern since there are too many unknowns. Clean results obtained from the two doses suggest real possibilities of undocumented and unidentified toxicities of rosiglitazone yet to come. One of important principles of pharmacology is to demonstrate dose- and time-dependency of drug toxicities. Therefore, the reviewer requested the sponsor the lowest dose(s) of rosiglitazone at which cardiac hypertrophy, hydrothorax, and/or anemia are produced on September 17, 1996(Please see Attachment #3). Moreover, the various toxicities that were manifest by the top dose of rosiglitazone appear as long-term clinical concern since AUC ratio could not be calculated or too low to draw any toxicologically viable conclusions. Taking all these together, the reviewer has insufficient evidence to predict long-term effects of rosiglitazone in human, based on existing animal toxicological data.

J. RECOMMENDATION: (Letter to the sponsor)

For initial toxicology studies one could choose rosiglitazone dosing-interval in log unit, particularly for in vitro tests. It is necessary, however, to have closer dosing intervals for quantitative determination of toxic profiles of the drug as shown below.

1. Please repeat a 26-Week toxicology study in CD rats, using rosiglitazone at doses of 10, 20 and 40 mg/kg/day including a control group.
2. One-year or 9-month toxicological study in dogs is also needed with rosiglitazone at doses of 10 and 20 mg/kg/day including a control group.
3. In performing the investigations above please pay special attention to multiples of clinical exposure at drug doses that produce atrial thrombosis, cardiac hypertrophy, hepatic enzyme elevation and hydrothorax including standard clinical chemistry readings. At the same time sex dependent metabolism and toxicities would be actively searched.

K. ATTACHMENTS:

1. Original IND Review (IND# [redacted]
2. Amendments dated 11/8/1994, 12/9/1994, 5/17/1995, 6/8/1995, 8/8/1995, 6/26/1996, 7/9/1996, 7/11/1996, 7/17/1996, 9/18/1966, and 11/8/1996.
3. A copy of FDA communication to the sponsor dated 9/19/1996.
4. Labeling Review

L. FINAL RECOMMENDATION:

Pharmacology recommends not to approve rosiglitazone (NDA 21071) for the proposed indication for long-term human use. However, the reviewer is willing to reconsider the status if the recommended studies were conducted properly.

Review Code: AE

/S/ [redacted]

Herman M. Rhee, Ph.D.
Pharmacologist

cc: Original NDA, HFD-510, HFD-345
Ronald Steigerwalt/H. Rhee/J. Weber

See attached memo from Team Leader

/S/ [redacted]

5/6/99

APPEARS THIS WAY ON ORIGINAL

H. Rhee

SEP 18 1996

Communication: 9/17/1996
Submission: 8/29/1996
Re: IND [redacted]

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
INFORMATION AMENDMENT #55

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]

Reference is made to our meeting with you for BRL49653C End of Phase II Evaluation, which took place on July 22, 1996.

We would like to have your responses on the following pharmacologic and toxicologic questions on BRL49653C.

1. Please provide pharmacokinetic, toxicokinetic and metabolic data for BRL49653C in species used for toxicology studies. Particularly identify the species, of which metabolic profile is relevant to human.

2. What was the exposure ratio(AUC) of the animals to human for the lowest doses at which cardiac hypertrophy, hydrothorax, and/or anemia are occurred?

3. What effort is currently directed to search for the understanding of mechanistic action(s) of this drug?

/S/ [redacted]
Herman M. Rhee, Ph. D.
/S/ [redacted]
9/18/96

cc:
Original IND, HFD-510
J. Rhee/R. Steigerwalt/H. Rhee

H. Rhee

Review Date: 1/7/1997
Submission Date: 11/8/1996
Re: IND [redacted]

JAN 29 1997

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
INFORMATION AMENDMENT #060

Sponsor: SmithKline Beecham Pharmaceuticals
King of Prussia, PA 19495 (610) 917-5302

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]

The sponsor provided the following data in response to our request(September 20, 1996).

Species and Sex	Lesion	Lowest Dose (mg/kg/day)	AUC (ug.h/ml)	Exposure Ratio*
Rat(M)	HTX	40	340	211
Rat(F)	HTX	40	680	422
Mouse(F)	CE	10	54	33
	DEP	20	112	70
Rat(M)	CE	5	63	39
	DEP	1	16	10
Dog(M,F)	CE	2	4	2
	DEP	2	4	2

CE, DEP, and HTX stand for cardiac enlargement, decreased erythrocyte parameters and hydrothorax, individually. *Based on human AUC=1.61 after 10-day treatment at 5 mg/day(study#002).

As to the mechanism of BRL49653C action, the sponsor provided on-going research. They are PPARγ mediated 1)Pre-adipocyte differentiation, 2) expression of genes associated with

glucose metabolism such as transporters, and 3) gene expression for lipogenesis. The sponsor had no definitive data on which species exhibits a metabolic profile most similar to man.

Recommendation(Letter to the sponsor):

1. If you select dog for nonrodent-12 month-study, the exposure ratio(animal to man) for high dose selection is recommended to be one order of magnitude greater than human exposure or to exhibit frank toxicity.

2. The sponsor attributed heart weight increases to fluid retention as a result of reduction in renal blood flow and sodium excretion. The reduced renal functions were secondary to increased sympathetic drive and heart rate as a result of the drug-induced hypotension. Have you measured renal catecholamine concentrations or renal sympathetic nerve activity in relevant animals? What is the primary mechanism of BRL49653C-induced peripheral vasodilation?

3. Do the changes in autonomic nervous system have anything to do with BRL49653C-induced clinical symptoms such as dry mouth, gastroesophageal reflex, and myalgia?

4. Please provide AUC ratios of animal to human in 2 year-rodent carcinogenicity study when the data are available.

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

cc:
Original IND, HFD-510
J. Rhee/R. Steigerwalt/H. Rhee

/S/ [redacted] 1/9/97

APPEARS THIS WAY ON ORIGINAL

H. Rhee

IND # [redacted] August 8,1996 AUG 20 1996

Sponsor: SmithKline Beecham Pharmaceuticals
King of Prussia, PA 19495 (610) 917-5302

Submission Date: 03/15/1996
Document Serial No: 044

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]

The sponsor submitted 11 preclinical studies in two volumes. All the studies were conducted at Huntingdon Research Centre, Cambridgeshire, or at SmithKline Beecham Pharmaceuticals, The Frythe, UK in accordance with the principles of the GLP. The main highlights of seven relevant studies are summarized briefly below.

1. A 4-Week Oral Range-Finding Study in Mice(SB Report#TF-1047/BRL-049658/1)

a. Methods: Eight CD-1 mice/sex/group were administered BRL49653C orally at doses of 0.4, 2.0, 10, or 20 mg/kg/day for 28 days and necropsies were performed on days 29 and 30.

b. Results: There were no treatment-related effects on any parameter except water intake which was 20% greater than controls in females given 20 mg/kg/day. Scapular brown adipose tissue weights were greater than controls(36-39%) in both sexes at the high dose. There were no sex-dependent changes in the pharmacokinetic parameters.

2. Decreased Insulin Receptor Phosphotyrosine Phosphatase(PTPase) Activity in Liver and Muscle from

Diabetic (db/db) Mice is NOT Altered by BRL49653(SB Report# PF-1007-BRL-049653/1).

a. Objective: PTPase activities were reduced in genetically-diabetic db/db insulin-resistant mice compared to non-diabetic controls. The purpose of this study was to determine the effects of BRL49653C on PTPase activity in muscle and liver.

b. Methods: PTPase was prepared from the liver and hindlimb muscle of female C57BL/KsJ db/db mice and lean control(+/?). The synthetic peptide(TRDIYETDYYRK) containing 30 mM Hepes, 0.3 mM EDTA, and 50 μM ATP(50 μCi of λ-32-ATP) was used as a substrate for ATPase as described(1).

c. Results: Liver insulin receptor phosphopeptide PTPase activity was reduced in diabetic(db/db) mice. PTPase activity was associated mainly with the particulate fraction. BRL49653 had no effects on PTPase activity as shown below.

Fraction	Mice	Control	BRL49653
Cytosolic	+/?	45.40±1.1	45.09±0.71
Cytosolic	db/db	18.84±1.5*	21.03±2.2*
Particulate	+/?	331.90±6.7	325.40±5.51
Particulate	db/db	212.13±12.2*	208.50±13.1*

*Indicates $P<0.05$, compared to the control(+/?) group.

3. Assessment of the Effects on Locomotor Activity in the Mouse(SB Report#TF-1031/BRL-049653/1)

a. Methods: Ten male ICR CD-1 mice were given BRL49653 orally at doses of 0.0, 0.4, 2.0, or 80.0 mg/kg. Another ten animals were administered 7.5 mg/kg of diazepam orally. The activity of each animal was determined for 1.5h after the administration.

b. Results: BLR49653 had no significant effect on spontaneous locomotor activity when compared to the vehicle, although diazepam caused a marked reduction in locomotor activity.