4. Assessment of Analgesic Activity in the Mouse(SB Report# TF-1030/BRL-049653/1)

a. Methods: Ten male ICR CD-1 mice/group were given BRL49653 orally at doses of 0, 0.4, 2.0 or 80 mg/kg. Another group of mice was given acetylsalicylic acid(100 mg/kg) intraperitoneally for writhing test and the last group was given morphine sulfate(50 mg/kg) orally for tail clip test.

b. Results: BRL49653C had no analgesic effects, although acetylsalicylic acid and morphine produced analgesic activity in the writhing and tail clip test respectively.

5. Assessment of Anticonvulsant Activity in the Mouse(SB Report# TF-1025/BRL-049653/1)

a. Methods: Ten male ICR CD-1 mice/group were given BRL49653 orally at doses of 0, 0.4, 2.0, or 80 mg/kg. Another two groups of animals were given phenobarbital orally as positive control groups. Anticonvulsant activity was assessed using the minimal metrazol test or the supramaximal electroshock test 45 minutes after the drug administration.

b. Results: BRL49653 produced an inhibition of the metrazol-induced tonic extensor reflex at the high dose. But the dose had no effect on the supramaximal electroshock test.

6. Effects of a Single Dose of BRL49653 on Mean Blood Pressure, Heart Rate and Plasma Renin Activity in Conscious, female SD Rats(SB Report# PF-1005/BRL-049653/1)

a. Methods: Four to five female SD rats were implanted arterial cannula a few days before experimentation. Each rat was given a single oral dose of 35.7 mg/kg of BRL49653 and determined mean blood pressure, heart rate and plasma renin activity(PRA) for 4 hours post drug.

b. Results: BRL49653 had no effects on BP or HR. But, it increased PRA at 4 hour after dosing(2.15 vs. 4.02 ng angiotensin I/ml/hr).

7. A long-term Study of the Renal and Metabolic Actions of Dietary Drug Administration to Male Zucker Fatty Rats(SB Report# PF-1006/BRL-049653/1)

a. Methods: Ten male Zucker fatty(fa/fa) rats/group were administered BRL49653 in feed at a dose of 50 μmol/kg for 18 weeks. Age-matched male Zucker lean(Fa/?) rats were used as control. Prior to commencing the experiment, and thereafter at intervals of 3-5 weeks, systolic BP was determined non-invasively from the tail of each rat.

b. Results: BRL49653 inhibited the rise in blood pressure in Zucker fatty rats, although the antihypertensive action has not been established. This drug also reduced fasted plasma insulin, total cholesterol and triglycerides. But, blood glucose and HbA_{1c} level were not affected by the drug.

8. Reference: 1. King, M.J. and Sale, G.J.(1988) Insulin-receptor phosphotyrosyl-protein phosphatases. Biochem J. 256, 893-902.

9. Comments: These 7 preclinical studies do not have direct and specific impacts on regulatory aspect of this drug.

10. Recommendation: N.A.I.

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted] 8/20/96

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

[redacted] APPEARS THIS WAY ON ORIGINAL

Study #	Species/Strain	Duration	Route	Low	Intermediate		High	Ratio	Comments
TF-1037	Mouse CD-1	13w	Diet	0.4	2	10	20	69(F)	no death RBC ↓ (38%)
RSD-100HKY/1	"	1Y	Diet	3			6		no death heart wt ↑ (23%)
	rat CD	4w	oral	.4	2		80		
TF 1023	rat CD	26W	oral	.2	1		40	m 30E F 40E	16%/42% 3m/5f ~~death~~ heart wt ↑ (61%)
RSD-10054M	[illegible] dog	4w					60		left vent. post wall thickness ↑ (55%)
TF-1022	Dog, beagle	26w	oral	.2	2	~~[illegible]~~	20	43X(m) 35X(F)	no death ↓RBC (30%) ↑ plasma [illegible] AKP ↑(5X) ALT(10X) = male
RSD-100 HJW/2	"	1Y		0.06	0.5		5		no death Hemosiderin ↑K%, m ALT ↑70% F

	4 W/C	
ALT	0.5	5
w	61%	83%

oral 13W 9:11:

8 [illegible] 2.97

H. Rhee

AUG 14 1996

OIC

IND # [redacted] July 11,1996

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

Submission Date: 04/21/1995
Document Serial No: 027

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 the following three reproductive toxicology studies in volumes 23 and 24.

1. A STUDY TO INVESTIGATE THE EFFECTS OF ORAL ADMINISTRATION ON THE FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE OF THE MALE RAT(SB Report No. TF-1021/BRL-049653/2)
2. ORAL STUDY OF FEMALE FERTILITY, EARLY EMBRYONIC AND EMBRYO-FOETAL DEVELOPMENT IN THE RAT(SB Report No. TF-1011/BRL-049653/1)
3. AN ASSESSMENT OF ORAL ADMINISTRATION IN RABBITS TO DETERMINE THE MAXIMUM TOLERATED DOSE AND SYSTEMIC EXPOSURE IN NON-PREGNANT FEMALES FOLLOWED BY A PRELIMINARY ASSESSMENT OF THE EFFECTS ON THE COURSE AND OUTCOME OF PREGNANCY(SB Report No. TF-1035/BRL-049653/1)

In addition, this amendment also contains a document which deals with the metabolism of BRL 9653C in male Sprague Dawley rat.

I. A STUDY TO INVESTIGATE THE EFFECTS OF ORAL ADMINISTRATION ON THE FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE OF THE MALE RAT(SB Report No. TF-1021/BRL-049653/2)

A. Methods: BRL 49653C(BatchHGC-E-01C) was administered orally to 26 male rats/group(unspecified strain) at doses of 0, 0.2, 1,0 and 40 mg/kg/day for 10 weeks. After 10 weeks of treatment, 22 males/group were paired on a one-to-one basis with untreated female Sprague Dawley(Crl:CD(SD)Br) rats. All females were killed on day 20 post coitum for examination of their uterine contents. But, males continued on treatment for up to 26 weeks.

B. Results: No deaths occurred at any dose levels. There were no adverse clinical observations during the 10-week pre-pairing treatment period in male rats. At the high dose, there was an increase in mean bodyweight(7%) and food(9%) and water (7 to 18%) intakes compared with control and slight reductions in hemoglobin and platelet counts were noted. The intermediate and low doses were unaffected. There were no effects on the fertility and general reproductive performance of the males and no toxicologically significant changes were observed in the pregnancies of their untreated partners.

C. Conclusion: BRL 49653C produced slight paternal toxicity at the high dose of 40 mg/kg/day but had no effect on male fertility and general reproductive performance in the rat.

II. ORAL STUDY OF FEMALE FERTILITY, EARLY EMBRYONIC AND EMBRYO-FOETAL DEVELOPMENT IN THE RAT(SB Report No. TF-1011/BRL-049653/1)

A. Methods: Twenty six female Sprague Dawley(Crl:CD(SD)BR) rats/group were administered BRL 49653C(Batch #HGC-E-01C) orally at doses of 0.2, 3.0 and 40 mg/kg/day for two weeks before pairing with untreated males. The treatment was continued to Day 17 post coitum(pc) and were necropsied on day 20 pc. The sponsor also performed toxicokinetic studies, using 4 selected animals.

B. Results: There were no deaths. Firm swellings in the scapular fat pads were noted in the majority of high dose females after four weeks of treatment.

At the high dose 5/26 females had periods of extended estrus and 7/26 were not pregnant. The majority of animals lost weight or had a minimal weight gain during the latter third of gestation, which might be due to a marked embryo-fetal loss(Table 1). One half of the high dose females exhibited red/brown vaginal discharge on one or more occasion from day 15 pc. There was also a reduction in the number of corpora lutea, a marked increase in pre- and, in particular, post-implantation loss. Ten out of 15 litters were totally resorbed as shown in table 2.

At 3 and 40 mg/kg/day, there were dose related increases in fetal immaturity and a 20-100% greater placental weight than the concurrent control. There were no effects on the incidence of major malformations. However, a small number of minor visceral anomalies/variants were more common than in the concurrent control. The most significant of these was no development of small renal papillae and kinked ureters. The pharmacokinetic data are summarized(Table 3).

C. Conclusion: At high dose of 40 mg/kg/day BRL49653C affected female reproductive function by distrupting estrous cycles, reducing pregnancy rate and decreasing embryo/fetal viability and growth. At the intermediate dose(3 mg/kg/day), there was a dose-related increase in fetal immaturity and in placental weight and slight changes to the fetal ureters. At that dose, there slight effects on the fetal renal papilla. The low dose(0.2 mg/kg/day)had no effects. The sponsor's interpretation of their data is essentially in agreement with the reviewer's conclusion.

III. AN ASSESSMENT OF ORAL ADMINISTRATION IN RABBITS TO DETERMINE THE MAXIMUM TOLERATED DOSE AND SYSTEMIC EXPOSURE IN NON-PREGNANT FEMALES FOLLOWED BY A PRELIMINARY ASSESSMENT OF THE EFFECTS ON THE COURSE AND OUTCOME OF PREGNANCY(SB Report No. TF-1035/BRL-049653/1)

A. Methods: Four female Zew Zealand White rabbits/group were administered BRL 49653C(Batch HGC-E-01C) orally at doses of 64, 96, or 128 mg/kg/day from Days 6 through 20 post coitus. The sponsor also performed pharmacokinetic studies, using unspecified number of not-pregnant rabbits.

B. Results: There were no deaths. There was a dose-related reduction in food intake at doses of 96 and 128 mg/kg/day of approximately 20 and 32% respectively. There was also a reduction in water intake at the high dose(about 12%). At the high dose there were reductions in fetal weight and increases in pre- and post-implantation losses compared with the historical control. All females were pregnant. External examination of the fetus revealed one minor visceral anamaly (kinked tail) at the high dose.

In the non-pregnant rabbits, the drug was rapidly absorbed and systemically available following administration at 64 mg/kg/day. Steady state appeared to have been achieved by day 6 which is consistent with a terminal phase half-life of 2 hours. It appeared that 128 mg/kg/day was the maximum tolerated dose.

C. Conclusion: The maximum tolerated oral dose of BRL 49653C was 128 mg/kg/day in the non-pregant rabbit. BRL 49653C at a range of doses between 2 and 100 mg/kg/day would be suitable for an embryo-fetal development study in rabbits.

IV. IDENTIFICATION OF METABOLITES AND PRELIMINARY INVESTIGATION OF THE METABOLITE PATTERN OF BRL49653 IN THE MALE SPRAGUE DAWLEY RAT FOLLOWING SINGLE ORAL ADMINISTRATION OF 14C-BRL49653C AT A TARGET DOSE OF 45 MG FREE BASE/KG(SB Report No.: BF-1026/BRL-049653/1)

A. Methods: Sprague Dawley rats were administered 14C-BRL49653C orally a single dose of 45 mg/kg. Collected samples of plasma, urine, bile and feces were analyzed by HPLC-MS and NMR spectroscopy.

B. Results: At least eight metabolites were detected in bile and six in urine. Metabolism involved ring oxidation, N-demethylation, N-dealkylation, sulfation and glucuronidation.

N-demethylated BRL 49653C was observed at low levels in urine, bile and feces. A phenoxyacetic acid formed via N-dealylation was observed as a major urinary metabolite and were observed to significant extents in urine, bile and plasma. Glucuronide metabolites were only observed in bile. BRL 49653C metabolism is summarized(Table 4).

C. Conclusion: Metabolic clearance of BRL49653C in the rat occurred predominantly by oxidation of the pyridinyl ring followed by sulfation or glucuronidation. N-dealkylation, eventually yielding a phenoxyacetic acid metabolite, as well as N-demethylation also occurred to significant extents. No oxidative metabolism of the thiazolidinedione portion of the molecule was observed.

V. Recommendation: NAI

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted] 8/14/95

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

[redacted] APPEARS THIS WAY ON ORIGINAL

TABLE Z

GROUP MEAN BODYWEIGHTS (g) OF PREGNANT FEMALES POST COITUM (F0-F1) (MEAN A)

STUDY NO: RRD128/F0 T94561/49653C/R/PO/FF+EFD(FEMALE) LITTER A

DAY		GROUP 1 CONTROL	GROUP 2 BRL 49653C 0.2 mg/kg	GROUP 3 BRL 49653C 3.0 mg/kg	GROUP 4 BRL 49653C 40 mg/kg
16	MEAN	382.5	375.6	378.4	358.9(^)
	S.D.	32.8	23.5	25.0	26.3
	N	21	20	21	15
17	MEAN	397.8	389.8	391.2	358.7***
	S.D.	34.3	24.2	25.8	31.4
	N	21	20	21	15
18	MEAN	417.3	408.9	407.0	357.3***
	S.D.	37.8	25.6	27.2	33.2
	N	21	20	21	15
20	MEAN	449.4	437.4	438.0	348.7***
	S.D.	39.7	27.8	30.2	41.1
	N	21	20	21	15
GUW	MEAN	83.34	90.74	86.71	22.22***
	S.D.	9.66	13.87	10.87	26.75
	N	21	20	21	14
ADJBW	MEAN	366.0	346.7*	351.3	329.4***
	S.D.	37.4	28.4	27.9	23.1
	N	21	20	21	14

N = Number of animals S.D. = Standard deviation

Data for groups 2, 3, 4 compared with data for group 1

Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test

NT not tested All other values not significant

MEAN A = Mean of all pregnant animals

GUW = Gravid uterus weight (g) ADJBW = Adjusted terminal bodyweight (g)

Table 2

Maternal Performance

Group:	**1**	**2**	**3**	**4**
Treatment:	**Control**		**BRL 49653C**	
mg/kg:		**0.2**	**3.0**	**40**

Criterion	Group 1	Group 2	Group 3	Group 4
Total Number of Females	22	26	26	26
Total Number of Females mated	22	26	26	26
Number allocated to Toxicokinetic Sub-Group (killed on day 13 pc)	0	4	4	4
Number not pregnant	-	0	0	1
Number with viable young	-	4	4	3
Number allocated to day 20 pc necropsy	22	22	22	22
Number not pregnant	1	2	1	7
Number with total resorption	0	0	0	10
Number with viable young	21	20	21	5

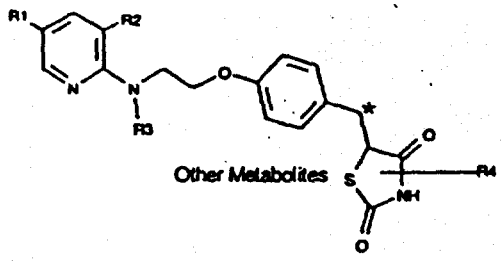
Table 3

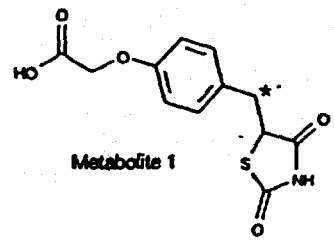
Summary of mean (s.d.) pharmacokinetic parameters for BRL 49653

Dose [mg/kg/day]	Cmax [ug/mL]	Tmax* [h]	AUC(0-t) [ug.h/mL]	T1/2 [h]
0.2	1.88 (0.57)	1.0 (1.0 - 1.0)	11.94 (2.84)	3.90 (0.94)
3.0	15.86 (2.05)	0.50 (0.50- 1.02)	58.50 (15.37)	2.53 (0.25)
40	76.78 (11.67)	1.02 (0.5-1.02)	596.83 (49.49)	2.39 (0.39)

* median [range]

TABLE 4





* Indicates position of the radiolabel

Metabolite No	R1	R2	R3	R4
BRL 49653	H	H	CH_3	-
2 **	H	H	H/Gluc?	Gluc?
3 **	H	O-Gluc	CH_3	-
4	OSO_3H	H	H	-
5	O-Gluc	H	CH_3	-
6	H	OSO_3H	H	-
8 **	H	OSO_3H	CH_3	-
9 **	H	OH	H	-
10	OSO_3H	H	CH_3	-
12	H	H	H	-

** Full structural information could not be obtained for these metabolites. The point of attachment of the glucuronic acid to metabolite 2 is unknown.

H. Rhee

JUL 9 1996

IND # [redacted]

July 9, 1996

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

Submission Date: 03/03/1995
Document Serial No: 025

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 one of genetic toxicology studies: "Chromosome Aberration Study in vitro with Cultured Human Lymphocytes". The other genetic toxicology studies (1.Bacterial mutation assay, 2. Unscheduled DNA synthesis in rat liver using an in vivo/in vitro procedure) were submitted to this division on Sept. 22, 1993 and subsequently reviewed by this division on Oct. 20, 1993.

Chromosome Aberration Study in vitro with Cultured Human Lymphocytes(SB Report No.: TF-1034/BRL-049653/1)

A. Methods: BRL49653C(Batch HGC-E-01C) was assayed for its ability to induce numerical and/or structural chromosome aberrations in cultured human lymphocytes. Based on solubility and toxicity data from a mouse lymphoma assay and a range-finding test, concentrations of BRL 49653C ranging from 60 to 240 μg/ml in the presence or in the absence of S9 were used. Forty-eight hours after initiation of cell division, cultures were exposed to control agents or BRL49653C treatments for 4h, followed by a period of treatment-free growth of 20 and 44h, after which slides were

prepared for analysis. Dimethylsulfoxide was used as the solvent, and the positive control in the presence of S9 was cyclophosphamide(7 μg/ml) and in the absence of S9 was chlorambucil(3 μg/ml). The mitotic indices of all cultures were determined and the lowest concentration of BRL49653C giving over 50% inhibition of mitotic index of the negative control, at 24 and 48h post-treatment with and without S9 was also checked.

B. Results: Mitotic index was inhibited by 50% with the drug at 180 μg/ml with and without S9, at both 24 and 48h post-treatment. All data from the negative and positive controls were within the 99% confidence limits determined from the accumulated historial data. Several, small non-significant increases in total damaged cells and polyploidy were observed at 180 μg/ml BRL49653C-treated cultures in the range-finding test.

Slight, but not statistically significant increases in total damaged cells were observed with 60 and 240 μg/ml BRL49653C in the confirmatory test, with S9. Overall, no increases in the frequencies of cells with numerical or structural chromosome aberrations, which fulfilled the criteria for results to be defined as positive, were observed after treatment with BRL 49653C in the presence or absence of S9.

C. Conclusion: BRL 49653C, in the presence and absence of S9, showed no evidence of induction of chromosomal effects in cultured human lymphocytes, in valid range-finding and confirmatory tests, at concentrations of between 60 and 240 μg/ml.

D. Recommendation: NAI

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

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

/S/ [redacted] 7/9/96

H. Rhee

JUL 10 1996

IND # [redacted] June 26, 1996

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

Submission Date: 10/14/1994
Document Serial No: 014

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 the following two studies: 1) A 26-Week Oral Repeat Dose Study in Rats Followed by a 12-Week Off-Dose Period(SB Report No. TF-1023/BRL-049653/1) and 2) A 26-Week Oral repeat Dose Study in dogs Followed by a 12-Week Off-Dose Period(SB Report No. TF-1022/BRL-049653). Both the studies were conducted in SmithKline Beecham Pharmaceuticals Research and Development, The Frythe, Welwyn, UK, according to GLP regulations.

A. A 26-Week Oral Repeat Dose Study in Rats Followed by a 12-Week Off-Dose Period(SB Report No. TF-1023/BRL-049653/1)

1. Method: Twelve Sprague-Dawley rats(Crl:CD(SD)BR)/sex/group were administered once daily by gavage at doses of 0, 0.2, 1.0, or 40 mg/kg/day for 26 weeks. A further 8 rats/sex in the control and high dose groups were treated for 26 weeks and then retained off-dose for 12 weeks before necropsy in week 39.

2. Results: Five females dosed at 40 mg/kg/day were found dead or were killed due to hydrothorax. Signs of labored breathing, reduced activity and cyanosis were noted prior to death. There was a dose-related increase in scapular brown fat pad weight in both sexes. The brown fat pad progressively reduced in all high dose animals during the off-dose period down to approximately half the size seen after 26 weeks treatment(Table 1).

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Samples Research
Species Relevancy

In the high dose group mean body weights were greater than controls by 11% in males(See table 2) and 9% in females(see table 3), which was accompanied by increased food and water intakes respectively of 10% and 13% in males. Ophthalmological examination revealed a dry or rough appearance to the surface of the cornea with mucoid flecks in the precorneal tear film of 4/18 males and 4/15 females dosed at the high dose in week 24. At the high dose reduction in erythrocyte parameters of about 13% in males and 25% in females was observed, which was reversible in 5 weeks off dose. Mean total protein was higher than control in weeks 9 and 14 (7 to 14%) with associated marginal increases in calcium in the high dose group. Urine output was moderately increased in the high dose females at week 25.

After 26 weeks, there were treatment related changes in several organ weights at the high dose, compared to controls both in males(See table 4 and 5) and females(Tables 6 and 7). Increased relative heart weights of 45% in males and 39% in females, increased liver weights of 15% and pituitary glana weights of 100% in females, a reduction in absolute testes, epididymides, prostate and seminal vesicle weights. In the intermediate and high dose males there was a 10% increase in relative kidney weights, with increases of 15% heart weight in males and 32% in females. Relative liver weights remained 15% higher in high dose females but there were no significant effects observed in the remaining organs with no related morphological changes in any of the organs.

Toxicokinetic data at Week 4 and 22 are summarized(Table 8).

3. Summary and Conclusion: Oral administration of BRL49653 to rats at dose of 0.2 mg/kg/day for 26 weeks practically had no effects. At dose of 1 mg/kg/day BRL49653C increased in fat deposition and morphology without significant adverse effects. High dose of 40 mg/kg/day, however, produced deaths related to hydrothorax, reduced erythrocyte parameters, changes in brown and white fat morphology, and increased heart weights. Most of the drug effects were reversible during the off-dose period.

B. A 26-Week Oral Repeat Dose Study in Dogs Followed by a 12-Week Off-Dose Period(SB Report No. TF-1022/BRL-049653)

1. Method: Twenty pure bred beagle dogs/sex/group were given BRL49653 once daily by capsule at doses of 0, 0.2, 2.0, or 20 mg/kg/day for 26 weeks. The post mortem examinations at the end of the treatment period, or after a 12 weeks off-dose, were performed in weeks 27 and 39 respectively.

2. Results:

a. Mortality: There were no deaths.

b. Body weight: There was a treatment related retardation of bodyweight gain at 2 and 20 mg/kg/day in males and females(Figures 1 and 2). At the end of treatment, group mean bodyweights were reduced by 7% to 8% in high dose animals. During the recovery period body weight gain was increased in high dose animals compared to controls.

c. Food consumption: There were no treatment related findings.

d. Electrocardiography: There were no rhythm or waveform abnormalities.

e. Hematology: No toxicologically significant changes were seen in dogs dosed at 0.2 mg/kg/day. Dogs at 2 mg/kg/day showed a marginal reduction in hemoglobin, packed cell volume, and RBC counts. At 20 mg/kg/day there was a progressive reduction in the parameters, which returned to normal after 7 weeks off-dose period.

f. Blood Chemistry: There were slight to marked increases in alanine aminotransferase in all high dose animals(Tables 9 and 10), all intermediate dose males and two intermediate dose females. The values were returned to normal by the end of the off-dose period.

g. Urinalysis: There were no significant findings.

h. Toxicokinetics: See the summary table 11.

i. Organ weights: The drug increased heart and liver weights at 20 mg/kg/day. Relative heart weights were increased by 44% and 38% in males and females respectively and liver weights by 52% and 27% respectively. The changes in organ weights are shown in tables 12-15. After 12 weeks off-dose period, liver weights were comparable to control while a few animals' hearts were remained increased.

j. Macro/microscopic observations: Fluid accummulation was noted in 3/8 animals from the high dose group. In the heart, there was mild to moderate hypertrophy in the left ventricle diagnosed by macroscopic examination(See table below). The hypertrophy was reversible after 12-week off-dose period.

Dose*	0.0	0.0	0.2	0.2	2.0	2.0	20	20
Sex	m	f	m	f	m	f	m	f
Mild	-	-	-	-	-	1	-	-
Moderate	-	-	-	-	1	-	4	2
Total	-	-	-	-	1	-	4	2

*The drug dose was in mg/kg/day. M & f stand for males and females, respectively.

In the spleen, treatment-related minimal to moderate hemopoiesis occurred in females dosed at 2.0 mg/kg/day and in both sexes treated at 20 mg/kg/day, which was also reversible.

3. Summary and Conclusions: The data indicate that BRL49653C toxicology depends highly on drug dose. At dose of 2 mg/kg/day there were little or no adverse effects. The ventricular myocardial hypertrophy was noted at 20 mg/kg/day, although there was no evidence of cellular changes and cardiac function. Other changes such as hematology, clinical chemistry and thymic atrophy were reversible during drug off-dose period.

C. Recommendation: NAI

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted]

7/10/96

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

Table 1 Incidence of Palpable Masses

Group 4 40 mg/kg/day FEMALES

ANIMAL NO.	TREATMENT PERIOD Week 1st mass noted	No. at week 27	Max. size (mm)/ site	OFF-DOSE PERIOD No. at week 38	Size (mm) /site
149	13	3	25 x 25 SC		
150	8	7	30 x 30 SC		
151	9	5	30 x 30 SC		
152	6	5	35 x 30 SC		
153	18	3	15 x 15 SC		
154	4	#			
155	6	#			
156	4	5	40 x 40 SC		
157	4	#			
158	6	5	42 x 40 SC		
159	*				
160	6	5	30 x 20 SC		
161	6	7	55 x 43 SC	5	30 x 20 SC
162	16	5	15 x 15 HL	2	10 x 10 HL
163	4	5	45 x 45 SC	5	25 x 30 SC
164	6	5	40 x 40 SC	3	12 x 15 SC
165	4	#			
166	6	5	30 x 30 SC	3	8 x 8 SC
167	11	3	20 x 20 SC	2	7 x 7 HL
168	4	#			
MEAN	7.2	4.9		3.2	
(N)	19	14		6	

SC = scapular area HL= Hindlimb

Died or was killed prior to end of treatment

* No masses evident

Table 2

INTER-GROUP COMPARISON OF BODY WEIGHTS (g)

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEKS		GROUP 1 CONTROL	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
	MALES				
21	MEAN	632.0	619.8	644.2	704.4***
	%DIFF	+0	-2	+2	+11
	S.D.	42.0	75.4	72.1	66.1
	N	20	12	11	19
22	MEAN	638.0	624.4	650.9	711.9***
	%DIFF	+0	-2	+2	+12
	S.D.	44.0	74.5	72.6	66.8
	N	20	12	11	19
23	MEAN	646.0	634.9	659.5	724.0***
	%DIFF	+0	-2	+2	+12
	S.D.	46.0	75.0	75.5	69.8
	N	20	12	11	19
24	MEAN	651.9	640.6	668.6	727.7***
	%DIFF	+0	-2	+3	+12
	S.D.	47.0	78.2	78.4	70.1
	N	20	12	11	18
25	MEAN	664.4	647.8	677.9	737.1**
	%DIFF	+0	-2	+2	+11
	S.D.	49.5	76.2	76.8	74.2
	N	20	12	11	18
26	MEAN	655.1	637.7	667.5	729.5**
	%DIFF	+0	-3	+2	+11
	S.D.	53.8	77.8	75.0	74.9
	N	20	12	11	18

Data for groups 2, 3, 4 compared with data for group 1
% differences calculated on unrounded data
Significance levels for t-Test: * P (= 0.05 ** P (= 0.01 *** P (= 0.001 () P) 0.05 for overall F test
NT not tested All other values not significant

Table 3

INTER-GROUP COMPARISON OF BODY WEIGHTS (g)

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEKS		GROUP 1 CONTROL	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
	FEMALES				
21	MEAN	352.0	364.7	359.8	379.9(*)
	%DIFF	+0	+4	+2	+8
	S.D.	31.1	40.4	30.3	37.9
	N	19	12	12	16
22	MEAN	351.4	362.0	360.4	374.8
	%DIFF	+0	+3	+3	+7
	S.D.	31.3	44.6	31.1	39.7
	N	19	12	12	16
23	MEAN	356.2	367.2	367.0	383.0(*)
	%DIFF	+0	+3	+3	+8
	S.D.	30.6	45.1	29.2	44.0
	N	19	12	12	16
24	MEAN	358.5	369.6	367.2	386.3(*)
	%DIFF	+0	+3	+2	+8
	S.D.	30.9	40.3	27.8	44.6
	N	19	12	12	16
25	MEAN	359.7	372.9	367.9	392.7(*)
	%DIFF	+0	+4	+2	+9
	S.D.	30.7	44.2	31.3	43.5
	N	19	12	12	15
26	MEAN	356.9	367.3	363.6	389.7(*)
	%DIFF	+0	+3	+2	+9
	S.D.	31.5	43.9	31.9	45.1
	N	19	12	12	15

Data for groups 2, 3, 4 compared with data for group 1
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

Table 4

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEK 27

PARAMETER			GROUP 1 BRL 49653C	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
MALES						
BODYWT		MEAN	626.2	623.2	652.5	703.4*
		%DIFF	+0	+0	+4	+12
(g)		S.D.	55.79	76.38	74.53	84.27
		N	12	12	11	11
BOTH ADRENAL		MEAN	0.0672	0.0613	0.0619	0.0636
		%DIFF	+0	-9	-8	-5
(g)		S.D.	0.0130	0.0079	0.0110	0.0127
		N	12	12	10	11
BOTH ADRENAL		MEAN	0.0107	0.0099	0.0094	0.0091(*)
		%DIFF	+0	-7	-12	-15
(%)		S.D.	0.0019	0.0014	0.0013	0.0020
		N	12	12	10	11
BRAIN		MEAN	2.411	2.392	2.355	2.372
		%DIFF	+0	-1	-2	-2
(g)		S.D.	0.1120	0.1297	0.0871	0.1057
		N	12	12	11	11
BRAIN		MEAN	0.3872	0.3880	0.3651	0.3405**
		%DIFF	+0	+0	-6	-12
(%)		S.D.	0.0309	0.0421	0.0428	0.0322
		N	12	12	11	11
HEART		MEAN	1.831	1.750	1.810	2.956***
		%DIFF	+0	-4	-1	+61
(g)		S.D.	0.2355	0.1991	0.2605	0.3808
		N	12	12	11	11
HEART		MEAN	0.2918	0.2826	0.2778	0.4231***
		%DIFF	+0	-3	-5	+45
(%)		S.D.	0.0180	0.0309	0.0273	0.0566
		N	12	12	11	11

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

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Table 5

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEK 27

PARAMETER			GROUP 1 BRL 49653C	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
MALES						
BOTH KIDNEYS		MEAN	4.113	3.950	3.854	4.169
		%DIFF	+0	-4	-6	+1
(g)		S.D.	0.4267	0.3600	0.4437	0.4347
		N	12	12	11	11
BOTH KIDNEYS		MEAN	0.6595	0.6371	0.5929**	0.5952**
		%DIFF	+0	-3	-10	-10
(%)		S.D.	0.0683	0.0435	0.0543	0.0457
		N	12	12	11	11
LIVER		MEAN	19.78	19.25	17.72	22.86*
		%DIFF	+0	-3	-10	+16
(g)		S.D.	2.944	2.343	2.948	3.352
		N	12	12	11	11
LIVER		MEAN	3.154	3.102	2.714**	3.253
		%DIFF	+0	-2	-14	+3
(%)		S.D.	0.3076	0.3034	0.3142	0.2832
		N	12	12	11	11
THYMUS		MEAN	0.1947	0.2252	0.2612	0.2386
		%DIFF	+0	+16	+34	+23
(g)		S.D.	0.0771	0.0878	0.1101	0.0672
		N	12	12	11	11
THYMUS		MEAN	0.0307	0.0362	0.0405	0.0342
		%DIFF	+0	+18	+32	+11
(%)		S.D.	0.0099	0.0126	0.0173	0.0089
		N	12	12	11	11
WHITE ADIPOSE		MEAN	11.49	12.64	14.53	11.46
		%DIFF	+0	+10	+26	+0
(g)		S.D.	4.083	4.062	4.880	3.596
		N	12	12	11	11
WHITE ADIPOSE		MEAN	1.805	2.001	2.204	1.627
		%DIFF	+0	+11	+22	-10
(%)		S.D.	0.5281	0.5004	0.6175	0.4295
		N	12	12	11	11

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P (= 0.05 ** P (= 0.01 *** P (= 0.001 () P) 0.05 for overall F test
NT not tested All other values not significant

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Table 6

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEK 27

PARAMETER		GROUP 1 BRL 49653C	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
FEMALES					
BROWN ADIPOSE	MEAN	0.5291	0.7957	0.9560	4.524***
	%DIFF	+0	+50	+81	+755
(g)	S.D.	0.2434	0.3041	0.3690	3.613
	N	11	12	12	9
BROWN ADIPOSE	MEAN	0.1570	0.2199	0.2702	1.232***
	%DIFF	+0	+40	+72	+685
(%)	S.D.	0.0663	0.0664	0.0953	0.9368
	N	11	12	12	9
PITUITARY	MEAN	0.0144	0.0180	0.0188	0.0317***
	%DIFF	+0	+25	+31	+120
(g)	S.D.	0.0028	0.0039	0.0052	0.0096
	N	11	12	12	9
PITUITARY	MEAN	0.0043	0.0052	0.0053	0.0086***
	%DIFF	+0	+20	+23	+100
(%)	S.D.	0.0008	0.0013	0.0015	0.0026
	N	11	12	12	9
BOTH OVARIES	MEAN	0.1150	0.2850	0.0859	0.0838
	%DIFF	+0	+148	-25	-27
(g)	S.D.	0.0408	0.6431	0.0284	0.0298
	N	11	12	12	9
BOTH OVARIES	MEAN	0.0342	0.0828	0.0243	0.0226
	%DIFF	+0	+142	-29	-34
(%)	S.D.	0.0111	0.1901	0.0078	0.0070
	N	11	12	12	9

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

Table 7

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GR0447 T93566/49653C/R/PO/RDS/26W

WEEK 27

PARAMETER			GROUP 1 BRL 49653C	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 1.00 MG/KG	GROUP 4 BRL 49653C 40.0 MG/KG
FEMALES						
BOTH KIDNEYS		MEAN	2.402	2.373	2.438	2.463
		%DIFF	+0	-1	+2	+3
(g)		S.D.	0.1386	0.2092	0.2046	0.1769
		N	11	12	12	9
BOTH KIDNEYS		MEAN	0.7221	0.6738(*)	0.6925	0.6721(*)
		%DIFF	+0	-7	-4	-7
(%)		S.D.	0.0397	0.0575	0.0437	0.0760
		N	11	12	12	9
LIVER		MEAN	10.98	10.82	10.96	13.98***
		%DIFF	+0	-1	+0	+27
(g)		S.D.	1.198	0.8696	1.507	1.368
		N	11	12	12	9
LIVER		MEAN	3.307	3.074	3.098	3.811**
		%DIFF	+0	-7	-6	+15
(%)		S.D.	0.4029	0.2706	0.2466	0.4599
		N	11	12	12	9
THYMUS		MEAN	0.1746	0.1789	0.1933	0.2133
		%DIFF	+0	+2	+11	+22
(g)		S.D.	0.0598	0.0511	0.0537	0.0724
		N	11	12	12	9
THYMUS		MEAN	0.0521	0.0504	0.0549	0.0577
		%DIFF	+0	-3	+5	+11
(%)		S.D.	0.0160	0.0136	0.0145	0.0183
		N	11	12	12	9
WHITE ADIPOSE		MEAN	5.548	9.089	6.989	5.109
		%DIFF	+0	+64	+26	-8
(g)		S.D.	3.478	6.577	2.889	3.800
		N	11	12	12	9
WHITE ADIPOSE		MEAN	1.641	2.426	1.974	1.378
		%DIFF	+0	+48	+20	-16
(%)		S.D.	1.002	1.405	0.7874	0.9940
		N	11	12	12	9

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

Table 8

Summary of mean (s.d.) pharmacokinetic parameters for BRL 49653

		WEEK 4				WEEK 22			
DOSE	SEX	Cmax (ug/mL)	Tmax* (hours)	AUC(0-t) (ug.h/mL)	T1/2 (hours)	Cmax (ug/mL)	Tmax* (hours)	AUC(0-t) (ug.h/mL)	T1/2 (hours)
0.2 mg/kg	MALE	1.32**	0.8**	3.97**	3.4**	1.08	0.5	4.63	3.5**
	(n=3)	(0.25)	(0.5-1.0)	(0.79)	(1.2)	(0.19)	(0.5-1.1)	(0.97)	(1.0)
	FEMALE	1.87	0.5	8.78	3.8	1.26	1.2	8.61	5.5
	(n=3)	(0.50)	(0.5-1.0)	(1.53)	(0.6)	(0.09)	(1.2-1.2)	(1.67)	(1.1)
1 mg/kg	MALE	6.43	0.8	17.95	1.9	4.46	0.5	15.78	2.8
	(n=3)	(1.53)	(0.5-1.0)	(7.59)	(0.3)	(0.39)	(0.5-1.1)	(3.88)	(0.4)
	FEMALE	7.33	1.0	20.38	3.0*	4.88	0.6	22.38	3.6
	(n=3)	(1.73)	(0.5-1.1)	(5.29)	(1.7)	(0.76)	(0.6-1.2)	(5.04)	(0.4)
40 mg/kg	MALE	106.38	1.0	364.77	2.4	83.42	1.2	330.91	2.6
	(n=3)	(28.67)	(0.5-1.0)	(56.54)	(0.4)	(3.44)	(1.1-1.2)	(48.08)	(0.3)
	FEMALE	125.21	1.0	574.35	3.2	107.00	1.2	652.69	3.3
	(n=3)	(2.96)	(1.0-1.0)	(82.37)	(0.9)	(13.62)	(1.2-1.2)	(188.88)	(1.1)

* - Median (range)
** - Mean or median of n=2

Table 9

ANALYTICAL RESULTS INTERGROUP COMPARISON

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

SUREMENT ODE	SEX: MALES WEEKS		GROUP 1 CONTROL	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 2.00 MG/KG	GROUP 4 BRL 49653C 20.0 MG/KG
:T	5	MEAN	42.8	42.8	68.0	95.3**
)/1		SD	23.5	14.9	24.0	27.9
		N	6	4	4	6
	12	MEAN	50.0	43.0	189.5	321.3**
		SD	40.4	16.9	155.0	224.0
		N	6	4	4	6
	17	MEAN	39.3	46.5	138.3	487.5**
		SD	20.9	13.9	103.6	349.3
		N	6	4	4	6
	19	MEAN	44.7	49.8	255.5	400.2**
		SD	18.9	20.5	306.4	196.5
		N	6	4	4	6
	25	MEAN	40.2	50.3	331.5	386.5*
		SD	16.8	17.4	430.9	209.2
		N	6	4	4	6
	32	MEAN	41.5			46.0
		SD	23.3			17.0
		N	2			2
	38	MEAN	41.5			42.5
		SD	19.1			17.7
		N	2			2
T	-1	MEAN	23.4	27.0	28.4	25.7
/1		SD	7.8	5.0	5.8	6.0
		N	12	8	8	12
	5	MEAN	25.3	29.0	38.3	28.8
		SD	4.0	5.2	26.7	5.3
		N	6	4	4	6

: Standard deviation N = Number of animals

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

Table / 0

A N A L Y T I C A L R E S U L T S I N T E R G R O U P C O M P A R I S O N

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

MEASUREMENT CODE	SEX: FEMALES WEEKS		GROUP 1 CONTROL	GROUP 2 BRL 49653C 0.200 MG/KG	GROUP 3 BRL 49653C 2.00 MG/KG	GROUP 4 BRL 49653C 20.0 MG/KG
ALT IU/l	5	MEAN	35.5	38.5	59.0	61.3
		SD	8.3	7.1	36.3	23.6
		N	6	4	4	6
	12	MEAN	36.2	40.0	74.3	117.2**
		SD	7.3	6.3	53.6	67.5
		N	6	4	4	6
	17	MEAN	35.3	43.8	84.0	163.5(*)
		SD	7.9	15.2	62.4	144.9
		N	6	4	4	6
	19	MEAN	35.5	41.8	66.5	160.8**
		SD	8.3	14.4	39.3	98.1
		N	6	4	4	6
	25	MEAN	36.2	47.0	113.0	199.0**
		SD	10.0	18.2	140.1	114.8
		N	6	4	4	6
	32	MEAN	41.0			50.5
		SD	8.5			21.9
		N	2			2
	38	MEAN	38.0			48.5
		SD	17.0			16.3
		N	2			2
AST IU/l	-1	MEAN	25.0	27.3	25.0	22.0
		SD	7.3	6.9	7.1	5.3
		N	12	8	8	12
	5	MEAN	21.8	24.8	22.0	20.8
		SD	5.9	3.7	3.5	5.4
		N	5	4	4	6

SD = Standard deviation N = Number of animals

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

Table 11

Summary of mean (s.d.) pharmacokinetic parameters for BRL 49653

		WEEK 4				WEEK 24			
DOSE	SEX	Cmax (ug/mL)	Tmax* (hours)	AUC(0-t) (ug.h/mL)	T1/2 (hours)	Cmax (ug/mL)	Tmax* (hours)	AUC(0-t) (ug.h/mL)	T1/2 (hours)
0.2 mg/kg	MALE	0.14	2.0	0.28	1.52**	0.19	1.0	0.54	1.75
	(n=3)	(0.03)	(1.0-2.0)	(0.09)	(0.41)	(0.05)	(0.52-2.0)	(0.06)	(0.18)
	FEMALE	0.18	2.0	0.37	ND	0.29	1.0	0.48	1.30
	(n=3)	(0.10)	(2.0-2.0)	(0.23)		(0.26)	(0.5-2.0)	(0.09)	(0.67)
2 mg/kg	MALE	2.92	0.5	4.38	1.26	2.18	1.0	4.14	1.51**
	(n=3)	(0.88)	(0.5-1.0)	(0.66)	(0.67)	(1.45)	(0.5-1.0)	(2.12)	(0.28)
	FEMALE	2.22	1.0	3.55	0.82	3.72	0.5	7.31	1.08
	(n=3)	(1.35)	(0.5-2.0)	(1.74)	(0.22)	(1.57)	(0.5-1.0)	(2.00)	(0.16)
20 mg/kg	MALE	22.83	1.0	40.48	1.57	27.15	1.0	69.90	2.36
	(n=3)	(13.93)	(0.5-1.0)	(21.00)	(0.80)	(4.84)	(1.0-1.0)	(11.74)	(0.46)
	FEMALE	22.06	2.0	47.63	1.26	20.44	1.0	56.59	2.71
	(n=3)	(2.52)	(0.5-2.0)	(5.03)	(0.73)	(10.85)	(1.0-2.0)	(31.60)	(0.31)

* - Median (range)
** - Mean or median of n=2
ND - not determined

SASORGWT-01/06 ORG101-03/01 10/12/93 Table /2

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

WEEK 27

PARAMETER			GROUP 1 CONTROL	GROUP 2 C49653/001 0.200 MG/KG	GROUP 3 C49653/001 2.00 MG/KG	GROUP 4 C49653/001 20.0 MG/KG
MALES						
BODYWT		MEAN	14.18	14.70	13.98	13.30
		%DIFF	+0	+4	-1	-6
(kg)		S.D.	3.355	2.804	0.8958	1.273
		N	4	4	4	4
BOTH ADRENAL		MEAN	1.193	1.217	1.366	1.424(*)
		%DIFF	+0	+2	+14	+19
(g)		S.D.	0.0839	0.1135	0.1458	0.2188
		N	4	4	4	4
BOTH ADRENAL		MEAN	0.0088	0.0085	0.0098	0.0108
		%DIFF	+0	-3	+11	+22
(%)		S.D.	0.0022	0.0018	0.0007	0.0018
		N	4	4	4	4
BRAIN		MEAN	84.98	79.40	83.85	81.38
		%DIFF	+0	-7	-1	-4
(g)		S.D.	7.725	10.17	1.828	3.349
		N	4	4	4	4
BRAIN		MEAN	0.6212	0.5520	0.6017	0.6144
		%DIFF	+0	-11	-3	-1
(%)		S.D.	0.1315	0.0993	0.0391	0.0360
		N	4	4	4	4
HEART		MEAN	105.4	108.8	141.1**	144.5**
		%DIFF	+0	+3	+34	+37
(g)		S.D.	15.59	15.93	8.642	9.409
		N	4	4	4	4
HEART		MEAN	0.7566	0.7498	1.010***	1.090***
		%DIFF	+0	-1	+34	+44
(%)		S.D.	0.0892	0.0968	0.0300	0.0483
		N	4	4	4	4

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

Table 13

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

WEEK 27

PARAMETER		GROUP 1 CONTROL	GROUP 2 C49653/001 0.200 MG/KG	GROUP 3 C49653/001 2.00 MG/KG	GROUP 4 C49653/001 20.0 MG/KG
MALES					
BOTH KIDNEYS	MEAN	56.35	59.18	61.43	61.80
	%DIFF	+0	+5	+9	+10
(g)	S.D.	12.61	8.583	10.89	7.867
	N	4	4	4	4
BOTH KIDNEYS	MEAN	0.3852	0.4060	0.4379	0.4664(°)
	%DIFF	+0	+5	+14	+21
(%)	S.D.	0.0395	0.0263	0.0571	0.0593
	N	4	4	4	4
LIVER	MEAN	346.3	364.5	415.0	501.6***
	%DIFF	+0	+5	+20	+45
(g)	S.D.	54.87	60.96	48.55	22.25
	N	4	4	4	4
LIVER	MEAN	2.492	2.503	2.966*	3.789***
	%DIFF	+0	+0	+19	+52
(%)	S.D.	0.3841	0.2654	0.2227	0.2445
	N	4	4	4	4
BOTH TESTES	MEAN	23.05	22.38	24.64	21.36
	%DIFF	+0	-3	+6	-7
(g)	S.D.	6.396	4.059	1.217	7.405
	N	4	4	4	4
BOTH TESTES	MEAN	0.1631	0.1531	0.1779	0.1580
	%DIFF	+0	-6	+9	-3
(%)	S.D.	0.0224	0.0131	0.0032	0.0406
	N	4	4	4	4
THYMUS	MEAN	8.463	9.445	8.173	6.868
	%DIFF	+0	+12	-3	-19
(g)	S.D.	2.752	3.685	1.462	2.486
	N	4	4	4	4
THYMUS	MEAN	0.0599	0.0673	0.0583	0.0525
	%DIFF	+0	+12	-3	-12
(%)	S.D.	0.0147	0.0301	0.0081	0.0216
	N	4	4	4	4

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

WEEK 27

PARAMETER	FEMALES	GROUP 1 CONTROL	GROUP 2 C49653/001 0.200 MG/KG	GROUP 3 C49653/001 2.00 MG/KG	GROUP 4 C49653/001 20.0 MG/KG
BODYWT	MEAN	12.85	13.35	12.53	11.68
(kg)	%DIFF	+0	+4	-3	-9
	S.D.	2.983	2.395	1.869	1.914
	N	4	4	4	4
BOTH ADRENAL	MEAN	1.381	1.448	1.399	1.668(*)
(g)	%DIFF	+0	+5	+1	+21
	S.D.	0.1468	0.1014	0.1679	0.1682
	N	4	4	4	4
BOTH ADRENAL	MEAN	0.0110	0.0111	0.0112	0.0144*
(%)	%DIFF	+0	+1	+2	+31
	S.D.	0.0019	0.0022	0.0009	0.0014
	N	4	4	4	4
BRAIN	MEAN	79.98	78.10	76.75	77.16
(g)	%DIFF	+0	-2	-4	-4
	S.D.	6.307	4.513	1.977	3.685
	N	4	4	4	4
BRAIN	MEAN	0.6407	0.5966	0.6229	0.6758
(%)	%DIFF	+0	-7	-3	+5
	S.D.	0.1067	0.0889	0.0898	0.1220
	N	4	4	4	4
HEART	MEAN	100.9	106.1	111.8	127.4(*)
(g)	%DIFF	+0	+5	+11	+26
	S.D.	17.34	15.50	19.88	15.30
	N	4	4	4	4
HEART	MEAN	0.7941	0.7993	0.8921*	1.097***
(%)	%DIFF	+0	+1	+12	+38
	S.D.	0.0614	0.0430	0.0759	0.0625
	N	4	4	4	4

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2. 3. 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant

 Table 15

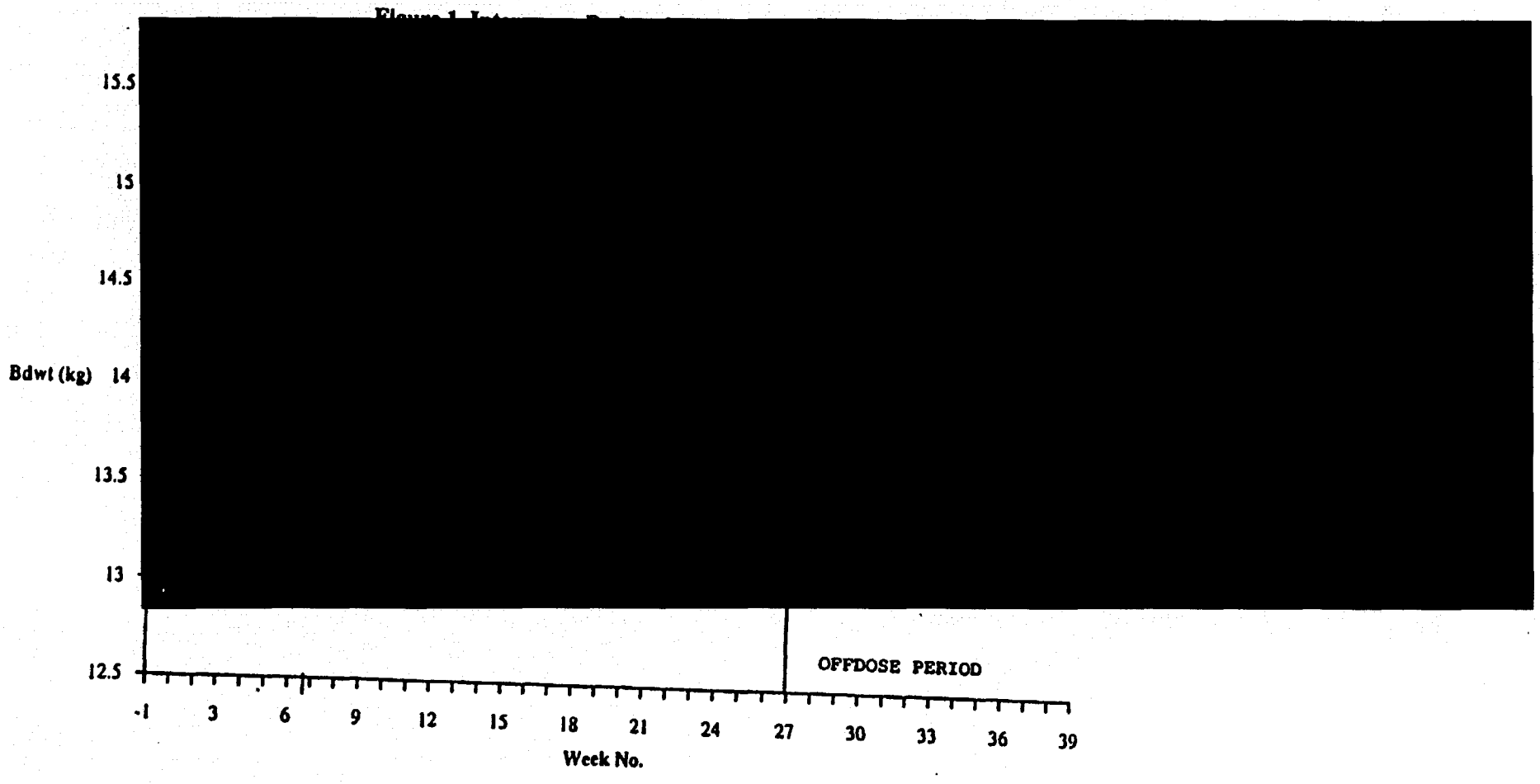
INTER-GROUP COMPARISON OF ABSOLUTE (g) AND RELATIVE (%) ORGAN WEIGHTS

STUDY NO: GD0435 T93567/49653C/D/PO/RDS/26W

WEEK 27

PARAMETER		GROUP 1 CONTROL	GROUP 2 C49653/001 0.200 MG/KG	GROUP 3 C49653/001 2.00 MG/KG	GROUP 4 C49653/001 20.0 MG/KG
FEMALES					
BOTH KIDNEYS	MEAN	52.28	50.33	51.00	51.80
(g)	%DIFF	+0	-4	-2	-1
	S.D.	13.80	10.87	7.139	7.284
	N	4	4	4	4
BOTH KIDNEYS	MEAN	0.4049	0.3766	0.4079	0.4450
(%)	%DIFF	+0	-7	+1	+10
	S.D.	0.0387	0.0321	0.0174	0.0161
	N	4	4	4	4
LIVER	MEAN	347.3	338.3	335.3	390.8
(g)	%DIFF	+0	-3	-3	+13
	S.D.	134.3	78.68	45.08	98.80
	N	4	4	4	4
LIVER	MEAN	2.625	2.544	2.685	3.322(*)
(%)	%DIFF	+0	-3	+2	+27
	S.D.	0.4604	0.4051	0.1778	0.4643
	N	4	4	4	4
BOTH OVARIES	MEAN	1.457	1.097	1.271	1.255
(g)	%DIFF	+0	-25	-13	-14
	S.D.	0.5659	0.1082	0.5061	0.3263
	N	4	4	4	4
BOTH OVARIES	MEAN	0.0113	0.0084	0.0100	0.0108
(%)	%DIFF	+0	-25	-11	-4
	S.D.	0.0031	0.0016	0.0026	0.0020
	N	4	4	4	4
THYMUS	MEAN	8.800	9.478	7.998	8.578
(g)	%DIFF	+0	+8	-9	-3
	S.D.	2.445	2.414	1.938	4.848
	N	4	4	4	4
THYMUS	MEAN	0.0700	0.0713	0.0644	0.0705
(%)	%DIFF	+0	+2	-8	+1
	S.D.	0.0224	0.0157	0.0163	0.0280
	N	4	4	4	4

T-TEST:- GROUP 1 COMPARED WITH GROUPS 2, 3, 4
% differences calculated on unrounded data
Significance levels for t-Test: * P <= 0.05 ** P <= 0.01 *** P <= 0.001 () P > 0.05 for overall F test
NT not tested All other values not significant


15.5
15
14.5
Bdwt (kg) 14
13.5
13
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OFFDOSE PERIOD
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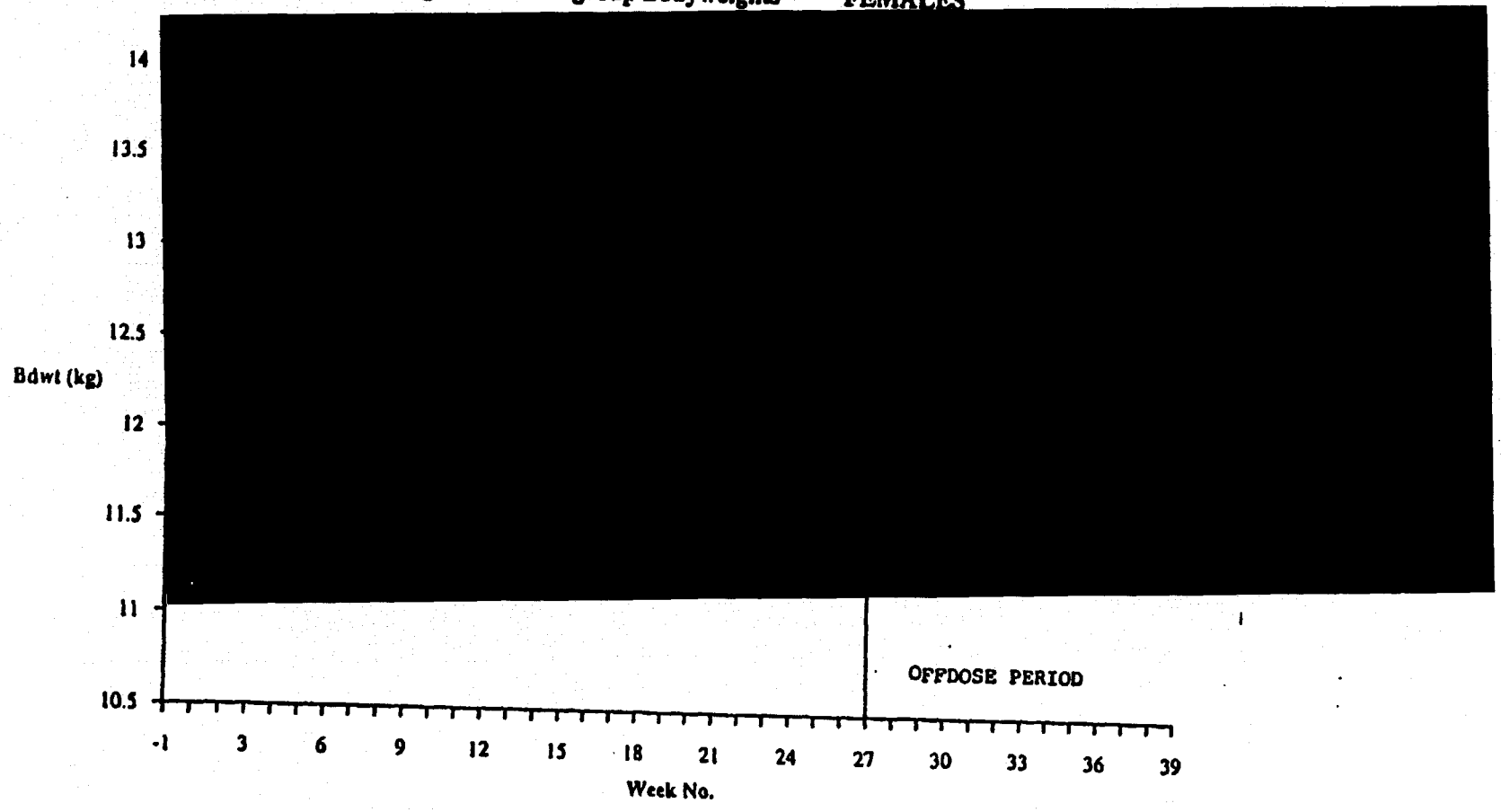
Week No.

Figure 2 Intergroup Bodyweights FEMALES



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IND #[redacted] June 8, 1995 OIC

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

Submission Date: 4/21/1995 JUN 9 1995
Document Serial No: 27

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 deals preclinical pharmacokinetic and reproductive toxicologic studies.

1. SR REPORT#TF-1021/BRL-049653/2: A STUDY TO INVESTIGATE THE EFFECTS OF ORAL ADMINISTRATION ON THE FERTILITY AND GENERAL REPRODUCTIVE PERFORMANCE OF THE MALE RAT

A. Methods: Twenty six male SD(Crl:CD(sd)Br) rats/groups were given BRL49653C (Refer as BRL hereafter) orally at doses of 0, 0.2, 1.0, and 40.0 mg/kg/day for 10 weeks prior to mating. Eight-eight female SD rats were paired on a one-to-one basis with the males for two days. The day on which mating was identified was termed day 0 post coitum. On day 20 post coitum all females were killed for conventional post mortem examination.

B. Results: There were no adverse clinical observations. Animals in the high dose group consumed more food than the animals in the control group(7 to 18%). Intermediate and low dose animals were unaffected by treatment. Terminal examination of females on Day 20 post coitum indicated that there were no toxicologically significant findings. The numbers of corpora lutea and uterine implantation sites, number of live fetuses, sex ratio, and fetal weight were similar in all groups. There were no treatment-related major malformations.

C. BRL 49653C produced slight paternal toxicity at a dose of 40 mg/kg/day but had no effect on male fertility and general reproductive performance in the rat.

2. SB REPORT NO. TF-1039/BRL-049653/1: ORAL STUDY OF FEMALE FERTILITY, EARLY EMBRYONIC AND EMBRYO-FETAL DEVELOPMENT IN THE RAT

A. Methods: Twenty-five virgin female SD(Crl:CD[sd]BR) rats/group were given BRL orally at doses of 0, 0.2, 3.0, and 40 mg/kg for two weeks before pairing with SD male rats. The drug treatment for the females was continued throughout the pairing period and up to and including day 17 post coitum.

B. Results:

1) Mortality and Clinical Signs: There was no mortality and no remarkable clinical signs were observed in the low and intermediate dose groups. Firm swellings in the scapular region were observed in 15/26 high dose females after 4 weeks of treatment, which was associated with larger brown fat pads at post mortem examination. One-half of high dose females had red/brown vaginal discharge on one or more occasions from day 15 post coitum.

2) Food and Water Consumption: There was no clear drug-related changes in the parameters except the fact that food intake was 8% higher than control value at the high dose during the first 8 days of treatment.

3) Effects on Estrus and Mating Performance: Five females out of 26 had periods of extended estrus during treatment and 8 animals out of 26 were not pregnant(Table 1).

4) Necropsy Findings: At the high dose, 14/22 females had prominent brown adipose tissue in the mid scapular region. At this dose 17 animals had vaginal discharge with a high level of post-implantation loss. In the low and intermediate dose groups, there were no remarkable necropsy findings.

5) Litter Data: At day 20 post-coitum, only 15/22 high dose females were pregnant. The corpora lutea of 6/15 pregnant animals could not be counted. At the intermediate dose, no significant changes were noted in the parameters. It appeared that the drug had little effect on sex ratio.

6) Malformations: No clear drug-related major malformations were noted since control as well as high dose groups showed a few malformations such as cleft palate, caudal vertebral agenesis and interventricular septal defects, etc. There were increased incidences of the following observations in the intermediate group compared with the control: no development of renal papilla, small renal papilla, kinked ureter and dilated ureter. In the high dose group, increased incidences of asymmetric ossification of sternebrae and one or more misaligned sternebrae(Table 2).

C. Conclusion: A dose of 40 mg/kg/day BRL affected female reproductive function by disrupting estrous cycles, reducing pregnancy rate and decreasing embryo/fetal viability and growth. At the intermediate dose of 3.0 mg/kg/day, there was a dose-related increase in fetal immaturity and in placental weight and slight changes to the fetal ureters. The low dose of 0.2 mg/kg/day was without effect, of which plasma concentration is expected to be 12 times of clinical doses(2 mg bid).

3. SB REPORT NO. BF-1025/BRL-049653/1: IDENTIFICATION OF METABOLITES AND PRELIMINARY INVESTIGATION OF METABOLITE PATTERN IN THE DOG FOLLOWING A SINGLE ORAL ADMINISTRATION OF [redacted]C-BRL AT A TARGET DOSE LEVEL OF 60 MG FREE BASE/KG

A. Methods: Two male [redacted]eagle dogs were dosed orally with capsules containing [redacted]C-BRL49653C at a dose of 60 mg/kg. Plasma was collected before dosing and at 1.5, 4, 8 and 24 h after. Urine and feces were collected until 144 h after dosing. Selected metabolites were isolated from urine or feces extracts for structural investigation by NMR spectroscopy.

B. Results: The results are summarized in table below and the structural relationship between metabolites is shown(fig. 1).

Metabolite #	Medium from which isolated	Metabolites identification
1	Urine	Carboxylic acid
4	Urine	N-desmethyl-3-O-sulfate
7	Feces	N-desmethyl-3-hydroxy
10	Feces	3-O-Sulfate
11	Feces	5-hydroxyl
12	Feces	N-desmethyl
13	Feces	3-hydroxyl
14	Feces	Unchanged BRL49653

C. Conclusion: Metabolic clearance of BRL49653 in the dog occurred by ring oxidation, N-demethylation, and sulfate conjugation. No metabolic changes occurred on the thiazolidinedione molecule.

4. RECOMMENDATION:

N.A.I.

5. Attachment: Tables 1 and 2, and Fig. 1.

/S/

Herman M. Rhee, Ph. D.

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

/S/

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Table 1

Group Distribution of Oestrous Cycles, Pre-Coital Interval, Mating Performance and Fertility Indices (%) (F0-F1)

Group:	1	2	3	4
Treatment:	Control		BRL 49653C	
mg/kg:		0.2	3.0	40

Distribution of Oestrous Cycles

Criterion	Group 1	Group 2	Group 3	Group 4
Number of Females	22	26	26	26
Baseline Period				
Regular	22/100.0%	26/100.0%	26/100.0%	25/96.2%
Irregular	0/-	0/-	0/-	0/-
Extended Oestrus	0/-	0/-	0/-	1/3.8%+
Treatment Period				
Regular	22/100.0%	24/92.3%	23/88.5%	17/65.4%
Irregular	0/-	0/-	0/-	2/7.7%
Acyclic	0/-	2/7.7%	3/11.5%	2/7.7%
Extended Oestrus	0/-	0/-	0/-	5/19.2%

+ = Animal also had irregular cycle

Distribution of Pre-Coital Interval

	Group 1		Group 2		Group 3		Group 4	
Number examined	22		26		26		26	
Oestrous cycle type during treatment	NO.	PCI	NO.	PCI	NO.	PCI	NO.	PCI
Regular	22	1-5	24	1-4	23	1-4	17	1-4
Irregular	0	-	0	-	0	-	2	1
Acyclic	0	-	2	14-18	3	13-19	2	12
Extended Oestrus	0	-	0	-	0	-	5	1-8

PCI = Pre-coital Interval Range (days)

Mating Performance and Fertility Indices

Criterion	Group 1	Group 2	Group 3	Group 4
Number of Females	22	26	26	25#
Number Mated	22	26	26	25#
Number Pregnant	21	24	25	18
Mated (%)	100.0	100.0	100.0	100.0
Fertility/Conception index (%)	95.5	92.3	96.2	72.0

= Excludes one female (number 82) which had a non-patent cervix

BEST POSSIBLE

TABLE 2

GROUP MEAN INCIDENCE OF MINOR SKELETAL ANOMALIES/VARIANTS (F0-F1)

GROUP	1		3		4	
TREATMENT	CONTROL		BRL 49653C		BRL 49653C	
mg/kg			3.0		40	
NUMBER EXAMINED	160		164		15	
NUMBER AFFECTED	NO.	%+	NO.	%+	NO.	%+
SKELETAL ANOMALIES/VARIANTS	120	74.1	120	72.7	13	95.6
DESCRIPTION	NO.	%+	NO.	%+	NO.	%+
SACRAL ARCHES						
No ossification of one or more arch(es)	2	1.2	3	1.8	0	-
Reduced ossification of one or more arch(es)	31	18.4	14	9.1	1	20.0
CAUDAL CENTRA						
Fewer than 3	4	2.6	13	7.7	2	40.0
VERTEBRAL COLUMN						
Pelvic shift - 27 presacral vertebrae	1	0.6	0	-	0	-
STERNEBRAE 1 - 4						
Bipartite 1st	0	-	1	0.7	0	-
Hemicentric 2nd	0	-	1	0.6	0	-
Asymmetric ossification of one or more	44	26.7	53	32.4	7	60.0
Irregular ossification of one or more	3	1.8	0	-	0	-
One or more misaligned	25	16.0	28	17.1	3	46.7
No ossification of one or more	7	4.5	2	1.3	1	2.2
Reduced ossification of one or more	1	0.6	7	4.2	0	-
One or more small	12	7.5	19	11.1	5	51.1
One or more semibipartite	2	1.1	13	7.3	3	28.9
One or more misshapen	1	0.6	4	2.4	0	-
Fewer than 4 sternebrae fully ossified$^{\alpha}$	56	34.8	80	48.1	10	88.9
RIBS						
Cervical rib	0	-	1	0.6	0	-
13th rib wavy	1	0.7	0	-	0	-
Rudimentary/short 14th rib	18	11.4	19	11.3	0	-
Unilateral/bilateral 14 ribs	18	11.4	20	12.0	0	-

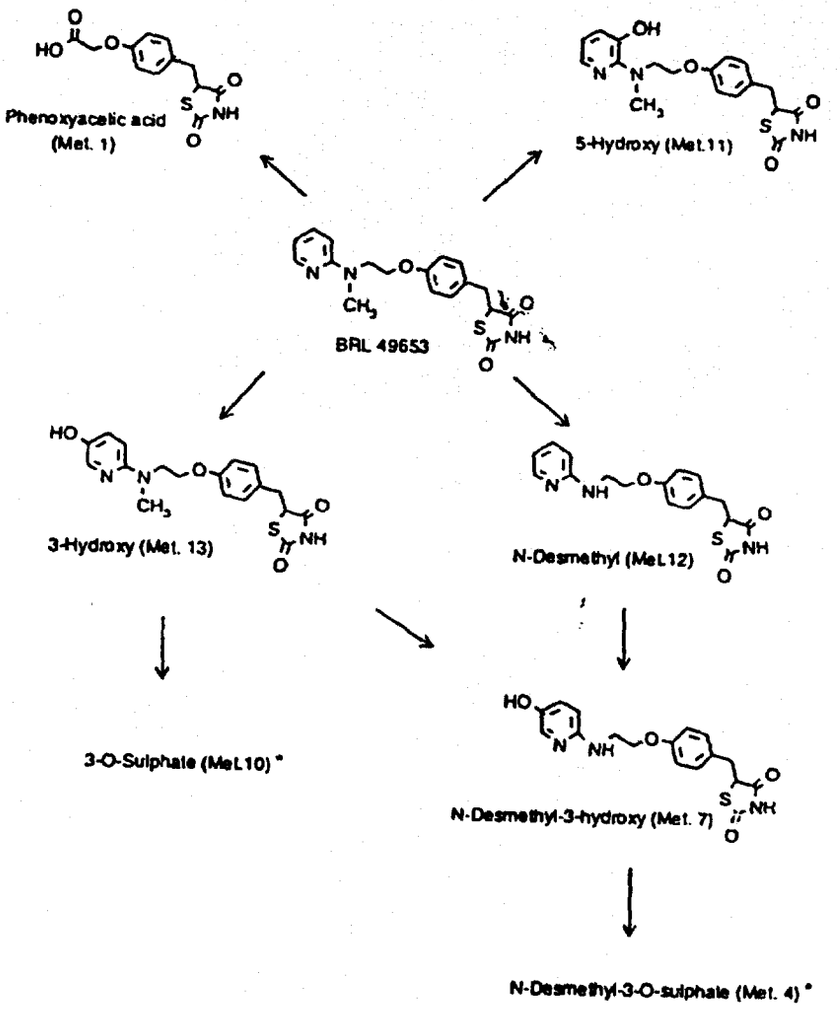
+ = Group Mean per Skeletally Examined foetuses

A foetus may appear in more than one category

α = Not considered an anomaly

Skeletal examination restricted to groups 1,3 and 4

Figure 1 Proposed metabolic pathways for BRL 49653 in the dog (asterisks indicate metabolites not isolated in this study).



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IND # [redacted] May 17, 1995

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

Submission Date: 3/3/1995
Document Serial No: 25

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 chemistry, clinical pharmacokinetic, and preclinical pharm/tox studies. Most of the amendment deals with chemical methods of [redacted] resolution.

1. SB REPORT NO. TF-1034/BRL-049653/L: CHROMOSOME ABERRATION STUDY IN VITRO WITH CULTURED HUMAN LYMPHOCYTES

A. Methods: BRL 49653C(Batch HGC-E-01C) was used to induce numerical and/or structural chromosome aberrations in vitro. Human lymphocytes were cultured for 48 hours after initiation of cell division, which were exposed to control agents or BRL49653c treatments for 4 hours. The concentrations of drug were 20, 36, 60, 108, 324 and 540 μg/ml for range finding studies and confirmatory test. DMSO was used as the solvent and negative control. The positive control in the presence of S9 was cyclophosphamide(7 μg/ml) and in the absence of S9 was chlorambucil(3 μg/ml).

B. Results and Conclusion: BRL 49653C treatment increased total damaged cells after 24 and 48 hours post-treatment, which was not significant. Higher values for polyploidy(4.7-fold the control, in the presence of S9 and 3.6 fold the control, in the absence of S9) were observed in the 24h post-treatment sample. There were no increases in the frequencies of cells with either numerical or structural chromosome aberrations which fulfilled the criteria for results to be defined as positive after BRL49653C treatment in the presence or absence of S9. The drug did not cause induction of chromosomal effects in cultured human lymphocytes.

2. SB REPORT NO. TF-1036/BRL-049653/1: MEASUREMENT OF THE EXTENT OF COVALENT BINDING TO HUMAN SERUM ALBUMIN IN VITRO

^{14}C-BRL 49653C(Batch no. 35432-67/2) in concentrations of 0.25 and 2.5 µg/ml was incubated with human serum albumin at 37°C for 6 or 24 hours. Portions of the solutions(15 ml) were dialyzed using Spectrapor membrane for a period of 24 hours against 200 volumes of fresh phosphate-buffered saline(PBS). Radioactivity in the medium and in the sample was determined by liquid scintillation analysis. The amounts of radioactivity remaining associated with the protein in the samples after trichloroacetic acid precipitation were considered bound fraction.

Results: At initial drug concentrations of 0.25 and 2.5 µg/ml, the mean proportion of drug irreversibly protein bound was 6.68% and 4.58% respectively after 6 hours' incubation, and 4.97% and 6.76% respectively after 24 hours' incubation.

3. SB REPORT NO. BF-1027/BRL-049653/1: QUANTITATIVE WHOLE-BODY AUTORADIOGRAPHY FOLLOWING ORAL ADMINISTRATION TO MALE PIGMENTED RAT

A. Methods: A group of 6 rats was dosed once orally with radioactive BRL. The animals were killed at 1, 4 and 24 h, 3, 10 and 35 day post-dose and rapidly frozen in a mixture of dry-ice/hexane. The frozen carcasses were prepared for whole-body autoradiographic procedures, using a validated image analysis system.

B. Results: BRL was rapidly absorbed and distributed into tissues. Highest concentrations of radioactivity in most tissues were observed at 1 h post-dose. Thereafter, concentrations of radioactivity declined and the majority of tissues had no quantifiable levels of radioactivity. Terminal half-lives of radioactivity in melanin containing tissues were significantly longer than most of other tissues.

4. SB REPORT NO. BF-1019/BRL-049653/1: EFFECT OF BRL 49653 ON THE HEPATIC LEVELS OF CYTOCHROME P450 AND RELATED PARAMETERS IN SD RATS AFTER ORAL ADMINISTRATION OF BRL AT 0, 0.4, 2 AND 80 MG/KG/DAY FOR 14 DAYS

A. Methods: Three male and three female rats were given BRL orally at doses of 0.4, 2, and 80 mg/kg/day for 14 days. Liver microsomes were prepared by centrifugation and cytochrome P450 levels and activities were measured. Ethoxyresorufin O-deethylase was used as a marker of CYP1A1, testosterone 16ß-hydroxylase for CYP2B, p-nitrophenol hydroxylase for CYP2E, testosterone 6ß-hydroxylase for CYP3A and lauric acid 12-hydroxylase for CYP4A.

B. Results: BRL caused no significant changes in total CYP450, CYP2B, CYP2E or CYP1A1 activities. But, at the high dose, CYP4A was increased 3.9 fold in males and 1.2 fold in females. CYP3A activity was also increased 2.9 fold in male animals.

5. SB REPORT NO. BF-1020/BRL-049653/1: PLASMA PHARMACOKINETICS OF BRL 49653 OVER A 24-HOUR PERIOD IN THE RAT FOLLOWING DIETARY ADMINISTRATION OF BRL FOR 14 DAYS

Four male rats/group were given BRL orally in the diet at doses of 0, 2, 5, and 10 mg/kg/day for 14 days. Blood samples were taken from 3 animals in each BRL treated dose group at various times during the 24 hours, which were analyzed for BRL by [redacted] detection.

Results are summarized below. [redacted] BEST POSSIBLE

Dose	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-24} (µg.h.ml)
2 mg/kg/day	1.36±0.21	8.1	21.9± 2.5
5 mg/kg/day	3.05±0.37	12.2	52.3± 8.1
10 mg/kg/day	5.27±1.08	12.2	84.7±29.8

This study was also performed in mice for comparison purpose under similar experimental conditions, of which results are summarized below.

2 mg/kg/day	0.53	4.03	6.8
5 mg/kg/day	1.24	4.14	18.7
10 mg/kg/day	2.84	4.26	44.1

6. SB REPORT #BF-1010/BRL-049653/1: THE PHARMACOKINETICS AND INTERCONVERSION OF SB206846 AND SB210232, THE ENANTIOMERS OF BRL 49653, AFTER ORAL ADMINISTRATION OF THE RACEMATE AND EACH OF THE ENANTIOMERS TO THE DOG AT TARGET DOSE LEVELS OF 0.4 AND 5.0 MG/KG

A. Methods: Two male beagle dogs were given BRL racemate and two enantiomers orally at a dose level of either 0.4 or 5.0 mg/kg. Serial blood samples were taken from the animals up to 8 hours after dosing and enantiomeric ratio was determined by a [redacted] with [redacted]

B. Results: Absorption of BRL and the two enantiomers was similar, with the C_{max} of the dosed entity being observed

within 3 hours in all cases. Pharmacokinetic parameters for the compounds were similar and both C_{max} and AUC for BRL increased approximately proportionately with increasing dose between 0.4 and 5.0 mg/kg. Interconversion was seen to occur and to favor SB206846[S(-)] and the apparent terminal phase half-lives were independent of dose level for BRL and the two enantiomers.

7. RECOMMENDATION:

N.A.I.

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted]

5/23

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

A. Rhee,
FEB 6 1995

IND # [redacted] January 30, 1995

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

Submission Date: 1/6/~~1995~~
Document Serial Nos: 019 and 020 Carcinogenicity

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 the draft two protocols for the mouse and rat carcinogenicity studies.

I. PROTOCOL FOR THE RATS

BRL 49653C: A Study of the tumorigenic Potential in Rats(Crl:CD(SD) BR) Following Prolonged Administration by Gavage (Study Code G95500/49653C/R/PO/CARC)

A. Introduction

This protocol details a 2-year carcinogenicity study in the rat using daily administration of BRL49653C by gavage. The study will be conducted within the Toxicology and Drug Metabolism and Pharmacokinetics Dept., UK SK Beecham Pharmaceuticals R & D, The Frythe, Hertfordshire.

B. Route of Administration

Oral administration by gavage.

C. Selection of Doses

The drug has been given to rats(Crl:CD(SD)BR) for 26 weeks at dose of 0.2, 1.0 and 40 mg/kg/day. Five out of 20 rats either died at the high dose or killed between weeks 16 and 24 because of fluid accumulation in the thoracic cavity. The high dose produced weight gain, reduced RBC(by 6-10% males, 10-20% females) and increases in heart(both sexes by 40%), liver(females by 15%) and pituitary(100% in females) weights.

There was a dose-related increase in scapular brown adipose tissue weight at all doses(417% in males and 685% in females at 40 mg/kg/day). Histological changes in adipocytes were present in rats given 1 mg/kg/day, but these effects were not observed at 0.2 mg/kg/day.

Toxicokinetic data after 4 and 26 weeks' treatment in the rat has shown that systemic exposure to the drug was dose proportional at doses up to 2 mg/kg/day in terms of AUC and C_{max}. In the Phase II clinical trials the maximum dose to be administered will be 2 mg bid, equivalent to a total dose of approximately 0.08 mg/kg/day. Comparison of the PK data shows that a daily dose of 2 mg/kg in the rat will provide a 33 fold multiple of plasma AUC over the maximum clinical dose.

The doses selected for this study are 0.05, 0.2, 1.0, and 2 mg/kg/day(Table 1).

D. Rationale for use of diet restriction

Restriction of food intake delays the onset of most spontaneous tumors and extends the lifespan of rodents. Thus, food intake will be restricted to about 70% of ad libitum intake, i.e. males will be fed 21 g/day and females 16 g/day.

E. Study methods

Conventional hematologic and histopathologic methods will be used.

II. BRL 49953C: A Study of the Tumorigenic Potential in the Mouse of its Prolonged Administration in the Diet(Study Code: G95501/49653C/M/DI/CARC)

A. Introduction

This protocol details a 2-year carcinogenicity study in the mouse(Crl:CD-1), using daily administration of BRL 49653C in the diet. The study will be conducted in the Toxicology and Drug Metabolism and Pharmacokinetics Dept., UK, SK Beecham Pharmaceuticals R&D, The Frythe, Hertfordshire.

B. Selection of doses and route of administration

Mice(Crl:CD-1) took the drug for 13 weeks in the diet at dose of 0.4, 2, 10 and 20 mg/kg/day. Hematologic parameters were reduced at the highest dose(8% of controls) along with slight reductions in reticulocyte and platelet counts. At 10 and 20 mg/kg/day, firm and palpable subcutaneous swellings

were due to hyperplasia of the adipocytes. There was a dose-related increase in scapular adipose tissue weight in females at 2 mg/kg/day. In males, there was a slight increase (approx. 10%) in kidney weights at 2 mg/kg/day and above. An increase of up to 16% in heart weights at 10 and 20 mg/kg/day was noted. Ovary weights were increased by 97% in high dose females due to a greater number of cystic ovaries compared to controls. There were no treatment related effects on body weight, food, or water consumption.

The severity of the effects present after 13 weeks of BRL in the diet at 20 mg/kg/day suggests that this dose would not be tolerated for prolonged treatment. At the dose of 2 mg/kg/day effects on fat morphology were seen in 3/8 females and extramedullary hemopoiesis in 1/8 males. Extraplolating from the 10 mg/kg dose suggests that 6 mg/kg will give a ratio of mouse to human AUC of 27.0(See table 1).

III. SUMMARY, COMMENTS, AND CONCLUSIONS

In the original IND, the sponsor indicated that the maximum clinical dose would be 5 mg/day. However, in the amendments 019 and 020, the sponsor calculated Rodent:Human AUC ratio, based on the experiment that the maximum dose was 4 mg/day. The sponsor assured to this reviewer that the dose(4 mg/day) will be the maximum human dose.

Thus, it appears that 2 mg/day gives an exposure ratio greater than 25 in rats. For mice, the sponsor revised its previous calculation and increased the dose from 5 mg/day to 6 mg/day, which gives an AUC ratio greater than 25.

IV. ATTACHMENT

Table 1.

V. RECOMMENDATION

The sponsor's doses are acceptable, which will be communicated by telephone.

/S/ [redacted]

Herman M. Rhee, Ph. D.

/S/ [redacted]

2/6

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

TABLE 1. Calculation of AUC Ratios, Rodent:Man

Rodent Toxicity Studies			Clinical Study 002	AUC Ratio
	Dose (mg/kg/day)	AUC (0-24)	AUC (0-inf) (1)	Rodent:Man
Mouse, M+F (2) [Week 8 data from 13 week dietary study]	0.4	1360	978	1.4
	2	8490	978	8.7
	6 (3)	26520	978	27.0
	10	44200	978	45.0
Rat, Males (4) [Day 24 data from 28-day gavage study]	2	32600 (5)	978	33.0
Rat, Males (4) [Week 4 data from 26 week gavage study]	0.05 (6)	993	978	1.0
	0.2	3970	978	4.1
	1	17950	978	18.4

(1) Calculated to approximate exposure at highest (4 mg/day) dose to be evaulated in efficacy trials: = 2 x mean AUC measured at 2mg dose on day 10, males and females combined.

(2) Mouse: Combined data for males and females used since no significant sex-difference was seen in pharmacokinetics.

(3) Calculated value: = 0.6 x mean AUC measured at 10 mg/kg.

(4) Rat: Mean AUC data from males only used since exposure generally lower in males--this provides *most conservative* AUC comparison to man.

(5) Mean value calculated from individual datapoints (*Median* AUC value was contained in original report).

(6) Calculated value: = 0.25 x mean AUC measured at 0.2 mg/kg.