3. TOXICOLOGY

Unless specified in the study, for all toxicological studies, the formulation of SR 47436 for oral administration was a suspension in a 10% aqueous solution of gum arabic, administered in a volume of 5-10 ml/kg.

3.1. *Acute Toxicity Studies*

3.1.1. Acute Oral Toxicity Study of SR 47436 in Rats and Mice (Report #RS0006920204/02, Study #TXA074). Vol. 23

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between November 19 and December 5, 1990.

Male and female Sprague-Dawley CD (SD) BR rats (from Charles River) were approximately 5 weeks of age and weighed 105-140 g (males) or 113-133 g (females) at the start of the study. The mice (Swiss OF1, from Charles River) were approximately 5 weeks of age and weighed 25-27 g (males) or 19-22 g (females) at the start of the study. SR 4746 (batch 90.00.5) was adminstered as a single dose by gavage. The animals were fasted before treatment, food being withdrawn 18 hr before treatment for the rats and 6 hr before treatment for the mice. It was redistributed about 4 hr after gavage for the rats and about 2 hr after gavage for the mice. For both species, groups of 5 animals per sex received 2000 mg SR 47436/kg or vehicle.

All animals were frequently observed for mortality and drug effects for 14 days following treatment. The body weights were recorded on days 1, 3, 8 and 15. A full necropsy was performed on all animals at the end of the observation period.

There were no deaths in the vehicle control or the drug treated groups of rats and mice. No overt signs of toxicity were noted for rats given 2000 mg/kg SR 47436. However, for mice, 1.5 hr after administration, piloerection was observed in all of the SR 47436 treated animals and somnolence was noted for 2/5 SR 47436 treated males and 3/5 SR 47436 treated females. These signs were noted on day 1 only. The body weight gain of treated animals was not modified by the treatment. At necropsy, no macroscopic findings attributed to SR 47436 administration were noted.

3.1.2. Acute Oral Toxicity Study of SR 47436/HCTZ in Mice (Report #RS0042960523/01, Study #93021) and Male Rats (Report #RS0042960730/01, Study #95047) Vol. 13

This GLP study was conducted by the Departments of Toxicology and Pathology, Pharmaceutical Research Institute, Bristol-Myers Squibb, New Brunswick, NJ between July 26 and August 10, 1993 in case of mice, and between September 18 and October 2, 1995 in case of rats.

The mice (CD-1 outbred albino, from Charles River) were approximately 4 weeks of age and weighed 19.3-22.1 g (males) or 16.5-19.8 g (females) at the start of the study. Male Harlan Sprague-Dawley (SD) BR rats (from Charles River) were approximately 6 weeks of age and weighed 134-169 g at the start of the study. SR 47436 (batches 93-04 and 93-06 for mice and rats, respectively), hydrochlorothiazide (HCTZ, batch 39610 and G297G for mice and rats, respectively), and/or the vehicle were each administered as oral doses (two treatments per animal on the same day) by gavage to groups of 5 male and 5 female mice (Table 3.1.2.1) or 5 rats (Table 3.1.2.2).

TABLE 3.1.2.1
SR 47436/ HCTZ: SINGLE DOSE ORAL TOXICITY STUDY IN MICE. STUDY DESIGN

Group number	Dose (mg/kg)		Dose (ml/kg)			Number of mice
	SR 47436	HCTZ	SR 47436	HCTZ	CMC, vehicle	
1	500	4000	5	20	-	5M, 5F
2	1000	4000	10	20	-	5M, 5F
3	2000	4000	20	20	-	5M, 5F
4	500	0	5	-	20	5M, 5F
5	1000	0	10	-	20	5M, 5F
6	2000	0	20	-	20	5M, 5F
7	0	4000	-	20	20	5M, 5F

Mice and not rats were fasted from approximately 1 hr before to 1 hr after treatment. All animals were frequently observed for mortality and drug effects on the day of dosing and twice daily during the 14 day observation period. The body weights were recorded before dosing and on days 2, 4, 7, 10 and 14 in mice, and 3, 5, 8, 11 and 15 in rats. A full necropsy was performed on all animals at the end of the observation period.

TABLE 3.1.2.2
SR 47436/ HCTZ: SINGLE DOSE ORAL TOXICITY STUDY IN RATS. STUDY DESIGN

Group number	Dose (mg/kg)		Dose (ml/kg)		
	SR 47436	HCTZ	SR 47436	HCTZ	1%CMC,Vehicle
1	750	62.5	7.5	1.25	-
2	1500	125	15	2.5	-
3	3000	250	30	5	-
4	750	125	7.5	2.5	-
5	1500	250	15	5	-
6	3000	500	30	10	-
7	750	0	7.5	-	2.5
8	1500	0	15	-	5
9	3000	0	30	-	10

There were no deaths or overt signs of toxicity in any of the groups of rats or mice. The body weight gain was not modified by the treatment in both species. At necropsy, no macroscopic or microscopic findings attributed to SR 47436/HCTZ administration were noted. It is concluded that there are no substantive toxicologic interactions between SR 47436 and HCTZ when the two drugs are co-administered as single oral doses to mice and rats at large multiples of the clinical dose.

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3.1.3. Acute Intraperitoneal Toxicity Study of SR 47436 in Rats and Mice (Report #RS0006920206/02. Study #TXA074). Vol. 23

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between November 19 and December 5, 1990.

Male and female Sprague-Dawley CD (SD) BR rats (from Charles River) were approximately 6 weeks of age and weighed 141-238 g (males) or 136-182 g (females) at the start of the study. The mice (Swiss OF1, from Charles River) were approximately 6 weeks of age and weighed 24-30 g (males) or 21-23 g (females). SR 4746 (batch 90.00.5) was suspended in water for injection with 10% gum arabic and administered by intraperitoneal injection. The animals were not fasted before treatment. For both sexes and species, dose levels administered were 0, 25, 200 and 2000 mg/kg. Each group consisted of 5 males and 5 females.

All animals were frequently observed for mortality and drug effects for 14 days following administration. The body weights were recorded on days 1, 3, 8 and 15. A full necropsy was performed on all animals.

No deaths occurred in the control or in the 25 and 200 mg/kg dosage groups. At 2000 mg/kg all the animals were found dead about 3 hr after treatment, following somnolence in both species and piloerection in the mouse. No clinical signs were observed during the study either in the control animals or in the treated rats at 25 and 200 mg/kg. Transient mild somnolence was observed in male mice given 200 mg/kg. Among groups that survived for 14 days after dosing, no body weight changes attributed to SR 47436 administration were noted. The macroscopic examinations performed on the animals found dead or sacrificed at the end of the study did not reveal any gross lesions attributed to treatment. In conclusion, in both species, the non lethal and absolute lethal doses were respectively, 200 and 2000 mg/kg; the LD_{50} lies between 200 and 2000 mg/kg.

3.1.4. Acute Intravenous Toxicity Study of SR 47436 in Rats and Mice (Report #RS0006931012/01, Study #TXA252), Vol. 23

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between September 19 and September 29, 1993.

Male and female Sprague-Dawley CD (SD) BR rats (from Charles River) were approximately 6 weeks of age and weighed 143-161 g (males) or 135-161 g (females) at the start of the study. The mice (Swiss OF1, from Charles River) were approximately 6 weeks of age and weighed 28-33 g (males) or 24-29 g (females). SR 4746 (batch J880N) was dissolved in water for injection containing potassium hydroxide, ethanol, alanine and mannitol and administered by intravenous route (caudal veins) at a constant volume (20 ml/kg, bolus injection). The animals were not fasted before treatment. For both sexes and species, dose levels administered were 0 and 50 mg/kg. Higher dosage could not be administered due to the poor solubility of the compound. Each group consisted of 5 males and 5 females.

All animals were frequently observed for mortality and drug effects for 15 days following administration. The body weights were recorded on days 1, 3, 8 and 15. A full necropsy was performed on all animals.

No deaths occurred in the control or in the 50 mg/kg dosage groups in both species. The maximal nonlethal dose was 50 mg/kg in mice and rats of both sexes. No clinical signs, body weight variations or macroscopic changes were noted in mice and rats of both sexes.

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3.2. *Subchronic and Chronic Toxicity Studies*

3.2.1. One Month Oral Toxicity Study of SR 47436 in Rats (Report #RS0006920214/01, Study #TSA797), Vol. 24

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between December 2, 1991 and January 8, 1992.

Male and female Sprague-Dawley CD (SD) BR rats (from Charles River) were approximately 9 weeks of age and weighed 280-404 g (males) or 195-256 g (females) at the start of the study. SR 47436 (batch 91.01) was administered once daily for 36 days at doses of 30, 70 or 150 mg/kg by oral gavage (5 ml/kg). The control group received the vehicle. Each group consisted of 10 male and 10 female rats. The animals were not fasted before treatment. The animals were housed singly. The doses were selected on the basis of a one week oral dose-range finding study in which two of three females given 900 mg/kg/day SR 47436 died. No overt signs of toxicity were noted at doses of up to 450 mg/kg/day. Macroscopic necropsy findings attributed to SR 47436 were limited to rats that died and consisted of black spots on the kidney surface and erosion or black areas on the stomach mucosa.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded on day -1, then weekly throughout the study. Individual food intake was measured weekly. Blood samples were withdrawn, prior to administration, from the retro-orbital sinus of all animals fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected on days -13 and 29, and -11 and 32, respectively. Blood samples were also collected at necropsy, on day 37 and 38, from 5 animals/sex/group for analysis of test compound and/or metabolite plasma levels. Urinalysis was performed on overnight urine samples collected from 5 animals/sex/group, on days -4 and 26. All surviving animals were killed on days 37 to 39. All animals were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology (Table 3.2.1.1).

Results

Two females, one each given 30 and 70 mg/kg/day, died accidentally (animal-handling accidents) on dosing day 32 and 29, respectively. Although hemoglobin levels, mean cell hemoglobin concentration and RBC counts were very slightly decreased, and mean corpuscular volume slightly increased (about 5% in all cases, $P < 0.05$) in females given 150 mg/kg/day, values remained within physiological limits, and the sponsor, therefore, considers them incidental findings.

TABLE 3.2.1.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION

	SAMPLES	E.M..	OW	LIGHT MIC.
Skin and Subcutaneous Tissues	1			1
Mammary Tissue	(1)			(1)
Liver	3	x	x	1
Gall Bladder	1		(x)	1
Spleen	1		x	1
Kidneys	2	x	x	1
Adrenals	2		x	2
Costochondral Joint	1			
Thymus	1		x	1
Heart	1		x	2
Lungs	3		x	2
Tracheobronchial Lymph Nodes	1			1
Urinary Bladder	1			1
Ovaries	2		x	2
Fallopian Tubes	2		x	2
Uterine Tubes	1		(x)	1
Uterine Cervix	(1)		(x)	1
Vagina	1			1
Testes	2		x	2
Epididymides	2			1
Seminal Vesicles	2		x	1
Prostate	1		x	1
Aorta	1			1
Sciatic Nerve	1			1
Popliteal Lymph Nodes	2			2
Femur and Bone Marrow	1			
Femur Diaphysis	1		x	
Crural Muscle	1			1
Pancreas	1			1
Esophagus	1			1
Stomach: Forestomach, Glandular Area	1 each, together			1 each, together
Duodenum	(1)			(1)
Jejunum	1			1
Ileum	1			1
Cecum	(1)			1
Colon	1			1
Rectum	1			1
Mesenteric Lymph Nodes	1			1
Salivary Glands	1			1
Thyroid Glands	2		x	2
Parathyroid Glands	(2)		(x)	(2)
Larynx	(1)			(1)
Cervical Esophagus and Trachea	1			1
Tongue	1			
Eyes	2		x	1
Harderian Glands	2			1
Brain	1		x	3
Pituitary	1		x	1
Inner Ear	1			
*Femoral Bone Marrow	1			1

Footnote for the Table 3.2.1.1:
*Isolated from the femur and bone marrow sample after a fixation period.
SAMPLES: the number of samples preserved from each organ or tissue
EM: organs sampled for electron microscopy
OW: organ weights- analysis of absolute and relative (organ weight/body weight) values. Organs between parentheses were weighed together with the preceding organs
Light MIC: the number of samples prepared for light microscopic examination

Treatment-related changes were observed in blood biochemistry parameters. Slight, dose-related, statistically significant elevations in blood glucose values compared with control group values were noted for all groups given SR 47436. Slightly increased urea levels in females given 70 and 150 mg/kg/day and in males given 150 mg/kg/day, associated with slightly increased creatinine levels in males receiving 150 mg/kg/day, were also observed. Mild to moderate increases in potassium levels were observed in all treated females and in males given 70 and 150 mg/kg/day (Table 3.2.1.2).

TABLE 3.2.1.2
SR 47436: NOTABLE CHANGES IN THE CLINICAL CHEMISTRY PARAMETERS

Sex	Males				Female			
Dose (mg/kg/day)	0	30	70	150	0	30	70	150
Glucose (mM)	5.08	5.54*	5.75***	6.08***	5.4	5.80*	6.11***	6.44***
Urea (mM)	5.48	5	5.04	6.62(1)	5.79	6.39	9.05*	10.67***
Creatinine (μM)	34.8	34.2	36.5	40.6***	44	39.6	44.9	48.3
Potassium (mM)	4.67	4.67	4.91	5.06	4.33	4.98	5	5.12*

(1) 14.16 in one animal, * P <0.05, ** P <0.01, *** P <0.001.

Sodium and chloride concentrations and excretions in urine were slightly decreased in all treated animals, though these variations were slightly more pronounced in females than in males (Table 3.2.1.3). These findings may indicate that SR 47436 produced alterations in kidney function. No findings attributed to SR 47436 were noted during macroscopic, and light and electron microscopic examination of kidney tissues. Renal proximal tubulopathy, mostly regenerative, in one high dose male, and multifocal chronic tubulointerstitial nephritis with tubular basophilia in one male and one female given 150 mg/kg/day were not attributed by the sponsor to treatment with SR 47436. These changes were considered isolated and spontaneous and were also observed in one concurrent control. No findings attributed to SR 47436 were noted for other tissues examined at necropsy. Also, no treatment-related organ or body weight effects were observed. The study failed to establish a non-toxic dose.

TABLE 3.2.1.3
SR 47436: NOTABLE CHANGES IN THE URINALYSIS PARAMETERS

Sex	Males				Females			
Dose (mg/kg/day)	0	30	70	150	0	30	70	150
Sodium concentration (mM)	27	23	23	17**	31	16**	19	13***
Sodium excretion (μmol)	366	255	322	248*	253	108	148	120
Chloride concentration (mM)	40	40	30	27	38	19(1)	14*(1)	9**
Choloride execretion (μmol)	536	452	438	423	308	153(1)	108(1)	92

(1) In one animal the value was situated below the detection limit (1 mM), * P <0.05, ** P <0.01, *** P <0.001.

There were small but significant differences in SR 47436 plasma concentrations between males and females for the 70 and 150 mg/kg/day doses (Table 3.2.1.4); SR 47436 concentrations were higher in males (P <0.05). With SR 47436 plasma concentration/dose ratios, highly significant differences were observed (P <0.001) between males and females. SR 47436 plasma concentrations observed were not consistent with dose proportionality for either males or females. No other pharmacokinetic parameters were evaluated in this study.

TABLE 3.2.1.4
GROUP SR 47436 PLASMA CONCENTRATIONS (MG/L)
MEAN ± STANDARD DEVIATION [MINIMUM - MAXIMUM]

		On Day 37/38	
Groups	Dosage (mg/kg/day)	Males (n = 5)	Females (n = 5)
0	0	0.007 ± 0.017 [0 - 0.037]	0
1	30	0.632 ± 0.109 [0.502 - 0.778]	0.756 ± 0.131 [0.626 - 0.909]
2	70	0.800 ± 0.135 [0.599 - 0.949]	0.640 ± 0.062 [0.543 - 0.710]
3	150	0.793 ± 0.068 [0.678 - 0.852]	0.673 ± 0.065 [0.574 - 0.730]

0 = Not detected

3.2.2. 26-Week Oral Toxicity Study of SR 47436 in Rats (Report #RS0006960118/01, Study #TXC949), Vols. 33-36

This GLP study was conducted by between December 22, 1994 and August 7, 1995.

Male and female Sprague-Dawley CD rats (from Charles River) were approximately 35 to 42 days of age and weighed 141-190 g (males) or 123-166 g (females) at the start of the study. Suspensions of SR 47436 (batches 4SNP021, 4SNP042, 4SNP063) were prepared in 10% aqueous gum arabic solution and administered orally by gavage (5 ml/kg), once daily for 26 weeks at doses of 250, 500 or 1000 mg/kg. Control animals received the vehicle. The doses were selected on the basis of a previous 26 week study performed at the same laboratory on the same strain of rats. In that study body weight gain for all female groups and for high dose males (90 mg/kg/day) was slightly lower than control (6 to 8%); effect reversible with discontinuation of dosing. At necropsy, low heart and thymus weights and high kidney weight were noted in animals receiving 90 mg SR 47436/kg/day. Hyperplasia/hypertrophy of the juxtaglomerular apparatus of the kidney was not observed in any dosage group. Because no significant toxic effects were noted in the first study, a second study was conducted at higher dose levels under the same conditions.

It was evident from the toxicokinetic data of the previous 6-month toxicity study as well as from other dose-ranging studies that measurable quantities of SR 47436 were present in plasma of control rats receiving the vehicle. Considering the housing conditions used in these studies, it was resolved by the sponsor and the contract laboratory that the cross-contamination of the controls was airborne (also see my 8/18/94 review of IND :). Thus, to eliminate a possible source of cross-contamination of test substance to control animals, the animals of the first control, group #1, were isolated from those of treated groups (#3 to #5) by housing them in a different room. The second control, group #2, was housed in the same room as the treated animals. In addition, each group was housed on a separate battery. The animals were housed five of one sex per cage, unless the number was reduced by mortality. The animals were not fasted before treatment.

Each group (main study) consisted of 20 male and 20 female rats. The high dose and both control groups contained an additional 10 males and 10 females for a 6 week recovery phase without dosing. In addition, five satellite animals per dose per sex per group were used for drug plasma concentration determinations performed before and 2, 4, 8 and 12 hour after dosing in weeks 5, 13 and 26 (1 animal/sex/dose/sampling time).

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded a week before treatment, on day 1 of treatment, then weekly throughout the treatment, recovery phase and before necropsy. Food supplied to each cage and that remaining was recorded a week before treatment and for each week throughout treatment and recovery. From these records, the mean weekly consumption per animal was calculated for each cage. Water

consumption was recorded for each cage of animals only during weeks 1, 14 and 25 of treatment. Ophthalmic examination was conducted on all animals before commencement and during weeks 12 and 24 of treatment. ECGs were done on 5 animals/sex/group before dosing and 2 and 24 hours after dosing during weeks 12 and 24. Blood samples were withdrawn from the retro-orbital sinus of all animals anesthetized and fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected during weeks -1, 13, 25 and 32. Urinalysis was performed on overnight urine samples collected from 5 animals/sex/group, before treatment, and in weeks 12, 24 and 32 of treatment. All surviving animals were killed in weeks 27 and 33 (reversibility phase animals). All animals were subjected to a complete necropsy that included detailed external examination and individual examination of several organs followed by histopathology.

The following organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy.

Adrenals
Brain
Heart
Kidneys
Liver
Lungs with mainstem bronchi
Ovaries
Pituitary
Prostate
Salivary glands - submandibular
Seminal vesicles
Spleen
Testes
Thymus
Thyroid with parathyroids, after fixation
Uterus with cervix

Samples of the following tissues were preserved in 4% neutral buffered formaldehyde, except eyes and optic nerves which were placed in Davidson's fluid and subsequently retained in 70% industrial methylated spirit, and testes and epididymides which were initially preserved in Bouin's fluid.

Adrenals
Brain
Cecum
Colon
Duodenum
Epididymides
Eesophagus
Eyes and optic nerves, left
Femoral bone and marrow
Heart
Ileum
Jejunum
Kidneys
Liver
Lungs with mainstem bronchi
Lymph nodes -mandibular, -mesenteric
Mammary gland - caudal
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary gland - submandibular, left
Sciatic nerve - left
Spinal cord
Spleen
Stomach - ketatinised, - glandular
Testes
Thymus
Thyroid with parathyroids
Trachea
Urinary bladder
Uterus with cervix
Vagina

Additional samples of kidney (cortex and medulla) and liver were taken from 3 male and 3 female animals in groups 1 (control isolated from rest of the groups), 2 and 5 at the 27 and 33 week sacrifices. Ultra-thin sections of the samples were prepared and examined by electron microscopy.

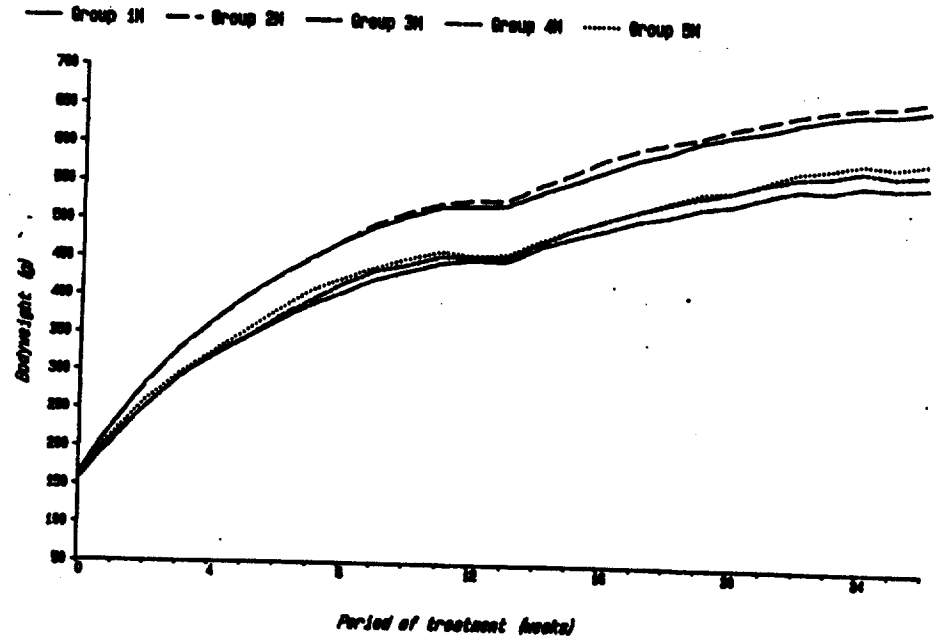
Results

There were no treatment-related changes. Eight animals died during the study (Table 3.2.2.1). These deaths, according to the sponsor, were considered unrelated to the administration of SR 47436.

TABLE 3.2.2.1
SR 47436: MORTALITY DATA

Doses (mg/kg/d)	Number of deaths M	F	Time	Probable cause of death
0	1/30	2/30	W8	Male found dead. No signs prior to death. Pale dark masses in the thorax involving the thymus, heart and lungs, and large and dark mandibular lymph nodes. Microscopic exam: chronic pericarditis in the heart, plasmacytosis in the mandibular lymph nodes, focal necrosis with inflammatory infiltrate in the liver, chronic pleuritis in thorax, lungs and thymus and chronic inflammation/abscesses in the peri-esophageal tissue.
			W13	Died after blood sampling. Incomplete collapse of the lungs and revealed alveolar hemorrhage in the lungs.
			W25	Died after blood sampling. Incomplete collapse of the lungs and revealed alveolar hemorrhage in the lungs and chronic myocarditis.
250	No deaths			
500	1/20	0/20	W7	gavage trauma
1000	2/30	2/30	W8	gavage trauma
			W15	1 male found dead, no previous signs of ill-health and no histophathological changes, cause of death not determined
			W26	1 female found dead. Signs before death included: firm and swollen area in the abdomen, piloerection, pallor and pale eyes. Findings at necropsy included: red fluid in the abdomen, abdominal distension, an enlarged swollen spleen and a pale and swollen liver with a ruptured area. Microscopic exam: malignant lymphoma in the adrenals, femur and marrow, liver and spleen, sinus lymphoma in the mesenteric lymph node, ovarian cysts in the left ovary and vascular mineralization in the lungs.
			W29	1 male found dead, a firm subcutaneous and movable mass on the upper ventral surface was noted at palpation from W25 of treatment, however, this subcutaneous mass not detected at necropsy due to partial canibalism, no histophathological changes, cause of death not determined.

The overall body weight gain of treated rats in comparison to controls during the dosing period was low ($P < 0.05$ to 0.01, Fig 3.2.2.1, Table 3.2.2.2). However, rats of the low dose group were the most severely affected and the data lacked a clear dosage-time response relationship. During the recovery phase, the body weight gain of males of the high dose group was significantly higher than that of the controls, while that of females was similar to that of the controls (Table 3.2.2.2). The decrease in body weight gain is attributed by the sponsor to a nonspecific toxic effect of the drug.



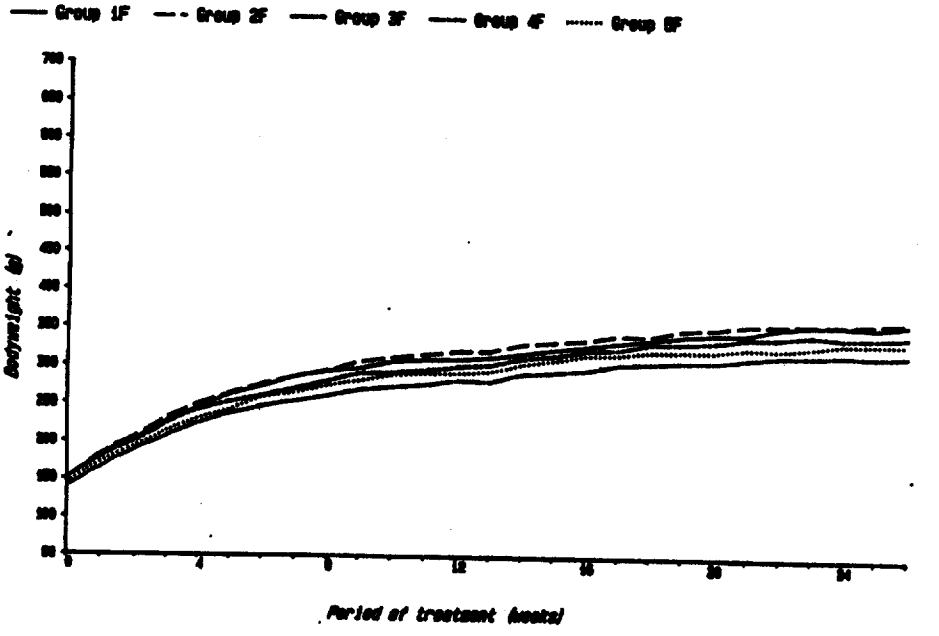


Fig. 3.2.2.1.: 26-week toxicity study of SR 47436 in rats. Group mean body weights of male (upper panel) and female (lower panel) rats *versus* time period in weeks.

TABLE 3.2.2.2

SR 47436: MEAN BODY WEIGHT DURING THE DOSING PERIOD AND THE OVERALL MEAN BODY WEIGHT GAIN - GROUP MEAN VALUES

Sex	Male					[redacted]
Dose (mg/kg/day)	0	0	250	500	1000	
Week 0	165	164	156	163	166	
Week 26	659	672	558	575	590	
Week 0-26*	494	508	402[2,3]	412[2,3]	425[2,3]	
Week 32	702	654			663	
Week 26-32*	39	28			82[2,3]	

*: overall mean body weight gain

[1]: significant when compared with group 1, P <0.05

[2]: significant when compared with group 1, P <0.01

[3]: significant when compared with group 2, P <0.01

The overall food intake for treated animals was slightly low (P >0.05) throughout the treatment period. There was no dosage-response relationship. During the recovery phase, there was a marginal increase in food intake for males of the high dose group. There were no electrocardiographic changes that could be attributed to treatment. Erythrocyte counts after 12 or 24 weeks of treatment in all dose groups were statistically lower than in controls. Slight but significant decreases in hemoglobin and mean cell hemoglobin concentration for all treated females and slightly high (P <0.05 to 0.001) mean cell hemoglobin and mean cell volumes for all treated males were noted. Platelet counts were statistically higher in treated groups when compared with controls but were not dosage-related. All these findings were reversible by week 32 (Table 3.2.2.3). Thus, hematological changes noted during treatment suggest some disruption of the hemopoietic process.

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TABLE 3.2.2.3.
SR 47436: NOTABLE CHANGES IN HEMATOLOGY PARAMETERS

Sex		Male					Female				
Dose mg/kg/day		Cl-1	Cl-2	250	500	1000					1000
Hemoglobin (gm/dl)	w -1	13.2	13.1	12.9	13.0	13.0					
	w 13	15.2[x]	15.5[a]	15.5	15.6	15.3					
	w 25	14.9	15.3	15.1	15.0	14.8[x]					
	w 32	15.7	15.4			15.3					
RBC ($x10^6/mm^3$)	w -1	6.33	6.37	6.20	6.38	6.36					
	w 13	8.64	8.80	8.4[cz]	8.41[cz]	8.4[cz]					
	w 25	8.66	8.81	8.42[cy]	8.33[bz]	8.31[bz]					
	w 32	9.09	8.91			8.77					
Mean cell hemoglobin (pg)	w -1	20.9	20.7	20.8	20.3[a]	20.4[a]					
	w 13	17.6	17.7	18.4[cz]	18.5[cz]	18.2[cz]					
	w 25	17.3	17.4	18.0[cy]	18.0[cy]	17.9[cy]					
	w 32	17.3	17.3			17.4					
Mean cell hemoglobin conc. (gm/dl)	w -1	32.9	33.0	32.9	32.9	32.9					
	w 13	34.8	34.7	34.7	35.0[cy]	35.0[y]					
	w 25	35.5[y]	35.2[b]	35.0[c]	35.2	35.5[y]					
	w 32	36.0	35.7			35.5[b]					
Mean cell volume (fl)	w -1	63.4	62.6	63.8	61.8[a]	62.2					
	w 13	50.5	51.0	53.1[cz]	52.8[cz]	52.0[bz]					
	w 25	48.6	49.4	51.5[cz]	51.2[cy]	50.4[cz]					
	w 32	48.0	48.5			49.2					
Platelets ($x10^3/mm^3$)	w -1	1237	1265	1285	1274	1307					
	w 13	937	1025	1088[b]	1069[a]	1120[cz]					
	w 25	986	1062	1081[a]	1096[a]	1086[a]					
	w 32	832	959			1025[a]					

Significant when compared with group 1: a- P <0.05; b- P <0.01; c- p <0.001.
Significant when compared with group 2: x- P <0.05; y- P <0.01; z- p <0.001.

Blood urea nitrogen increased significantly in all treated groups when compared with the controls in week 13 and a clear dosage-relationship was evident. High urea concentrations were noted in week 25 in all treated male groups and in females receiving 500 or 1000 mg/kg/day. A significant increase in serum creatinine concentration was noted for high dose males in weeks 13 and 25 and for both sexes receiving 500 or more mg/kg/day in week 13 of treatment. In both sexes, serum phosphorus levels were significantly higher than control at weeks 13 and 25 (dose-response evident only at 13 weeks) (Table 3.2.2.4). After 12 and 24 weeks of treatment, slightly low total protein concentrations for treated males and high potassium concentrations for all treated female groups were recorded. Total bilirubin increased for groups receiving 500 or more mg/kg/day at week 13 and for females receiving 1000 mg/kg/day at week 25 (Table 3.2.2.4). Calcium concentrations were marginally low ($P < 0.05$ to 0.001) in all treated groups relative to controls but lacked any clear dosage-relationship. Similarly, compared to controls, slightly but significantly high glucose concentrations were observed for male groups receiving 500 or more mg/kg/day at week 13. Isolated statistically significant variations in some blood chemistry parameters were considered by the sponsor to be of no toxicological significance since they lacked any clear dosage-relationship or were confined to one sex or sampling occasion or were dependent on which control groups were used in the analysis. All these changes were reversible.

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TABLE 3.2.2.4
EFFECT OF SR 47436 ON BLOOD CHEMISTRY DURING 6 MONTHS TOXICITY STUDY IN RATS
(Results are expressed as group mean values)

Sex		Male					Female				
Dose (mg/kg/day)		Cntl-1	Cntl-2	250	500	1000	Cntl-1	Cntl-2	250	500	1000
Urea (mmol/l)	w -1	3.8	3.5	3.4	3.5	3.6	4.1	3.8	3.7	3.8	3.8
	w 13	5.4	5.5	8.3^{cy}	10.6^{cz}	10.7^{cz}	5.9	6.1	8.7^{cz}	10.2^{cz}	10.7^{cz}
	w 25	4.8	5.1	7.1^{cy}	8.7^{cz}	8.0^{cz}	6.4	6.2	6.9	8.0^{cz}	9.0^{cz}
	w 32	4.6	4.7			5.0	6.0	5.7			5.6
Creatinine (µmol/l)	w -1	38.9^{z}	35.3^{c}	36.4^{b}	35.9	34.0^{c}	39.5	37.4	36.4	36.4^{b}	36.8^{b}
	w 13	50.0^{z}	54.0^{c}	54.0^{b}	56.0^{c}	57.0^{cz}	62.0	65.0^{c}	62.0	68.0^{c}	69.0^{cz}
	w 25	52.0^{z}	54.0^{a}	53.0	56.0^{c}	59.0^{cz}	64.0	69.0^{cy}	70.0^{cz}	68.0^{c}	73.0^{cz}
	w 32	53.0	54.0			53.0	62.0	61.0			62.0
Glucose (mM)	w -1	3.5	3.8	4.1^{a}	4.8^{cy}	4.1^{a}	3.4	4.2	4.0	4.0	4.7
	w 13	6.1	6.3	6.5	6.7^{a}	7.1^{cz}	6.6	6.4	6.0^{c}	6.4	6.9
	w 25	7.3	7.6	7.1^{x}	7.6	7.5	6.6	7.2^{b}	7.0	6.9	6.8
	w 32	5.9	5.9			6.0	6.1	6.8^{c}			6.6
Bilirubin (µM)	w -1	1.7	1.7	1.9	2.1	1.7	2.1	2.5	2.5	2.9	2.9
	w 13	2	3	3	3^{cy}	4^{cz}	5	6	5	7^{c}	6^{c}
	w 25	2	2	2	3.0^{cz}	2.0	3	3	3	2	4^{cz}
	w 32	1	2			2	3	3			2
K^+ (mM)	w -1	3.8	3.8	3.8	3.9	3.9	4.0	3.9	3.9	4.2^{c}	3.9
	w 13	4.4	4.3	4.1	4.4	4.3	3.5	3.5	3.9^{c}	3.8^{c}	3.9^{c}
	w 25	3.6^{z}	3.8^{b}	3.9^{c}	4.1^{cy}	3.8^{c}	3.3	3.3	3.5^{c}	3.7^{c}	3.8^{cz}
	w 32	4.3^{y}	3.7^{b}			3.8^{a}	3.5	3.2			3.5
P (mM)	w -1	3.2	3.2	3.1^{a}	3.2	3.2	3.1	3.1	3.0^{c}	3.3^{c}	3.2
	w 13	2.2	2.3	2.4^{b}	2.5^{cz}	2.5^{cz}	1.9	1.9	2.1^{c}	2.2^{cz}	2.2^{cz}
	w 25	1.8^{z}	2.0^{c}	2.1^{cx}	2.2^{cz}	2.1^{c}	1.6	1.6	1.6	1.8^{c}	1.8^{c}
	w 32	1.8	1.9			2.0^{cz}	1.4	1.5			1.5

Significant when compared with group 1: a- $p < 0.05$; b- $p < 0.01$; c- $p < 0.001$
Significant when compared with group 2: x- $p < 0.05$; y- $p < 0.01$; z- $p < 0.001$

Urinary sodium and chloride concentration and excretion were significantly higher than control in high dose animals at week 24. In addition, urinary potassium output and concentration were higher than control for males at week 12 and for both sexes at week 24. There were no other findings considered to be related to treatment with SR 47436. During the recovery phase only marginally high ($P < 0.05$) urinary potassium concentration and excretion were still noted in high dose males (Table 3.2.2.5)

TABLE 3.2.2.5.

SR 47436: NOTABLE CHANGES IN THE URINALYSIS PARAMETERS

Sex		Male					Female				
Dose mg/kg/day		Cl-1	Cl-2	250	500	1000	Cl-1	Cl-2	250	500	1000
Sodium conc. (mmol)	d -1	0.37	0.31	0.27	0.35	0.31	0.25	0.31	0.25	0.27	0.28
	d 12	0.50	0.68	0.59	0.90[a]	0.66	0.37	0.43	0.32	0.37	0.46
	d 24	0.52	0.86	0.67	0.49	0.99[a]	0.39	0.66	0.54	0.53	0.96
Sodium excretion (mmol/l)	d -1	75.0	84.0	64.4	90.6	77.0	77.8	92.8	79.6	79.8	89.6
	d 12	57.0	56.5	42.8	50.4	51.0	59.2	52.2	46.8	51.8	67.2
	d 24	31.2	43.8	35.4	28.2[x]	42.6	40.0	49.4	50.2	56.0[x]	74.4[y]
Chlorides conc. (mmol)	d -1	0.48	0.38	0.36[a]	0.41	0.39	0.33	0.39	0.34	0.35	0.29
	d 12	0.38	0.44	0.60	0.87[ax]	0.70	0.33	0.38	0.38	0.38	0.49
	d 24	0.61	0.65	0.80	0.57	1.03[ax]	0.32	0.47	0.48	0.37	0.55
Chlorides excretion (mmol/l)	d -1	100.2	103.8	86.8	109.6	104.8	100.6	119.6	108.2	99.6	95.2
	d 12	42.8	37.0	40.8	49.6	53.8	38.4	47.6	45.6	48.0	62.2
	d 24	37.2	33.2	42.0	34.0	44.8[x]	32.8	42.4	45.8	39.2	48.2
Potassium conc. (mmol)	d -1	0.63	0.56	0.47[b]	0.47[b]	0.47[b]	0.37	0.48	0.45	0.47	0.36
	d 12	0.97	1.07	1.53[a]	1.88[cy]	1.73[bx]	0.66	0.63	0.89	0.77	0.82
	d 24	1.39	1.29	1.68	1.62	2.17[by]	0.74	0.98[y]	1.09[y]	1.07[y]	1.60[y]
	d 32	1.39	1.25			1.56[x]	0.88	0.78			0.70
Potassium excretion (mmol/l)	d -1	130.0	156.5	117.1	126.9	134.4	110.6	130.0	120.0	125.9	118.0
	d 12	110.0	92.4	110.2	113.0	134.6[ay]	110.4	80.0	106.9[y]	108.4	126.1
	d 24	84.4	70.1	91.0[x]	95.6[y]	93.8[y]	75.2	73.0	107.4[y]	107.8[y]	80.9
	d 32	56.0	54.3			66.4[ay]	54.4	56.1			54.7

Significant when compared with group 1: a- $P < 0.05$; b- $P < 0.01$; c- $p < 0.001$.
Significant when compared with group 2: x- $P < 0.05$; y- $P < 0.01$; z- $p < 0.001$.

At the 26 week sacrifice, absolute decreases (18%) in weight were noted for hearts of all treated groups when compared to control, although no clear dosage-relationship was evident. Relative kidney weights were dose-related (17 to 21% higher than control). Relative liver weights of the 500 and 1000 mg/kg/day female groups were slightly higher (10%) than control. In the absence of any histopathologic findings, these organ weight findings are unlikely to be of any toxicologic significance. Low absolute and relative thymus weights (39% and 37%, respectively, below control) were noted for treated males but were not dosage-dependent. For animals killed after the 6 week recovery period, slightly high absolute and relative kidney weights were noted for males receiving 1000 mg/kg/day (Table 3.2.2.6). Otherwise, organ weights of the recovery group animals did not differ from control.

TABLE 3.2.2.6

SR 47436: GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS (gm) FOR ANIMALS KILLED AFTER 26 AND 33 WEEKS OF TREATMENT

Dose, mg/kg/day		Control		Control		250		500		1000	
Sex		M	F	M	F	M	F	M	F	M	F
Body weight, g		650	358	692	357	553[b]	312[by]	574[b]	338	585[b]	529[ax]
B.wt- recovery		690	363	655	372					657	335
Heart	Abs wt.	1.87	1.26	1.96	1.28	1.55[b]	1.05[by]	1.55[b]	1.14[y]	1.54[b]	1.12[by]
	Rel wt.	0.29	0.35	0.284	0.36	0.28	0.34	0.27	0.34	0.264[a]	0.341
Kidney	Abs wt.	3.80	2.34	4.402	2.45	3.74[a]	2.28	4.03	2.51	4.14[a]	2.52
	recovery	3.94	2.40	3.86	2.50					4.34[a]	2.36
	Rel wt.	0.587	0.654	0.584	0.685	0.68[by]	0.74[by]	0.71[by]	0.75[by]	0.71[by]	0.77[by]
	recovery	0.572	0.664	0.590	0.692					0.67[ax]	0.704
Liver	Abs wt.	23.3	13.5	24.3	13.3	19.9[by]	11.9[y]	21.5[a]	13.9	21.4[a]	13.6
	Rel wt.	3.58	3.77	3.52	3.70	3.63	3.81	3.74	4.12[y]	3.66	4.13[y]
Thy-mus	Abs wt.	0.52	0.36	0.61	0.40	0.37[by]	0.33[y]	0.40[by]	0.37	0.37[by]	0.33[y]
	Rel wt.	0793	.1003	.0871	.1126	.0667[by]	.1067	0685[by]	.1087	.0636[by]	.0996

Significant when compared with group 1: a- P <0.05; b- P <0.01.
Significant when compared with group 2: x- P <0.05; y- P <0.01.

There were no macroscopic changes that could be attributed to treatment with SR 47436. Microscopic findings considered to be related to treatment were confined to the kidneys. Hyperplasia/hypertrophy of the juxtaglomerular apparatus was observed in 3/19, 6/19 and 6/20 males receiving 250, 500 and 1000 mg/kg/day, respectively, and in 1/20 and 3/19 females receiving 500 and 1000 mg/kg/day, respectively. On completion of the recovery phase, hyperplasia of the

juxtaglomerular apparatus was noted in 1/8 males and 1/9 females receiving 1000 mg/kg/day. It was absent in all control animals. According to the sponsor, the microscopic changes in the kidney are due to an adaptive response to the action of SR 47436 on the RAS. A higher incidence of hemorrhage of the thymus in high dose males (4/8) sacrificed after 6 weeks of recovery compared to controls (2/39) is considered by the sponsor a chance occurrence and not related to treatment. There were no ultra-structural changes that were considered to be related to the administration of SR 47436.

Toxicokinetic investigations showed that control animals housed in the same study room as treated animals (group #2) were exposed at relatively constant level to test substance (AUC about one tenth that calculated for the low dose group). Plasma levels of SR 47436 were lower in females than in males (Table 3.2.2.7). SR 47436 was not detected in plasma samples withdrawn from control animals housed in a separate room (group #1). No significant effects were noted in group #2 when compared with group #1. There is no explanation from the sponsor as to how control animals housed in the same room as treated animals showed measurable quantitites of SR 47436 in their plasma. The airborne contamination seen with the rats only suggests that the compound may find a way into the rats respiratory tract.

TABLE 3.2.2.7
SR47436 PLASMA LEVELS IN CONTROL GROUP HOUSED IN THE STUDY ROOM

Sampling time	Males			Females		
	Range (mg/l)	Mean ± S.D.	AUC 0-24h (mg.h/l)	Range (mg/l	Mean ± S.D.	AUC 0-24h (mg.h/l)
Week 5		0.108 ± .026	2.466		0.086 ± .018	2.090
Week 13		0.136 ± .038	3.192		0.065 ± .020	1.597
Week 26		0.132 ± .052	3.187		0.047 ± .012	1.123

SR 47436 was detected in all treated rats in which the assay was conducted. Plasma levels of SR 47436 were higher in females than in males except during week 26 in mid dose animals. Peak concentration was attained within 2-4 hours of dosing (Table 3.2.2.8).

TABLE 3.2.2.8
SR47436 TOXICOKINETIC PARAMETERS IN TREATED ANIMALS

Sampling time	Dose mg/kg/d	tmax (h)		Cmin (mg/l)		Cmax (mg/l)		AUC 0-24h (mg.h/l)	
		Males	Females	Males	Females	Males	Females	Males	Females
Week 5	250	2	2	0.65	0.68	2.06	5.30	25.1	30.6
	500	4	2	0.94	0.74	5.69	8.76	43.1	95.8
	1000	2	2	1.28	0.64	14.1	35.4	76.4	140
Week 13	250	2	2	0.65	1.62	2.40	15.1	26.3	48.3
	500	4	4	1.14	0.53	9.83	51.5	57.5	255
	1000	4	2	1.36	0.59	57.5	100	311	240
Week 26	250	2	2	0.37	0.24	2.47	43.4	23.3	127
	500	2	2	0.85	0.20	5.77	78.3	41.2	268
	1000	2	2	0.60	0.43	58.6	88.3	148	226

In summary, 26-weeks of repeated administration of SR 47436 to rats resulted in clinical chemistry and hematological changes affecting renal function and the hematopoietic system. The principal target organ effect, hyperplasia of the juxtaglomerular apparatus in the kidney (which has been observed with other angiotensin II receptor antagonists), was considered by the sponsor to have resulted from exaggerated pharmacological activity of the test substance.

3.2.3. Six-Month Oral Toxicity Study of SR 47436/HCTZ in Rats (Report #RS0042960530/03, Study #93037), Vols. 14-17

This GLP study was conducted by the Departments of Toxicology and Pathology, Pharmaceutical Research Institute, Bristol-Myers Squibb, New Brunswick, NJ between December 7 and June 20, 1994.

Male and female Harlan Sprague-Dawley outbred albino rats were approximately 5 weeks of age and weighed 102-133 g (males) or 97-120 g (females) at the start of the study. Suspensions of SR 47436 (batches 93-04, 93-06) and HCTZ (batch 48192), alone or in a 1:1 combination in 1% carboxymethylcellulose, were administered orally by gavage (4.5 ml/kg), to five groups of 20 rats/sex/group, once daily for 6 months. Animals received SR 47436 in combination with HCTZ at doses of 10/10 and 90/90 mg/kg or SR 47436 or HCTZ alone, at doses of 90 mg/kg daily. The control group received the vehicle at 4.5 ml/kg. The doses were selected on the basis of a 6-month study with SR 47436 alone, performed on the same strain of rats at doses of 10, 30, and 90 mg/kg/day (see section 3.2.2). In that experiment, mean body weight gain for all female and all high dose male groups was slightly lower than that of controls (-6 to 8%) throughout the study period. At necropsy, low heart and thymus weights and high kidney weight were noted at 90 mg/kg/day. Hyperplasia/hypertrophy of the juxtaglomerular apparatus of the kidney was not observed in any dosage group. Thus, daily doses of 10 and 90 mg SR 47436/kg/day were chosen for use in the combination toxicity study. A 1:1 ratio of doses of SR 47436 and HCTZ was selected to provide the maximum exposure of the animals to the two drugs when they are administerred in combination. Animals were not fasted before treatment and were housed individually in stainless steel cages.

Observations and Measurements

All animals were observed at least twice daily for mortality and at least once daily for changes in condition and behavior. The body weights were recorded at least twice each week. Individual 24-hour food intake was determined weekly. Indirect systolic blood pressure was determined approximately 1 to 2 hr after a daily dose during weeks 15 and 24 of treatment in 5 males and 5 females/group selected from the rats used for clinical-pathology testing. Blood samples for hematology and clinical chemistry tests (except for coagulation tests) were obtained from the cut tip of the tail of 10 animals/sex/group (those not used for toxicokinetic evaluations) prior to administration of a daily dose during weeks 12 and 24. Blood samples for coagulation tests were obtained by cardiac puncture, just prior to necropsy. Reticulocyte and bone-marrow smears from animals from all groups were examined microscopically as part of the hematology evaluation. Urinalysis was performed on 24-hour urine samples collected from 10 animals/sex/group in weeks 13 and 25 of treatment. Concentrations of SR 47436 and/or HCTZ were determined in plasma samples from 10 animals/sex/group (those not used for clinical pathology tests) obtained at 1, 2, 4, 6, 7, and 24 hr after the first dose (day 1) and after a daily dose during the third and sixth months. Each rat was bled from the cut tip of the tail at two time points (i.e., 4 males and 4 females/group at 1 and 6 hr, 3 males and 3 females/group at 2 and 8 hr, and 3 males and 3 females/group at 4 and 24 hr). All animals were subjected to a complete necropsy that included external examination and

individual examination of several organs followed by histopathology. Table 3.2.3.1 lists the organs and/or tissues examined.

TABLE 3.2.3.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION[a]

Adrenal glands*	Prostate gland (weighed with seminal vesicles)
Aorta	Salivary glands
Bone and bone marrow (sternum/femur/rib)	Sciatic nerve
Brain*	Seminal vesicles
Epididymides	Skeletal muscle (thigh and diaphragm)
Esophagus	Skin (inguinal region)
Eyes	Small intestine: duodenum, ileum, jejunum
Heart*	Spinal cord (whole cord collected)
Kidneys*	Spleen*
Large intestine: cecum, colon, rectum	Stomach
Liver (left and median lobes)*	Testes*
Lungs	Thymus*
Lymph nodes (mesenteric, cervical)	Thyroid gland*
Mammary gland	Tongue
Occular accessory glands (Harderian glands)	Trachea
Ovaries with fallopian tubes*	Urinary bladder
Pancreas	Uterine with cervix*
Parathyroid gland	Vagina
Pituitary gland*	Tissues/organs with gross lesions

a: samples prepared for light microscopic examination and did not involve electron microscopy; * : organs weighed.

Results

All rats survived to scheduled sacrifice. The results are summarized in Table 3.2.3.2. Group mean body weights are shown in Fig 3.2.3.1. Drug-related microscopic changes were observed in the kidney and stomach. The juxtaglomerular cell hypertrophy/hyperplasia correlated with the increased kidney weights only in animals given high doses of SR 47436/HCTZ. This kidney lesion is attributed to the pharmacologic effects of SR 47436 on the RAS. Additional drug-related changes in the kidney included a statistically significant decrease in the incidence of progressive murine nephropathy in females receiving low doses of SR 47436/HCTZ and 90 mg SR 47436/kg/day and a decrease in the severity of progressive murine nephropathy in males receiving the high dose of SR 47436 alone or in combination with HCTZ. The decreased incidence and severity of progressive murine nephropathy in the kidneys, a naturally occurring disease of the rats, was attributed to the pharmacologic effects of SR 47436. ACE inhibitors have also been shown to reduce the incidence and severity of this disease. The increased incidence of coagulative necrosis and/or ulceration in the stomach of rats given the low dose of SR 47436, and given the high dose of SR 47436 alone or in cimbination with HCTZ indicates that SR 47436 was the more irritative of the two drugs under

the conditions of the present study.

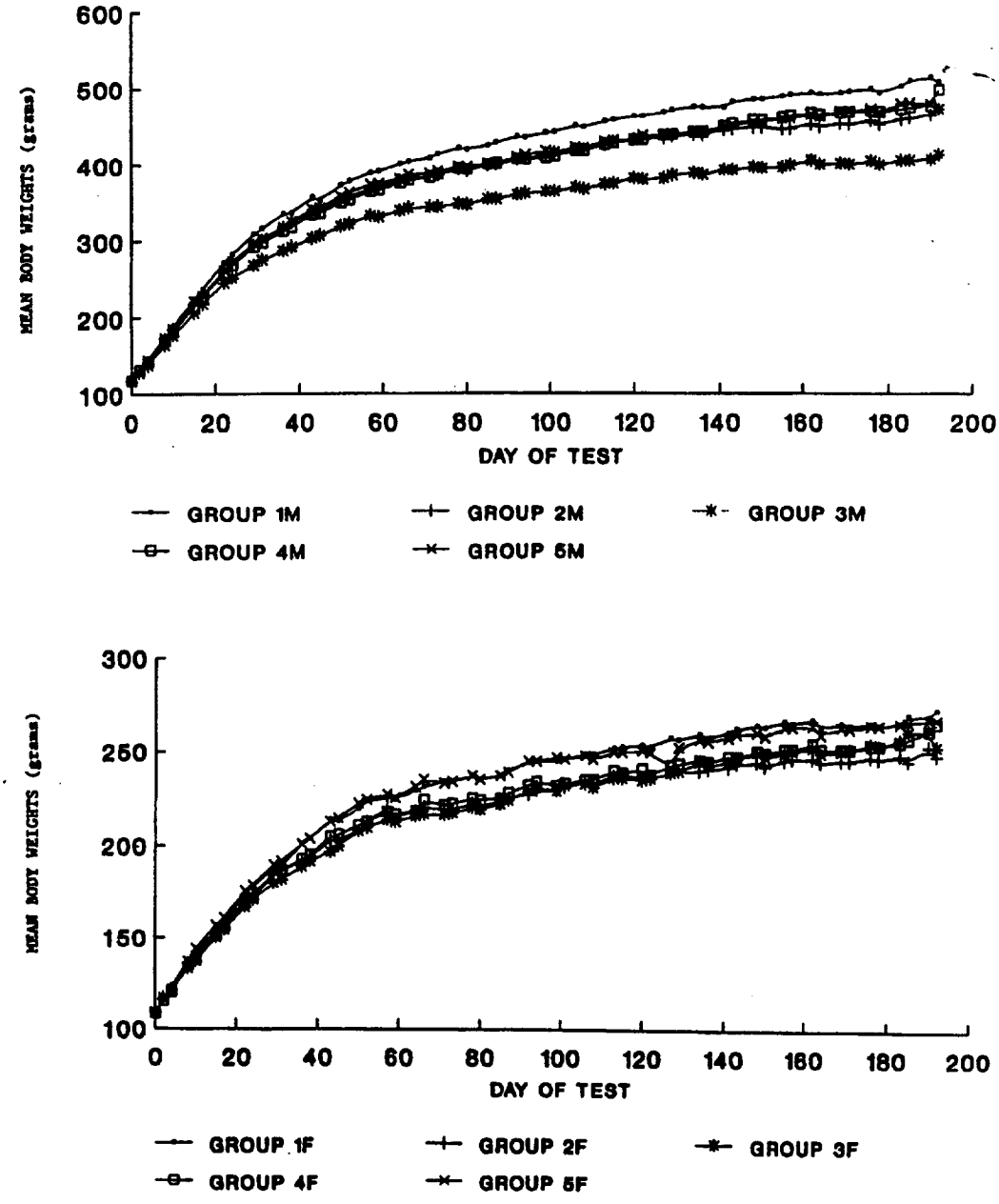

Fig. 3.2.3.1.: 6-month toxicity study of SR 47436/HCTZ. Group mean body weights *versus* period of treatment of male (upper panel) and female (lower panel) rats.

TABLE 3.2.3.2
SR 47436/HCTZ: RESULTS OF 6 MONTH ORAL TOXICITY IN RATS
(all findings relative to concurrent control)

DRUG REGIMEN→	10 SR / 10 HCTZ	90 SR / 90HCTZ	90 SR 47436	90 HCTZ
Clinical signs	No treatment-related change			
Mortality	None			
Ophtalmology	No treatment-related change			
Blood pressure Decrease in systolic pressure	Moderate	Marked	Moderate	-
Bodyweight Decreased gain[a]	Slight, significant in males	Slight (females) or moderate (males*)	Slight, significant in males	Slight (in males*)
Food intake	Occasionally decreased[a]			
Hematology Decreased Hemoglobin, PCV, RBC	-	Slight (in females)*	Slight (in males)*	-
Serum Chemistry - Increase in Urea	-	Slight	-	Very slight (in males - W24)
- Decrease in Triglycerides	Slight (in males)	Slight to moderate (mainly in males)*	Slight	-
- Decrease in Cholesterol	Slight (in females - W24)*	Slight to moderate (in males)*	Slight	-
- Increase in SAP	-	Slight (in males - W24)*	-	-
- Increase in Chlorides	-	-	Slight*	-
- Decrease in K	Slight (in males - W12)*	Slight (in males)*	-	Slight to moderate*
- Decrease in P	Slight (in males - W12)*	Slight (in males - W12)*	Slight (in males - W12)*	Slight to moderate (W12)*
- Decrease in Ca	-	Slight (in males - W12)*	-	Slight (in males - W12)*
Urinalysis - Increased urine output, decreased specific gravity	-	Slight - W25*	-	-
- Increase in urine pH	-	Slight - W25*	-	-
- Protein concentration	Moderately lower*	Markedly lower*	Moderately lower*	Higher (females)*

DRUG REGIMEN→	10 SR / 10 HCTZ	90 SR / 90HCTZ	90 SR 47436	90 HCTZ
Necropsy: Organ weight				
Heart	Decrease, absolute & relative wt.*	Decrease, absolute & relative wt.*	Decrease, absolute & relative wt.*	-
Kidney	-	Increase (in ♀) absol. & rel wt.*	-	-
Liver	Decrease (in ♂) absol & rel wt.*	Decrease (in ♂) absol. & rel wt.*	Decrease (in ♂) absol & rel wt.*	-
Macroscopy				
Glandular stomach (discolored mucosa)	2/40 animals	7/40 animals	4/40 animals	1/40 animals
Microscopy				
Focal coagulative necrosis or ulceration of the stomach	1M, 1F/40 animals	5M, 2F/40 animals	2M, 2F/40 animals	1F/40 animals
Hypertrophy/hyperplasia of juxtaglomerular cells	minimal to mild	minimal to mild	minimal to mild	-
Decreased incidence and/or severity of progressive nephropathy	in females	in males	in both males and females	-

- indicates effects or changes not seen in animals of that group
W: week, M: male, F: female; a: nondose-dependent; *$p < 0.05$

Exposure to SR 47436 and hydrochlorothiazide increased as the administered dose increased. For SR 47436, the increase was less than proportional to dose, and for HCTZ, the increase was generally near or greater than proportional to dose. Exposure to SR 47436 was not affected by coadministration of hydrochlorothiazide; however, exposure to hydrochlorothiazide was greater when it was given in combination with SR 47436 than when given alone. In general, exposures to both drugs were higher at 3 and 6 months than on day 1. For SR 47436, females had slightly greater exposure than males given the same dose. For HCTZ, no substantive gender differences were noted. As in a previous 26-week study (section 3.2.2) SR 47436 was detected in plasma samples from control animals. Of a total of 80 samples collected from control rats, only three samples on day 1 and 8 samples in the third month had detectable levels of SR 47436 (concentrations ranged from 102 to 758 ng/ml). However, in the 6th month, 31 out of 40 samples had measurable levels of SR 47436 (Table 3.2.3.3). Exposure to SR 47436 in the control group in the 6th month was less than 1/6 and 1/15, respectively, of that in the low and high dose combination groups.

TABLE 3.2.3.3
TOXICOKINETIC PARAMETERS OF SR 47436 AND HYDROCHLOROTHIAZIDE IN RATS GIVEN SR 47436 ALONE OR IN COMBINATION WITH HCTZ

Dose, mg/kg/day		Control		SR/HTZ, 10/10		SR/HTZ, 90/90		SR or HTZ, 90	
Sex		M	F	M	F	M	F	M	F
SR 47436									
Cmax (µg/ml)	Day 1	-	[illegible]	0.7	[illegible]	3.1	[illegible]	2.9	[illegible]
	Month 3	-	[illegible]	1.9	[illegible]	2.9	[illegible]	4.1	[illegible]
	Month 6	0.3	[illegible]	1.7	[illegible]	4.8	[illegible]	3.2	[illegible]
$AUC_{(0-T)}$ (µg.h/ml)	Day 1	-	[illegible]	13.0	[illegible]	22.4	[illegible]	27.5	[illegible]
	Month 3	-	[illegible]	29.2	[illegible]	37.2	[illegible]	41.6	[illegible]
	Month 6	3.7	[illegible]	24.1	[illegible]	58.3	[illegible]	36.2	[illegible]
HYDROCHLOROTHIAZIDE									
Cmax (µg/ml)	Day 1	-	[illegible]	0.4	[illegible]	6.0	[illegible]	4.5	[illegible]
	Month 3	-	[illegible]	1.1	[illegible]	4.3	[illegible]	4.2	[illegible]
	Month 6	-	[illegible]	1.0	[illegible]	5.6	[illegible]	5.4	[illegible]
$AUC_{(0-T)}$ (µg.h/ml)	Day 1	-	[illegible]	1.2	[illegible]	21.0	[illegible]	15.9	[illegible]
	Month 3	-	[illegible]	5.2	[illegible]	32.3	[illegible]	27.1	[illegible]
	Month 6	-	[illegible]	3.5	[illegible]	81.7	[illegible]	38.6	[illegible]

$AUC_{(0-T)}$: AUC from time (0) to the time (T) of the last measurable concentraion

In summary, daily treatment with SR 47436 in combination with HCTZ at doses of 10/10 and 90/90 mg/kg or SR 47436 or HCTZ alone, at doses of 90 mg/kg daily, was associated with nondose-dependent slight to moderate decreases in body weight gain, and with occasional decreases in food consumption. Anemia was predominantly observed in high dose combination treated females and to some extent in males given the high dose of SR 47436 alone. Alterations in serum chemistry values were observed mainly in males receiving the high dose SR 47436/HCTZ combination. At necrospy, decreased heart weights, decreased liver weights, juxtaglomerular apparatus hypertrophy/hyperplasia and reduced incidence of progressive nephropathy were observed in all treated groups except those given HCTZ alone. Discoloration of the glandular stomach, noted in all treated groups, correlated with focal coagulative necrosis or ulceration of the mucosa, with an incidence slightly higher in rats given the high dose combination. Exposure to SR 47436 was not affected by coadministration of hydrochlorothiazide; however, exposure to hydrochlorothiazide was greater when it was given in combination with SR 47436 than when it was given alone. In addition, SR 47436 was detected in plasma samples from control animals.

3.2.4. One Month Oral Toxicity Study of SR 47436 in Monkeys (Report #RS0006920212/01, Study #TSA796), Vol. 27

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between November 19, 1991 and December 19, 1991.

The male and female Cynomolgus monkeys, *Macaca fascicularis* (from Mauritius) weighed 2.4-4.06 kg, and 2.09-3.37 kg (age not given since they were collected from the wild), respectively, at the start of the study. SR 47436 (batch #91.01) was administered (not clear whether the animals were fasted) once daily for 28 days at doses of 10, 30 or 90 mg/kg by oral gavage (5 ml/kg). The control group received the vehicle. Each group consisted of 3 male and 3 female monkeys. The animals were housed singly. The doses were selected on the basis of a one week oral dose-range finding study (groups of one male and one female monkey at doses of up to 600 mg SR 47436/kg/day) in which there were no mortalities and no overt signs of toxicity. A slightly elevated blood urea for all SR 47436-treated groups, slightly high creatinine levels in males given 300 and 600 mg/kg/day, and slightly lower spleen weights for all groups given test compound compared to control group values were attributed to SR 47436 administration. No findings attributed to SR 47436 were noted during macroscopic or microscopic examination of tissues.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. Electrocardiography was performed on days 1 and 25, before and 2 hr after test compound administration in all animals. The body weights were recorded on day -1, then weekly for the duration of the study. Individual food intake was measured daily. Blood samples were withdrawn, prior to administration, from the femoral vein of all animals fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected on days -7, 8 and 29. The plasma concentrations of active renin and angiotensin II were determined from blood samples taken from all animals on days -1, 8 and 29. For toxicokinetics study, blood samples were collected on days 10 and 30 from all animals, before and 1 hr after administration of SR 47436/vehicle. Urinalysis was performed on all animals, on overnight urine samples collected on days -4 and 28. All surviving animals were killed on day 31 or 32. All animals were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology (Table 3.2.4.1).

Results

No deaths or treatment-related changes occurred in the study. There were no variations in ECG records, body weight gain or food intake that could be attributed to SR 47436 administration. Hemoglobin concentration on day 8 in high dose animals was statistically lower than in controls but remained within physiological limits. Further, no differences were noted on day 29. Isolated statistically significant variations in some blood chemistry parameters were considered by the

TABLE 3.2.4.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION

	SAMPLES	E.M.	OW	LIGHT MIC.
Skin and Subcutaneous Tissues	1			1
Mammary Tissue	1			1
Liver	3	x	x	1
Gall Bladder	(1)		(x)	(1)
Spleen	1		x	1
Kidneys	2	x	x	1
Adrenals	2		x	2
Costochondral Joint	1			
Sternebre	1			
Thymus	1		x	1
Heart	1		x	2
Lungs	3		x	2
Tracheobronchial Lymph Nodes	1			1
Urinary Bladder	1			1
Ovaries	2		x	2
Fallopian Tubes	2		x	2
Corpus Uteri	(1)		(x)	(1)
Uterine Cervix	(1)		(x)	1
Vagina	1			1
Testes	2		x	2
Epididymides	2			1
Seminal Vesicles	2		x	1
Prostate	1		x	1
Aorta	1			1
Sciatic Nerve	1			1
Popliteal Lymph Nodes	2			2
Femur and Bone Marrow	1			
Crural Muscle	1			1
Pancreas	1			1
Esophagus	1			1
Stomach: Cardia, Fundus, Pyloric Area	1 each			1 each
Duodenum	(1)			1
Jejunum	1			1
Ileum	1			1
Cecum	(1)			1
Colon	(1)			1
Rectum	1			1
Mesenteric Lymph Nodes	1			1
Parotid Glands	1			1
Submandibular Glands	1			1
Thyroid Glands	2		x	2
Parathyroid Glands	(2)		(x)	(2)
Larynx	1			1
Trachea	(1)			1
Tongue	1			
Eyes	2		x	1
Optic Nerve	(2)			1
Brain	1		x	3
Pituitary	1		x	1
Spinal Cord	1			3
Inner Ear	1			
*Femoral Bone Marrow	1			1

Footnote for the Table 3.2.4.1:
*Isolated from the femur and bone marrow sample after a fixation period.
SAMPLES: the number of samples preserved from each organ or tissue
EM: organs sampled for electron microscopy
OW: organ weights- analysis of absolute and relative (organ weight/body weight) values. Organs between parentheses were weighed together with the preceding organs
Light MIC: the number of samples prepared for light microscopic examination

sponsor to be of no toxicological significance. They were: slight increase in creatinine levels in low dose animals on day 29, a mild and non-dose related decrease in triglyceride levels in all treated groups compared to controls on day 29 (in fact, control values were high on day 8 and day 29 compared to day -7 because of two animals which were in poor general health), and a slight increase in GPT levels in animals given 30 mg/kg/day compared to control on day 8. Urine specific gravity and sodium and chloride concentration and excretion in urine were slightly lower for the group given 90 mg/kg/day SR 47436 than for the control group. However, the values remained within physiological limits.

No treatment-related changes were observed in the weights of the organs studied. There were no drug-related macroscopic findings at necropsy. Microscopic findings were: foci of mineralization in the papilla of kidneys in one male and one female given 10 mg/kg/day, and in one high dose female; and endomyocardial fibrosis in one male given 30 mg/kg/day. The sponsor considers these findings as spontaneous pathology of the species. When hyperplasia of the juxtaglomerular apparatus was noted in the monkeys from the 6-month chronic study at dose levels of 10, 30 and 90 mg/kg/day (see section 3.2.6), the sponsor performed additional histopathological examination of the kidneys of all animals on this one month study using three additional stains (Masson's trichome, PAS and Bowie's). Dose-related hyperplasia of the juxtaglomerular apparatus was observed from 30 mg/kg/day upwards, in both sexes. It was characterized by hyperplasia and hypertrophy of the muscle fibres of afferent and efferent arterioles and by an increase in the number of secretory granules within these cells.

There was a significant dose- and time-dependent relationship for active renin (Fig. 3.2.4.1) and, to a lesser extent, for plasma angiotensin II (Table 3.2.4.2, Fig. 3.2.4.2).

TABLE 3.2.4.2
SR 47436: MEAN PLASMA ANGIOTENSIN II AND ACTIVE RENIN CONCENTRATIONS (pg/ml) IN MONKEYS

Doses	Angiotensin II			Active Renin		
(mg/kg/day)	Day-1	Day 8	Day 29	Day-1	Day 8	Day 29
0	8.2	13.7	9.3	79.5	88.7	70.8
10	18.7	170.5	261.0	105.3	953.5	1552.0
30	11.7	203.5	278.7	98.2	3561.3	4791.0
90	19.0	380.5	433.5	97.7	3890.3	9746.0

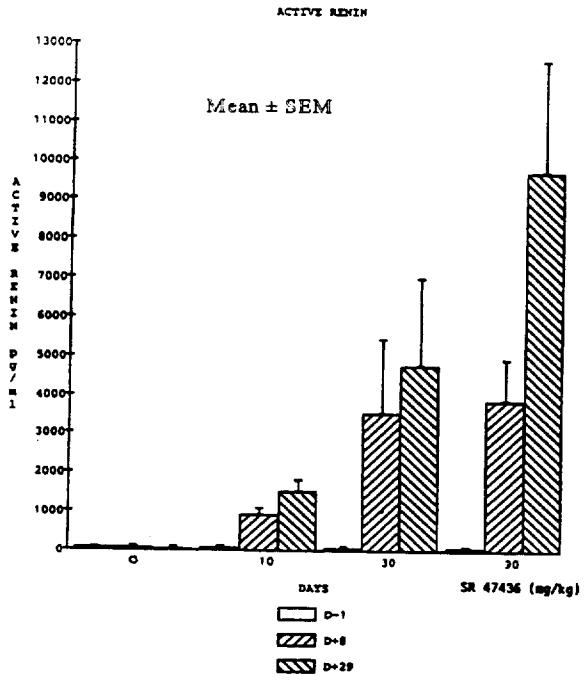

Figure 3.2.4.1.: Active renin plasma concentrations in monkeys after oral administration of SR 47436

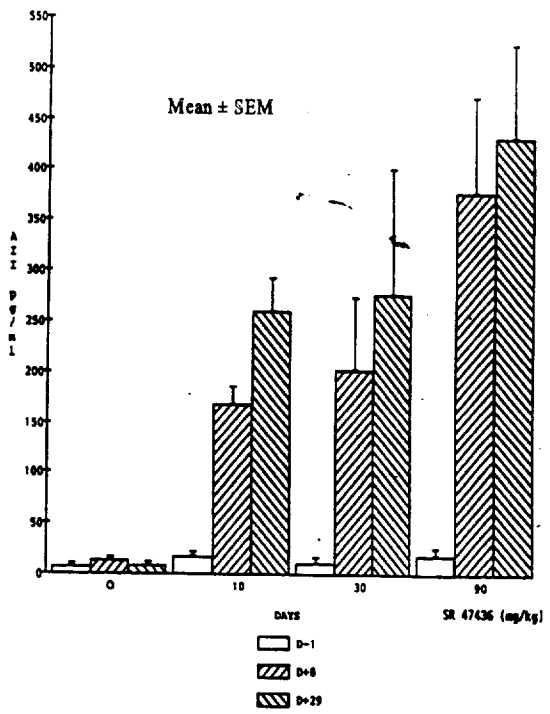

Figure 3.2.4.2.: Angiotensin II plasma concentration in monkeys after oral administration of SR 47436

SR 47436 plasma concentrations determined before and 1 hr after administration on days 10 and 30 did not increase dose proportionately (Table 3.2.4.2). No statistically significant differences between dosage groups were observed. No other pharmacokinetic parameters were evaluated in this study.

TABLE 3.2.4.3

SR 47436: MEAN GROUP SR 47436 PLASMA CONCENTRATIONS (MG/L) OBSERVED ON DAYS 10 AND 30. VALUES ARE EXPRESSED AS MEAN ± SD [MINIMUM-MAXIMUM].

Groups n = 6	Dosage (mg/kg/day)	Day 10		Day 30	
		Before Admin.	1 Hour After Admin.	Before Admin.	1 Hour After Admin.
0	0	0	0	0	0
1	10	0.073 ± 0.035 [0.037 - 0.119]	0.689 ± 0.247 [0.325 - 1.020]	0.092 ± 0.031 [0.043 - 0.137]	0.736 ± 0.270 [0.546 ± 1.269]
2	30	0.254 ± 0.120 [0.128 - 0.427]	1.678 ± 1.059 [0.760 - 3.633]	0.259 - 0.112 [0.099 - 0.382]	2.752 ± 1.476 [1.241 - 5.509]
3	90	0.393 ± 0.145 [0.235 - 0.577]	4.404 ± 4.043 [0.744 - 9.628]	0.762 ± 0.274 [0.270 - 1.010]	2.697 ± 1.490 [1.450 - 4.822]

0 = Not detected

3.2.5. Second One Month Oral Toxicity Study of SR 47436 in Monkeys (Report #RS0006940406/01, Study#TSA898). Vol. 28

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between October 1, 1993 and November 4, 1993.

The male and female Cynomolgus monkeys, *Macaca fascicularis* (from Mauritius) weighed 2.2-3.3 kg, and 2.0-2.6 kg (age not given since they were collected from the wild), respectively, at the start of the study. SR 47436 (batch #93.06) was administered (not clear whether the animals were fasted) once daily for 33 or 34 days at doses of 250, 500 or 1000 mg/kg by oral gavage (10 ml/kg). The control group received the vehicle. Each group consisted of 3 male and 3 female monkeys. The animals were housed singly. The doses were selected on the basis of previous studies in which few toxic signs were observed up to 600 mg SR 47436/kg/day after one week of administration, and up to 90 mg/kg/day after 1 (section 3.2.4) or 6 months (section 3.2.6) of administration.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. Electrocardiography was performed on days 1 and 29, 2 to 4 hr after test compound administration in all animals. The body weights were recorded on day -1, then weekly throughout the study. Individual food intake was measured daily, from which mean individual daily values were calculated on a weekly basis. Blood samples were withdrawn, prior to administration, from the femoral vein of all animals fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected on days -9, 7 and 28. Urinalysis was performed on all animals, on overnight urine samples collected on days -1 and 27. All surviving animals were killed on days 34 and 35. All animals were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology. Table 3.2.3.1 lists the organs and/or tissues examined. For toxicokinetics study, blood samples were collected from a femoral vein on day 33 from all drug-treated animals, before and 1, 2, 4 and 6 hr after administration of SR 47436.

Results

A high dose male was found dead on day 28 after a 3-day period of weakness and sleepiness. This animal presented an abscess on the right humeral distal epiphysis, very dark adrenals and mesenteric lymph nodes and very irregular stomach mucosa. One high dose female was sacrificed *in extremis* on day 28, after presenting with prostration, weakness, palor and hypothermia following blood sampling in the morning. It appeared very thin with moderate pale discoloration of the mucosa. There were no other treatment-related physical signs or gross lesions in any of the dose groups.

TABLE 3.2.5.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION

	SAMPLES	E.M..	OW	LIGHT MIC.
Skin and Subcutaneous Tissues	1			1
Mammary Tissue	1			1
Liver	3	x	x	1
Gall Bladder	1		(x)	1
Spleen	1		x	1
Kidneys	2	x	x	1
Adrenals	2		x	2
Costochondral Joint	1			
Thymus	1		x	1
Heart	1		x	2
Lungs	3		x	2
Tracheobronchial Lymph Nodes	1			1
Urinary Bladder	1			1
Ovaries	2		x	2
Fallopian Tubes	2		x	2
Corpus Uteri	1		(x)	1
Uterine Cervix	1		(x)	1
Vagina	1			1
Testes	2		x	2
Epididymides	2			1
Seminal Vesicles	2		x	1
Prostate	1		x	1
Aorta	1			1
Sciatic Nerve	1			1
Popliteal Lymph Nodes	2			2
Femur and Bone Marrow	1			
Crural Muscle	1			1
Pancreas	1			1
Esophagus	1			1
Stomach: Cardia, Fundus, Pyloric Area	1 each			1 each
Duodenum	1			1
Jejunum	1			1
Ileum	1			1
Cecum	1			1
Colon	1			1
Rectum	1			1
Mesenteric Lymph Nodes	1			1
Parotid Glands	1			1
Submandibular Glands	1			1
Thyroid Glands	2		x	2
Parathyroid Glands	2		(x)	2
Larynx	1			1
Trachea	1			1
Tongue	1			
Eyes	2		x	1
Optic Nerve	2			1
Brain	1		x	3
Pituitary	1		x	1
Spinal Cord	1			3
Inner Ear	1			
*Femoral Bone Marrow	1			1
Proximal Radial Epiphysis	1			1

Footnote for the Table 3.2.5.1:
*Isolated from the femur and bone marrow sample after a fixation period.
SAMPLES: the number of samples preserved from each organ or tissue
EM: organs sampled for electron microscopy
OW: organ weights- analysis of absolute and relative (organ weight/body weight) values. Organs between parentheses were weighed together with the preceding organs
Light MIC: the number of samples prepared for light microscopic examination

Electrocardiographic changes:
(a) Control, animal #1M: slight left ventricular enlargement on days 1 and 29
(b) Control, animal #6F: right bundle branch block on days 1 and 29.
(c) 250 mg/kg/d, animal #10F: left ventricular enlargement particulary marked and associated with noticeably increased P wave duration and PR interval on day 1, increased QRS interval on day 29, and inverted T wave on days 1 and 29.
(d) 500 mg/kg/d, animal #15M: slight left ventricular enlargement on day 29 only.
(e) 500 mg/kg/d, animal #17F: right bundle branch block on days 1 and 29.

There were no variations in body weight gain or food intake that could be attributed to SR 47436 administration. Hemoglobin, hematocrit (PCV) and red blood cell count were slightly decreased on day 28 (-20% at the most) in a dose-related pattern at 250 or more mg/kg/day in males and 500 or more mg/kg/day in females. A slight increase in neutrophil counts was observed in one female each of control and low dose groups, one mid dose male and one male and one female from high dose group. Such an increase in neutrophil counts could be attributed to inflammatory changes (due to repeated withdrawl of blood), however, such changes could not be identified in all these animals. Thus, the cause has not been established by the sponsor. A significant increase in platelet counts was observed on day 28 in one female receiving 250 mg/kg/day and in all females from 500 or more mg/kg/day. In males, a statistically nonsignificant increase in platelets counts was observed in one each in mid and high dose groups only. A non-dose-dependent increase in fibrinogen levels in both sexes at 500 or more mg/kg/day is attributed to drug treatment. The data was significant on day 28 of measurement for males given 500 mg/kg/day and for females given 1000 mg/kg/day.

Statistically significant changes in some blood chemistry parameters were observered in treated groups. Slight to moderate increases in urea levels at doses of 250 or more mg/kg/day, in a time- and dose-dependent manner, and associated with slight increases in creatinine levels, were noted (Table 3.2.5.2).

TABLE 3.2.5.2.
SR 47436: NOTABLE CHANGES IN THE CLINICAL CHEMISTRY PARAMETERS

Sex		Male				Female			
Dose (mg/kg/day)		Control	250	500	1000	Control	250	500	1000
Urea (mmol/l)	Day -9	6.72	6.39	6.75	7.23	6.60	7.84	7.42	7.14
	Day 7	6.57	8.22	9.18	15.09*	6.92		9.01	9.42
	Day 28	6.85	15.72*	12.28*	31.77	7.63	14.38	17.68*	19.63*
Creatinine (µmol/l)	Day -9	85.3	95.0	91.3	83.3	88.4	89.5	81.9	79.8
	Day 7	85.3	109.0*	99.3	118.0*	89.0	85.7	85.1	85.7
	Day 28	89.3	128.7*	100.7	164.5	86.7	106.0	97.63	94.5

*P <0.05 compared to control

Sodium and chloride concentrations and excretions in urine were decreased in all treated groups. The decrease in chloride concentration and both sodium and chloride excretion were statistically significant only in males, whereas the decreases in sodium concentration were statistically significant only in females (Table 3.2.5.3). These findings suggest that SR 47436 alters kidney function. Additionally, two high dose animals that were found dead or sacrificed *in extremis* on day 28 had glycosuria.

TABLE 3.2.5.3.
SR 47436: NOTABLE CHANGES IN THE URINALYSIS PARAMETERS

Sex		Male				Female			
Dose mg/kg/day		Control	250	500	1000	Control	250	500	1000
Sodium conc. (mM)	d -1	38	47	50	46	63	70	81	54
	d 27	55	32	27	19	50	18*	30*	22*
Chlorides conc.(mM)	d -1	25	21	29	37	22	23	24	24
	d 27	36	13*	15	4.4*	22	5	17	10
Sodium excretion (µM)	d -1	2970	4408	2447	3733	5277	3657	3738	4860
	d 27	8720	2360*	2050*	2160*	5277	1093	2487	2657
Chlorides excretion (µM)	d -1	1927	1998	1373	2333	1853	1110	864	2160
	d 27	5657	903*	1067*	1100*	2153	383	1537	1260

*P <0.05 compared to control

In a high dose female monkey, sacrificed moribund on day 28, all organ weights were decreased

with the exception of adrenal gland weight, which can be related to health deterioration rather than to drug treatment. A low dose female showed markedly increased heart and lung weights (approximately 2-fold), which is consistent with ECG modifications and histological changes. A slightly increased (both absolute and relative) liver weight was recorded in females receiving 500 (+16% relative) or 1000 (+19% relative) mg/kg/day ($P < 0.05$). Absolute and relative decreases in thymus weight were observed in a few monkeys given 500 or more mg/kg/day. Additionally, decreases in ovarian and uterus weights were observed in monkeys dosed at 500 or more mg/kg/day (Table 3.2.5.4). Changes in thymus, ovary and uterus weights were not statistically significant due to high standard error and small sample size (n=2 or 3). Thus, sponsor considers these findings as variations that bear no toxicological significance.

TABLE 3.2.5.4
SR 47436: GROUP MEAN ABSOLUTE AND RELATIVE ORGAN WEIGHTS (gm)

Dose, mg/kg/day		Control		250		500		1000	
Sex		Male	Female	Male	Female	Male	Female	Male	Female
Body weight, g		3143	2610	3003	2613	2870	2807	3150	2585
Liver	Abs wt.	81.52	62.85	67.72	59.27	69.12	64.10	82.06	73.53
	Rel wt.	25.85	23.88	22.90	22.69	24.26	27.81	26.03	28.45*
Thymus	Abs wt.	0.2426	0.1903	0.2061	0.1729	0.1320	0.1888	0.1205	0.1970
	Rel wt.	0.779	0.715	0.709	0.662	0.460	0.673	0.381	0.576
Ovary	Abs wt.		0.283		0.326		0.241		0.180
	Rel wt.		0.110		0.126		0.0858		0.07
Uterus	Abs wt.		0.76		0.58		0.435		0.47
	Rel wt.		0.276		0.205		0.155		0.18

*P <0.05 compared to control

Macroscopic examination in one low dose female revealed a thickened and reduced lumen of the left ventricle of the heart corresponding to extensive endocardial fibrosis and hypertrophy of cardiomyocytes observed by light microscopy. It also had intraalveolar siderophage infiltration in the lung (cardiac lung). This animal presented marked ECG changes on days 1 and 29 with no obvious aggravation between the two days. Since these findings were observed at the lowest dose level in one animal only, the sponsor contends that this animal could have had pre-existing lesions. There were no treatment-related findings in any other animals.

Microscopically, slight or moderate hyperplasia of the juxta-glomerular apparatus, non-dose related and characterized by hyperplasia and hypertrophy of the modified smooth muscle cells (renin secretory cells) of afferent and efferent arterioles, was observed in all treated groups. Similar observations were also made in an earlier one month toxicity study in monkeys given 30 or more mg/kg/day (see section 3.2.4). Besides this, a few monkeys receiving 250 or more mg/kg/day

exhibited one or several foci or areas of mycocardial fibrosis in the left ventricle or at the tip of the inner part of the ventricular myocardium (Table 3.2.5.5). Health deterioration changes such as reduction or absence of subcutaneous fat tissue, decrease in hepatocyte cytoplasmic margination (indicating a decrease in glycogen storage), moderate, subchronic or chronic thymic involution, slight testicular atrophy and moderate atrophy of the bone marrow were observed in a few animals, especially from high dose group (Table 3.2.5.5). The high dose male found dead on day 28 presented with marked granulomatous synovitis at the elbow, slight edema of the heart (left atrio-ventricular groove and epicardium of the tip of the heart) and lung (perivascular edema) and with lymph node post-reactive germinal centers. According to the sponsor, this animal presented with intercurrent infectious pathology (abscess), which could have contributed, in addition to treatment, to health deterioration and death. Another high dose female that was sacrificed moribund on day 28 presented with slight or moderate subchronic inflammation of the digestive mucosa with congestion of the duodenal and ileal villi, an area of ulceration and moderate subchronic inflammatory cell infiltration of the ileal submucosa, and moderate subacute inflammatory cell infiltration of the mesenteric lymph nodes. The sponsor contends that this inflammation could be the result of treatment with SR 47436 and probably participated in this animal's health deterioration.

TABLE 3.2.5.5.
SR 47436: LIGHT MICROSCOPIC EXAMINATION.

Dose mg/kg/day	Control		250		500		1000	
Sex	M	F	M	F	M	F	M	F
No of animals at the end of study	3	3	3	3	3	3	2	2
Found dead [1]/scrificed moribund[2]							1[1]	1
Kidney: juxtaglomerular hyperplasia	-	-	3	3	3	3	1 2	1 2
slight multifocal chronic inflammatory cell infiltration in the cortex	-	1			1	2		2
Heart: myocardial fibrosis	-	-	-	1	1	1	1 -	1 1
Health deterioration:								
fat tissue atrophy	-	-	-	-	-	-	1 1	1 1
hepatocyte margination	3	3	3	3	3	3	- 2	2 2
thymic involution, subchronic/chronic	-	-	-	-	-	-	-* 1	1 1
testicular atrophy	-	-	-	-	-	-	1 -	1 1
bone marrow atrophy	-	-	-	-	-	-	- -	1 1

*thymus missing in this animal

The absorption of SR 47436 was slow and reached Tmax between 2.5 and 3.5 hr. The systemic exposure increased with the increase in dose but not in a proportional manner (Table 3.2.5.6). There was no difference in the systemic exposure between male and female animals. However, the plasma levels presented a high inter-individual variability.

TABLE 3.2.5.6
SR 47436 PHARMACOKINETIC PARAMETERS ON DAY 33 (MEAN ± STANDARD DEVIATION)

Dose (mg/kg/day)	N	tmax (h)	Cmax (mg/l)	Cmin (mg/l)	AUC (0-24h) (mg.h/l)
250	6	3.0 ± 1.1	28.1 ± 19.0	1.05 ± 0.37	107.7 ± 58.2
500	6	2.5 ± 1.0	57.1 ± 46.5	1.47 ± 0.59	329.4 ± 270.4
1000	4	3.5 ± 1.0	106.4 ± 51.2	1.49 ± 0.83	570.4 ± 446.5

In summary, monkeys were administered SR 47436 at dosages of 250, 500 and 1000 mg/kg/day for 1 month. One high dose animal was found dead and one was sacrificed *in extremis* before the end of the study. The monkey that died showed signs of an intercurrent infectious pathology, whereas subchronic gastroenteritis and changes in coagulation parameters were observed in the monkey that was sacrificed *in extremis* (increase in platelet counts and fibrinogen levels were also found in other treated animals). In the absence of another explanation, these changes could be attributed to the SR 47436 treatment, which contributed to health deterioration leading to premature sacrifice or death. Additionally, SR 47436 treatment was associated with adverse findings affecting kidney, heart, erythrocyte, leukocyte, platelet and fibrinogen parameters at all dose levels. Adverse heart findings may not be drug-related since control animals showed ECG changes with the same incidence as treated animals.

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3.2.6. Six Month Oral Toxicity Study of SR 47436 in Monkeys (Report #RS0006930526/01, Study #TXC841). Vol. 37-38

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between August 11, 1992 and April 8, 1993.

The male and female Cynomolgus monkeys, *Macaca fascicularis* (from Charles River, USA) weighed 2.0-4.0 kg, and 2.0-3.0 kg (age not given since they were collected from the wild), respectively, at the start of the study. SR 47436 (batch #92.02) was administered (not clear whether the animals were fasted) once daily for 191 to 196 days at doses of 10, 30 or 90 mg/kg by oral gavage (5 ml/kg). The control group received the vehicle. Each group consisted of 5 male and 5 female monkeys. The high dose and control groups contained an additional 3 males and 3 females for a 6 week recovery phase without dosing. The animals were housed singly. The doses were selected on the basis of a previous study performed at the same laboratory on the same strain of monkeys for a month (see section 3.2.4). The only adverse effect observed in this study was the presence of hyperplasia of the juxtaglomerular apparatus in both sexes at 30 or more mg/kg/day.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. Electrocardiography was performed on all animals before dosing (day -4), and after dosing on days 95 and 186. The body weights were recorded on day -1, then weekly for the duration of the study. Individual food intake was measured daily. Blood samples were withdrawn, prior to administration, from the femoral vein of all animals fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected on days -12, 91, 184 and 239. Samples of blood collected on day 184 were also used to determine the levels of thyroxine (T4) and triiodothyronin (T3). The plasma concentrations of active renin, angiotensinogen and angiotensin II were determined from blood samples taken from all animals before drug or vehicle administration on days 126 and 163. For toxicokinetics study, blood samples were collected on day 190 from 3 animals/sex/group before and 2, 4, 6 and 24 hr after administration of SR 47436. Urinalysis was performed on all animals, on overnight urine samples collected on days -6/-5, 99 and 179/182. All surviving animals were killed between day 192 and day 197 (end of treatment) or on day 241 for the recovery phase. All animals were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology. Table 3.2.6.1 lists the organs and/or tissues examined.

Results

No deaths occurred in the study. One high dose male presented with variations in the ECG record on day 186, indicating biventricular enlargement. At necropsy, this animal showed an increase in heart weight associated with thickening of the myocardium, and light microscopic examination revealed multifocal fibrous and necrotic myocarditis. However, before beginning of the study,

TABLE 3.2.6.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION

	SAMPLES	E.M..	OW	LIGHT MIC.
Skin and Subcutaneous Tissues	1			1
Mammary Tissue	1			1
Liver	3	x	x	1
Gall Bladder	(1)		(x)	(1)
Spleen	1		x	1
Kidneys	2	x	x	1
Adrenals	2		x	2
Costochondral Joint	1			
Sternebrae	1			
Thymus	1		x	1
Heart	1		x	2
Lungs	3		x	2
Tracheobronchial Lymph Nodes	1			1
Urinary Bladder	1			1
Ovaries	2		x	2
Fallopian Tubes	2		x	2
Corpus Uteri	(1)		(x)	(1)
Uterine Cervix	(1)		(x)	1
Vagina	1			1
Testes	2		x	2
Epididymides	2			1
Seminal Vesicles	2		x	1
Prostate	1		x	1
Aorta	1			1
Sciatic Nerve	1			1
Popliteal Lymph Nodes	2			2
Femur and Bone Marrow	1			
Crural Muscle	1			1
Pancreas	1			1
Esophagus	1			1
Stomach: Cardia, Fundus, Pyloric Area	1 each			1 each
Duodenum	(1)			1
Jejunum, Ileum, Rectum	1 each			1 each
Cecum	(1)			1
Colon	(1)			1
Mesenteric Lymph Nodes	1			1
Parotid Glands	1			1
Submandibular Glands	1			1
Thyroid Glands	2		x	2
Parathyroid Glands	(2)		(x)	(2)
Larynx	1			1
Trachea	(1)			1
Tongue	1			
Eyes	2		x	1
Optic Nerve	(2)			1
Brain	1		x	3
Pituitary	1		x	1
Spinal Cord	1			3
Inner Ear	1			
Femoral Bone Marrow	1			1
Proximal Radial Epiphysis	1			1

Footnote for the Table 3.2.6.1:
SAMPLES: the number of samples preserved from each organ or tissue
EM: organs sampled for electron microscopy
OW: organ weights- analysis of absolute and relative (organ weight/body weight) values. Organs between parentheses were weighed together with the preceding organs
Light MIC: the number of samples prepared for light microscopic examination

the ECG parameters were modified and were at the upper limit of the physiological range. Considering this, the sponsor asserts that SR 47436 was not the cause of the myocarditis but may have interfered with its development. In other animals, the values of ECG parameters were within the range of physiological variability. A gradual dose-related decrease in body weight gain was observed in animals dosed at 30 or more mg/kg/day (Fig. 3.2.6.1). The decrease was slight to moderate at mid dose and was not significant. It was statistically significant at the end of treatment in high dose animals (Table 3.2.6.2). These variations completely reversed within 1 or 2 weeks following cessation of treatment. The variations in food intake seen occassionally were within physiological limits and not treatment related.

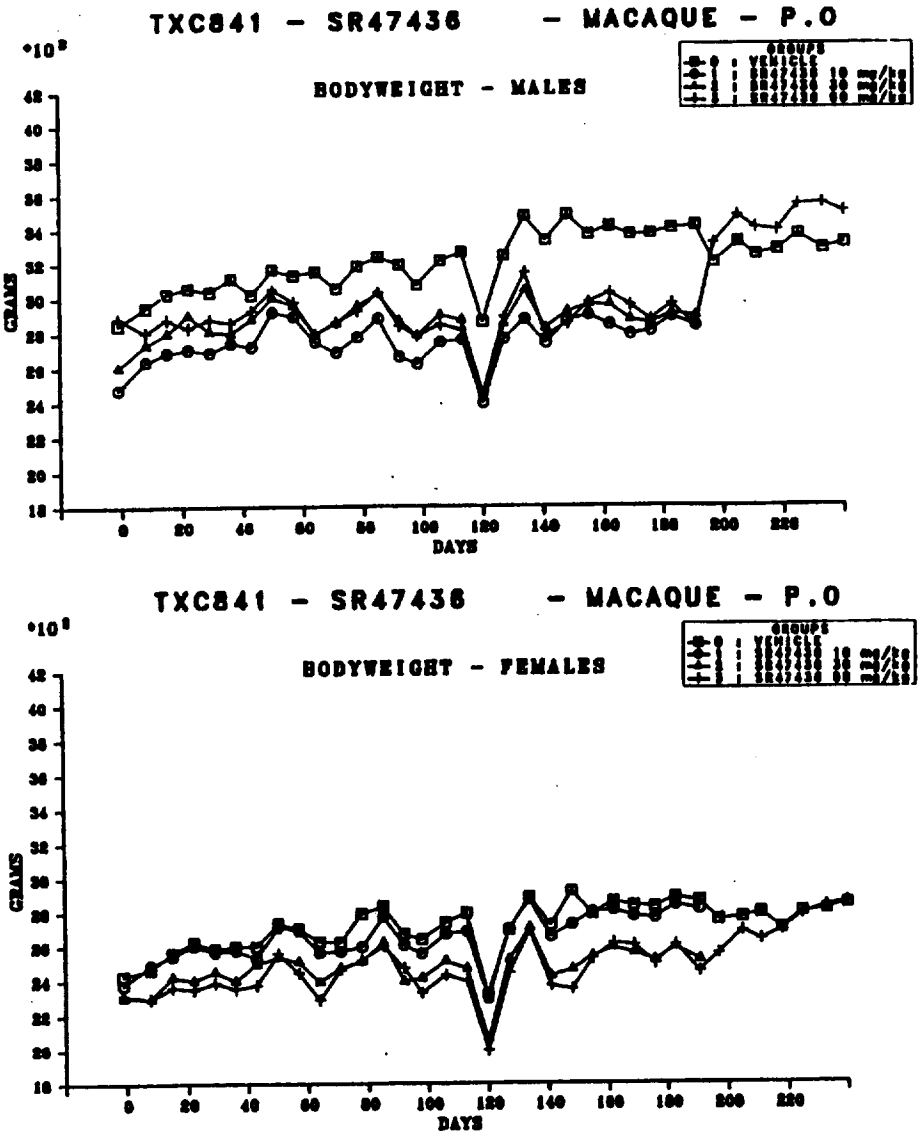

Fig. 3.2.6.1.: 6-month toxicity study of SR 47436. Group mean body weights *vs* treatment period in male (upper panel) and female (lower panel) monkeys.

TABLE 3.2.6.2
SR 47436: MEAN CHANGE IN BODYWEIGHT (%) FOR EACH GROUP (BETWEEN THE BEGINING AND THE END OF TREATMENT)

Dose (mg/kg/d)	Control		10		30		90	
Sex	M	F	M	F	M	F	M	F
Wt. Variations, %	+19	+18	+15	+19	+11	+9	-1**	+6**

** $0.001 < p \leq 0.01$

Dose-related decreases in hemoglobin levels, erythrocyte counts and hematocrit were noted in females receiving 10 or more mg/kg/day and in males at 30 or more mg/kg/day. Differences from control were slight, 10 to 16% at the most, and totally reversible at the end of the recovery period. Minor differences from baseline in clinical chemistry, urinalysis and thyroid hormones were observed in all treated groups and were considered due to individual variation and to have no relation to treatment.

SR 47436 induced a strong dose-related decrease in angiotensinogen plasma levels. Concomitantly, dose-related angiotensin II accumulation was observed with a tendency to increase over time. Furthermore, renin increases were always much higher than angiotensin II increases. Inhibition of RAS, regardless of the mechanism of action (here A-II receptor antagonism), triggers renin release. In addition, inverse correlations were found between angiotensinogen and renin plasma concentrations, and between angiotensinogen and angiotensin II plasma concentrations. On the other hand, a direct correlation was found between angiotensin II and renin plasma levels (Table 3.2.6.3).

TABLE 3.2.6.3
EFFECTS OF SR 47436 ON PLASMA LEVELS OF ANGIOTENSINOGEN (AG), ANGIOTENSIN II (AII) AND ACTIVE RENIN (AR) DURING 6 MONTHS TOXICITY STUDY IN MONKEYS

Dose (mg/kg/day)	AG (μM) 4 months	AII (pg/ml) 4 months	AII (pg/ml) 5.5 months	AR (pg/ml) 4 months	AR (pg/ml) 5.5 months
0	0.49 ± 0.03	10 ± 1	10 ± 2	80 ± 8	88 ± 12
10	0.27 ± 0.02	92 ± 12[a]	122 ±15[ad]	1154 ± 293[a]	1276 ± 212[a]
30	0.15 ± 0.03[a]	163 ± 35[a]	230 ± 6[ad]	5744 ± 2065[ab]	7488 ± 2267[ab]
90	0.06 ± 0.01[ab]	247 ± 40[ab]	289 ± 34[ab]	15824 ± 3250[abc]	18713 ± 391[abc]

a: $p < 0.05$ *vs* control, b: $p < 0.05$ *vs* 10mg/kg/d, c: $p < 0.05$ *vs* 30mg/kg/d, d: $p < 0.05$ *vs* the same group at 4 months.

The high dose male with variations in ECG record showed a marked increase in heart weight (+100%). This animal had marked multifocal fibrous and necrotic myocarditis, localized in the left ventricle and papillary muscles. There were no other organ weight or macroscopic changes in the treated groups that could be attributed to treatment with SR 47436. Dose-related slight to moderate hyperplasia of the juxtaglomerular apparatus was observed in most of the treated animals (Table 3.2.6.4). It was partially reversible as it was less frequent in high dose animals following the

recovery phase of the study. This finding was not associated with changes in plasma or urine biochemistry. Electron microscopic examination of the kidney showed a dose-related increase in the number of juxtaglomerular arterioles showing smooth muscle cells in active phase of renin granule synthesis. This change was still visible at the end of the recovery phase, but to a lower extent. It was absent in all control animals. According to the sponsor, the microscopic changes observed in the kidney are due to an adaptive response to the action of SR 47436 on the RAS.

TABLE 3.2.6.4.
SR 47436: LIGHT MICROSCOPIC EXAMINATION.

Dose (mg/kg/day)	Control				10				30				90			
Sex	M		F		M		F		M		F		M		F	
No of animals at the end of study	5	3[1]	5	3[1]	5		5		5		5		5	3[1]	5	3[1]
Atrophy of the cutaneous fat tissue	-	-	1		2		1		-		2		4	1		
Hyperplasia of the juxtaglom apparatus																
very slight	-	-			1		3		1		2		-	-		
slight	-	-			3		1		4		2		3	2*	2	3*
moderate	-	-			-				-		1		2	-	3	

[1]: animals in the recovery phase, *: without smooth muscle cell hypertrophy nor increased secretory granules.

Unlike in rats, SR 47436 was not detected in plasma samples of control animals. Irrespective of the dosage, Cmax was always observed 2 hr after treatment. Accumulation of the parent compound was indicated since SR 47436 was quantifiable in plasma before and 24 hr after administration on day 190. A marked inter-individual variability in SR 47436 plasma concentration was observed. No inter-sex difference was noted. Cmin, Cmax and $AUC_{(0\text{-}24h)}$ increased with the dose administered but plasma drug levels were not consistent with dose proportionality (Table 3.2.5.5).

TABLE 3.2.5.5
SR 47436 PHARMACOKINETIC PARAMETERS OF TREATED GROUPS ON DAY 190

Dose (mg/kg/d)	tmax (hours) Male	Female	Cmax (mg/l) Male	Female	Cmin (mg/l) Male	Female	AUC (0-24h) (mg.h/l) Males	Females
10	2 ± 0	2 ± 0	0.83 ± 0.34	0.77 ± 0.27	0.09 ± 0.04	0.15 ± 0.07	6.96 ± 0.74	6.92 ± 2.24
30	2 ± 0	2 ± 0	1.22 ± 0.22	0.93 ± 0.35	0.16 ± 0.06	0.18 ± 0.06	12.82 ± 6.07	9.93 ± 3.92
90	2 ± 0	2 ± 0	2.53 ± 0.95	2.14 ± 0.61	0.65 ± 0.49	0.45 ± 0.14	26.12 ± 16.03	20.55 ± 3.34

In summary, monkeys were administered SR 47436 at dosages of 10, 30 and 90 mg/kg/day for 6 months with a 6 week recovery phase. Doses of 10 or more mg/kg/day induced histological renal lesions due to the pharmacological activity of the compound; a slight dose-related toxic effect characterized by decreased weight gain and slight anemia, reversible on cessation of treatment, was noted at 30 or more mg/kg/day.

3.2.7. Six-Month Oral Toxicity Study of SR 47436/HCTZ in Monkeys (Report #RS0042960522/01, Study #94016). Vols. 18-19.

This GLP study was conducted by

for Bristol-Myers Squibb, New Brunswick, NJ between July 18, 1994 and January 17, 1995.

The male and female Cynomolgus monkeys, *Macaca fascicularis* (from) weighed 2.2-4.2 kg, and 2.2-2.9 kg, respectively, and were approximately 3 years of age at the start of the study. Suspensions of SR 47436 (batches 4SNP031, 3SNP006) and HCTZ (batch 48192), alone or in a 1:1 combination in 1% sodium carboxymethylcellulose, were administered orally by naso-gastric intubation (4.5 ml/kg), to five groups of 4 rats/sex/group, once daily for 6 months. Monkeys received SR 47436 in combination with HCTZ at doses of 10/10 and 90/90 mg/kg or SR 47436 or HCTZ alone, at doses of 90 mg/kg daily. The control group received the vehicle at 4.5 ml/kg. Animals were not fasted before treatment and were housed individually in elevated metal grid cages. The doses were selected on the basis of a previous 6-month oral administration study of SR 47436 alone in the same strain of monkey at daily dosages of 10, 30 and 90 mg/kg (see section 3.2.6). In that study, 10 or more mg/kg/day induced renal lesions. In addition, a slight dose-related toxic effect characterized by decreased weight gain and slight anemia, reversible on cessation of treatment, was noted at 30 or more mg/kg/day.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. Ophthalmoscopic examination was performed pretest (day -13), and at termination. Electrocardiography and indirect blood pressure were recorded for all animals before dosing (day -12), and 1 to 2 hr after dosing at 1, 3 and 6 months of treatment. Body weights and food consumption were recorded a week prior to treatment initiation and weekly thereafter for the duration of the study. Blood samples were withdrawn, prior to administration, from the femoral veins of 4 monkeys/sex/group fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected on days -12, 29, 85 and 177. For toxicokinetics study, blood samples were collected as above on day 1, 88 and 180 from 4 animals/sex/group, 1, 2, 4, 8 and 24 hr after administration of test substance(s). Samples from control group were not analyzed. Animals were fed after the 2 hr blood collection. Urinalysis was performed on overnight urine samples collected on days -11, 32, 86 and 179. All surviving animals were killed on day 184/185 (end of treatment). All animals were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology. Table 3.2.7.1 lists the organs and/or tissues examined.

TABLE 3.2.7.1
TISSUES/ORGANS SAMPLED FOR WEIGHT AND HISTOPATHOLOGICAL EXAMINATION

	SAMPLES	OW	LIGHT MIC.
Adrenal glands	2	x	2
Aorta, abdominal	1		1
Bone (sternum/femur)	2		2
Bone marrow (sternum/femur)	2		2
Brain	1	x	1
Epididymides	2		2
Esophagus	1		1
Eyes with optic nerve	2		2
Gall bladder	1		1
Heart	3	x	3
Intestine: cecum, colon, duodenum, ileum, jejunum, rectum	1		1
Kidneys	2	x	2
Liver	2	x	2
Lungs with mainstem bronchii	2		2
Mammary gland	1		1
Lymph nodes (mesenteric, mediastinal)	1		1
Muscle (*Biceps femoris*)	1		1
Ovaries with fallopian tubes	4	x	4
Pancreas	1		1
Pituitary gland	1	x	1
Prostate gland (weighed with seminal vesicles)	1	x	1
Salivary glands (submandibular)	2		2
Sciatic nerve	1		1
Seminal vesicles	1	x	1
Spinal cord (cervical, thoracic, lumbar)	1		3
Spleen	1	x	1
Stomach	3		3
Testes	2	x	2
Thymus	1		1
Thyroid/Parathyroid Glands	4	x	4
Trachea	1		1
Urinary bladder	1		1
Uterine cervix	3		3
Vagina	1		1
Tissues with macroscopic findings including tissues masses	x		x

SAMPLES: the number of samples preserved from each organ or tissue, OW: organ weights- analysis of absolute and relative (organ weight/body weight) values, Light MIC: the number of samples prepared for light microscopic examination, No electron microscopy.

Results

All animals survived to term. There was no variation in ECG records that could be attributed to treatment. Blood pressure values in animals receiving SR 47436, either alone or in combination with HCTZ, were frequently lower than concurrent control control values. In animals receiving the high dose combination, the blood pressure was moderately lower, and in animals receiving the low dose combination it was mild to moderately lower, as compared to control values. The decrease in blood pressure was mild in animals receiving SR 47436 alone and there was no change in blood pressure of animals treated with HCTZ alone. Body weights and body weight gains of males receiving the high dose of SR 47436/HCTZ at the end of 26 weeks of treatment were decreased by 20% and 72%, respectively, as compared to control (Fig 3.2.7.1). However, body weights and body weight gains of all other groups of treated males were comparable to control values. Mean body weight gains also decreased in females receiving SR 47436 alone or in combination with HCTZ (Fig 3.2.7.2)(Table 3.2.7.2). Occasional sporadic decreases in food consumption were observed in all groups of treated females when compared to control values.

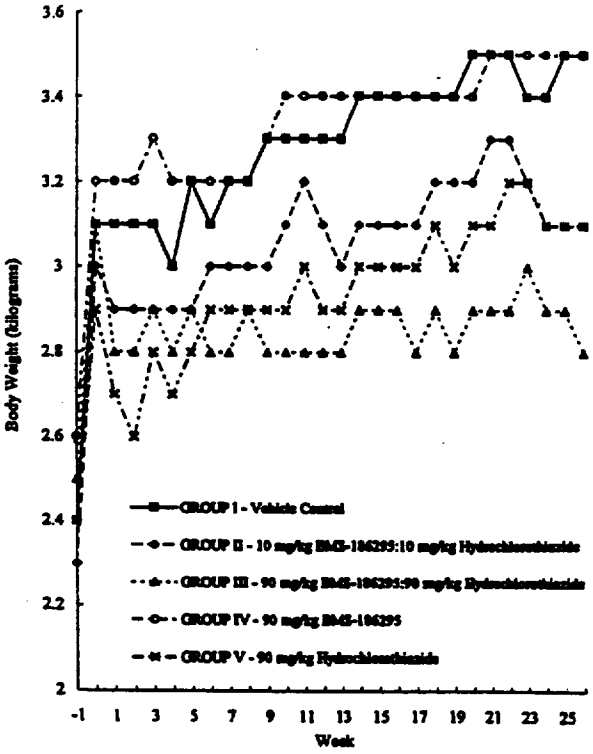

Fig. 3.2.7.1.: 6-month toxicity study of SR 47436/HCTZ. Group mean body weights in male monkeys.

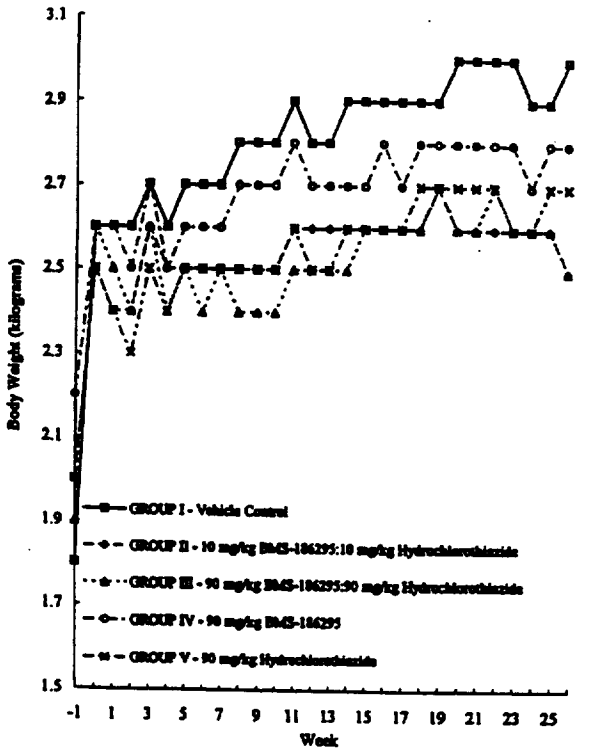

Fig. 3.2.7.2.: 6-month toxicity study of SR 47436/HCTZ. Group mean body weights in female monkeys.

TABLE 3.2.7.2
BODY WEIGHT AND BODY WEIGHT GAIN - GROUP MEAN VALUES (KG) - AT THE END OF THE STUDY

Dose mg/kg/day	Males			Females		
	Initial b.wt.	b.wt. wk 26	b.wt. gain	Initial b.wt.	b.wt. wk 26	b.wt. gain
0	2.4	3.5	1.1	1.8	3.0	1.2
SR/HCTZ, 10/10	2.3	3.1 (-11.4%)*	0.8 (-27%)	2.0	2.5 (-17%)	0.5 (-58%)
SR/HCTZ, 90/90	2.5	2.8 (-20%)	0.3 (-73%)	1.9	2.5 (-17%)	0.6 (-50%)
SR 47436, 90	2.6	3.5 (0 %)	0.9 (-18%)	2.2	2.8 (-7%)	0.6 (-50%)
HCTZ, 90	2.4	3.1 (-11.4%)	0.7 (-36%)	2.0	2.7 (-10%)	0.7 (-42%)

*: % when compared to control (no statistical analyses performed by the sponsor)

Mean hemoblogin, hematocrit and erythrocyte values were mildy to moderately decreased (approximately 8%) compared to control values for male and female high dose combination groups throughout the study ($p < 0.05$ at termination). Significant ($p < 0.5$ to 0.01) increases in mean blood urea nitrogen and creatinine compared to control group were noted for male and female high dose combination groups at all intervals. Mean sodium, potassium, and chloride values were slightly to moderately lower than concurrent control values ($p < 0.5$) throughout the study for all groups dosed with the combination and for the group receiving HCTZ alone (except for sodium). The decreases were not dose-related. Urinalysis did not reveal any adverse treatment-related effects.

At necropsy, absolute and relative heart weights were statistically significantly lower (dose-dependent) than control weights for all treated female groups. In males, the decrease was slight and not statistically significant. Since decreased heart weight has been reported for other antihypertensive agents, including ACE inhibitors and angiotensin II receptor antagonists, the sponsor concludes that the effect represents an expected physiological response to an antihypertensive agent. Additional support for their position comes from normal ECG recordings and the absence of morphological abnormalities of the heart. Dose-related hyperplasia/hypertrophy of the juxtaglomerular apparatus was seen in the kidneys of all monkeys treated with SR 47436. The severity (slight to moderate) was similar in monkeys treated with 90 mg/kg SR 47436 alone and in combination with 90 mg/kg of HCTZ (Table 3.2.7.3). This finding was absent in monkeys receiving HCTZ alone and in the control animals. Electron microscopic examination of the kidney was not done. As noted in earlier studies with SR 47436 in monkeys (see section 3.2.6) and rats (see section 3.2.2), this is an expected finding for this class of drug and is considered to represent a pharmacological response.

TABLE 3.2.7.3.
LIGHT MICROSCOPIC EXAMINATION OF THE KIDNEYS

Dose mg/kg/day	Control		10 SR/10 H		90 SR/90 H		90 SR		90 HCTZ	
Sex	M	F	M	F	M	F	M	F	M	F
No of animals examined	4	4	4	4	4	4	4	4	4	4
hyperplasia of the juxtaglom apparatus										
total	0	0	4	4	4	4	4	4	0	0
minimal	0	0	4	3	0	0	0	0	0	0
slight	0	0	0	1	2	2	2	3	0	0
moderate	0	0	0	0	2	2	2	1	0	0

Toxicokinetic analysis showed that Cmax and AUC for SR 47436 (Table 3.2.7.4) and HCTZ (Table 3.2.7.5) increased as the dose increased, but in a manner less than proportional to the increase in dose. AUC values at the third and sixth months were comparable to those at day 1, suggesting there was no accumulation of either drug with repeated administration. There were no gender-related differences noted with either drug. Exposure to SR 47436 was not affected by co-administration of HCTZ; however, exposure to HCTZ was about 60% greater when it was given in combination with SR 47436 than when it was given alone (Table 3.2.7.5). Regardless of the duration of dosing, Tmax generally occurred within 2 hours.

TABLE 3.2.7.4.
MEAN (SD) TOXICOKINETIC PARAMETERS OF SR 47436 AFTER THE FIRST DOSE ON DAY 1 AND DURING THE THIRD AND SIXTH MONTH IN MONKEYS GIVEN SR 47436 ALONE OR IN COMBINATION WITH HYDROCHLOROTHIAZIDE (TOXICOLOGY STUDY NO. 94016)

Treat-ment¶	Cmax (ng/ml)			Tmax (h)*			$AUC_{(0\text{-}24)}$ (ng.h/ml)		
	Day 1	Month 3	Month 6	Day 1	Month 3	Month 6	Day 1	Month 3	Month 6
SR/HCTZ 10/10, F									
SR/HCTZ 10/10, M	662.07 (245.31)	620.97 (204.04)	645.26 (264.34)	2.00 (1.00,4.00)	1.00 (1.00,1.00)	1.5 (1.00,24.0)	8877.16 (4411.26)	4839.30 (1316.34)	9137.69 (7680.03)
SR/HCTZ 90/90, F									
SR/HCTZ 90/90, M	5854.55§ (3779.55)	6068.83 (8142.60)	3380.01 (985.36)	2.00§ (1.00,2.00)	1.00 (1.00,2.00)	1.00 (1.00,1.00)	52117.40§ (33297.59)	21889.15 (11516.95)	27389.54 (15338.66)
SR 47436 90, F									
SR 47436 90, M	15017.67 (10253.54)	5829.98 (3109.65)	19883.27 (16030.67)	1.50 (1.00,2.00)	1.50 (1.00,2.00)	1.50 (1.00,2.00)	46540.20 (34812.41)	22352.42 (8332.19)	41357.47 (17630.02)

§N=3. One monkey (#3112, high dose male) had unusually high plasma concentrations of SR 47436 on day 1 and was excluded from mean (SD) calculations.

TABLE 3.2.7.5

MEAN (SD) TOXICOKINETIC PARAMETERS OF HCTZ AFTER THE FIRST DOSE ON DAY 1 AND DURING THE THIRD AND SIXTH MONTH IN MONKEYS GIVEN HCTZ ALONE OR IN COMBINATION WITH SR 47436 (TOXICOLOGY STUDY NO. 94016)

Treatment¶	Cmax (ng/ml)			Tmax (h)*			$AUC_{(0-24)}$ (ng.h/ml)		
	Day 1	Month 3	Month 6	Day 1	Month 3	Month 6	Day 1	Month 3	Month 6
SR/HCTZ 10/10 Female	830.51 (172.54)	[illegible] (254.27)	1280.76 [illegible]	3.00 [illegible]	1.50 (1.00,2.00)	2.00 [illegible]	[illegible]	[illegible]	[illegible]
SR/HCTZ 10/10 Male	701.12 (236.80)	903.15 (274.97)	1156.74 (729.59)	2.00 (2.00,4.00)	1.50 (1.00,2.00)	1.50 (1.00,2.00)	5817.17 (1812.59)	4738.72 (2509.07)	7081.90 (2931.49)
SR/HCTZ 90/90 Female	4450.22 (799.82)	4638.21 (1725.85)	7356.03 [illegible]	[illegible]	2.00 (1.00,2.00)	2.00 [illegible]	[illegible]	[illegible]	[illegible]
SR/HCTZ 90/90 Male	3327.83 (882.70)	4439.77 (2629.32)	6718.63 (1906.95)	4.00 (2.00,4.00)	2.00 (2.00,2.00)	2.00 (2.00,4.00)	37412.36 (19609.03)	26065.26 (13614.61)	40612.26 (7888.74)
HCTZ 90 Female	3377.06 (592.35)	2831.82 (258.15)	3911.06 (2035.40)	2.00 (2.00,2.00)	1.50 (1.00,2.00)	2.00 (1.00,2.00)	[illegible]	[illegible]	[illegible]
HCTZ 90 Male	4009.24 (1183.84)	3286.27 (1298.39)	3676.00 (1576.03)	3.00 (1.00,4.00)	2.00 (1.00,2.00)	2.00 (2.00,2.00)	25094.40 (11143.98)	29530.17 (19657.61)	28471.66 (12366.06)

¶SR/HCTZ 10/10 indicates a 1:1 combination of SR 47436/hydrochlorothiazide at 10 mg/kg/day dose level,
SR/HCTZ 90/90 indicates a 1:1 combination of SR 47436/hydrochlorothiazide at 90 mg/kg/day dose level,
SR 90 or HCTZ 90 indicates SR 47436 or hydrochlorothiazide alone at 90 mg/kg/day dose level.
* Median (minimum, maximum)

In summary, monkeys were administered SR 47436/HCTZ at dosages of 10/10 and 90/90 mg/kg/day, and each drug alone at 90 mg/kg/day for 6 months. Decreases in blood pressure and heart weight and hyperplasia/hypertrophy of the juxtaglomerular apparatus were the result of the pharmacological activity, rather than the toxicity, of SR 47436. However, moderate decreases in body weight and body weight gain, mild to moderate anemia, significant increases in mean blood urea nitrogen and creatinine reflect toxic effects of SR 47436.

3.2.8. 52-Week Oral Toxicity Study of SR 47436 in Monkeys (Report #RS0006960507/01, Study #TXC950). Vol. 39-40

This GLP study was conducted by between November 3, 1994 and November 7, 1995.

The male and female Cynomolgus monkeys, *Macaca fascicularis* (from weighed 1.40-2.49 kg, and 1.20-2.25 kg, respectively, and were between 11 and 39 months of age at the start of the study. Suspensions of SR 47436 (batches 4SNP021, 4SNP042,4SNP062, 4SNP063,5SNP515) were prepared in 10% aqueous gum arabic solution and administered orally by gavage (5 ml/kg), once daily for 52 weeks at doses of 20, 100 or 500 mg/kg. Animals were fasted before treatment. Each group consisted of 5 male and 5 female monkeys. The animals were housed in groups of two or three of same sex in twin or triple cages. The doses were selected on the basis of previous studies performed on the same strain of monkeys (however, these studies were not conducted at this laboratory). A one month toxicity study (see section 3.2.5) performed at dosages of 250, 500 and 1000 mg/kg/day revealed myocardial fibrosis, anemia and hyperplasia of the juxtaglomerular apparatus at all dosages and a general deterioration in the health of animals that received 1000 mg/kg/day. A six month study (section 3.2.6) performed at dosages of 10, 30 and 90 mg/kg/day revealed a dosage-related decrease in erythrocytic parameters and hyperplasia of the juxtaglomerular apparatus at all dosages and a dosage-related decrease in body weight gain in animals that received 30 or more mg/kg/day.

Observations and Measurements

All animals were observed at intervals throughout the working day for mortality and drug effects. Electrocardiography was performed on all animals before dosing (week -1), and 2 and 24 hr after dosing during weeks 2, 13, 26 and 52. At the same times, indirect blood pressure (and pulse rate) was measured for each animal, using a pressure cuff secured around the base of the tail. Body weight and food consumption were recorded at weekly intervals before and during the treatment period. Ophthalmic examination was done a week before and during weeks 12, 25, 38 and 51 of treatment. Blood samples were withdrawn, prior to administration, from the femoral vein of all animals fasted overnight. Hematology and clinical chemistry parameters were examined from blood samples collected during weeks -1, 13, 25, 39 and 51 of treatment (before dosing on each occasion). Urinalysis was performed on all animals, on overnight urine samples, under conditions of food and water deprivation, 13 days before commencement of treatment and during weeks 12, 25, 38 and 51 of treatment. For toxicokinetics study, blood samples were collected from 3 animals/sex/group, during weeks 4, 27 and 52, at 2 and 24 hr after administration of SR 47436. Terminal necropsies commenced on day 1 of week 53 and were completed on day 6 of week 53. All animals killed and animals that died were subjected to a complete necropsy that included external examination and individual examination of several organs followed by histopathology.

The following organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a

percentage of the bodyweight recorded immediately before necropsy.

Adrenals
Brain
Epididymides
Heart
Kidneys
Liver
Lungs
Ovaries
Pituitary
Prostate with urethra sample
Seminal vesicles
Spleen
Testes
Thymus
Thyroid with parathyroids
Uterus with cervix

Samples of the following tissues were preserved in 4% neutral buffered formaldehyde, except eyes and optic nerves which were placed in Davidson's fluid and subsequently retained in 70% industrial methylated spirit, and testes and epididymides which were initially preserved in Bouin's fluid.

Adrenals
Aorta, thoracic
Brain
Bronchi
Cecum
Colon
Duodenum
Epididymides
Esophagus
Eyes and optic nerves
Femoral bone
Gall bladder
Heart
Ileum
Jejunum
Kidneys
Liver
Lungs
Lymph nodes -
-submandibular
-mesenteric
-bronchial
Mammary glands
Ovaries
Pancreas
Pituitary
Prostate with urethra
Rectum
Salivary gland -
submandibular
Sciatic nerve, left
Seminal vesicles
Skeletal muscle-thigh, left
Skin
Spinal cord
Spleen
Stomach -fundus
-pylorus
Testes
Thymus
Thyroid with
parathyroids
Tongue
Trachea
Urinary bladder
Uterus with cervix
Vagina

Microscopic examination was performed on the tissue sections listed above, taken from all animals. Findings were reported as "present" or assigned a severity grade. Ultra-thin sections of the samples of kidney (cortex and medulla) taken from all animals were prepared and examined by electron microscopy.

Results

One male receiving 100 mg/kg/day died during week 16 of treatment. A day before, it was noted to be hunched, underactive and reluctant to use its right arm. The following morning it was observed to be underactive and prostrate, with labored respiration, dilated pupils and appeared to have mild convulsion and stopped breathing. Macroscopic findings at necropsy comprised multiple fibrous adhesions between the heart, lungs, thymus and musculature of the rib cage. Histopathology revealed a diffuse pericarditis and pleuritis with patchy pneumonia. The sponsor considers this death to be incidental and unrelated to treatment with test substance. There were no other deaths in the study.

Treatment-related signs associated with the dosing procedure were restricted to salivation, observed throughout the study from week 20 of treatment in high dose animals, a higher incidence in females than in males. The salivation was probably due to the taste of the test substance which is known to have an irritant effect on the GIT and the tongue when administered at high concentrations. Tail lesions in 4 high dose males started with abrasions/encrustations, followed by swellings or sores at the tip of the tail and progressed to ulceration and necrosis of the tip. Occasional swellings and abrasions were noted on the tails of some of the high dose females. The tissue damage seen on the tails was possibly related to the low blood pressure and the amount of blood reaching the tip of the tail was insufficient for cell survival. ECG recordings did not reveal any disturbances that could be attributed to treatment with SR 47436. In mid and high dose group animals, blood pressure significantly decreased throughout the dosing period. The effect was more pronounced at 2 than at 24 hr after dosing and tended to be time and dose-related (Table 3.2.8.1).

TABLE 3.2.8.1.
EFFECT OF SR 47436 ON INDIRECT BLOOD PRESSURE IN 52-WEEK ORAL TOXICITY STUDY IN MONKEYS (GROUP MEAN VALUES)

Sex		Male				Female			
Dose mg/kg/day		Ctl	20	100	500	Ctl	20	100	500
Week -1	-	145	130	130[a]	125[a]	135	130	130	135
Week 2	2hr	120	115	115	110	135	125	110	110
	24hr	125	120	125	115	145	125	110	105
Week 13	2hr	135	110[b]	105[b]	85[c]	140	110	105	95
	24hr	135	125	105[b]	105[b]	125	115	105	105
Week 26	2hr	125	120	100[c]	90[c]	130	120	105	90
	24hr	130	125	105[a]	95[b]	140	135	120	105
Week 52	2hr	125	110	85[c]	90[c]	120	115	105	85
	24hr	120	115	90[c]	85[c]	115	110	105	90

Significant when compared with control: [a]- P <0.05; [b]- P <0.01; [c]- p <0.001.

The overall bodyweight gain of females was dose-dependently low with the difference from control reaching statistical significance at 100 or more mg/kg/day. In the case of males, the bodyweight gain was dose-dependently low at 100 or more mg/kg/day but statistically significant only at 500 mg/kg/day. The mean body weight gains of the rest of the treated groups were similar to that of the control (Fig. 3.2.8.1). The overall quantity of food consumed by high dose group males was marginally low, but not statistically significantly lower than that of the control group.

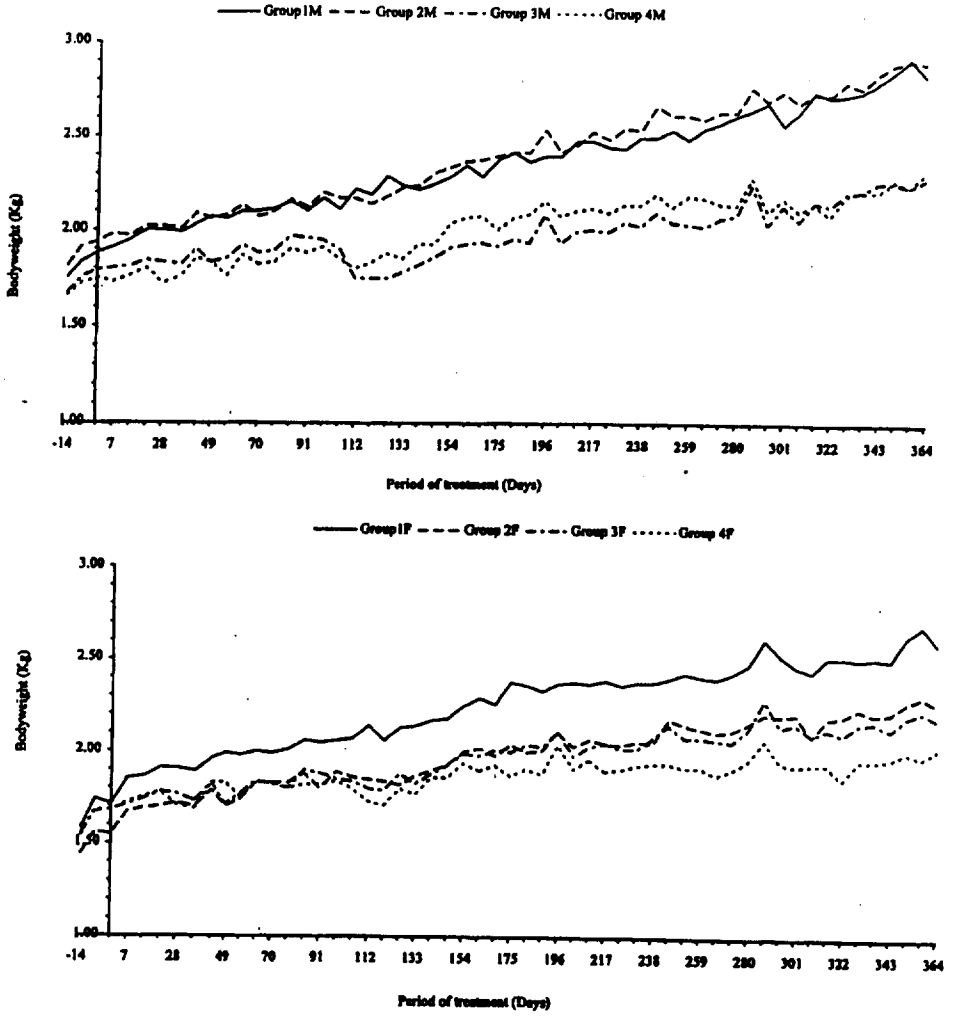

Fig. 3.2.8.1.: 52-week toxicity study of SR 47436. Group mean body weights *versus* treatment period in male (upper) and female (lower panel) monkeys.

As in previous studies with monkeys, all animals in the high dosage group (500 mg/kg/day) exhibited moderate anemia characterized by lower packed cell volumes, hemoglobin concentrations and erythrocyte counts at all times of measurment. Slight decreases in erythrocyte counts or hemoglobin concentrations, or both, were also observed in females receiving 100 mg/kg/day at weeks 13, 39 and 51 of treatment (Table 3.2.8.2). Statistically significant increases in blood urea and creatinine levels were noted for high dose animals and tended to be time-related. A moderate but significant increase in creatinine concentration was also observed in females receiving 100 mg/kg/day during weeks 13 and 25 of treatment. These changes were attributed to microscopic changes recorded in the kidneys (see below). Significantly low phosphorus levels were noted in high dose animals during weeks 13, 25 (in females only), 39 and 51 of treatment. Additionally, higher calcium concentrations ($p < 0.05$) were observed in females receiving 500 mg/kg/day during weeks 25, 39 and 51 of treatment and in males at this dosage

TABLE 3.2.8.2.
SR 47436: NOTABLE CHANGES IN HEMATOLOGY PARAMETERS

Sex		Male				Female			
Dose (mg/kg/day)		Ctl	20	100	500	Ctl	20	100	500
Packed cell volume	w -1	0.38	0.39	0.40	0.39	0.40	0.40	0.41	0.39
	w 13	0.40	0.40	0.37[a]	0.37[a]	0.41	0.39	0.38[a]	0.36[b]
	w 25	0.44	0.42	0.43	0.39[a]	0.44	0.43	0.42	0.39[a]
	w 39	0.43	0.41	0.41	0.37[a]	0.43	0.41	0.40	0.39[a]
	w 51	0.44	0.43	0.42	0.37[b]	0.45	0.43	0.42	0.39[b]
Hemoglobin (gm/dl)	w -1	10.9	11.6	11.3	11.1	11.3	11.2	11.5	11.3
	w 13	12.0	12.0	11.2	10.9[a]	12.3	11.7	11.3[a]	11.2[b]
	w 26	12.9	12.6	12.4	11.4[b]	12.9	12.7	12.2	11.4[a]
	w 39	12.6	12.2	12.0	10.8[b]	12.6	12.1	11.8	11.5[a]
	w 51	12.8	12.6	12.2	10.3[c]	13.0	12.4	11.9[a]	11.3[b]
RBC ($x10^6/mm^3$)	w -1	6.01	6.24	6.09	5.77	6.15	6.03	5.97	6.07
	w 13	6.75	6.65	6.05	5.71[b]	6.71	6.40	5.92[b]	6.06[a]
	w 25	7.25	6.96	6.96	5.88[c]	7.05	7.03	6.46	6.24[a]
	w 39	7.05	6.71	6.64	5.57[b]	6.88	6.57	6.06[a]	6.26[a]
	w 51	7.12	6.97	6.68	5.44[c]	7.06	6.74	6.19[a]	6.12[a]
Mean cell hemoglobin conc. (gm/dl)	w -1	29.0	29.0	29.0	28.0	28.0	28.0	28.0	28.9
	w 13	29.8	30.4	3o.7	29.6	29.8	30.0	29.9	30.9[a]
	w 25	29.5	29.7	28.8	28.9	29.4	29.2	28.8	29.2
	w 39	29.7	30.0	29.5	28.8[a]	28.5	29.0	28.5	29.1
	w 51	29.2	29.1	28.8	28.0[b]	29.2	29.8	29.6	29.5
Mean cell volume (fl)	w -1	64.0	63.0	65.0	68.0[a]	65.0	67.0	69.0	64.0
	w 13	59.8	59.7	60.6	64.8[a]	61.2	6.1	64.1	60.1
	w 25	60.4	61.0	61.8	66.9[b]	62.0	62.0	65.8	62.6
	w 39	60.5	60.8	61.2	67.4[c]	62.6	61.8	66.0	62.2
	w 51	61.6	62.4	63.3	67.7[b]	64.5	63.9	67.5	63.7

Significant when compared with group 1: [a]- $P < 0.05$; [b]- $P < 0.01$; [c]- $p < 0.001$.

TABLE 3.2.8.3.
SR 47436: NOTABLE CHANGES IN CLINICAL CHEMISTRY PARAMETERS

Sex		Male				Female			
Dose (mg/kg/day)		Ctl	20	100	500	Ctl	20	100	500
Urea (mmol/l)	w -1	7.7	6.7	5.5	5.1	6.8	6.7	5.6	6.6
	w 13	8.6	7.2	7.7	10.9	7.2	7.1	8.2	9.1
	w 25	6.2	5.8	6.9	12.7[a]	5.6	5.2	7.0	11.2
	w 39	5.8	6.3	8.9	12.0[b]	5.6	5.6	8.6	11.9
	w 51	5.0	5.8	7.4	19.4[c]	5.8	5.3	7.5	16.8
Creatinine (µmol/l)	w -1	63	62	62	60	62	67	67	67
	w 13	66	60	63	70	62	60	73[a]	72
	w 25	72	69	74	82[a]	65	69	75[b]	82
	w 39	68	63	71	87[c]	67	63	70	78
	w 51	75	70	75	116[a]	78	72	79	95
Phosphorus (mmol/l)	w -1	2.50	2.20	2.40	2.10	2.70	2.50	2.60	2.50
	w 13	2.48	2.23	2.30	1.75[c]	2.50	2.43	2.34	1.99
	w 25	2.4	2.49	2.36	2.29	2.30	2.26	2.21	1.99
	w 39	2.32	2.35	2.35	1.85[c]	2.19	2.19	2.03	1.71
	w 51	2.09	2.17	2.11	1.41[c]	2.01	1.99	2.07	1.36
Calcium (mmol/l)	w -1	2.50	2.60	2.50	2.60	2.50	2.60	2.70	2.70
	w 13	2.48	2.35[a]	2.34[a]	2.49	2.44	2.43	2.49	2.52
	w 25	2.43	2.41	2.44	2.56	2.37	2.50	2.50	2.63
	w 39	2.47	2.40	2.34	2.56	2.41	2.46	2.45	2.59
	w 51	2.38	2.38	2.42	2.76[a]	2.48	2.48	2.39	2.83

Significant when compared with group control: [a]- P <0.05; [b]- P <0.01; [c]- p <0.001.

during week 51 of treatment only (Table 3.2.8.3). There were no treatment-related changes in the urinalysis.

Dose-related decreases ($p < 0.05$) in absolute and relative heart weights were observed at mid and high doses in females. In males, the absolute decrease in heart weight was significant for the high dose group only. However, absence of any microscopic changes suggest that this finding probably has no toxicological significance. Statistically, a significant dose-related increase in relative (but not absolute) kidney weight was noted for females at 100 or more mg/kg/day and for males given 500 mg/kg/day. A low absolute and relative thymus weight (neither statistically significant) in high dose animals may reflect a response to stress.

Macropathological examination at necropsy indicated abrasions, swelling and/or encrustations on the stumps of the tails of all but one of the males receiving 500 mg/kg/day. According to the sponsor, the tissue damage seen on the tails was considered to be directly related to the low blood pressure, i.e., the amount of blood reaching the tip of the tail was insufficient for cell survival and consequently the tails became necrotic. Dark mesenteric lymph nodes were noted in three males receiving 500 mg/kg/day and in a male and a female receiving 20 mg/kg/day. As in previous toxicity studies with monkeys, hyperplasia/hypertrophy of the juxtaglomerular apparatus was noted in all mid and high dose animals and in 2 each/sex in low dose groups. The following histopathological changes in the kidneys were observed (Table 3.2.8.4).

TABLE 3.2.8.4
HISTOPATHOLOGICAL EXAMINATION OF THE KIDNEYS

Dose (mg/kg/day)	Control		20		100		500	
	M		M		M		M	
Number examined	5		5		5		5	
No. with focal cortical lymphocytic infiltrate	1		4		3		4	
No. with tubular dilatation	0		0		1		3	
No. with interstitial inflammation	0		0		0		2	
No. with cortical mineral deposits	0		0		0		1	
No. with tubular epithelial brown pigment deposition	0		0		0		1	
No. with hypertrophy/hyperplasia of juxtaglomerular apparatus	0		2		5[b]		5[b]	
No. with papillary mineralization	0		0		1		0	

b: significant when compared with control, $p < 0.01$

The higher plasma urea and creatinine concentrations, low phosphorus and high calcium concentrations and increase in kidney weights were considered to be related to microscopic changes

observed in the kidneys. Hypertrophy/hyperplasia of the juxtaglomerular apparatus in the kidney is considered to be due to an adaptive response to the action of SR 47436 on the RAS.

SR 47436 was not detected in plasma samples of control animals. Plasma concentrations at 2 hr after dosing increased with the dose, while concentrations at 24 hr were similar at 100 and 500 mg/kg/day. The results suggest that animals were under a relatively constant exposure to the drug during the treatment period. A marked inter-individual variability in SR 47436 plasma concentration was observed. No inter-sex difference was noted (Table 3.2.8.5).

TABLE 3.2.8.5
SR 47436 PLASMA CONCENTRATIONS (MEAN ± SD) AT 2 AND 24 HR OF DOSING

Sampling period	Dose mg/kg/d	C2h (mg/l)		C24h (mg/l)	
		Males	Females	Males	Females
Week 4	20	0.66 ± 0.32	0.57 ± 0.19	0.08 ± 0.03	0.10 ± 0.04
	100	1.35 ± 0.10	3.22 ± 1.58	0.41 ± 0.07	0.24 ± 0.08
	500	35.80 ± 34.27	11.09 ± 14.05	0.58 ± 0.01	0.67 ± 0.20
Week 27	20	0.68 ± 0.34	0.56 ± 0.16	0.09 ± 0.04	0.10 ± 0.05
	100	1.80 ± 1.10	1.37 ± 0.29	0.53 ± 0.26	0.16 ± 0.10
	500	19.59 ± 15.09	2.50 ± 0.22	0.38 ± 0.05	0.15 ± 0.02
Week 52	20	0.98 ± 0.52	0.83 ± 0.19	0.15 ± 0.09	0.08 ± 0.03
	100	1.87 ± 0.76	1.98 ± 0.52	0.44 ± 0.14	0.22 ± 0.07
	500	21.59 ± 26.33	6.65 ± 28.52	1.68 ± 1.28	0.50 ± 0.46

In summary, oral administration of SR 47436 to monkeys at doses of 20, 100 and 500 mg/kg/day for 52 weeks was associated with a dose-dependent decrease in body weight gain in mid and high dose animals. Mild anemia and low heart weight observed in mid and high dosage groups is thought to be related to the pharmacological action of drugs of this class and is normally observed in studies with ACE inhibitors. Microscopic examination revealed hyperplasia/hypertrophy of the juxtaglomerular apparatus in all treated groups, which may have triggered higher plasma urea and creatinine concentrations, low phosphorus and high calcium concentrations. These changes are considered to be due to an adaptive response to the action of SR 47436 on the RAS. Thus, most of the changes observed in this study are considered to be due to the pharmacological activity of the drug.

3.3. *Special Toxicity Studies*

3.3.1. Evaluation of Phototoxicity and /or Photoallergy Effects of SR 47436 in the guinea pig. (Report #RS0006920120/01, Study #PH0100). Vol. 69

This GLP study was conducted by the Department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between November 14, 1991 and January 9, 1992. The aim of the study was to evaluate the potential photosensitizng effect of SR 47436 in the guinea pig. The experiment is based upon the notion of minimal erythema dose (MED), which is the smallest quantity of sun energy inducing a perceptible erythema in man or animal. The MED method is well adapted to phototoxicity and photoallergy studies since a conspicuous photoincrease in erythema appears when the compound is phototoxic and/or photoallergic.

Experiments consisted of two treatment and irradiation phases separated by a treatment-free interval of approximately 3 weeks. The first phase evaluated phototoxicity and induction of photoallergy (induction phase). Two groups of ten male Hartley guinea pigs (10 weeks old, 500 g) received either 100 mg/kg SR 47436 or vehicle (10% gum arabic solution) by gavage on 6 (days 1,2, 5, 6, 7, 8) out of 8 consecutive days. One hour after dosing, an area of depilated skin was exposed to light in the total solar spectrum followed by light in the UVA and UVB spectra. The amount of irradiation was selcted to deliver a MED. A contralateral area of depilated skin was unexposed to irradiation and served as a control measure. Twentyfour hours after irradiation, erythema, cutaneous eruption, edema, and hyperkeratosis were rated to determine phototoxicity.

The same group of guinea pigs were used for the second phase of the treatment. Both groups of guinea pigs received single oral doses of 100 mg/kg SR 47436. Areas of depilated skin were exposed to irradiation in the UVA and UVB spectra at separate sites. Observations (erythema and edema) were made 24 and 48 hr postirradiation and responses were graded. A second photoallergy test was performed after a 2 week treatment-free interval. Cutaneous responses to irradiation were rated to evaluate the development of photoallergy.

No evidence of phototoxicity or photoallergy was noted in either period for guinea pigs given 100 mg/kg SR 47436 orally.

3.4. *Carcinogenicity Studies*

3.4.1. 13-Week Oral Range-Finding Toxicity Study of SR 47436 in Mice (Report #RS0006930928/01, Study #DDO505), Vol. 47

This GLP study was conducted by for Sanofi Recherche, France between March 23, 1993 and June 28, 1993. This study was conducted to aid in the selection of dosages for an oncogenicity study in this species.

Male and female Swiss CD-1 mice (from Charles River) were approximately 21 to 28 days of age and weighed 24-35 g (males) or 20-26 g (females) at the start of the study. SR 47436 (batch 92.03) was administered orally by gavage in a 10% aqueous suspension of gum arabic (5 ml/kg), once daily for 13 weeks at doses of 15, 50, 150 or 500 mg/kg. Animals were not fasted before treatment. Control animals received the vehicle. Each group consisted of 26 male and 26 female mice. The animals were housed 3 to 4 of same sex per cage.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded a week before treatment, on day 1 of treatment, then weekly throughout the treatment period. Food was supplied to each cage and that remaining was recorded for each week throughout the treatment period. From these records, the mean weekly consumption per animal was calculated for each cage. Blood samples were withdrawn from the retro-orbital sinus of all animals under anesthesia. Animals were not fasted prior to sampling. Hematology parameters were examined from blood samples collected in week 13 of treatment (before dosing) from 6 male and 6 female animals with the highest animal numbers in each group. For toxicokinetics study, blood samples were collected in week 13 from 20 mice/sex/group (with the lowest animal numbers in each group) at 1, 2, 4, 8 and 24 hr after administration of SR 47436/vehicle. Clinical blood chemistry and urinalysis were not performed. All animals were subjected to a detailed necropsy. The following organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy.

Brain
Heart
Kidneys
Liver
Lungs with mainstem bronchi
Pituitary
Seminal vesicles
Spleen
Testes
Uterus with cervix

Samples of the following tissues from all animals were preserved in 4% neutral buffered formaldehyde, except eyes, optic nerves and Harderian glands which were placed in Davidson's fluid. Bone marrow smears, taken from all animals killed were air-dried and fixed in methanol. Samples of any abnormal tissues were also retained.

Adrenals
Aorta
Brain
Cecum
Colon
Duodenum
Epididymides
Eesophagus
Eyes and optic nerves
Femoral bone
Gall bladder
Harderian glands
Heart
Ileum
Jejunum
Kidneys
Liver
Lungs with mainstem bronchi
Lymph nodes -mandibular, -mesenteric
Mammary glands - caudal, -cranial
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary gland -submandibular,
Sciatic nerves
Seminal vesicles.
Skeletal muscle -thigh
Skin
Spinal cord
Spleen
Sternum
Stomach - ketatinised, -glandular
Testes
Thymus
Thyroid with parathyroids
Tongue
Trachea
Urinary bladder
Uterus with cervix
Vagina

Results

There were no deaths and there were no clinical signs attributed to the treatment. Mean body weight gain and food consumption were not affected by treatment and although blood sampled after 13 weeks of dosing showed a number of minor inter-group differences in hematological values, these were not considered treatment-related as they were confined to one sex or lacked a dose-response relationship. At necropsy, the mean absolute and relative heart weights of both male and female groups were lower than control, reaching significance only in males given 150 mg/kg/day ($P \leq 0.05$) but in all treated females ($P \leq 0.01$) (Table 3.4.1.1). The mean liver weight was slightly higher (8-20%, $p<0.05$) in males at 150 and 500 mg/kg/day (relative but not absolute weight at the lower dose) and in high dose females, and the mean kidney weight (both absolute and relative) was slightly decreased (10%, $p<0.05$) in high dose males (Table 3.4.1.1). There were no changes in the weights of other organs of treated animals compared to that of control. Macroscopic examinations did not show any treatment-related differences and microscopic examinations were not performed.

SR 47436 was not detected in plasma samples of control mice. For animals receiving SR 47436, irrespective of the sex, Cmax was observed 1 hr after treatment and Cmin was equal to 0 mg/l. Both Cmax and AUC values increased with the dose but lacked dose-proportionality. Further, both Cmax and AUC values at all dose levels were higher for females than for males (Table 3.4.1.2).

TABLE 3.4.1.1
ORGAN WEIGHT VARIATION IN THE 13-WEEK DOSE-RANGE FINDING STUDY IN MICE
GROUP MEAN VALUES (G) AT THE END OF TREATMENT

Dose, mg/kg/day		Control		15		50		150		500	
Sex		M	F	M	F	M	F	M	F	M	F
Body weight, g		39.8	30.9	37.6	29.5	40.3	30.0	39.1	28.0[a]	39.4	30.7
Heart	Abs wt.	0.235	0.199	0.224	0.175[b]	0.226	0.175[b]	0.208[a]	0.166[b]	0.212	0.159[b]
	Rel wt.	0.596	0.652	0.597	0.596	0.561	0.586[a]	0.534[a]	0.598	0.540	0.524[b]
Kidney	Abs wt.	0.605	0.382	0.574	0.379	0.605	0.377	0.566	0.356	0.545[a]	0.374
	Rel wt.	1.532	1.252	1.531	1.292	1.503	1.263	1.450	1.280	1.384[a]	1.227
Liver	Abs wt.	2.07	1.66	1.97	1.58	2.13	1.61	2.19	1.56	2.51[b]	2.10[b]
	Rel wt.	5.207	5.354	5.233	5.351	5.262	5.365	5.606[a]	5.596	6.354[b]	6.849[b]

Significant when compared with control: a, P <0.05; b, P <0.01.

TABLE 3.4.1.2
SR 47436 PHARMACOKINETIC PARAMETERS DETERMINED IN WEEK 13
(24-hour sampling time also used as pre-dose (0-hour) sampling time)

Doses	t_{max} (h)		C_{max} (mg/l)		C_{min} (mg/l)		$AUC_{(0\text{-}24h)}$ (mg•h/l)	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
15	-	1	UV	0.32	< LOD	< LOD	-	0.41
50	1	1	0.14	0.29	< LOD	< LOD	0.32	0.69
150	1	1	3.86	21.95	< LOD	< LOD	5.00	24.57
500	1	1	13.73	38.03	< LOD	< LOD	20.04	93.70

UV : unexpected value (8.1 mg/l); - : not reported; LOD : below limit of detection (0.02mg/l)

In summary, although the test material appeared to be well absorbed at all dosage levels, no clear evidence of toxicity of SR 47436 was elicited.. Thus, evidence of a maximum tolerated dosage (MTD) in CD-1 mice was not attained.

3.4.2. 13-Week Oral Range Finding Toxicity Study of SR 47436 in Mice (Second Study) (Report #RS0006931220/01, Study #DDO522). Vol. 48

This GLP study was conducted by) for Sanofi Recherche, France between August 2, 1993 and November 2, 1993. Since the first dose-range finding study (section 3.4.1) failed to identify an MTD, the sponsor repeated the study with higher dosages of SR 47436 to aid in the selection of dosages for an oncogenicity study in mice.

Male and female Swiss CD-1 mice (from Charles River) were approximately 21 to 28 days of age and weighed 24-31 g (males) or 19-26 g (females) at the start of the study. Suspensions of SR 47436 (batches 92.03 and 93.05) prepared in a 10% aqueous solution of gum arabic were administered orally by gavage (10 ml/kg), once daily for 13 weeks at doses of 1000 or 2000 mg/kg; animals were not fasted before treatment. Control animals received the vehicle, 10% aqueous solution of gum arabic. Each group consisted of 20 male and 20 female mice. The animals were housed 4 of same sex per cage.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded a week before treatment, on day 1 of treatment, then weekly throughout the treatment period. Food was supplied to each cage and that remaining was recorded for each week throughout the treatment period. Water consumption was recorded during weeks 8 and 13 of treatment for each cage of animals. From these records, the mean weekly consumption per animal was calculated for each cage. Plasma concentrations of SR 47436 were determined in week 13, in blood samples collected from the retro-orbital sinus of all mice (under anesthesia) at 1, 2, 4, 8 and 24 hr after administration of SR 47436/vehicle. Immediately after blood collection, all animals were killed and subjected to a detailed necropsy. The following organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy.

Brain
Heart
Kidneys
Liver
Lungs with mainstem bronchi
Seminal vesicles
Spleen
Testes
Uterus with cervix

Samples of the following tissues were preserved in 4% neutral buffered formaldehyde, except eyes, optic nerves and Harderian glands which were placed in Davidson's fluid. Bone marrow smears, taken from all animals killed were air-dried and fixed in methanol. Samples of of any abnormal tissues were also retained. Microscopic examination was performed on all these tissues for all animals killed or dying during the study. For surviving animals, microscopic examination was performed only on the thymus, spleen, liver and kidneys for 10 males and 10 females from the control group and from the 1000 mg/kg/day group at study termination. Findings were reported as

"present" or assigned a severity grade.

Adrenals
Aorta
Brain
Cecum
Colon
Duodenum
Epididymides
Esophagus
Eyes and optic nerves
Femoral bone
Gall bladder
Harderian glands
Heart
Ileum
Jejunum
Kidneys
Liver
Lungs with mainstem bronchi
Lymph nodes -mandibular, -mesenteric
Mammary glands - caudal, -cranial
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary gland -submandibular,
Sciatic nerves
Seminal vesicles
Skeletal muscle -thigh
Skin
Spinal cord
Spleen
Sternum
Stomach - keratinised, -glandular
Testes
Thymus
Thyroid with parathyroids
Tongue
Trachea
Urinary bladder
Uterus with cervix
Vagina

Results

The dose of 2000 mg/kg resulted in the deaths of 3 males (weeks 5, 12 and 13) and 7 females (weeks 4, 6, 7, 8, 9, 11 and 13). One additional high dose male in week 5 and one female control in week 8 died due to misdosing. Histological examination of these high dose animals revealed acute tubular necrosis of kidneys, focal necrosis of the liver and single cell necrosis of the spleen and thymus (Table 3.4.2.1). No significant treatment-related changes were observed in any other organs or tissues of animals found dead. Analysis of organ weights from decedent animals that received 2000 mg/kg/day revealed lower heart weights and liver weights than expected, and also slightly reduced seminal vesicle weights for males. Signs of reaction to treatment included thinness, underactivity, salivation, reduced body temperature, piloerection, partially closed eyes, hunched posture and/or abnormal breathing. Most of these signs were observed during weeks 11 through termination in 4 high dose males and 2 high dose females.

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TABLE 3.4.2.1
MORTALITY AND CAUSE OF DEATH

Dose (mg/kg/d)	Sex	N	Wk of death	Cause of death (and major lesions)
0	M	0/20	-	No deaths
	F	1/20	8	1: accidental (esophagus perforation)
1000	M	0/20	-	No deaths
	F	0/20	-	No deaths
2000	M	4/20	5	1: accidental (esophagus perforation)
				1: undetermined (no lesion could account for death)
			12	1: possibly drug related (acute renal tubular necrosis)
			13	1: possibly drug related (pale materials in GIT and gas in the duodenum, single cell necrosis of the spleen and thymus, hyperplastic JG apparatus)
	F	7/20	4	1: possibly drug related (acute renal tubular necrosis, single cell necrosis of the thymus)
			6	1: possibly drug related (acute renal tubular necrosis, single cell necrosis of the spleen and thymus)
			7	1: possibly drug related (acute renal tubular necrosis, abnormal pale material in the GIT, interstitial pneumonitis, single cell necrosis of the spleen)
			8	1: possibly drug related (pale material in the GIT, hyperplastic JG apparatus)
			9	1: possibly drug related (acute renal tubular necrosis, pale material in the GIT, interstitial pneumonitis, single cell necrosis of the spleen)
			11	1: possibly drug related (focal liver necrosis, single cell necrosis of the spleen abnormal pale material in the GIT, hyperplastic JG apparatus)
			13	1: possibly drug related (acute renal tubular necrosis, focal liver necrosis)

Mean body weight gains were lower in all treated groups when compared to control but differences attained statistical significance ($p \leq 0.05$) only in treated males (Table 3.4.2.2). The food intake of control and treated animals was generally similar. A dosage-related increased water intake was noted for all treated groups during weeks 8 and 13 of treatment; the effect was greater in females than in males.

TABLE 3.4.2.2
BODY WEIGHT CHANGES IN THE 13-WEEK DOSE-RANGE FINDING STUDY
GROUP MEAN VALUES (G), STANDARD DEVIATION

Dose, mg/kg/day	Control		1000		2000	
Sex	M	F	M	F	M	F
Initial bodyweight	27.5 ± 1.65	22.1 ± 1.67	26.8 ± 2.05	21.7 ± 1.11	26.9 ± 2.05	22.1 ± 1.15
Bodyweight on W13	41.3 ± 3.60	33.1 ± 4.45	38.5 ± 3.91	30.6 ± 2.92	38.2 ± 3.18	32.9 ± 2.30
Average % difference from control			-6.8%	-7.5%	- 7.5 %	- 0.6 %
Body wt. gain*, W 0-13	13.9 ± 2.65	11.1 ± 3.88	11.7 ± 2.97[a]	8.9 ± 2.80	11.6 ± 2.89[a]	10.9 ± 2.21

* of survivors at study termination. Significant when compared with control: a, $p<0.05$

At termination, significantly lower heart weights (10-20%) and higher liver weights (50 to 95% with dose-related effect) in all treated groups and lower seminal vesicle weight (15 to 30%) in all treated male groups were documented (Table 3.4.2.3). A slight but significantly lower mean absolute kidney weight was observed only for males dosed at 1000 mg/kg/day and the difference from control was considered incidental by the sponsor since a significant difference was not found for males dosed at 2000 mg/kg/day. Significant differences were not observed for the weights of other organs of treated animals compared to control. Treatment-related macroscopic findings were confined to decedent high dosage mice. Treatment-related microscopic changes from all decedent animals receiving 2000 mg/kg/day were confined to the kidneys, spleen and thymus (see Table 3.4.2.1). Microscopic examination of low dose animals killed at termination revealed, in kidneys, basophilic and dilated cortical tubules, vacuolation and tubular epithelial cells (latter only in males) and hyperplasia of the juxtaglomerular apparatus. The incidence of these findings was greater in females. None of these tissues from the animals in the high dose group were subjected to microscopic examination.

TABLE 3.4.2.3
ORGAN WEIGHT VARIATION IN THE 13-WEEK DOSE-RANGE FINDING STUDY
GROUP MEAN VALUES (G) AT THE END OF TREATMENT

Dose, mg/kg/day		Control		1000		2000	
Sex		M	F	M	F	M	F
Body weight, g		39.9	32.0	37.3[a]	29.2[a]	36.8[a]	31.7
Heart	Abs wt.	0.229	0.191	0.176[b]	0.140[b]	0.179[b]	0.170
	Rel wt.	0.576	0.604	0.473[b]	0.483[b]	0.487[b]	0.539
Kidney	Abs wt.	0.585	0.383	0.494[b]	0.363	0.522	0.377
	Rel wt.	1.472	1.209	1.332	1.248	1.412	1.189
Liver	Abs wt.	1.90	1.59	2.63[b]	2.26[b]	3.41[b]	2.880[b]
	Rel wt.	4.751	4.957	7.050[b]	7.727[b]	9.232[b]	9.061[b]
Seminal vesicles	Abs wt.	0.413		0.332[b]		0.290[b]	
	Rel wt.	1.0401		0.8918[a]		0.7928[b]	

Significant when compared with control: a, $P < 0.05$; b, $P < 0.01$.

As in the previous dose-range finding study, SR 47436 was not quantified in plasma samples of control mice. For animals receiving SR 47436, irrespective of the sex, Cmax was observed 1-2 hr after treatment. Both Cmax and AUC values increased with the dose but lacked dose-proportionality. Further, both Cmax and AUC values at all dose levels were higher for females than for males (Table 3.4.2.4).

TABLE 3.4.2.4
SR 47436 PHARMACOKINETIC PARAMETERS IN WEEK 13

Dose mg/kg/day	tmax (h)		Cmin		Cmax (mg/l)		AUC $_{(0\text{-}24h)}$ (mg.h/l)	
	Males	Females	Males	Females	Males	Females	Males	Females
1000	2	1	0	0.052	28.25	61.34	104.84	136.48
2000	1	1	0	1.467	46.54	92.47	226.69	269.07

In summary, administration of SR 47436 to mice for 13 weeks at dosages of 1000 and 2000 mg/kg/day resulted in the deaths of 3 males and 7 females in the high dose group. Histological examination revealed acute tubular necrosis of kidneys in 5 of 7 decedent females and 1 of 4 decedent males at 2000 mg/kg/day. Though the mean body weight gains were lower for all treated groups relative to control, the differences were statistically significant only for treated males. Additionally, in a comparison of organ weights of surviving treated and control mice, mean heart weight was reduced (10 to 20%, $p<0.05$) and mean liver weight increased (50 to 95% with dose-related effect, $p<0.05$) in all treated groups, and mean seminal vesicle weight was increased (15 to 30%, $p<0.05$) in all treated male groups. On the basis of the reduced survival and renal pathology observed at 2000 mg/kg/day, this dose was considerably in excess of the MTD. Thus, 1000 mg/kg/day was considered to be the maximum tolerated dose.

3.4.3. 104-Week Oral Carcinogenicity Study of SR 47436 in Mice (Report #RS0006960716/01, Study code: SNF/045-CAR016). Vols. 49-55

This GLP study was originally conducted by , for Sanofi Recherche, France between March 9, 1994 and March 13, 1996. The aim of the study was to assess the oncogenic effects of SR 47436 during its repeated daily oral administration to CD-1 mice for 104 weeks.

Male and female CD-1 mice (from Charles River) were approximately 21 to 28 days of age and weighed 24.6 to 35.1 and 18.7 to 26.4 g, respectively, at the start of the study. Suspensions of SR 47436 (batches 4SNP002, 4SNP013,4SNP006, 4SNP042, 4SNP063, 4SNP062 and 5SNP515) in 10% aqueous solution of gum arabic were administered orally by gavage (5 ml/kg), once daily for 104 weeks at doses of 100, 300 or 1000 mg/kg. Animals were not fasted before treatment. The CD-1 strain was selected because of the historical control data available in the contract laboratory and its established susceptibility to known carcinogens. Control animals received the vehicle. It was evident from the toxicokinetic data from previous rat toxicity studies (see sections 3.2.4, 3.2.7), including dose-ranging studies, that measurable quantities of SR 47436 were present in plasma of vehicle control rats in those studies. It was resolved by the sponsor and the contract laboratory that the cross-contamination of the controls was airborne (see my review of INL dated 8/18/94). Thus, to eliminate a possible source of cross-contamination of test substance to control animals in the mouse carcinogenicity study, the animals of the first control group (group #1) were isolated from the treated animals (groups 3 to 5) by housing them in a different room. The second control group (group #2) was housed in the same room as the treated animals. They were housed four of one sex per cage, unless this number was reduced by mortality. Allocation of animals to various groups are shown in Table 3.4.3.1.

TABLE 3.4.3.1
104-WEEK CARCINOGENICITY STUDY IN MICE: COMPOSITION OF TREATMENT GROUPS

Group	Treatment	Dosage (mg/kg/ day)	Number of Animals			
			Main Study		Satellite Study	
			Male	Female	Male	Female
1	Vehicle control	0	56	56	24	24
2	Vehicle control	0	56	56	24	24
3	SR 47436	100	56	56	24	24
4	SR 47436	300	56	56	24	24
5	SR 47436	1000	56	56	24	24

Observations and Measurements

Mice and their cage-trays were observed at least twice daily for evidence of reaction to treatment or ill-health. Detailed observations (including palpation for the presence of masses) were made according to the following schedule, week 1: daily, weeks 2 to 4: twice daily, weeks 5 to 13: once each week, weeks 14 to 46: once each fortnight, week 47 and up: once each month. The body weight of each animal was determined on the day that treatment commenced, at weekly intervals for the first 14 weeks of treatment, every 2-weeks after that and before necropsy. The mean weekly food consumption per mouse was calculated for each cage throughout the treatment period. At study end (104 weeks) blood was withdrawn from the retro-orbital sinus (under anesthesia) of all surviving mice and each animal killed *in extremis* or for humane reasons, and examined for erythrocyte and total leucocyte counts. In addition, blood from 10 male and 10 female mice with the highest animal numbers remaining in each group were examined for complete hematological parameters. Serum drug levels were determined from blood samples taken from satellite animals from control and treated groups at 1 and 3 hr after dosing in weeks 13, 27 and 53 (3/sex/group/time; different animals sampled at different times).

All except satellite animals were subjected to a detailed necropsy that included weighing of major organs, with tissues of these and other organs fixed for histopathological examination. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy. Samples of the tissues listed below were preserved in 4% neutral buffered formaldehyde, except eyes and optic nerves which were placed in Davidson's fluid. Bone marrow smears, taken from all animals killed were air-dried and fixed in methanol. Samples of of any abnormal tissues were also retained for hisopathological examination. Microscopic examination was performed on all these tissues for all animals killed or dying during the study. Findings were reported as "present" or assigned a severity grade.

Adrenals
Aorta-thoracic
Brain§
Cecum
Colon
Duodenum
Epididymides
Eesophagus
Eyes and optic nerves, left
-right[a]
Femoral bone and marrow
Gall bladder
Harderian glands[a]
Heart§
Ileum
Jejunum
Kidneys§
Liver§
Lungs with mainstem
bronchi§
Lymph nodes -mandibular,
-mesenteric
Mammary gland - caudal,
-cranial[a]
Ovaries
Pancreas
Pituitary
Prostate
Rectum
Salivary gland
-submandibular, left
- right[a]
Sciatic nerve-left
-right[a]
Seminal vesicles§
Skeletal muscle -thigh
Skin
Spinal cord
Spleen§
Sternum
Stomach - keratinised,
-glandular
Testes§
Thymus
Thyroid with parathyroids
Tongue
Trachea
Urinary bladder
Uterus with cervix§
Vagina

§: Organs weighed
a: These tissues were not processed histologically, but held in fixative for future requirements.

Unless otherwise noted, group mean values or incidences for the treated groups were compared against combined controls.

Results

A total of 182 males and 171 females died or were killed during the 104 week treatment period (Table 3.4.3.2). Statistically, there was a significant difference in the mortality rates across the groups. For males, there were pairwise significances between the combined control group and the mid and high dosage groups and a significant positive trend. For females, there was a pairwise significance between the combined control group and the high dosage group, a significant positive trend and a significant departure from trend. We disagree with the sponsor's argument that, as there was a lack of treatment-related target organ toxicity, the increased incidence of mortality in the highest dosage group is unlikely to be related to drug treatment. We conclude that 1000 mg/kg/day is, or possibly exceeds, the maximum tolerated dose of SR 47436 in the CD-1 mouse. It should be noted that, despite the mortality effect of drug treatment, the survival rate (for each male and female group) was greater than 50% at 78 weeks into treatment and at least 25% of males and 20% of females survived to the terminal sacrifice after 104 weeks.

TABLE 3.4.3.2
INCIDENCE OF MORTALITY: MOUSE CARCINOGENICITY STUDY

Group/sex	1M	2M	3M	4M	5M	1F	2F	3F	4F	5F
Dosage mg/kg/day	0	0	100	300	1000	0	0	100	300	1000
Group size	56	56	56	56	56	56	56	56	56	56
Mortality:										
Week 26	0	1	0	0	1	1	0	0	3	10
% survival	100	98	100	100	98	98	100	100	95	82
Week 52	3	2	2	2	8	4	2	5	6	16
% survival	95	96	96	96	86	93	96	91	89	71
Week 78	7	11	9	11	21	12	10	11	11	25
% survival	88	80	84	80	63	79	82	80	80	55
Termination	29	35	36	42	40	27	36	30	33	45
% survival	48	38	64	25	29	52	36	46	41	20

A high incidence of perigenital staining (yellow) with urine was seen in the majority of high dosage males at the 4th month of treatment. As treatment progressed, yellow staining of the hindlimbs and ventral body surface in several of these males, and yellow staining of the dorsal surface in a few

animals were also noted. These signs were also observed from 8th month of treatment in several males receiving 300 mg/kg/day. The staining could be related to an increase in urinary output due to disturbance in kidney function or an alteration in the RAAS that regulates water retention. However, these were not measured in this study. There were no other signs of reaction to treatment. The total number of palpable swellings was less in all treated groups relative to controls and also the number of animals bearing them was relatively low for all dose groups compared to control animals. This could be a consequence of the higher mortality in the high dosage groups. Also, the mean time of onset of the first tumor in these animals was slightly later than for the controls (Table 3.4.3.3).

TABLE 3.4.3.3
PALPABLE SWELLINGS - GROUP DISTRIBUTION, MULTIPLICITY AND MEAN TIME OF ONSET

Dosage (mg/kg/day)	Multiplicity†				One or more¶	Total number of swellings	Mean time of onset*
	0	1	2	3 or more			
0M	31	15	5	5	25	40	45
0M	34	11	8	3	22	37	58
100M	29	17	7	3	27	43	63
300M	35	18	3	0	22	24	71
1000M	44	10	2	0	12	14	67
0F	48	8	0	0	8	8	90
0F	44	7	4	1	12	19	80
100F	48	7	1	0	8	9	91
300F	50	6	0	0	6	6	61
1000F	52	3	0	1	4	6	71

† Expressed as number of animals bearing the indicated number of swellings.
¶ Total number of animals bearing at least one swelling
* In weeks to onset of first recorded swelling, including those found at necropsy examination.

The overall mean body weight gains of all treated female groups were consistently lower than those of the controls, though no dosage relationship could be seen. At fifty-two weeks, females receiving 100 or 300 mg/kg/day had mean body weight gains 14% below control and high dose females had a mean body weight gain 19% below control. At 104 weeks, these decreases from control body weight gain rose to 19, 22 and 17%, respectively (Table 3.4.3.4, Fig. 3.4.3.1). The high mortality (80% at termination) combined with a large reduction in body weight gain (10-44%) throughout the study suggests that for females, the high dose exceeded the MTD. In contrast, the body weight gains of treated males were not affected (Fig. 3.4.3.2). However, SR 47436 affected the survival of male mice to a statistically significant degree (71% mortality *vs* 57% mortality for combined controls) suggesting the high dose possibly exceeded the MTD for males as well. The food consumption of males and females in the high dose group was slightly higher compared to controls throughout the treatment period.

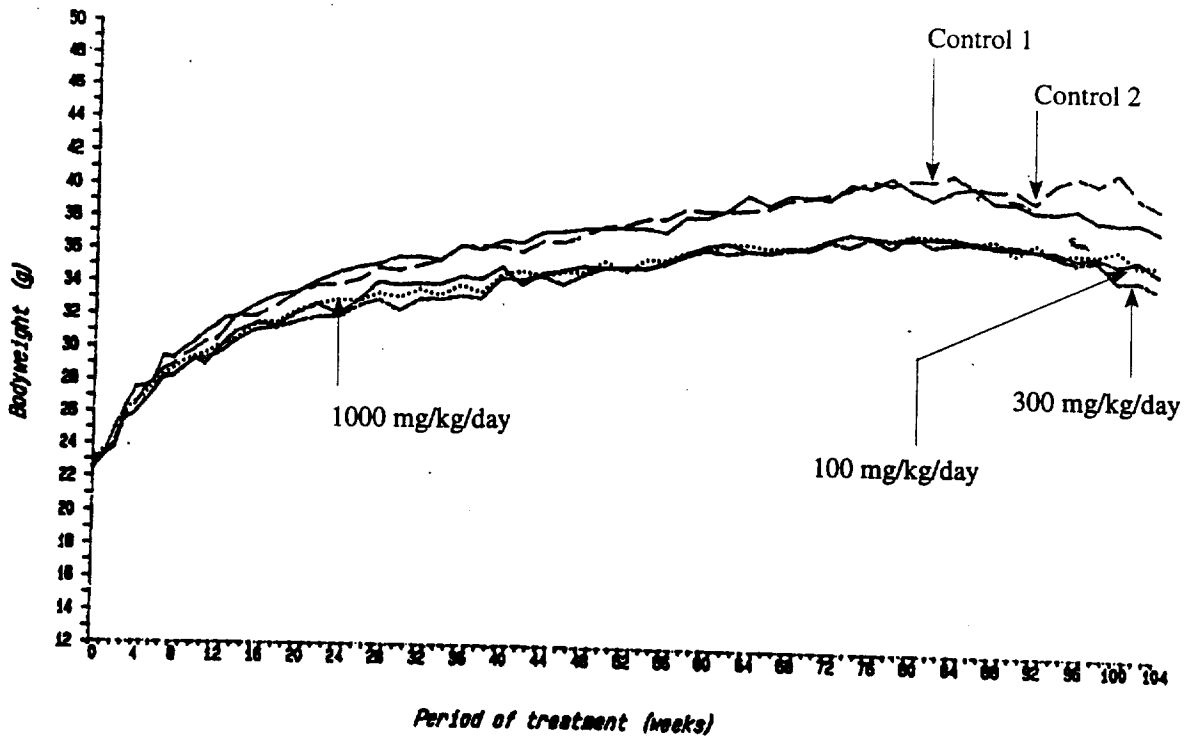

Figure 3.4.3.1.: Group mean body weight curves for female mice

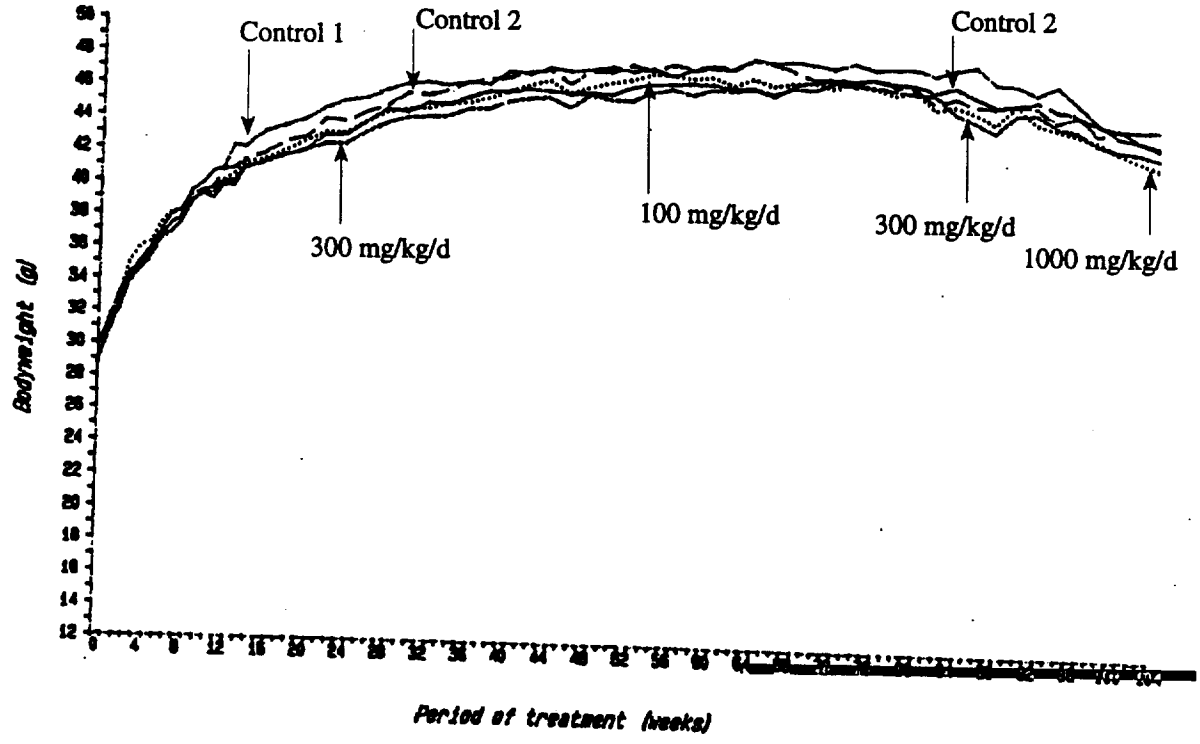

Figure 3.4.3.2.: Group mean bodyweight curves for male mice

TABLE 3.4.3.4
MEAN BODY WEIGHT GAINS FOR VARIOUS STUDY INTERVALS

Wk	0-13		0-26		26-52		0-52		52-78[f]	0-78		78-104[f]	0-104	
Gp[c]	g[a]	%[b]	g[a]	%[b]	g[a]	%[b]	g[a]	%[b]	g[a]	g[a]	%[b]	g[a]	g[a]	%[b]
1M	12.4	-	15.5	-	2.0	-	17.5	-	0.9	18.3	-	-2.5	14.0	-
F	8.6	-	11.7	-	2.7	-	14.5	-	2.9	18.0	-	-2.2	14.8	-
2M	11.0	-	14.7	-	3.4	-	18.1	-	-0.5	17.3	-	-3.3	14.0	-
F	8.5	-	11.6	-	3.7	-	15.2	-	2.9	18.1	-	-2.8	17.0	-
3M	11.1	0	15.1	0	2.7	0	17.7	-1	0.5	18.4	+3	-2.4	16.0	+14
F	7.9	-8	10.5	-10	2.5	-22	12.8[e]	-14	2.0	14.7[e]	-19	-0.2[e]	12.9[d]	-19
4M	11.2	0	14.4	-5	2.3	0	16.7	-6	1.2	17.9	+1	-2.9	14.4	+3
F	7.5[d]	-12	10.2[d]	-12	2.5	-22	12.8[e]	-14	1.9[d]	14.3[e]	-21	-1.0	12.4[e]	-22
5M	11.6	0	15.0	-1	3.0	0	18.1	+2	-0.2	17.7	-1	-3.7	13.4	-4
F	7.7	-10	10.2[d]	-12	1.8[e]	-44	12.1[e]	-19	2.1	14.0[e]	-22	0.2[e]	13.2	-17

a: mean body weight gain for the group in grams; b: % of combined controls; c: groups, 1and 2: controls, groups 3, 4, and 5 are respectively, 100, 300 and 1000 mg SR 47436/kg/day; significant when compared with combined controls, d: $p < 0.05$, e: $p < 0.01$. f: % of combined controls not calculated for this period.

All high dose animals exhibited moderate anemia during the 104 weeks of treatment, characterized by lower packed cell volumes, hemoglobin concentrations and erythrocyte counts. The data was, however, statistically significant ($p < 0.001$) for high dose females only. Additionally, in all treated animals examined for RBC and WBC counts only, both of these parameters decreased, however, there was no clear dosage relationship. Increased ($p < 0.01$) platelet counts were noted for males receiving 1000 mg/kg/day.

At the 104 week sacrifice, absolute and relative liver weights of males receiving 100 or 300 mg/kg/day and all animals receiving 1000 mg/kg/day were significantly higher ($p < 0.05$) than those of the combined controls. Both absolute and relative seminal vesicle weights were low ($p < 0.01$) in high dose males relative to combined controls. There were no associated histopathological changes in these organs. In addition, high relative kidney weights were noted in females that received 300 ($p < 0.05$) or 1000 ($p < 0.01$) mg/kg/day. The increase was not significant in high dose males.

Gross pathology findings which were considered to be related to treatment were confined to the kidneys, GIT and the urinary bladder.

Mid and high dose females that were killed or died before term showed a slightly higher incidence of distension of the small intestine relative to combined controls. Also, a similar incidence of distension of the colon was observed in these high dose females. In animals killed at term, a high incidence ($p < 0.01$) of pale and granular kidneys was noted for high dose males and for all treated females. Additionally, a high incidence ($p < 0.05$ to 0.01) of cystic kidneys were observed for high

dose males and for mid dose females. Slightly higher ($p < 0.01$) incidences of liver masses for males receiving 300 mg/kg/day and of distension of the urinary bladder for males receiving 1000 mg/kg/day (relative to control incidence) were also noted. Abnormally shaped uterus was noted ($p < 0.05$) in a number of females dosed at 300 or more mg/kg/day.

Regarding *microscopic pathology*, the sponsor's analysis revealed no evidence of a statistically significant increasing trend in the incidence of any neoplasm that could be attributed to treatment with SR 47436 in either sex of mice that were killed or died during the treatment period, or killed at term (see appendix I for tumor type and site). Summary of tumor incidence is given in Tables 3.4.3.5., 3.4.3.6. and 3.4.3.7.

TABLE 3.4.3.5
SUMMARY OF NEOPLASTIC FINDINGS FOR ALL MICE
(Numbers in parentheses indicate % incidence)

Dose, mg/kg/d	CONTROL 1		CONTROL 2		100		300		1000	
Sex	M	F	M	F	M	F	M	F	M	F
# examined	56	56	56	56	56	56	56	56	56	56
# *Mice with neoplasms*										
Malignant	23 (41)	23 (41)	28 (50)[a]	31 (55)[b]	31 (55)	26 (46)	29 (52)	22 (39)	16 (29)	18 (32)
Benign	28 (50)	17 (30)	17 (30)	17 (30)	28 (50)	21 (37)	26 (46)	19 (34)	19 (34)	14 (25)
Malignant or benign	42 (75)[c]	29 (52)	36 (64)	36 (64)[a]	47 (84)	36 (64)	41 (73)	33 (59)	33 (59)	25 (45)
Total # of neoplasms										
Malignant	27 (48)	28 (50)	31 (55)	38 (68)	38 (68)	29 (52)	32 (57)	23 (41)	17 (30)	21 (37)
Benign	34 (61)	21 (37)	19 (34)	24 (43)	35 (62)	22 (39)	33 (59)	21 (37)	24 (43)	15 (27)
Malignant or benign	61 (109)	49 (87)	50 (89)	62 (111)	73 (130)	51 (91)	65 (116)	44 (79)	41 (73)	36 (64)
# of tumors/ animal[1]	0-3	0-4	0-4	0-5	0-3	0-2	0-4	0-3	0-4	0-3

Cochran-Armitage trend test:
None of the tumors is significant for the one tailed test for increasing incidence of tumours with dosage. However, the two tailed test shows some significant differences due to the decreased incidence of tumors at the highest dosage:
a = $p < 0.05$ (two tailed test only) versus Control Group 2; b = $p < 0.01$ (two tailed test only) versus Control Group 2
c = $p < 0.05$ (two tailed test only) versus Control Group 1. 1: see table below for further details.

TABLE 3.4.3.6
OCCURRENCE OF PRIMARY TUMORS IN MICE

Dose, mg/kg/day	Control 1		Control 2		100		300		1000	
Sex	M	F	M	F	M	F	M	F	M	F
Animals initially in the study	56	56	56	56	56	56	56	56	56	56
# examined microscopically	56	56	56	56	56	56	56	56	56	56
# of animals with 1 tumor, *total*	26	16	22	17	26	21	24	25	27	15
Found dead	2	2	3	3	2	1	2	4	3	7
Moribund sacrifice	10	7	11	11	12	10	10	10	11	3
Accidental death	1	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	13	7	7	3	12	10	6	11	7	5
# of animals with 2 tumors, *total*	17	8	5	15	16	15	10	5	5	9
Found dead	2	-	1	4	1	1	3	-	1	-
Moribund sacrifice	8	3	2	7	8	9	5	2	1	7
Accidental death	2	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	5	5	1	4	6	5	3	3	2	2
# of animals with 3 tumors, *total*	5	3	3	2	5	-	5	3	3	1
Found dead	1	-	1	-	1	-	1	-	1	-
Moribund sacrifice	1	-	1	-	3	-	2	2	2	1
Accidental death	1	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	2	3	2	2	1	-	1	1	1	-
# of animals with 4 tumors, *total*	2	2	1	1	2	-	2	-	2	-
Found dead	1	-	1	-	1	-	1	-	1	-
Moribund sacrifice	1	2	1	-	1	-	1	-	1	-
Accidental death	1	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	-	-	-	1	-	-	-	-	-	-
# of animals with 5 tumors, *total*	1	-	1	1	1	-	1	-	1	-
Found dead	1	-	1	-	1	-	1	-	1	-
Moribund sacrifice	1	-	1	-	1	-	1	-	1	-
Accidental death	1	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	-	-	-	1	-	-	-	-	-	-
# of animals with tumors										
Found dead	6	2	7	7	4	2	10	4	11	7
Moribund sacrifice	21	12	14	18	23	19	19	14	12	10
Accidental death	1	-	1	-	1	-	1	-	1	-
Scheduled sacrifice	18	15	13	11	20	15	16	15	16	7

TABLE 3.4.3.7
104-WEEK ORAL CARCINOGENICITY STUDY OF SR 47436 IN MICE (REPORT #RS0006960716/01)
INCIDENCE OF NEOPLASTIC CHANGES*

Treatment	Control 1		Control 2		SR 47436					
Dose levels (mg/kg/day)	0		0		100		300		1000	
Sex	M	F	M	F	M	F	M	F	M	F
Adrenals (No. examined)	55	56	55	56	56	56	56	56	56	56
Phaechromocytoma						1				
Cortical adenoma										1
Cortical adenocarcinoma										
Brain (No. examined)	56	56	56	56	56	56	56	56	56	56
Astrocytoma										
Meningioma										
Duodenum (No. examined)	55	55	49	56	55	54	53	54	52	55
Adenoma										
Adenocarcinoma								1		
Epididymides (No. examined)	56		56		56		56		56	
Adenoma			1							
Femur (No. examined)	56	56	56	56	56	56	56	56	56	56
Osteoma		1				1				
Haemangiosarcoma				1						
Heart (No. examined)	56	56	56	56	56	56	56	56	56	56
Haemangiosarcoma		1								
Kidney (No. examined)	56	56	56	56	56	56	56	56	56	56
Renal carcinoma				1						
Liver (No. examined)	56	56	55	56	56	56	56	56	56	56
Hepatocellular adenoma				1		1		1		1
Haemangioma										
Hepatocellular carcinoma										1
Haemangiosarcoma										1
Lung (No. examined)	56	56	56	56	56	56	56	56	56	56
Pulmonary adenoma		7		3		5		5		6
Bronchioalveolar carcinoma		2		4		3		3		1
Ovaries (No. examined)		55		56		56		56		56
Adenoma		6		2		1		1		1
Luteoma				2						
Sertoli cell adenoma								1		
Granulosa-theca cell adenoma						1				1
Granulosa-theca cell carcinoma		1								
Pancreas (No. examined)	56	56	56	56	56	56	56	56	55	55
Islet cell adenoma										

* Animals bearing tumors of specific tissues

TABLE 3.4.3.7
Incidence of neoplastic changes in mice (continued)

Treatment	control 1		control 2		SR 47436					
Dose levels (mg/kg/day)	0		0		100		300		1000	
Sex	M	F	M	F	M	F	M	F	M	F
Parathyroids (No. examined)	49	51	44	47	51	47	51	49	49	47
Adenoma	1									
Pituitary (No. examined)	56	56	56	56	56	55	56	56	55	56
Adenoma				1		2				
Seminal vesicles (No. examined)	56		56		56		56		56	
Adenoma									1	
Leiomyosarcoma					1					
Skin (No. examined)	56	56	56	56	56	56	56	56	56	56
Sarcoma							1			
Spleen (No. examined)	56	56	56	56	56	56	56	56	56	54
Haemangioma				1			1			
Haemangiosarcoma		2		1		1	1		1	1
Stomach (No. examined)	56	56	54	56	56	55	55	55	55	56
Squamous cell carcinoma			1							
Submandibular gland (No. examined)	55	56	56	56	56	56	56	56	55	56
Adenocarcinoma						1				
Testis (No. examined)	56		56		56		56		56	
Interstitial cell tumour	1								1	
Thymus (No. examined)	56	56	56	56	56	56	56	56	56	56
Thymoma				1						
Thyroids (No. examined)	56	56	56	56	56	56	56	56	56	56
Follicular cell adenoma				1						
Uterine cervix (No. examined)		56		53		55		55		55
Polyp								1		
Leiomyoma				1				1		
Sarcoma						1		1		1
Uterus (No. examined)		56		55		56		56		56
Polyp		6		6		6		8		4
Adenoma						1				
Leiomyoma		1		4		1		2		1
Adenocarcinoma				1						
Leiomyosarcoma		2						1		
Haemangiosarcoma		2		2		1		2		2
Vagina (No. examined)		55		55		55		55		52
Haemangiosarcoma								1		

TABLE 3.4.3.7

Incidence of neoplastic changes (continued)

Treatment	control 1		control 2		SR 47436					
Dose levels (mg/kg/day)	0		0		100		300		1000	
Sex	M	F	M	F	M	F	M	F	M	F
Haematopoietic tumour (No. examined)	56	56	56	56	56	56	56	56	56	56
Malignant lymphoma	6	10	8	14	5	13	7	9	2	9
Granulocytic leukemia	1	1	1	1	2			1		1
Histiocytic sarcoma	1	2	1	2	1	3		2	1	1
Abdomen (No. examined)	1	2	1					3	1	1
Neuroendodermal tumour			1							
Abdominal fat (No. examined)	1	2	3		3	1	1	2	2	3
Haemangioma			1							
Harderian gland left (No. examined)	2		1	2	1	1	4		0	
Adenoma	2				1	1	3			
Adenocarcinoma				1						
Harderian gland right (No. examined)	1		2		2	1	1			
Adenoma	1		1		2	1	1			
Mammary gland cranial (No. examined)		1	2		4			1		
Adenocarcinoma								1		
Mammary gland other (No. examined)		3	1	5		4				3
Adenocarcinoma		3	1	4		4				3
Adenoacanthoma				1						
Musculo-skeletal (No. examined)	2		2	4	1	2	3			4
Ameloblastoma			1							
Sarcoma			1			1				
Osteosarcoma				1						
Skin - other (No. examined)	10	9	20	8	26	8	9	11	11	5
Papilloma			1		1		1	1		
Keratoacanthoma				1						
Benign basal cell tumour	1									
Haemangioma										
Lipoma					1					
Sarcoma	3	1	3	3	2		7	1	1	
Carcinoma		1		1						
Haemangiosarcoma			1							
Tail (No. examined)				1	1	2		2	1	1
Sarcoma						1				

Although a significant positive trend was noted for males with adrenal cortex adenomas (Table 3.4.3.8), the sponsor does not consider this finding as biologically significant because there was no dosage relationship and the highest incidence in any group (5.36% in the high dose group) was only slightly above historical control range (0-3.3%); furthermore, there were no adenocarcinomas of the adrenal cortex in treated animals (one observed in a control animal).

TABLE 3.4.3.8
NEOPLASTIC FINDINGS: ADRENAL CORTEX ADENOMAS AND ADENOCARCINOMAS IN MALE MICE

Dose, mg/kg/day	Adenoma			Adenocarcinoma
	Tumor incidence	Statistical tests		Tumor incidence
		Trend	Pairwise	
Control 1	0/55			1/55
Control 2	0/55			0/55
100	2/56			0/56
300	0/56			0/56
1000	3/56	p=0.014	p=0.0014	0/56
Historical control, %	1.14 (0-3.3%)			0.00

Additionally, a significant positive trend was noted for males with combined malignant/benign hepatocellular tumors. The sponsor does not consider this finding as biologically significant because "there was no dosage relationship, the incidence for the high dose group was not different from that of controls, there was no significant trend for each tumor taken separately, and the incidence in each treated group was not significantly different from historical incidence (Table 3.4.3.9)." This statement is correct since the Center's guidance says that trends for common tumors are not considered to be significant unless $p<0.005$.

TABLE 3.4.3.9
NEOPLASTIC FINDINGS: HEPATOCELLULAR ADENOMAS AND CARCINOMAS IN MALE MICE

Dose, mg/kg/day	Adenoma		Carcinoma		Combined	
	incidence	trend test	incidence	trend test	incidence	trend test
Control 1	11/56		5/56		15/56	
Control 2	5/55		5/55		9/55	
100	9/56		10/56		19/56	
300	14/56		7/56		21/56[a]	
1000	10/56	p=0.0305	7/56	p=0.0913	17/56	p=0.0024
Historical control, %	16.15 (6.7 - 30.8)		9.51 (3.3 - 15.0)		25.0 (16.7 - 42.3)	

a: pairwise test : P = 0.0046

Non-neoplastic pathology considered to be related to treatment was seen in the kidneys and was characterized by nephropathy, hyperplasia of juxtaglomerular apparatus, perivascular chronic inflammation, cortical cysts, dilatation of Bowman's capsules and medullary tubules. There was a treatment-related increase in the incidence of amyloidosis in a number of tissues at various dosages, including adrenals, GIT, heart, kidneys, liver, ovaries, thyroid and parathyroids. The sponsor observes that amyloidosis is a common change seen in ageing mice. The reason for the apparent increase in a number of tissues with amyloid per animal, particularly in high dosage animals, is unclear but they doubt its toxicological significance. Other significant findings related to treatment were increased incidence of focal inflammation with associated hepatocytic degeneration in liver, clear cell foci in the liver, acute keratitis in the eyes, thick and viscous bile in the gall bladder, auricular thrombi in the heart, alveolar hemorrhage and accumulations of alveloar macrophages in the lungs, and spermatoceles in the testes. The findings are listed in the following table 3.4.3.10 (see next page).

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TABLE 3.4.3.10
INCIDENCE OF NON-NEOPLASTIC LESIONS: 104-WEEK CARCINOGENICITY STUDY IN MICE

Organ and lesion	Control 1		Control 2		100 mg/kg/d		300 mg/kg/d		1000 mg/kg/d	
	M	F	M	F	M	F	M	F	M	F
Number examined[1]	55	56	55	56	56	56	56	56	56	56
Adrenals: cortex-amyloidosis	3	3	7	7	7	6	9	12[a]	12[a]	12[a]
-focal cortical hypertrophy	7	0	11	0	11	1	11	4[a]	4	1
Adrenals: medulla-amyloidosis	0	0	0	2	0	0	0	1	3[a]	2
Cecum: amyloidosis	1/56	0	1/51	1	3	3/54	5[a]/53	3/55	10[c]/51	7[b]/54
Colon: amyloidosis	1	1	1/54	1	1	1/55	3	0	7[b]/53	6[a]
Duodenum: amyloidosis	1	3/55	3/49	8	7[a]/55	6/54	6	12/54	14[c]/52	18[c]/55
Gall bladder: inspissated bile	0/51	0	0/49	0/52	0/51	0/52	0/48	0/51	3[a]/54	0/51
Heart: auricle- amyloidosis	1/56	0	0/56	0	1	0	0	0	5[a]	0
-auricular thrombus	1/56	0	0/56	2	0	0	3	2	4[a]	0
ventricle- amyloidosis	2/56	1	3/56	6	10[b]	1	7	6	14[c]	9
Ileum: amyloidosis	12/53	13/55	15/49	15	14/54	15/53	13/47	21	15/49	18/54
villus atrophy	0/53	0/55	0/49	0	0/54	0/53	0/47	0	0/49	4[a]/54
Jejunum: amyloidosis	4	3/55	8/52	9	9	10/54	13[a]/52	12/54	16[b]/51	17[b]/54
villus atrophy	0	0/55	0/52	0	0	0/54	0/52	0/54	0/51	4[a]/54
Kidneys: nephropathy	5/56	9	6/56	9	8	32[c]	11	41[c]	22[c]	21[b]
amyloidosis	7	10	12	13	15	14	15	20[a]	15	17
hyperplasia of JG	0	1	1	0	0	17[c]	0	40[c]	22[c]	49[c]
perivascular chronic inflammatory cells	8	20	14	25	18	39[c]	20[a]	35[b]	33[c]	23
medullary dilated tubules w/protein casts	1	0	0	0	0	2	0	6[b]	3	4[a]
papillary mineralization	0	0	1	0	2	3[a]	2	5[b]	5[a]	6[b]
cortical cysts	14	2	11	3	12	12[b]	15	9[a]	23[a]	5
dilated Bowman's capsules	12	3	8	5	6	39[c]	4	31[c]	18[a]	10
focal infarcts	2	3	3	8	3	4	4	2	8[a]	1
Lymph node- mandibular amyloidosis	0/56	1	0/56	1	1	0	2	1	3[a]	3
- mesenteric amyloidosis	4/55	2	3/56	3	7	1	6	8[a]	7	6
Liver: focal inflammation, degeneration	4/56	4	6	4	6	5	5	8	7	8
amyloidosis	0/56	2	1	3	1	1	2	6	5[a]	9[a]
Left eye[2] : acute keratitis	0/56	0/54	0/51	1	0/55	0/54	1/52	0	3[a]/51	0/52
Lungs: alveolar hemorrhage	3/56	2	5/56	4	5	5	6	4	12[a]	5
-alveolar macrophages	2/56	0	1/56	7	0	1	2	3	7[a]	4
Ovaries: amyloidosis	-	3/55	-	6	-	6	-	11[a]	-	7
Pancreas: amyloidosis	0/56	0	0	2	0	0	1	2	3[a]	2
Parathyroid: amyloidosis	1/49	3/51	2/44	7/47	5/51	7/47	7[a]/51	9/49	12[c]/49	10/47
Rectum: amyloidosis	1/56	0	0/56	0	1	0	2	3[a]	0/55	4[a]
Stomach: amyloidosis	0/56	0	1/54	5	3	2/55	5[a]/55	9[a]/55	10[c]/55	12[b]
Thyroids: amyloidosis	0/56	5	7/56	9	11[a]	8	11[a]	15[a]	15[c]/55	15[a]

1: unless otherwise specified; 2: right eye not examined microscopically; a: $p<0.05$, b: $p<0.01$, c: $p<0.001$ (significant when compared with combined controls).

Toxicokinetics: In control group 2, SR 47436 was detected in three (out of 42) samples obtained 3 hours after dosing in week 53. SR 47436 was not detected in animals from the first control group housed in a separate room. SR 47436 was detected in all treated animals and levels were much higher 1 hour than 3 hours after dosing and increased with the dose except for males in week 13 (Table 3.4.3.11). SR 47436 was rapidly absorbed (Tmax between 0.5 and 2 hr) and rapidly cleared from the plasma. High interanimal variability was observed and no sex differences could be documented.

TABLE 3.4.3.11.
MEAN SR 47436 PLASMA CONCENTRATIONS: 104-WEEK CARCINOGENICITY STUDY IN MICE

	Dose	C_{1h} (mg/l)		C_{3h} (mg/l)	
	(mg/kg/day)	Males	Females	Males	Females
Week 13	**100**	18.03	9.82	0.05	0.21
	300	6.67	13.95	2.30	2.52
	1000	58.54	52.56	9.88	12.77
Week 27	**100**	0.37	13.42	0.06	0.17
	300	9.53	30.62	2.43	0.54
	1000	10.62	37.99	11.60	4.09
Week 53	**100**	2.22	4.26	0.11	0.43
	300	19.98	28.07	2.81	2.05
	1000	20.43	53.45	9.87	12.72

In summary, in the 104-week mouse carcinogenicity study, oral administration of SR 47436 at dose levels up to 1000 mg/kg/day elicited no clinical signs of toxicity but affected survival of the highest male and female dosage groups, thus demonstrating that 1000 mg/kg/day was, or exceeded, the maximum tolerated dose. The survival rate was greater than 50% after 78 weeks of treatment and at least 25% of males and 20% of females in each group survived to the terminal sacrifice after 104 weeks (which was more than adequate to assess the carcinogenic potential of SR 47436). The mean overall body weight gains of all treated female (but not male) groups were consistently lower than those of the control, though no dosage relationship could be seen. All high dose animals exhibited moderate anemia. At terminal sacrifice, absolute and relative liver weights of males receiving 100 or 300 mg/kg/day and males and females receiving 1000 mg/kg/day were significantly higher than those of the combined controls. Both absolute and relative seminal vesicle weights were low ($p < 0.01$) in high dose males relative to combined controls. There were no associated histopathological changes in these organs. In addition, high relative kidney weights were noted in females that received 300 or more mg/kg/day with non-neoplastic histopathologic findings observed in the kidneys of all groups of treated females and high dose males. There was a treatment-related increase in the incidence of amyloidosis in a number of tissues, including adrenals, GIT, heart, kidneys, liver, ovaries, thyroid and parathyroids. There were no neoplastic findings considered to be related to treatment. Toxicokinetics performed during this study revealed a dosage-related exposure of the animals to SR 47436. A very low amount of test substance was detected in a few blood samples from the control group housed along with the drug-treated groups.

3.4.4. 13-Week Oral Range Finding Toxicity Study of SR 47436 in Rats (Report #RS0006931103/01, Study #DDO504). Vol. 58

This GLP study was conducted by for Sanofi Recherche, France between January 26, 1993 and April 30, 1993. This study was conducted to aid in the selection of dosages for an oncogenicity study in this species.

Groups of 15 male and 15 female HanIbm Wistar rats, 5 weeks old and weighing 95-136 g (males) or 80-130 g (females), received oral doses of 0, 15, 50, 150 or 500 mg SR 47436 (batch 92.03)/kg/day by gavage for 13 weeks. Test substance was suspended in a 10% aqueous gum arabic. Control animals received the vehicle. The animals were housed five of one sex per cage and were not fasted before treatment.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded a week before treatment, on day 1 of treatment, then weekly throughout the treatment period. Food was supplied to each cage and that remaining was recorded for each week throughout the treatment period. From these records, the mean weekly consumption per animal was calculated for each cage. For toxicokinetics study, blood samples were withdrawn from the retro-orbital sinus of all (non-fasted anesthetized) animals during week 13 of treatment at 1, 2, 4, 8 and 24 hr after administration of SR 47436/vehicle (n=3/sex/sampling time, animals were not reused). Hematology, clinical chemistry and urinalysis were not performed. All animals were subjected to a detailed necropsy. Selected organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy. Samples of the following tissues were collected and preserved for future microscopic examination.

Adrenals§
Aorta-thoracic
Brain§
Cecum
Colon
Duodenum
Epididymides§
Eesophagus
Eyes and optic nerves
Femoral bone and stifle jt
Gall bladder
Harderian glands
Heart§
Ileum
Jejunum
Kidneys§
Lachrymal glands
Liver§
Lungs with mainstem bronchi§
Lymph nodes -mandibular, -mesenteric
Mammary glands - caudal, -cranial
Ovaries§
Pancreas
Pituitary§
Prostate§
Rectum
Salivary glands -submandibular§
Sciatic nerves
Seminal vesicles§
Skeletal muscle -thigh
Skin
Spinal cord
Spleen§
Sternum and marrow
Stomach - ketatinised, -glandular
Testes§
Thymus§
Thyroid with parathyroids§
Tongue
Trachea
Urinary bladder
Uterus with cervix§
Vagina

§: Organs weighed

Results

One control female died immediately following blood sampling and the death was considered to be related to the stress of that sampling. There were no deaths or signs attributed to treatment. Mean body weight gain was statistically significantly and dose-dependently decreased in treated males receiving 50 or more mg/kg/day compared to the controls. This was more noticeable towards the end of the study in high dose males (Table 3.4.4.1). The difference was associated with a marginally lower food intake in males (-5 to -9% relative to control). No statistically significant differences were observed in treated females.

TABLE 3.4.4.1
BODY WEIGHT- GROUP MEAN VALUES AND BODY WEIGHT GAIN (G) AT THE END OF THE STUDY

Doses	Males			Females		
	Initial b.wt.	b.wt. at wk 13	b.wt. gain	Initial b.wt.	b.wt. at wk 13	b.wt. gain
0	112	390	279	104	236	132
15	110	372 (- 5 %)*	262 (-6%)*	104	223	119
50	114	354 (- 9 %)	240[a] (-14%)	104	228	123
150	113	350 (- 10 %)	238[a] (-15%)	105	230	125
500	112	344 (- 12 %)	232[b] (-17%)	105	231	126

*: % when compared to control. a: P <0.05; b: P <0.01.

At necropsy, the mean absolute and relative heart weights of both male and female groups given 50 or more mg/kg/day were significantly lower than control (maximal change, -23% at 500 mg/kg/day in males). Absolute and/or relative increases in kidney weights were noted for both males and females at all dose levels relative to control (maximal change, +34% for high dose females). The mean relative liver weight of females receiving 500 mg/kg/day also was significantly higher (+18.5%) than control (Table 3.4.4.2). Macroscopic examination of organs did not reveal any treatment related changes. Microscopic examinations were not performed.

The individual plasma concentrations observed at each sampling time showed a large variability, mainly during the first 4 hours at 150 and 500 mg/kg/day for both sexes. Major inter-sex differences were observed at 50 mg/kg/day (Cmax), 150 mg/kg/day (Cmax and $AUC_{(0\text{-}24h)}$) and 500 mg/kg/day (Cmax and $AUC_{(0\text{-}24h)}$). Mean Cmax was observed 2 hr after treatment in males and 1 or 2 hr after treatment in females. Plasma concentrations were much higher in females than in males at doses >50 mg/kg/day. Mean Cmax and $AUC_{(0\text{-}24h)}$ observed in week 13 increased with increasing dose but were not proportional to dose (Table 3.4.4.3). Unchanged compound was also detected in all blood samples taken from controls (concentrations lower than in treated animals). The sponsor and the contract laboratory believe that the contamination was airborne.

TABLE 3.4.4.2
ORGAN WEIGHT VARIATION IN THE 13-WEEK DOSE-RANGE FINDING STUDY IN RATS
GROUP MEAN VALUES (G) AT THE END OF TREATMENT

Dose, mg/kg/day		Control		15		50		150		500	
Sex		M	F	M	F	M	F	M	F	M	F
Body weight, g		388.5	237.5	364.2	223.1	349.6	226.1	346.2[a]	230.1	338.2[a]	229.5
Heart	Abs wt.	1.18	0.85	1.06	0.76[a]	0.96[b]	0.71[b]	1.02[a]	0.76[a]	0.91[b]	0.71[b]
	Rel wt.	0.307	0.359	0.290	0.346	0.275[a]	0.316[a]	0.294	0.332	0.269[a]	0.31[b]
Kidney	Abs wt.	2.25	1.59	2.14	1.62	2.34	1.75[a]	2.29	1.76[a]	2.26	1.79[b]
	Rel wt.	0.583	0.669	0.589	0.728[b]	0.672[b]	0.775[b]	0.665[b]	0.765[b]	0.671[b]	0.783[b]
Liver	Abs wt.	13.7	8.80	12.8	8.50	12.5	8.70	12.8	9.00	11.7[a]	9.60
	Rel wt.	3.51	3.68	3.52	3.83	3.58	3.86	3.71	3.89	3.48	4.16[a]

Significant when compared with control: a, P <0.05; b, P <0.01.

TABLE 3.4.4.3
SR 47436 PHARMACOKINETIC PARAMETERS ON WEEK 13 IN THE 13-WEEK DOSE-RANGE FINDING STUDY IN RATS

Doses (mg/kg/day)	tmax (h)		Cmax (mg/l)		Cmin (mg/l)		AUC $_{(0\text{-}24h)}$ (mg.h/l)	
	Males	Females	Males	Females	Males	Females	Males	Females
Control	24	24	0.310	0.298	0.199	0.196	Not calculated	
15	2	2	1.10	1.10	0.53	0.40	17.06	16.28
50	2	1	1.46	2.27	0.74	0.71	22.34	23.57
150	2	1	3.36	19.44	0.70	0.66	25.73	56.92
500	2	2	20.60	258.77	1.04	0.93	60.48	473.36

Based on the above findings, it can be concluded that the maximum tolerated dose (MTD) was not attained or identified in the female HanIbm Wistar rat.

3.4.5. 2-Week Oral Range Finding Toxicity Study of SR 47436 in Rats (Report #RS0006951120/01, Study #DDO521). Vol. 57

This GLP study was conducted by

for Sanofi Recherche, France between August 2, 1993 and August 16, 1993. Because limited toxicity was observed in the first dose-range finding study (section 3.4.4), this additional 2-week study was conducted to aid the selection of dosages for an oncogenicity study in this species.

Groups of 5 male and 5 female HanIbm Wistar rats, 5 weeks old and weighing 100-118 g (males) or 87-110 g (females), received oral doses of 0 or 1000 mg SR 47436 (batch 92.02)/kg/day by gavage for 2 weeks. Test substance was suspended in a 10% aqueous gum arabic. Control animals received the vehicle. The animals were housed five of one sex per cage and were not fasted before treatment.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded on day 1 of treatment, then twice weekly throughout the treatment period. Food supplied to each cage and that remaining was recorded for each week throughout the treatment period. From these records, the mean weekly consumption per animal was calculated for each cage. All animals were subjected to a detailed necropsy. Selected organs, taken from each animal, were dissected free of adjacent fat and other contiguous tissue and the weights recorded. The weight of each organ was expressed as a percentage of the bodyweight recorded immediately before necropsy. Samples of the following tissues were collected and preserved for future microscopic examination.

Adrenals§
Aorta-thoracic
Brain§
Cecum
Colon
Duodenum
Epididymides§
Eesophagus
Eyes and optic nerves
Femoral bone and stifle jt
Harderian glands
Heart§
Ileum
Jejunum
Kidneys§
Lachrymal glands
Liver§
Lungs with mainstem bronchi§
Lymph nodes -mandibular, -mesenteric
Mammary glands - caudal, -cranial
Ovaries§
Pancreas
Pituitary§
Prostate§
Rectum
Salivary glands -submandibular§
Sciatic nerves
Seminal vesicles§
Skeletal muscle -thigh
Skin
Spinal cord
Spleen§
Sternum and marrow
Stomach - keratinised, -glandular
Testes§
Thymus§
Thyroid with parathyroids§
Tongue
Trachea
Urinary bladder
Uterus with cervix§
Vagina

§: organs weighed

Results

There were no deaths and no signs that could be attributed to treatment. Mean body weight gain of treated rats was low throughout the 14 days of treatment relative to control. The decrease was approximately 23% for males and 21% for females and the data is statistically significant (Table 3.4.5.1). The food intake of treated animals was slightly low relative to control animals for both weeks of the treatment period; however, the difference from control is not statistically significant.

TABLE 3.4.5.1
BODY WEIGHT- GROUP MEAN VALUES AND BODY WEIGHT GAIN (GM) AT THE END OF THE STUDY

Doses	Males			Females		
	Initial b.wt.	b.wt. on day 14	b.wt. gain	Initial b.wt.	b.wt. on day 14	b.wt. gain
0	107	173	66	106	144	38
1000	110	161	51[b] (-23 %)*	93	123	30[a] (-21%)*

*: compared to control. a: P <0.05; b: P <0.01.

At necropsy, the mean absolute and relative heart weights for treated females were lower (-20%, p<.05) than control. Compared to controls, relative increases (+9%, p<.05) in kidney weights in males, and slight decreases (p<0.05) in both absolute and relative liver weights in both males and females were noted. Slight decreases (p<0.05) in absolute thymus and spleen weights relative to control were observed in treated males. Macroscopic examination of organs did not reveal any treatment related changes. Microscopic examinations were not performed.

Administration of SR 47436 at the dose level of 1000 mg/kg/day for 14 days resulted in a reduction body weight gain relative to that recorded in control males and females. Additionally, significantly low liver weights in males and females, low spleen and thymus weights in males, and low heart weights in females were noted. The study demonstrates that 1000 mg/kg/day was at, or exceeded, the maximum tolerated dosage in both males and females.

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