3.4.6. 104-Week Oral Carcinogenicity Study of SR 47436 in Rats (Report #RS0006960715/01, Study code: SNF/044-CAR015). Vols. 59-67

This GLP study was originally conducted by) for Sanofi Recherche, France between December 29, 1993 and May 17, 1996. The aim of the study was to assess the oncogenic effects of SR 47436 during its repeated daily oral administration to Wistar Rats for 104 weeks.

Male and female HanIbm Wistar rats were approximately 5 weeks old and weighed 89 to 164 and 84 to 145 g, respectively, at the start of the study. The HanIbm Wistar strain was selected because of the historical control data available at the contract laboratory and its established susceptibility to known carcinogens. Suspensions of SR 47436 (batches 93-06, 4SNP002, 4SNP013, 4SNP006,4SNP021, 4SNP035, 4SNP042, 4SNP063, 4SNP062, 5SNP515 and 5ARL005 (one day only)) in a 10% aqueous solution of gum arabic were administered orally by gavage (5-10 ml/kg), once daily for 104 weeks, at doses of 5, 50 or 500 mg/kg/day for males and 5, 50, 500, 1000 or 2000 mg/kg/day for females. Animals were not fasted before treatment. Control animals received the vehicle. Allocation of animals to various groups is shown in Table 3.4.6.1.

TABLE 3.4.6.1.
COMPOSITION AND IDENTITY OF TREATMENT GROUPS

Group	Treatment	Dosage (mg/kg/ day)	Number of Animals			
			Main Study		Satellite Study[e]	
			Male	Female	Male	Female
1	Vehicle control-1[a]	0	55	55	0	0
2	Vehicle control-2[b]	0	55	55	5	5
3	SR 47436	5	55	55	5	5
4	SR 47436	50	55	55	5	5
5	SR 47436	500	55	55	5	5
6	SR 47436	1000	0	55	0	5
7[c]	SR 47436[c]	2000[c]	0	55	0	5
8	Vehicle control-3[d]	0	55	55	5	5

a: First control group isolated from drug-treated groups during week 14 of treatment and housed with group 8.
b: Second control group housed along with drug treated groups.
c: Group 7 animals received test substance for 14 weeks only, after which they were prematurely sacrificed due to high mortality (see text for details).
d: Additional control group set up at week 21 and housed with group 1 in a separate room from drug-treated animals.
e: After 80 weeks of treatment for groups 2 to 6, and 60 weeks of treatment for group 8, all surviving satellite animals were killed without necropsy.

All animals were housed in Techniplast® ventilated cabinets in order to reduce the possiblility of cross contamination between groups. Measurable quantities of SR 47436 (0.139 - 0.395 mg/L) had been observed in plasma of control rats receiving the vehicle as part of a 3-month dose-ranging study at doses of 0, 15, 50, 150, and 500 mg/kg/day (section 3.4.4, and also reported in other studies, see sections 3.2.4, 3.2.7). After an extensive review of data and discussion, the sponsor and the contract laboratory believe that the contamination is airborne; in addition, SR 47436 is known to be extremely electrostatic. To test this mechanism for the contamination, a separate 10-day study was conducted in rats under identical conditions as the original range-finding study (at doses of 0, 0, 5, 50, and 500 mg/kg/day). In this 10-day study, one of two control groups was isolated from treatment room air in separate cage enclosures equipped with filters. Following 10 days of exposure, plasma samples were collected and assayed for SR 47436. Drug (~0.030 mg/L) was detected in samples from unprotected control animals. However, plasma samples from protected control animals revealed no significant contamination by SR 47436. Based on these findings, measures were taken to protect against airborne contamination in the 2-year study. Each specific dose group was housed in filtered, ventilated enclosures which isolate each cage rack from room air. Despite these measures, toxicokinetic evaluations at day 30 indicated SR 47436 was present in plasma samples of control animals (group 2) (mean 0.03 mg/L determined at 2 hr following dosing, see below for details under toxicokinetcs). In addition, satellite animals receiving the vehicle and sentinel animals (kept within the treatment room for evaluation of viral load) which were not dosed with vehicle revealed trace amounts of SR 47436 (~0.025 mg/L and ~0.014 mg/L, respectively). These groups were protected in separate cage rack enclosures within the same room as treated animals. Subsequent analysis of swabbed samples from cage racks, walls, cages, etc. in the test room have revealed traces of SR 47436, in keeping with a contamination through the air. All samples were evaluated by an HPLC assay. These measurements were confirmed with a radioimmunoassay.

In contrast, sentinel animals caged in a separate room without the use of a cage rack enclosure were not contaminated. Based on these findings, groups 1 and 8 were moved to a room separate from that housing animals receiving SR 47436, group 1 animals being relocated during week 14 of treatment and joined by group 8 animals six weeks later on their commencement of treatment. The purpose of including three control groups [two of which were housed in a separate room (groups 1 and 8) from treated animals] was to ensure that there were two control groups with a reduced possibility of cross-contamination with the test substance. All cages carried protective covering over the cage lid.

Observations and Measurements

All animals were observed at least twice daily for mortality and drug effects. The body weights were recorded on the day that treatment commenced and at weekly intervals for the first 14 weeks, once fortnightly thereafter and before necropsy. 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. With the exception of group 8, water consumption was recorded over a 2-day period for the three cages of rats with the lowest number in

each group during week 8 of treatment. During week 103 of treatment (before dosing) blood samples were obtained from the retro-orbital sinus of all surviving rats (in anesthetized state), with the exception of group 7; those animals were sampled after 14 weeks due to premature sacrifice. Samples from 10 surviving animals/group/sex with highest animal numbers were examined for all hematological parameters, while the samples from the remaining rats were analyzed for RBC and WBC only. Blood chemistry analysis was limited to high dose (2000 mg/kg/day) females (group 7) after 13 weeks of treatment. Serum drug levels were determined from blood samples taken from 3 male and 3 female satellite animals with the highest animal numbers of groups 2, 3, 4, 5, 6, 7 (female only) and 8 (female only) at 2 and 24 hr during weeks 5, 13, 27 and 53 of drug treatment (same animals used at all timepoints and weeks). Blood samples were also taken from group 8 males in week 38. Since SR 47436 was detected in the plasma of control animals from group 2 during week 5 of treatment, blood samples were taken from 3 male and 3 female main study control group 1 animals with the highest animal numbers in week 18 (2 hr timepoint only), and 2 animals from group 7 in week 13 (same animal for 2 and 24 hr timepoints). Blood was collected from these animals 2 hr after administration of vehicle and approximately 3 weeks after moving into a separate room. In addition, the following samples were taken to analyze the presence of test substance as a source of contamination through the air. (a) water samples from the water bottle of one cage from each of groups 2F and 7F (sampling interval not given); (b) fecal samples from one cage from each of groups 2M, 2F and 3F (sampling interval not given); (c) during week 13 of treatment, the three cages with the lowest cage numbers of groups 5M and 7F were swabbed with cotton wool soaked in methanol immediately prior to cage washing and again on completion of cage washing; (d) the inlet and extract filters from the cabinets of groups 2M and 2F and the extract filters from the cabinets of groups 3M, 3F and 5M during week 12 of treatment; (e) the extract filter from the cabinet of group 7F during week 14 of treatment.

All animals (from main study groups) 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 tissues to be examined for histopathology were preserved in 4% neutral buffered formaldehyde, except for 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. Microscopic examination was performed on all tissues listed below from all male rats of groups 1, 2, 5 and 8, and from all female rats of groups 1, 2, 6 and 8 sacrificed at the termination of the study; from all female rats of group 7 killed after 14 weeks of treatment; and from all rats killed or dying during the study. Microscopic examination was also performed on kidneys and pitutitary glands of males and females; the mammary glands, adrenals and uteri of females and the hearts of males, from all rats of the low and intermediate dosage groups sacrificed at term. Microscopic examinations were also performed on all masses, the lymph nodes draining the regions adjacent to those masses, and on any other grossly abnormal tissues.

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

¶Tissues preserved, but not examined, §: organs weighed

Results

A total of 30 females receiving 2000 mg/kg/day (group 7) died or were killed during the 14 week treatment period as compared to one control female from group 1 which died during the same period. Of the 30 decedent females, 19 were found dead and 11 were killed in extremis, the largest incidence of deaths occurring between weeks 7 and 12 of treatment. Incidence and time of death and/or sacrifice for group 7 are: 1 each in weeks 2, 5, 6, 7; 4 each in weeks 8, 9, 10; 7 in week 11; 2 each in weeks 12 and 14; and 3 in week 13. All of these deaths were considered to be related to treatment. In view of the high mortality, all surviving animals in this group were killed after completing 14 weeks of treatment. In the remaining 7 groups, to term, a total of 70 males and 106 females died or were killed during the treatment period (Table 3.4.6.2). A significant increasing trend (Tarone's linear trend test, $p < 0.001$) was observed for deaths in the case of females only. Compared to controls, mortality was higher amongst females receiving 1000 or more mg/kg/day. There was no treatment-related target organ toxicity to account for the increased mortality.

Signs of reaction to treatment included pre- and postdose salivation from day 7 of treatment, in females receiving 1000 or more mg/kg/day. Other signs associated with the high dose in females were piloerection, partially closed eyelids, hunched posture, abnormal respiration and underactivity from week 3, with increased incidence as the study progressed and associated with other sporadic signs (irregular respiration, ataxia, vocalisation, prostration, peripheral vasodilation and closed eyes). Routine examination of females receiving 2000 mg/kg/day identified a number of signs (such as thinness, reduced body temperature, hair loss, dull eyes) indicative of a general deterioration in health condition. The majority of these signs were also seen in females dosed with 1000 mg/kg/day during the first 12 months of treatment. Isolated incidences of salivation and piloerection were also recorded for males receiving 500 mg/kg/day. The total number of palpable swellings and the number of animals bearing them was lower when compared with the controls for females receiving 500 or 1000 mg/kg/day. Males were considered unaffected.

TABLE 3.4.6.2
INCIDENCE OF MORTALITY: RAT CARCINOGENICITY STUDY

Group/sex	1M	2M	3M	4M	5M	8M	1F	2F	3F	4F	5F	6F	8F
Dosage (mg/kg/d)	0	0	5	50	500	0	0	0	5	50	500	1000	0
Group size	55	55	55	55	55	55	55	55	55	55	55	55	55
Mortality:													
Week 26	1	1	0	0	0	0	1	0	1	0	0	7	0
% survival	98	98	100	100	100	100	98	100	98	100	100	87	100
Week 52	1	2	1	3	0	0	1	0	1	1	0	9	0
% survival	98	96	98	95	100	100	98	100	98	98	100	84	100
Week 78	6	3	5	4	4	4	4	2	3	2	5	11	3
% survival	89	95	91	93	93	93	93	96	95	96	91	80	95
Termination	12	11	14	9	14	10	11	11	14	8	17	28	17
% survival	78	80	75	84	75	82	80	80	75	85	69	49	69

The overall body weight gains of high dose (2000 mg/kg/day) females were 83 and 74% ($p < .01$) of control groups 1 and 2, respectively. In females receiving 50, 500 or 1000 mg/kg/day, the overall body weight gains relative to combined controls were, respectively, 85, 85 and 74%. The decrease was mainly due to lower body weight gains during the 2nd year of study. Mean body weight gains for the high dose females in the first and second years of study were, respectively, 12.4% and 25.4% below the mean gain of the combined controls (Fig. 3.4.6.1). The mean body weight gain of males receiving 50 or 500 mg/kg/day was lower than that of the controls. However, from week 52 onwards, the mean body weight gain of males in these groups was slightly higher than that of the controls (Table 3.4.6.3). Mean body weight gains for the high dose males at weeks 52 and 104 were, respectively, 14.4% and 5.9%, below the gain of the combined controls (Fig. 3.4.6.1). The amount of food consumed by high dose females during the first three weeks of treatment was lower than that of the controls. Thereafter it was similar or slightly higher than that of the controls. The food intake for the rest of the treated groups was essentially similar to that of control animals throughout the treatment period. The water intake of females receiving 1000 or more mg/kg/day was markedly higher than control during week 8 of treatment. No other treated groups were affected.

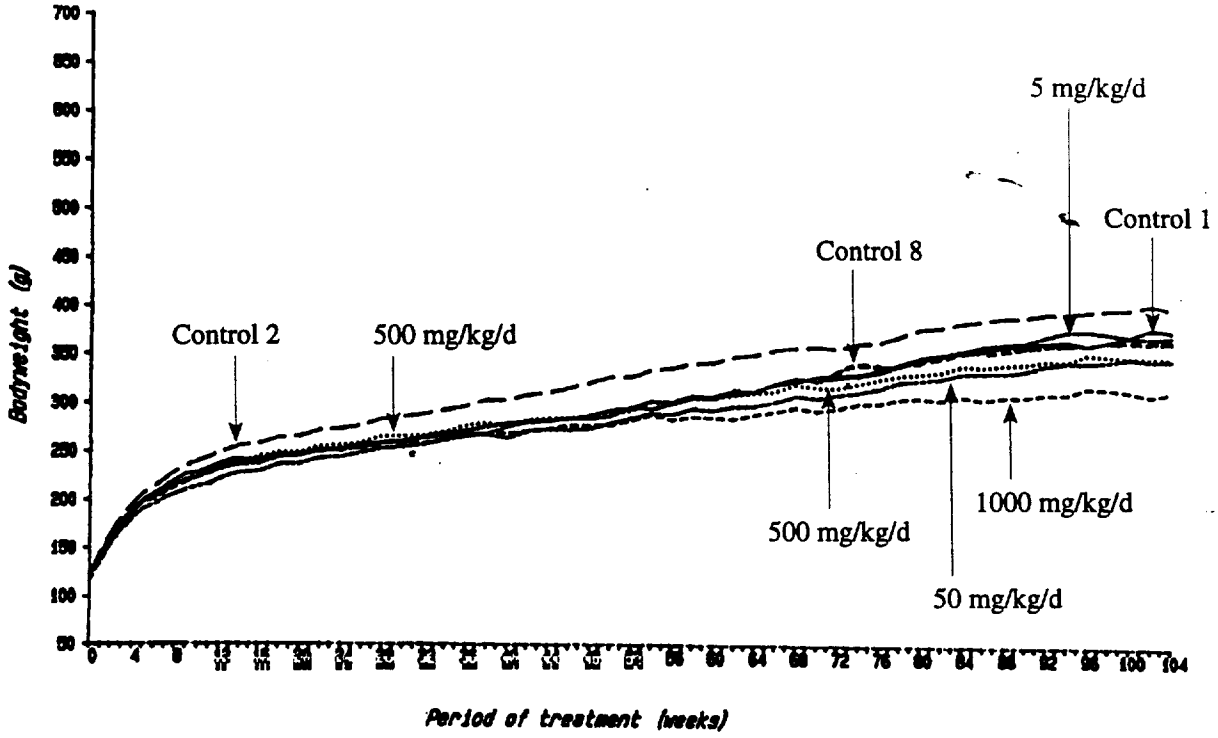

Figure 3.4.6.1.: Group mean bodyweight curves for female rats

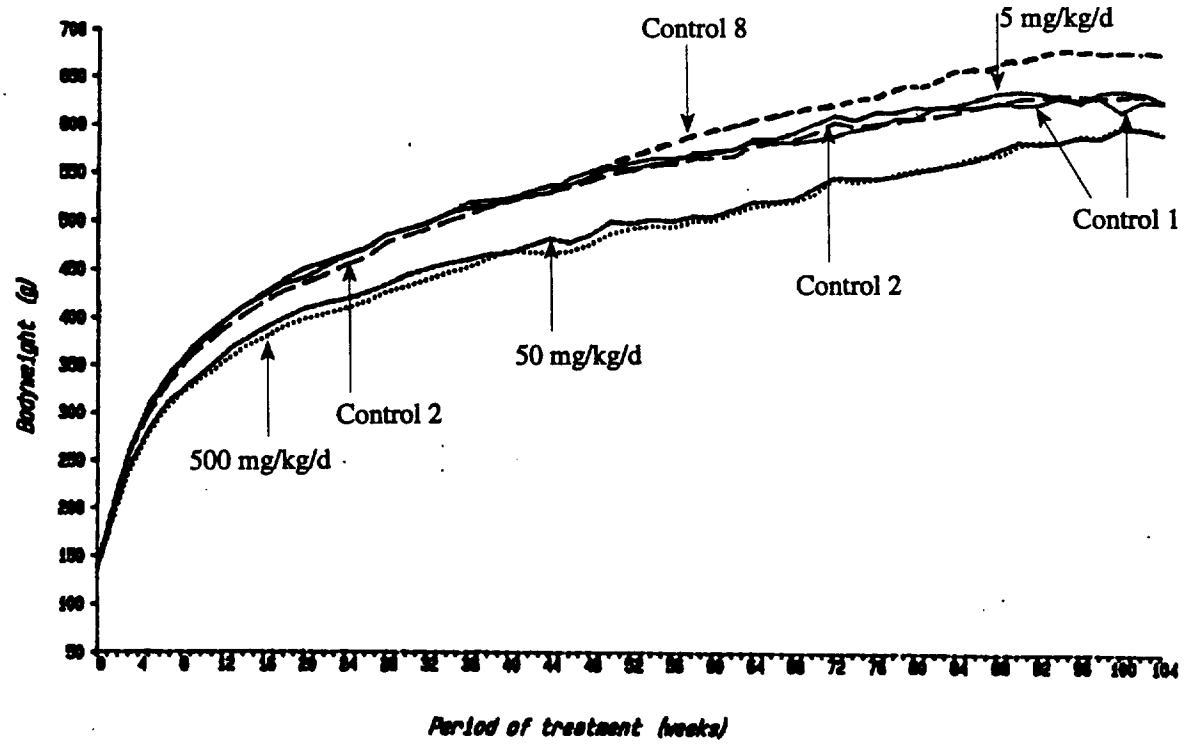

Figure 3.4.6.2.: Group mean bodyweight curves for male rats

TABLE 3.4.6.3

BODYWEIGHT GAIN OF SURVIVORS - GROUP MEAN VALUES (G) IN THE 104-WEEK ORAL CARCINOGENICITY STUDY IN RATS

Week number	mg/kg/day	control-1	control-2	5	50	500	control-3	control-1	control-2	5	50	500	1000	control-3
	Gp # Sex	1M	2M	3M	4M	5M	8M	1F	2F	3F	4F	5F	6F	8F
0-13	N	55	55	55	55	55	55	54	55	55	55	55	52	55
	Mean	262z	259z	265z	233cez	223cfz	304cf	118fx	130c	112fz	104cfz	113fz	119fx	127a
	SD	39.3	28.7	38.9	34.1	38.0	35.8	14.9	18.4	17.3	14.1	14.9	17.5	13.1
13-26	N	54	54	55	55	55	55	54	55	54	55	55	48	55
	Mean	67	67	69	56bez	57bez	72	13fz	24cx	23cx	24cy	22cz	19cez	28cd
	SD	16.4	16.5	16.7	14.7	17.7	16.8	8.5	10.8	10.4	7.4	8.0	8.3	10.7
26-52	N	54	53	54	52	55	55	54	55	54	54	55	46	55
	Mean	90	92	84	74cfz	77bey	91	43d	51ay	38f	36f	34af	28cfy	39e
	SD	28.6	22.5	28.8	18.6	24.0	26.0	21.8	23.1	16.5	14.8	17.0	15.9	22.7
52-78	N	49	52	50	51	51	51	51	53	52	53	50	44	52
	Mean	57x	53y	59	52y	61	70ae	48	48	46	39adz	40y	25cfz	54
	SD	28.8	31.0	26.1	30.8	18.2	32.2	26.7	22.5	19.9	16.5	25.4	21.0	28.2
78-104	N	43	44	41	46	41	45	44	44	42	47	40	28	38
	Mean	16	21	19	47be	42ae	35	34	32	35	26	25	23	25
	SD	60.8	38.7	43.4	36.8	33.4	36.9	37.5	34.6	37.9	18.1	34.2	20.8	22.1
0-104	N	43	44	41	46	41	45	44	44	42	47	40	28	38
	Mean	488z	494z	495z	459z	461z	575cf	255	280	251d	227afy	227afy	198cfz	267
	SD	113.8	87.9	84.8	86.2	92.4	116.9	64.9	52.1	65.8	38.1	49.5	34.9	64.0

SD: Standard deviation; N: Number of animals.

Significant when compared with Group 1: a - $p<0.05$; b - $p<0.01$; c - $p<0.001$.

Significant when compared with Group 2: d - $p<0.05$; e - $p<0.01$; f - $p<0.001$.

Significant when compared with Group 8: x - $p<0.05$; y - $p<0.01$; z - $p<0.001$.

High dose females that were killed *in extremis* (not at week 14) showed slightly low RBC. Slight decreases in RBC and hemoglobin were also noted in females receiving 50 or more mg/kg/day and in males receiving 500 or more mg/kg/day in week 103. Several high dose females killed after 13 weeks of treatment showed elevated alkaline phosphatase, alanine and aspartate amino-transferase activities, urea, creatinine and glucose concentrations relative to controls. Triglyceride and cholesterol concentrations appeared low.

At the 14 week sacrifice, the mean absolute and relative adrenal, kidney and liver weights of high dose females were high relative to control. In this same group, the absolute and relative spleen and thymus weights were low. At the 104 week sacrifice, low mean absolute heart and lung weights were noted for treated animals receiving 50 or more mg/kg/day (lungs for males affected only at 500 mg/kg/day). High mean liver weights were observed for groups receiving 50 or more mg/kg/day and high kidney weights were recorded for males given 500 mg/kg/day and for all treated females (Table 3.4.6.4). Liver and heart weight findings were not associated with any histopathological findings.

Several of the decedent females given 2000 mg/kg/day showed, on autopsy, distention of the stomach with a pale, caseous material, which was occasionally found in the esophagus, and dark, liquefied contents in the cecum. Other macroscopic findings observed, at low incidence, in these decedents females, were pale kidneys, enlarged adrenals, pale liver, thickening of the stomach and cecum wall, areas of change in the stomach and cecum wall, darkening of the cecum wall and distention of the cecum. Thin appearance was also observed in most rats of the 2000 mg/kg group. Several of the females receiving 2000 mg/kg/day and sacrificed after completion of 14 weeks of treatment showed fluid distention of the uterus.

There were no clear treatment-related macroscopic changes noted among other animals killed or dying during the 104 weeks of treatment. For animals killed after 104 weeks of treatment, a decreased number of masses in the mammary area was recorded for all females at dosages of 50 or more mg/kg/day. Also, the number of pituitary masses in females receiving 1000 mg/kg/day was significantly reduced. When all animals (survivors and decedents) were considered together, there was an increased incidence ($p < .05$) of areas of change in the lungs of females that received 1000 mg/kg/day (15 of 55 animals) when compared to combined controls (13 of 165). There were no other changes considered to be treatment-related.

TABLE 3.4.6.4
ORGAN WEIGHT VARIATION IN THE 104-WEEK ORAL CARCINOGENICITY STUDY IN RATS
GROUP MEAN VALUES (GM) AT THE END OF TREATMENT

Group		1		2		3		4		5		6	7	8	
Dose, mg/kg/day		Control-1		Control-2		5		50		500		1000	2000*	Control-3	
Sex		M	F	M	F	M	F	M	F	M	F	F	F	M	F
Terminal Body wt, g		616	370	617	384	620	354^{d}	588^{y}	337^{f}	590^{y}	343^{ae}	299^{cfz}	213.3	665.8	360
N		43	44	44	44	41	41	46	47	41	38	27	25	45	38
Adrenals	Abs wt.	0.085	0.064	0.063	0.064	0.061	0.067	0.061	0.058^{adx}	0.061	0.063	0.063	0.088	0.065	0.074
	Rel wt.	0.0137	0.0177	0.0103	0.0173	0.0099	0.0194	0.0105	0.0175	0.0104	0.0187	0.0212^{be}	0.0415	0.0098	0.0207
Heart	Abs wt.	1.55	1.17	1.59	1.18^{x}	1.52	1.10^{d}	1.42^{acx}	1.07^{af}	1.40^{adz}	1.11^{d}	1.05^{bf}	0.81	1.61	1.11^{d}
	Rel wt.	0.255	0.320	0.259	0.315	0.248	0.316	0.243	0.322	0.240^{d}	0.328	0.355^{adx}	0.383	0.245	0.317
Kidney	Abs wt.	2.79	2.01^{f}	2.85	2.19^{cy}	2.94	2.14^{a}	3.03	2.09	3.06^{be}	2.22^{cy}	2.21^{bx}	1.57	2.98	2.02^{e}
	Rel wt.	0.459	0.557	0.466	0.584	0.478^{x}	0.614^{bx}	0.517^{x}	0.625^{cy}	0.529^{cfz}	0.659^{cex}	0.745^{cfx}	0.738	0.450	0.570
Liver	Abs wt.	17.0^{x}	11.2	16.9^{x}	12.0	17.0^{x}	11.4	17.7	10.8^{e}	17.4	12.0	11.3	11.6	18.8^{ad}	11.0
	Rel wt.	2.76	3.06	2.75	3.18	2.77	3.22^{a}	3.03^{cfx}	3.23	2.97^{ad}	3.52^{cdx}	3.80^{cfz}	5.44	2.81	3.03
Lungs & bronchi	Abs wt.	2.46	1.87	2.52	1.94^{x}	2.55	1.92	2.37	1.68^{af}	2.20^{ae}	1.65^{bf}	1.61^{cfx}	1.17	2.38	1.78^{d}
	Rel wt.	0.410	0.516	0.416^{x}	0.518	0.417^{x}	0.550	0.413^{x}	0.505	0.380	0.490	0.540	0.549	0.367^{d}	0.513
Spleen	Abs wt.	1.15^{y}	0.89	1.25	0.83	1.23	0.84	1.33^{a}	0.79	1.16^{x}	0.81	0.71^{cex}	0.464	1.31^{ab}	0.81
	Rel wt.	0.1885	0.2457	0.2044	0.2193	0.2002	0.2378	0.2293^{b}	0.2359	0.1966	0.2412	0.2379	0.2188	0.1997	0.2253
Thymus	Abs wt.	0.58^{d}	0.29^{d}	0.39^{ax}	0.36^{a}	0.46	0.50	0.46	0.31	0.49^{e}	0.36	0.24^{f}	0.274	0.48^{d}	0.46
	Rel wt.	0.0980	0.0763^{d}	0.0621	0.0938^{a}	0.0737	0.1347	0.0771^{e}	0.0943^{a}	0.083^{fx}	0.1050	0.0804	0.1292	0.0707	0.1201

Significant when compared with group 1: a, P <0.05; b, P <0.01; c, P <0.001.
Significant when compared with group 2: d, P <0.05; e, P <0.01; f, P <0.001.
Significant when compared with group 8: x, P <0.05; y, P <0.01; z, P <0.001.
*: No statistical analysis performed by the sponsor.

Regarding *microscopic pathology*, a statistically significant positive trend was noted by the sponsor for uterine adenocarcinoma (Table 3.4.6.5). However, the sponsor hesitates to link this trend to treatment with SR 47436 because: "(a) there was no significant pairwise difference between high dose and control groups, (b) there was no clear dose response relationship, and (c) the incidence in the highest dosage group was within the historical control range for this tumor type" (Table 3.4.6.5). Further, FDA's analysis produced no statistically significant trends or pairwise comparisons. The reason for this: the FDA reviewer assigned 0.005 as the critical p-value for significance (criterion used by FDA for trend tests when control rate exceeds one percent) whereas, the sponsor used 0.05. None of the groups reached the critical p value. However, it should be noted that the historical control data (0-2%) of the laboratory ____, in which the work was originally conducted had only two control groups (the other laboratory had 4 control groups).

TABLE 3.4.6.5
NEOPLASTIC FINDINGS: UTERINE ADENOCARCINOMA IN FEMALE RATS: 104 WEEK CARCINOGENICITY STUDY IN RATS

Dose, mg/kg/day	Control-1	Control-2	5	50	500	1000	Control-3
Group	1	2	3	4	5	6	8
Incidence[1]	3/54	0/55	0/55	3/55	6/55	4/54	0/54
% Incidence	5.55%	0%	0%	5.45%	10.91%	7.41%	0%
Statistical tests							
1. Cochran-Armitage test		p<0.05					p<0.05
2. Fisher exact pairwise					p<0.05[c]		
Historical controls (%)	2 - 8 % at Huntingdon Research Center 0 - 2 % at Pharmaco LSR						

[1] # rats affected / # rats examined

[c] p= 0.027 against control group 2 and control group 3

Otherwise, the sponsor's and the FDA's analyses revealed no statistically significant increased trend in the incidence of any neoplasm that could be attributed to treatment with SR 47436 for either sex of rats that were killed or died during the treatment period, or killed after 104 weeks of treatment. Summary of tumor incidence is given in Tables 3.4.6.6, 3.4.6.7 and 3.4.6.8.

The other types of tumors observed in this study were those expected for rats of this strain and age and they occurred with similar incidences in control and treated groups except for mammary fibroadenomas and pituitary adenomas which appeared to be decreased in females receiving 1000 mg/kg/day.

TABLE 3.4.6.6
SUMMARY OF NEOPLASTIC FINDINGS FOR ALL RATS: 104 WEEK CARCINOGENICITY STUDY IN RATS
(Numbers in parentheses indicate % incidence)

Group	1		2		3		4		5		6	8	
Dose, mg/kg/d	CONTROL-1		CONTROL-2		5		50		500		1000	CONTROL-3	
Sex	M	F	M	F	M	F	M	F	M	F	F	M	F
# examined	55	55	55	55	55	55	55	55	55	55	55	55	55
# Rats with neoplasms													
Malignant	12(22)	6 (11)	10(18)	10(18)	8(15)	10(18)	4(7)	10(18)	9(16)	11(20)	8(15)	8(15)	8(15)
Benign	37(67)	41(75) [a]	37(67)	45(82) [b]	29(53)	41(75)	16(29)	35(64)	26(47)	72(76)	29(53)	36(65) [b]	38(69)
Malignant or benign	42(76)	43(78)	43(78)	48(87) [b]	32(58)	43(78)	19(35)	41(75)	33(60)	48(87)	33(60)	40(73) [a]	42(76)
Total # of neoplasms													
Malignant	12	6	10	11	8	11	4	10	10	11	9	8	8
Benign	59	62	59	73	36	65	17	54	39	64	37	58	58
Malignant or benign	62	68	69	84	44	76	21	64	49	75	46	66	66
# of tumors/animal	0-3	0-4	0-4	0-5	0-4	0-2	0-3	0-4	0-4	0-4	0-3	0-4	0-5

Cochran-Armitage trend test:
None of the tumours is significant in 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 tumours at the highest dosage:
a = $p < 0.05$ (two tailed test only)
b = $p < 0.01$ (two tailed test only)

TABLE 3.4.6.7
OCCURRENCE OF PRIMARY TUMORS IN 104 WEEK CARCINOGENICITY STUDY IN RATS

Group	1		2		3		4		5		6	8	
Dose, mg/kg/day	Control-1		Control-2		5		50		500		1000	Control-3	
Sex	M	F	M	F	M	F	M	F	M	F	F	M	F
Rats initially in the study	55	55	55	55	55	55	55	55	55	55	55	55	55
# examined microscopically	55	55	55	55	55	55	55	55	55	55	55	55	55
# of rats with 1 tumor, *total*	26	24	22	25	21	21	14	24	23	28	21	21	28
Found dead	2			3		3		1	3	3	7	2	2
Moribund sacrifice	8	5	7	3	7	3	6	5	5	8	3	4	11
Accidental death													
Scheduled sacrifice	16	19	15	19	14	15	11	18	15	17	11	15	15
# of rats with 2 tumors, *total*	12	14	17	14	10	14	2	13	6	15	11	14	8
Found dead				1	1	2				1	2		
Moribund sacrifice	2	4	1	2	4	3	1	1	1	1	2	2	1
Accidental death													
Scheduled sacrifice	10	10	16	11	5	9	2	12	6	13	7	12	7
# of rats with 3 tumors, *total*	4	4	3	6	1	5		2	2	3	1	3	3
Found dead													1
Moribund sacrifice				1	1	1						1	
Accidental death													
Scheduled sacrifice	4	4	3	5		4		2	1	3	1	2	2
# of rats with 4 tumors, *total*		1	1	2		3		2	2	2		2	2
Found dead													
Moribund sacrifice			1	1		1			2	2		1	1
Accidental death													
Scheduled sacrifice		1		1		2		2				1	1
# of rats with 5 tumors, *total*													1
Found dead													
Moribund sacrifice													
Accidental death													
Scheduled sacrifice													1
# of rats with tumors													
Found dead	2			4	1	5		1	3	4	9	2	3
Moribund sacrifice	10	9	9	7	12	8	6	6	8	11	5	7	13
Accidental death													
Scheduled sacrifice	30	34	34	37	30	30	15	34	29	33	19	31	26

TABLE 3.4.6.8
104-WEEK ORAL CARCINOGENICITY STUDY OF SR 47436 IN RATS (REPORT #RS0006960715/01)
INCIDENCE OF NEOPLASTIC CHANGES*

Treatment	control-1		control-2		control-3		SR 47436						
Group	1		2		8		3		4		5		6
Dose levels (mg/kg/day)	(0)		(0)		(0)		(5)		(50)		(500)		(1000)
Sex	M	F	M	F	M	F	M	F	M	F	M	F	F
Adrenals (No. examined)	55	54	55	55	55	55	14	55	10	55	55	55	55
Cortical adenoma	1	1			1			1		3		3	
Benign phaechromocytoma	1	2	2		1			1	1	1		2	
Malignant phaechromocytoma	1		1					1		1	1		1
Brain (No. examined)	55	54	55	55	55	55	16	22	10	11	55	22	54
Benign granular cell tumour	1	2	1		2	1					1		1
Malignant granular cell tumour											2		
Malignant oligodendroglioma						1							
Malignant astrocytoma	1		1			1					1		
Choroid plexus carcinoma					1							1	
Femur (No. examined)	55	54	55	55	55	54	14	14	9	8	55	17	55
Osteosarcoma													1
Jejunum (No. examined)	55	54	55	55	55	55	14	13	9	9	52	18	47
Leiomyosarcoma										1			
Kidney (No. examined)	55	54	55	55	55	55	55	55	55	55	55	55	55
Haemangioma			1										
Mesenteric L.N. (No. examined)	55	54	55	55	55	54	18	17	14	14	55	20	55
Haemangioma	8	4	10	4	10	3	4		4	2	9	4	2
Haemangiosarcoma	3		1			1		1	1	1	1	1	
Liver (No. examined)	55	54	55	55	55	55	19	15	14	12	55	23	55
Cholangioma				1						1		1	1
Hepatocellular adenoma				1	1	2		1				1	1
Cholangiocarcinoma		1											
Hepatocellular carcinoma					1								
Mammary gland (No. examined)	55	54	55	55	55	55	24	55	19	55	55	55	55
Fibroadenoma		6		5	1	9		6		5		2	
Adenocarcinoma		1				1		2					
Ovaries (No. examined)		54		55		55		18		15		22	55
Benign sertoliform tumour												1	
Benign granulosa cell tumour				1								2	
Leiomyosarcoma								1					
Pancreas (No. examined)	55	54	55	55	55	55	14	13	9	8	55	17	55
Islet cell adenoma	2		2	1	2	1							
Exocrine cell adenoma					1								
Islet cell carcinoma					1		2				2		1
Exocrine cell carcinoma						1							
Parathyroids (No. examined)	53	53	53	55	53	52	13	13	9	7	53	15	53
Adenoma	6		3	2	3								
Pituitary (No. examined)	55	54	55	55	54	54	55	55	55	55	54	55	54
Pars intermedia adenoma			1	1	1				1			1	
Pars distalis adenoma	18	31	12	25	15	34	20	32	8	27	17	31	18
Pars distalis carcinoma		2											1

*Animals bearing tumors of specific tissues

TABLE 3.4.6.8

Incidence of neoplastic changes in rats (continued)

Treatment	control-1		control-2		control-3		SR 47436						
Group	1		2		8		3		4		5		6
Dose levels (mg/kg/day)	(0)		(0)		(0)		(5)		(50)		(500)		(1000)
Sex	M	F	M	F	M	F	M	F	M	F	M	F	F
Prostate (No. examined)	55		55		55		14		11		54		
Adenoma	1												
Seminal vesicles (No. examined)	55		55		55		14		9		55		
Cystadenoma			1										
Leiomyosarcoma							1						
Spleen (No. examined)	55	54	55	55	55	55	16	14	12	9	55	18	55
Haemangioma							1						
Haemangiosarcoma	1												
Stomach (No. examined)	55	54	55	55	55	55	14	15	15	8	53	18	55
Squamous cell papilloma					1								
Submandibular gland (No. exam.)	55	54	55	55	55	55	14	12	9	7	55	16	53
Malign. myoepithelial tumour										1			
Testes (No. examined)	55		55		55		23		17		55		
Benign interstitial cell tumour			1		1		1						
Thymus (No. examined)	55	53	55	55	55	55	16	18	9	10	55	20	55
Benign thyrogl. duct tumour											1		
Benign Thymoma				1		4		4		2		2	1
Malignant Thymoma		1	1										
Sarcoma													
Thyroids (No. examined)	54	53	55	55	56	55	21	14	10	8	55	18	53
Follicular cell adenoma	5	2	3		1		1				1	2	
Parafollicular cell adenoma	7	3	2	5	6	6	1	1			2	1	4
Follicular cell carcinoma		1	1		1			1		1		1	
Parafollicular cell carcinoma						1							
Tongue (No. exam.)	54	52	55	55	55	55	21	14	9	7	55	16	54
Granular cell tumour										1			
Uterine cervix (No. examined)		54		54		55		16		9		17	54
Stromal polyp						3		1					
Endometrial sarcoma												1	
Uterus (No. examined)		54		54		55		55		55		55	54
Stromal polyp		1		5		4		7		9		3	5
Adenoma								2		2			
Leiomyoma						1							
Adenocarcinoma				3						3		6	4
Leiomyosarcoma										1			
Malignant schwannoma		1											
Vagina (No. examined)		54		55		54		14		8		18	54
Benign granular cell tumour												1	
Haemopoietic tumour													
Malignant lymphoma			2		2	1	2	1			1		1
Histiocytic sarcoma				1	1		2	1					
Large granul. lympho. leukem.								1					

TABLE 3.4.6.8
Incidence of neoplastic changes in rats (continued)

Treatment	control-1		control-2		control-3		SR 47436						
Group	1		2		8		3		4		5		6
Dose levels (mg/kg/day)	(0)		(0)		(0)		(5)		(50)		(500)		(1000)
Sex	M	F	M	F	M	F	M	F	M	F	M	F	F
Abdomen (No. examined)	1	1	1	0	2	1	1	1	0	0	0	3	2
Sarcoma					1								
Mesotheliosarcoma	1					1	1						
Leiomyosarcoma			1										
Abdominal fat (No. examined)	5	3	1	5	2	5	2	3	3	3	2	3	4
Haemangioma													1
Iliac lymph node (No. examined)	6	1	6	8	5	5	6	6	4	3	3	0	0
Haemangioma				1									
Mammary gl. -cranial (No. exam.)	1	11	1	11	1	15	0	14	0	7	2	6	4
Fibroadenoma		5		5		2		6		1		4	1
Adenocarcinoma				1		3		2					
Mammary gl. other (No. exam.)	0	0	0	2	0	1	0	1	0	1	0	1	2
Fibroadenoma				1		1		1		1		1	1
Adenocarcinoma				1									
Musculo-skeletal (No. examined)	0	1	2	0	0	0	0	1	0	0	0	2	0
Lipoma												1	
Haemangiosarcoma												1	
Sarcoma		1	1										
Nasal cavity (No. examined)	0	0	1	0	0	0	0	0	0	0	0	0	0
Carcinoma			1										
Skin - other (No. examined)	12	8	17	9	20	7	22	8	13	8	16	2	6
Papilloma					2	2	1						
Keratoacanthoma	5		2		4		1				3		1
Benign basal cell tumour					2			1					
Benign granular cell tumour			1		1								
Sebaceous adenoma			1										
Benign trichoepithelioma									1				
Fibroma	1		1				3		1		2		
Lipoma			1	1	1			1	1		3		
Haemangioma							1						
Squamous cell carcinoma										1			
Fibrosarcoma					1				1		1		
Liposarcoma					1						1		
Haemangiosarcoma			1										
Sarcoma			1										
Tail (No. examined)	1	0	1	1	1	0	1	0	1	0	0	0	0
Papilloma				1	1								
Fibrosarcoma			1										

Non-neoplatic histopathology considered to be related to treatment was seen primarily in the kidneys of rats receiving 500 or more mg/kg/day.

Females at 2000 mg/kg/day: Findings related to treatment were seen mainly in the liver, kidney, GIT and thymus. In the liver, evidence of necrosis of individual hepatocytes and centriacinar hepatocytic fatty vacuolation was noted. In the kidney, there were juxtaglomerular apparatus hypertrophy, basophilic cortical tubules, dilated cortical tubules, generalized vacuolation and/or degeneration of tubules, cortical tubular necrosis, intratubular proteinaceous casts and hypertrophy of medullary tubular epithelium. GI findings included erosion, acute inflammation and mucosal hyperplasia of the glandular stomach and mucosal/submucosal acute inflammation, erosion and mucosal/submucosal edema of the small intestine. Animals killed prematurely or dying before term showed mucosal/submucosal acute inflammation, edema and erosion of the large intestine. In the thymus, lymphoid atrophy and lymphocytolysis were observed. Chronic inflammation of the tongue was noted in a number of animals.

Males and Females at doses ≥500 mg/kg/day: There were a number of significant findings in the kidneys of rats killed or dying during the treatment period or killed after 104 weeks of treatment. Incidence of findings in the kidneys of male and female rats are given in Table 3.4.6.9. The increased incidence of perivascular inflammatory infiltrate and basophilic tubules in the cortex of the kidneys could reflect mild renal tubular damage associated with changes in the hemodynamics of the kidney while renal tubule hyperplasia, seen mainly in high dosage animals, may represent a compensatory response to such damage. However, there were no kidney tumors. The findings were similar to those observed in the 104 week mouse carcinogenicity study (see Table 3.4.3.10). A decreased incidence of some findings was seen in other tissues such as a reduction in the incidence of secretory activity and acinar hyperplasia of the caudal mammary gland and a reduced incidence of hemorrhagic degeneration of the adrenal cortex (Table 3.4.6.9). The later finding reflects an association with a long term reduction in blood pressure. There were no other findings which were considered to be treatment-related.

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TABLE 3.4.6.9
INCIDENCE OF NON-NEOPLASTIC LESIONS: 104-WEEK CARCINOGENICITY STUDY IN RATS

Organ and lesion	Control 1		Control 2		5¶		50		500		1000	Control 3	
	M	F	M	F	M	F	M	F	M	F	F	M	F
Number examined[1]	55	55	55	55	55	55	55	55	55	55	55	55	54
Adrenals cortex: -hemorrhagic degeneration	0	28	1	24	0	37[d]	0	23[y]	3	7[cdz]	11[bdz]	2	37
Heart ventricle -chronic myocarditis/fibrosis	34	12[d]	34	3[ax]	29	2	20[ady]	0	22[adx]	2	2[bx]	35	10/53
Kidneys:													
-perivascular chronic inflamtn	15[x]	7[x]	17[y]	14[x]	14[x]	6[x]	16[x]	7[x]	35[cax]	24[cx]	22[bx]	5	0
-basophilic cortical tubules	6	5	9	5	5	2	6	4	27[cdy]	15[ad]	11	11	6
-renal tubule hyperplasia	2	0	2	1	1	0	3	2	11[adz]	3	4	0	1
-pelvic epithelial mineralizn	12	45	21	44	17	37	9[d]	31[bd]	5[fx]	29[bx]	15[cfx]	16	38
-pelvic calculus/calculi	14	18	9	18	20[dy]	14	8	12	3[b]	14	21	7	11
-dilated cortical tubules	0	6[x]	2	2	1	2	0	1	7[ax]	4	8[y]	0	0
-dilated collecting ducts	0	4	0	0	1	0	0	2	3	1	6[d]	0	0
Lymph node mesenteric, -hyperplasia	6	6	12[y]	8/54	3/18	5/17	3/14	2/14	10[y]	5/20	14[y]	2	3
Caudal mammary gland -secretory activity/ductular dilatation	1	20	0	23	1	18	0	12[d]	0	15	12[d]	1	16
-acinar hyperplasia	0	26	0	26	1	24	0	25	0	15[ad]	17	0	17
Spleen: -hemosiderosis	3	7	2	6	2/16	5/14	1/12	1/9	4	8/18	16[d]	0	13
-atrophy of white pulp	0	3	0	4	2/16	1/14	0/12	0/9	0	0/18	6[x]	1	0
Stomach:-glandular region: dilated glands	2	3	2	3	0/14	1/15	0/15	0/8	3/53	1	6[x]	1	0

¶: dose in mg/kg/day, 1: unless otherwise specified
Significant when compared with group 1: a: $p<0.05$, b: $p<0.01$, c: $p<0.001$
Significant when compared with group 2: d: $p<0.05$, e: $p<0.01$, f: $p<0.001$
Significant when compared with group 8: x: $p<0.05$, y: $p<0.01$, z: $p<0.001$

Toxicokinetics: Exposure of control group 2 (housed along with drug treated groups) to SR 47436 was first observed in week 5 and was confirmed in week 13 (see Table 3.4.6.10). As a result of this, the first control group (group 1) was moved to a separate room in week 14, a new control group (group 8) was added to the study on week 21 and caged within the same room as the isolated control group #1. The second control group (group 2) which remained in the same room as drug-treated animals, was exposed to SR 47436 constantly, throughout the study. SR 47438 was not detected in samples of group 1. SR 47436 was also never detected in animals of group 8, except in one animal at 2 hr sampling in week 27. However, the presence of drug was not confirmed in subsequent time-periods (weeks 38 and 53) and, therefore, was not considered by the sponsor to

reflect a contamination of the animal.

SR 47436 was detected at very low levels in the animals of control group 2 (remained in the same room as treated groups) at all weeks of measurement except in 2 female animals in week 13 with a mean level corresponding to approximately 10% of that of the low dose treated group (Table 3.4.6.10). In treated animals, plasma concentrations of SR 47436 increased with the administered dose in non-proportional fashion over the dose range studied. It was higher in female than in male rats and a large inter-individual variability was observed. Concentrations at 24 hours suggest that steady state conditions were achieved by week 5 (Table 3.4.6.10).

TABLE 3.4.6.10

MEAN SR 47436 PLASMA CONCENTRATIONS: 104-WEEK CARCINOGENICITY STUDY IN RATS

Sampling Time	Group	Dose (mg/kg)	C_{2h} (mg/l)		C_{24h} (mg/l)	
			Males	Females	Males	Females
Week 5	Group 2	0	0.03 ± 0.01	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
	Group 3	5	0.58 ± 0.15	0.56 ± 0.09	0.38 ± 0.10	0.42 ± 0.06
	Group 4	50	1.16 ± 0.43	3.15 ± 0.95	0.42 ± 0.02	0.65 ± 0.11
	Group 5	500	16.41 ± 5.41	91.28 ± 36.48	0.97 ± 0.13	2.03 ± 1.77
	Group 6	1000	-	129.20 ± 70.20	-	3.91 ± 3.49
	Group 7	2000	-	498.63 + 78.47	-	8.84 ± 8.91
Week 13	Group 2	0	0.05 ± 0.01	0.02 ± 0.02	0.07 ± 0.01	0.03 ± 0.03
	Group 3	5	0.59 ± 0.04	0.65 ± 0.08	0.49 ± 0.20	0.58 ± 0.10
	Group 4	50	1.42 ± 0.37	3.32 ± 2.15	0.58 ± 0.09	0.60 ± 0.10
	Group 5	500	25.89 ± 19.24	87.28 ± 44.15	0.90 ± 0.05	1.12 ± 0.20
	Group 6	1000	-	78.15 ± 64.65	-	1.89 ± 0.70
	Group 7	2000	-	298.74 ± 206.41	-	3.79 ± 1.04
Week 27	Group 2	0	0.01 ± 0.01	0.07 ± 0.06	0.02 ± 0.01	0.04 ± 0.03
	Group 3	5	0.70 ± 0.11	0.54 ± 0.12	0.46 ± 0.12	0.48 ± 0.11
	Group 4	50	2.88 ± 0.26	6.01 ± 4.11	0.61 ± 0.11	0.96 ± 0.29
	Group 5	500	103.05 ± 26.49	255.44 ± 112.24	1.17 ± 0.27	1.80 ± 0.80
	Group 6	1000	-	217.84 ± 137.69	-	3.44 ± 4.66
	Group 7	2000	-	-	-	-
Week 53	Group 2	0	0.01 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.10 ± 0.06
	Group 3	5	0.80 ± 0.12	0.72 ± 0.16	0.99 ± 0.49	0.42 ± 0.07
	Group 4	50	4.56 ± 2.45	9.08 ± 3.17	1.22 ± 0.65	0.72 ± 0.35
	Group 5	500	34.96 ± 13.15	141.71 ± 73.82	1.32 ± 0.34	4.83 ± 5.20
	Group 6	1000	-	171.51 ± 69.86	-	4.84 ± 1.88
	Group 7	2000	-	-	-	-

3.5. *Mutagenicity Studies*

3.5.1. SR 47436: Ames Reverse-Mutation Assay in Salmonella typhimurium (Report #RS0006910319/01 and RS0006920416/01; Study #CEL497 and CEL663). Vol. 46

These are two GLP studies conducted in the laboratory of Genetic Toxicology, department of Toxicology, Sanofi Recherche, Montpellier Cedex, France between February 27, 1990 and March 27, 1992.

The Ames test permits the detection of gene mutations induced by the test compound or its metabolites in histidine-requiring strains of *Salmonella typhimurium*. In the presence of a genotoxic agent, tester strains revert from histidine dependence (auxotrophy) to histidine independence (prototrophy). SR 47436 was evaluated for its ability to increase the reversion frequency at the histidine locus in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA 98 and TA100 in the presence and absence of a rat liver metabolic activation system (S-9 mix).

In order to select an appropriate range of test compound concentrations for the genotoxicity test, the bacterial toxicity of SR 47436 (batches 90.00 and 90.00bis) was assessed in tester strain TA100 by evaluating the effects of the drug on the growth of the background lawn and/or frequency of spontaneous revertants. SR 47436 was dissolved in dimethylsulfoxide and tested at concentrations of 100, 500, 1000, 2500 and 5000 µg/plate in the absence and presence of S-9 mix (from rat liver). Results of this test indicated that SR 47436 was not toxic to the strain at doses less than 2500 µg/plate. A marked toxic effect was observed at 5000 µg/plate with metabolic activation whereas, the effect was less pronounced without metabolic activation. At 2500 µg/plate, a slight toxic effect was noted only without metabolic activation.

In the mutation assay, the test and control articles were evaluated in triplicate cultures with all five tester strains in each study in the presence and absence of S-9 mix. The S-9 mixture included 6% (v/v) Aroclor 1254-induced male Sprague-Dawley rat liver homogenate with the appropriate buffer and cofactors. SR 47436 was evaluated at five dose levels, viz., 100, 500, 1000, 2500, and 5000 µg/plate. The reference positive controls used were: sodium azide (in TA1535 and TA100 strains), 9-aminoacridine (in TA1537), 2-nitrofluorene (in TA1538 and TA98) in the absence of S-9, and 2-aminoanthracene in all five tester strains in the presence of S-9 mix. For all strains, exposure to SR 47436 resulted in revertant frequencies similar to or less than observed in the concurrent solvent (DMSO) control cultures. A slight toxic effect (inhibited growth of TA1537, TA1538 and TA98) was observed only at 5000 µg/plate without metabolic activation. Positive controls induced a marked increase in the number of revertants per plate.

SR 47436 was reevaluated (at conc. of 250, 500, 1000, 2500 and 5000 µg/plate, batch 91.01) in a confirmatory assay (RS0006920416/01) under almost identical conditions (TA 102 instead of TA1538), with similar results. All positive and negative control values in both assays were within acceptable limits. Thus, SR 47436 had no genotoxic activity on *Salmonella typhimurium*.

3.5.2. SR 47436: *In vitro* DNA Repair Assay on Rat Hepatocytes (Report #RS860900903/CB1 and RS0006920421/01; Study #CEL476 and CEL622). Vol. 46

These two GLP studies were conducted in the laboratory of Genetic Toxicology, department of Toxicology, Sanofi Recherche, Montpellier Cedex, France between March 13, 1990 and April 17, 1992. The method detects unscheduled DNA synthesis (UDS) due to a lesion induced by a chemical or physical agent, by measuring the incorporation of tritiated thymidine in the DNA of non replicating rat hepatocytes in primary culture.

Collagenase dispersed hepatocytes were prepared from a male Fisher rat (320 g) following perfusion of the liver with Hank's saline solution. Liver cells were incubated with SR 47436 (batch 90.00), positive control (2-aminofluorene) or solvent control in the presence of 10 µCi/ml tritiated thymidine. SR 47436 was dissolved in dimethylsulfoxide and tested at concentrations of 5, 10, 50, 100, 250 and 500 µg/ml. The top concentration to assess the potential unscheduled DNA repair synthesis was selected according to the cytotoxicity of the compound. After 18 to 20 hr of incubation at 37°C, cells were washed and observed under a microscope. Morphology, attachment, and spreading out of cells, as well as any potential cytotoxic effect were noted. Then the cells were washed, fixed and autoradiographic experiments were performed. Each cell was examined microscopically and nuclear and cytoplasmic grains counted to measure the incorporation of thymidine. A compound is considered as positive when the number of nuclear grains is different in treated cultures and controls, when the mean number of "net" nuclear grains is above 3, and when the percentage of cells in repair is above 20%.

SR 47436 induced a marked toxic effect at concentrations of 100 or more µg/plate. At 50 µg/ml, this effect was less marked. At lower concentrations, no cytotoxic effect was observed. Consequently, test concentrations for the DNA repair synthesis study were 5, 10 and 50 µg/ml. In cells treated with SR 47436, the mean incorporation of tritiated thymidine in the nuclei was similar to that observed in solvent control cells and lower than that observed in cytoplasms. The positive control (2-aminofluorene) induced incorporation of many nuclear grains, indicating an intense DNA repair synthesis. In conclusion, SR 47436 did not induce DNA repair synthesis in the *in vitro* hepatocytes/DNA repair assay at concentrations of 5, 10 or 50 µg/ml.

The second study (RS0006920421/01) was conducted using a different batch of SR 47436 (#91.01) at concentrations of 1, 5, 10, 25, 50 and 75 µg/ml and similar results were reported. Thus, SR 47436 had no genotoxic activity in the *in vitro* DNA repair assay.

3.5.3. SR 47436: *In vitro* Gene Mutation Test With Chinese Hamster V79 Fibroblasts (Report #RS0006920422/01; Study #6TG074/080), Vol. 46

This GLP study was conducted in the laboratory of Genetic Toxicology, department of Toxicology of Sanofi Recherche, Montpellier Cedex, France between January 10 and March 20, 1992.

The assay system detects a gene mutation (base pair substitutions, frameshift mutations and deletions induced by the test substance) from the parental type to the mutant form which gives rise to a change in an enzymatic protein, HGPRT (hypoxanthine guanine phosphoribosyl transferase). The gene for HGPRT is located on the X chromosome and the role of the HGPRT enzyme is to induce the biosynthesis of purine nucleotides by converting hypoxanthine and guanine to the corresponding nucleoside 5'-monophosphate. Purine analogues such as 6-thioguanine (6-TG) are also converted, but to toxic ribonucleotides, which kill cells with normal enzyme activity. Conversely, mutant cells with a HGPRT-deficient genotype due to a mutation induced by a genotoxic agent can proliferate in a medium containing 6-thioguanine because of their ability to synthesize that required purine via the *de novo* pathway from 5'-ribose phosphate, amino acids and ATP. Experimentally, mutagenic effects are manifested by the appearance of cells resistant to 6-thioguanine (6-TG) and can be quantified by comparison of the numbers of 6-TG resistant colonies in the treated and control cultures. The experiment was conducted both in the presence and absence of rat-liver postmitochondrial fraction S-9 (prepared from rats injected with Aroclor 1254) and co-factors in order to ensure that any mutagenic effect of metabolites of the test compound would also be detected.

Two independent studies (#6TG074 and #6TG080) were performed under the same experimental conditions with the same batch of SR 47436 (batch 91.01).

V-79 cell cultures (Chinese hamster lung cells, fibroblast-like morphology) were exposed to the following concentrations of SR 47436 in DMSO: first study, 5, 10, 25, 50, 100, 250 and 500 µg/ml; and second study, 25, 50, 100, 250 and 500 µg/ml. The positive control used in the non-activated part of the experiment was ethylmethansulfonate (3 and 6 mM). In the presence of S-9 mix, benzopyrene (10 and 20 µg/ml) was used. An untreated cell culture and a solvent (DMSO) control were included in each mutation assay. V-79 cell populations were in exponential phase of growth irrespective of treatments. Subcultures of each culture were prepared to maintain exponential growth (3 or 4 days after the treatment). After 5 days, colonies were stained and counted. During this period cells could recover and divide to express the mutant phenotype. The number of colonies which developed in these cultures reflected the viability at the end of treatment. The high density cultures were subjected to the mutant selection procedure by supplementing the growth medium with 5 µg/ml 6-TG. Only cells mutated at the HGPRT locus could survive the 6-TG treatment. The number of colonies formed in the petri dishes during 7 days of incubation at 37°C reflected the overall number of mutations induced by the treatment with the test substance or the mutagen (i.e. positive control). The mutant colonies were counted manually and results are expressed as 'mean mutant frequency'.

Results: SR 47436 was found to be cytotoxic at 250 µg/ml and above with and without metabolic activation. The mutation frequency values for cultures treated with varying concentrations of SR 47436 were in the same range as untreated cell and control cultures in both studies. However, in the first study, cultures treated with DMSO and without S-9 mix had a low mutation frequency relative to untreated control. As a result of this, in comparison with DMSO control, SR 47436 treated cultures showed a statistically significant mutation frequency at all tested concentrations (Table 3.5.3.1). Since the mutation frequencies were not significant when compared with untreated cultures, these results were not considered biologically significant. The statistically significant (P <0.02) increase recorded at 50 µg/ml with S-9 mix in the first study was again considered by the sponsor to be of no toxicological significance since mutation frequency was not increased at higher concentrations of SR 47436 (Table 3.5.3.2) and not replicated in the second study (see below). However, the mutation frequency was not within the historical control range.

TABLE 3.5.3.1

IN VITRO GENE MUTATION ASSAY (WITHOUT METABOLIC ACTIVATION) AT THE LOCUS HGPRT IN CHINESE HAMSTER V79 FIBROBLASTS

		CFE I	CFE II	Viable cells x 10^6	nb 6TG mutants	M.F. x 10^6	Statistic test p	
Group 0 CC		1.24	0.93	3.71	69	18.62	< 0.001***	(1)
Group 1 DMSO		1.20	0.81	3.25	12	3.70		
Group 2 SR47436 25 microg/ml		0.92	1.07	4.26	45	10.56	0.10 NS 0.04 *	(0) (1)
Group 3 SR47436 50 microg/ml		0.83	1.02	4.09	68	16.64	0.71 NS 0.002**	(0) (1)
Group 4 SR47436 100 microg/ml		0.92	1.10	4.41	58	13.16	0.33 NS 0.009**	(0) (1)
Group 5 SR47436 250 microg/ml		1.06	0.90	3.61	61	16.88	0.71 NS 0.002**	(0) (1)
Group 6 SR47436 500 microg/ml		0.61	1.02	4.09	71	17.35	0.85 NS < 0.001***	(0) (1)
Group 7 EMS 3 mM		0.89	1.07	4.29	345	80.36	< 0.001*** < 0.001***	(0) (1)
Group 8 EMS 6 mM		0.82	1.00	3.99	753	188.88	< 0.001*** < 0.001***	(0) (1)
Historical negative control (DMSO)	n					14		
	mean					6.68 ± 3.64		
	range					1.43 - 11.4		

CFE: the colony-forming efficiency given as the ratio of clones formed to cells seeded; this ratio was evaluated twice, one after treatment (CFE I) and another after mutant phenotype expression (CFE II); MF: mutant frequency; (0): Statistic test / group 0; (1): Statistic test / group 1; NS: non significant; * : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

TABLE 3.5.3.2

IN VITRO GENE MUTATION ASSAY (WITH METABOLIC ACTIVATION) AT THE LOCUS HGPRT IN CHINESE HAMSTER V79 FIBROBLASTS

	CFE I	CFE II	Viable cells x 10^6	nb 6TG mutants	M.F. x 10^6	Statistic test p	
Group 0 CC	1.05	1.03	4.10	81	19.76	0.03*	(1)
Group 1 DMSO	1.15	1.14	4.56	62	13.60		
Group 2 SR47436 25 microg/ml	0.96	0.77	3.08	33	10.71	0.51 NS	(1)
Group 3 SR47436 50 microg/ml	0.89	0.94	3.77	99	26.28	0.02*	(1)
Group 4 SR47436 100 microg/ml	0.98	1.07	4.29	55	12.82	0.83 NS	(1)
Group 5 SR47436 250 microg/ml	1.04	1.09	4.35	54	12.40	0.83 NS	(1)
Group 6 SR47436 500 microg/ml	1.07	1.34	5.37	66	12.28	0.83 NS	(1)
Group 7 BaP 10 microg/ml	0.70	1.04	4.15	226	54.41	< 0.001***	(1)
Group 8 BaP 20 microg/ml	0.81	1.03	4.13	302	73.06	< 0.001***	(1)
Historical negative control (DMSO) n					15		
Historical negative control (DMSO) mean					7.29±5.2		
Historical negative control (DMSO) range					0.8-19.70		

(1): Statistic test / group 1; NS: non significant; * : $p<0.05$; ** : $p<0.01$; *** : $p<0.001$.

In the second study, like in the first study, no increase in the mutation frequencies was observed (with or without S-9 mix) in the range of tested concentrations. The very slight statistically significant increase observed at 50 µg/ml in the absence of S-9 mix had no biological significance since mutation frequency was not increased at higher concentrations of SR 47436 (Table 3.5.3.3) and was within the historical control values. The slight increase in mutation frequency observed at 50 µg/ml in the presence of S-9 mix in the first study was not reproducible (Table 3.5.3.4). The positive controls significantly and consistently increased mutant frequencies in both studies. Thus it is concluded that SR 47436 has no genotoxic activity whether in the presence or absence of S-9 mix, up to a concentration of 500 µg/ml.

TABLE 3.5.3.3

IN VITRO GENE MUTATION ASSAY (WITHOUT METABOLIC ACTIVATION) AT THE LOCUS HGPRT IN CHINESE HAMSTER V79 FIBROBLASTS (SECOND STUDY)

	CFE I	CFE II	Viable cells x 10^6	nb 6TG mutants	M.F. x 10^6	Statistic test, p	
Group 0 CC	0.58	0.88	3.51	23	6.56	0.54 NS	(1)
Group 1 DMSO	0.61	0.93	3.73	29	7.78		
Group 2 SR47436 25 microg/ml	0.77	0.78	3.10	24	7.74	1.00 NS	(2)
Group 3 SR47436 50 microg/ml	0.60	1.02	4.07	54	13.28	0.004 **	(2)
Group 4 SR47436 100 microg/ml	0.70	0.94	3.76	37	9.84	0.43 NS	(2)
Group 5 SR47436 250 microg/ml	0.77	0.93	3.73	39	10.45	0.23 NS	(2)
Group 6 SR47436 500 microg/ml	0.38	0.00 +	0.00	0	0.00	1.00 NS	(2)
Group 7 EMS 3 mM	0.79	1.01	4.02	369	91.79	< 0.001***	(2)
Group 8 EMS 6 mM	0.72	1.08	4.33	1132	261.63	< 0.001***	(2)
Historical negative control (DMSO) n					19		
Historical negative control (DMSO) mean					7.15±3.3		
Historical negative control (DMSO) range					1.43-11.4		

+ technical problem (1): Statistic test / group 1; (2): Statistic test / group 0 & 1; NS: non significant;
* : p<0.05; ** : p<0.01; *** : p<0.001.

TABLE 3.5.3.4

IN VITRO GENE MUTATION ASSAY (WITH METABOLIC ACTIVATION) AT THE LOCUS HGPRT IN CHINESE HAMSTER V79 FIBROBLASTS (SECOND STUDY)

	CFE I	CFE II	Viable cells	nb 6TG	M.F.	Statistic	
Group 0 CC	0.67	0.90	3.23	24	7.42	0.001 **	(1)
Group 1 DMSO	0.82	0.88	3.18	52	16.35		
Group 2 SR47436 25 microg/ml	0.81	0.95	3.42	62	18.13	0.71 NS	(1)
Group 3 SR47436 50 microg/ml	0.68	0.97	3.09	35	11.33	0.30 NS	(1)
Group 4 SR47436 100 microg/ml	0.81	1.12	4.04	50	12.38	0.41 NS	(1)
Group 5 SR47436 250 microg/ml	0.81	1.00	3.61	57	15.78	0.84 NS	(1)
Group 6 SR47436 500 microg/ml	0.41	1.03	4.10	45	10.98	0.21 NS	(1)
Group 7 BaP 10 microg/ml	0.95	1.19	4.75	307	64.68	< 0.001***	(1)
Group 8 BaP 20 microg/ml	0.67	0.95	3.42	229	66.96	< 0.001***	(1)
Historical negative control (DMSO) n					21		
Historical negative control (DMSO) mean					7.86 ± 5.0		
Historical negative control (DMSO) range					0.8 -19.70		

(1): Statistic test / group 1; (2): Statistic test / group 0 & 1; NS: non significant; * : p<0.05; ** : p<0.01; *** : p<0.001.

3.5.4. SR 47436: *In Vitro* Chromosomal Aberration Test in Human Lymphocytes (Report #RS0006930719/01; Study #MAF007). Vol. 46

This GLP study was conducted in the laboratory of for Sanofi Recherche, Montpellier Cedex, France between November 9, 1992 and March 18, 1993. The aim of the study was to evaluate the ability of the test compound to induce chromosomal aberrations using human lymphocytes cultured *in vitro*, with and without metabolic activation.

Methods: Two human lymphocyte cultures were prepared from blood taken from two different donors, a man and a woman. Lymphocytes were induced to divide in culture by phytohemagglutinin A for 48 hr. After the incubation period, solvent, positive control or SR 47436 (batch 92.02) was added with or without exogenous metabolic activation (S-9 fraction from Aroclor 1254-induced rat liver). Positive controls were mitomycin C (0.25 µg/ml) and cyclophosphamide (50 µg/ml) in the absence and presence of S-9 mix, respectively. Test substance dose levels for chromosome analysis were selected by evaluating the effect of SR 47436 on mitotic index. After the mitotic index (number of cells in mitosis X 100 ÷ total number of cells examined) has been determined, the highest dose selected for the assay generally produces a reduction by about 50% in the mitotic index compared with the control or is the dose which, when added to the culture medium, produces slight precipitation. In the present study the following concentrations of SR 47436 were used: 0.3, 1, 3, 10, 30, 100 and 300 µg/ml.

Cells were exposed to SR 47436 or positive control in the absence and presence of S-9 mix for 24 and 1 hr, respectively. In the assay without metabolic activation, 22 hr after the beginning of treatment, i.e., 2 hours before harvesting the cells, colcemide was added (0.1 ug/ml) to arrest dividing cells in metaphase (24 + 0 hours). At the end of 24 hr, cultures were centrifuged, cells suspended, spread on slides and stained. Two hundred cells were analyzed per concentration. The cells were examined under the microscope and screened for chromosome abnormalities (gaps, breaks, translocations, etc.). A second sampling was studied 48 hr after the treatment (24 + 24). Cultures treated with S-9 mix were incubated for 1 hr with SR 47436 or positive control (treatment was limited to 1 hr due to toxic effects of S-9 mix on the cells). At the end of incubation, the cells were washed three times and incubated for a further 24 hr (1 + 24). Twenty-two hours later, colcemide was added and the cells harvested 2 hr after the addition of colcemide. A second sampling was done 47 hr after 2nd wash/incubation.

Results: The mitotic index of human lymphocytes treated with different concentrations of SR 47436 at the 24 and 48 hr sampling times in the presence and absence of S-9 mix is reported in Table 3.5.4.1. A relatively high cytotoxicity of SR 47436 was demonstrated in the absence of S-9 mix where mitotic index at 300 µg/ml decreased to 65.2% (survival 34.8%) of solvent control. According to the study protocol, the mitotic index should be greater than 50%. The two lower concentrations, 100 and 30 µg/ml, induced 29% and 8% mitotic inhibition, respectively. On the other hand, weak or no cytotoxic effects of SR 47436 at concentrations up to 300 µg/ml were observed in the presence of S-9 mix.

TABLE 3.5.4.1
EFFECTS OF SR 47436 ON THE MITOTIC INDEX OF HUMAN LYMPHOCYTES
(Mitotic index is expressed as % of Control)

Treatment		No S-9 mix		With S-9 mix	
		24 hr	48 hr	24 hr	48 hr
SR 47436 µg/ml	3	107.1	Not studied	101.3	Not studied
	10	109.2	Not studied	100	Not studied
	30	91.5	103.2	84	84.2
	100	70.9	96.8	92	93.4
	300	34.8	61.9	85.3	84.2
Mitomycin C, 0.25 µg/ml		24.8	Not studied	-	-
Cyclophosphamide, 50 µg/ml		-	-	20	Not studied

According to the study protocol, a test compound is found to demonstrate clastogenicity against human lymphocytes if it results in a statistically significant increase in the frequency of breaks per cell relative to control or in the number of cells with aberrations (excluding gaps), if this increase amounts to at least a doubling of the control value and if the genotoxicity detected shows a dose-effect relationship.

Treatment of cultures at 300 µg/ml in the absence of S-9 mix resulted in a statistically significant ($p < 0.001$) increase in aberrant cell frequency. This increase was supported by a high frequency of chromatid breaks, a great number of cells with more than 10 aberrations and several pulverized cells (Table 3.5.4.2). The sponsor argues that this positive response was observed at relatively high levels of mitotic inhibtion (65.2%) and it could be attributed to indirect mechanisms possibly due to a toxic effect. At 100 µg/ml, a slight increase in the frequency of cells with structural chromosome abserrations was also observed. This increase was mainly due to the presence of breaks and to an increased number of acentric chromosomes in only one (of two) cultures treated with SR 47436. Though the values slightly exceeded the normal range of aberrant cells in both cultures treated at this concentration, no statistical significance was found. Thus, the sponsor states that the response had no biological relevance. The lowest dose, 30 µg/ml had no effect (Table 3.5.4.2). At the 48 hr sampling time, none of the tested concentrations increased aberrant cell frequency.

3.5.8. SR 47436/HCTZ: *In vitro* Gene Mutation Test With Chinese Hamster Ovary (Report #RS0042960530/02; Study #94652). Vol. 21

This GLP study was conducted by the department of Genetic Toxicology of Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY between June and October 1994.

The assay system detects a gene mutation (base pair substitutions, frameshift mutations and deletions induced by the test substance) from the parental type to the mutant form which gives rise to a change in an enzymatic protein, HGPRT (hypoxanthine guanine phosphoribosyl transferase). The gene for HGPRT is located in the X chromosome and the role of HGPRT enzyme is to induce the biosynthesis of purine nucleotides by converting hypoxanthine and guanine to the corresponding nucleoside 5'-monophosphate. The purine analogues such as 6-thioguanine (6-TG) are also converted, but to toxic ribonucleotides, which kill cells with normal enzyme activity. Mutations at the HGPRT locus are measured by scoring colonies resistant to the lethal actions of 6-thioguanine. Conduct of this study included three phases: a dose range finding study, a full mutation assay and a confirmatory assay. The experiment was conducted both in the presence and absence of rat-liver postmitochondrial fraction S-9 (prepared from rats injected with Aroclor 1254 at 500 mg/kg) and co-factors in order to ensure that any mutagenic effect of metabolites of the test compound would also be detected. A solvent (DMSO) control were included in each mutation assay.

Chinese hamster ovary fibroblasts were exposed to the following concentrations of SR 47436 (batch 93.06)/HCTZ (lot #48192) in the dose range finding study: 1/1, 10/10, 50/50, 100/100, 200/200, 300/300, 400/400 and 500/500 µg/ml. The relative cell survival (%) values observed in the range finding study were 93, 86, 57, 24, 9, and 1% with S-9 mix and 87, 78, 73, 72, 15, and 6% without S-9 mix for concentrations of 50/50, 100/100, 200/200, 300/300, 400/400 and 500/500 µg/ml, respectively. Thus, the dose selections for the full mutation assay were 50/50, 100/100, 200/200, 300/300 µg/ml with S-9 mix and 100/100, 200/200, 300/300, and 400/400 µg/ml without S-9 mix. The positive control used in the non-activated part of the experiment was mutagen ethylmethansulfonate (200 µg/ml). In the presence of S-9 mix, benzopyrene (2 µg/ml) was used.

Results

SR 47436/HCTZ was found cytotoxic at concentrations above 50/50 µg/ml. The mean relative cell survival values for concentrations of 100/100, 200/200, and 300/300 µg/ml with S-9 mix were 76%, 59%, and 30% and for concentrations of 300/300, and 400/400 µg/ml without S-9 mix were 89%, and 72%, respectively. The mutation frequency values for cultures treated with varying concentrations of SR 47436/HCTZ were in the same range as the untreated control culture. For confirmatory assay, higher doses of test substances were included in order to achieve sufficient cytotoxicity. In the concentration range used, 300/300, 400/400 and 500/500 µg/ml, both in the presence and absence of S-9 mix, the mean relative cell survival values were only 0-5%. Thus an insufficient cell population was present for passage through mutation expression and the mutation

data was not available for these cultures. In the remaining SR 47436/HCTZ-treated cultures, no significant elevations in mean mutation frequency were observed relative to the negative control cultures. The validity of each mutation study was demonstrated by positive controls which resulted in significant elevations in mutant frequencies when compared to concurrent negative control cultures.

In conclusion, SR 47436/HCTZ, when tested to cytotoxic concentrations, did not produce significant elevations in mean mutant frequencies relative to solvent controls. Hence, SR 47436/HCTZ is not mutagenic to CHO cells at the HGPRT locus.

3.5.9. SR 47436/HCTZ: *In Vitro* Chromosomal Aberration Test in Human Lymphocytes (Report #RS0042960530/01; Study #94659). Vol. 21

This GLP study was conducted by the department of Genetic Toxicology of Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY between May and September 1994. The aim of the study was to evaluate the ability of the test compound to induce chromosomal aberrations using human blood lymphocytes cultured *in vitro*, with and without metabolic activation.

Methods

Whole blood cultures were established from blood taken from two healthy volunteers (sex not identified). Phytohemagglutinin was added to stimulate the lymphocytes to divide. An Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9 mix, 2%) was used for metabolic activation. Blood cultures were treated with solvent (DMSO), SR 47436 (batch 93.06)/HCTZ (lot #48192) or positive controls (mitomycin C, - S-9; cyclophosphamide, + S-9) and the following scheme illustrates the treatment pattern used in the study. SR 47436/HCTZ was always added in the ratio of 1:1. Duplicate cultures from each of two blood donors (i.e., 4 plates/group) were used for each treatment group.

TABLE 3.5.9.1
EXPERIMENTAL DESIGN

Treatment regime	Range finding		Main study	
	No S-9 24 + 0 hr¶	+ S-9 5 + 19 hr¶	No S-9 24 + 0 hr¶	+ S-9 5 + 19 hr¶
Untreated control	-	-	-	-
Solvent control	50 µl	50 µl	50 µl	50, 100 µl
SR 47436/HCTZ (1:1)* µg/ml	2, 20, 100, 200, 400 600, 800, 1000		25, 50, 100, 200	100, 200, 400, 800
Mitomycin C	-	-	0.1 µg/ml	
Cyclophosphamide	-	-	-	4 µg/ml

¶ Cultures were exposed to test articles for 24 hr without and 5 hr with S-9 mix. The later cultures, at the end of 5 hr, were washed and centrifuged followed by an additional 19 hr of incubation. 21 hr after the addition of test substances, colcemid, 0.1 µg/ml was added to both type of cultures to arrest the dividing cells in metaphase.
* Doses (µg/ml) expressed as total containing equal parts of SR 47436 and HCTZ

Results

In the dose range finding study, SR 47436/HCTZ at a concentration of 400/400 µg/ml, without S-9, induced greater than 50% mitotic inhibition (mitotic index 0.8% *versus* 17.9% for control). No dividing cells were observed at 500/500 µg/ml. With S-9 mix (5 hr treatment), the mitotic index at 500/500 µg/ml was only 1.3% *versus* a negative control index of 19.9%. Based on these results the top doses selected for the main study were 100/100 µg/ml and 400/400 µg/ml in the absence and presence of S-9, respectively (see Table 3.5.9.1). The positive controls, as expected, caused an

increase in frequency of cells with chromosome aberrations.

A slight increase (above untreated or DMSO concurrent control) in the frequency of cells with chromosome aberrations was observed in cells treated with SR 47436/HCTZ at one of the intermediate doses, 25/25 µg/ml, in the absence of S-9. However, such an increase was not observed at doses above or below this concentration. Cultures exposed to SR 47436/HCTZ in the presence of S-9 showed an increase in aberrant cell frequencies over concurrent controls at the highest dose evaluated (200/200 µg/ml). But this dose resulted in a 50% reduction in mitotic index (7.3% *versus* 14.4% for negative control (Table 3.5.9.2). Thus, it can be concluded that SR 47436/HCTZ is non-clastogenic when tested to cytotoxic concentrations in primary human lymphocytes.

TABLE 3.5.9.2
CLASTOGENIC EFFECT OF SR 47436/HCTZ ON CULTURED HUMAN BLOOD LYMPHOCYTE

TREATMENT			ABERRATIONS							
	MITOTIC INDEX MEAN±S.E.M	CELLS SCO-RED	CELLS WITH ABERRATIONS		CHROMATID		CHROM		>10/ CELL	TOTAL ABS
			MEAN% ± SEM.	ABS/CELL±SEM	BKS	EXC	BKS	EXC		
BMS-186295/HCTZ: 24 HOUR EXPOSURE WITHOUT S-9										
UNTREATED	14.2%±0.3%	200	0.5%±0.5%	0.01±0.01	0	0	1	0	0	1
DMSO 50ul	14.4%±0.3%	200	1.5%±0.9%	0.02±0.01	1	0	2	0	0	3
25 µg/ml•	13.4%±0.5%	200	2.0%±1.2%	0.02±0.01	1	0	3	0	0	4
50 µg/ml	14.3%±0.5%	200	5.0%±1.3%	0.05±0.01	4	0	5	1	0	10
100 µg/ml	12.7%±0.09%	200	0.5%±0.5%	0.01±0.01	1	0	0	0	0	1
200 µg/ml	7.3%±0.6%	200	1.0%±1.0%	0.01±0.01	0	0	2	0	0	2
MITOMYCIN C										
100 ng/ml	6.8%±0.1%	200	34.5%*±1.3%	0.53±0.02	34	14	56	0	1	105
BMS-186295/HCTZ: 5-HOUR EXPOSURE WITH S-9										
S-9 ONLY	13.6%±0.9%	200	4.5%±3.2%	0.05±0.04	2	1	7	0	0	10
DMSO +S-9 50ul	13.1%±0.7%	200	1.5%±0.9%	0.02±0.01	3	0	0	0	0	3
DMSO +S-9 100ul	9.5%± 0.2%	200	2.5%±1.3%	0.03±0.02	5	0	1	0	0	6
100 µg/ml	10.7%± 0.5%	200	3.5%±1.7%	0.04±0.02	4	0	4	0	0	8
200 µg/ml	10.0%± 0.5%	200	2.5%±1.9%	0.03±0.01	1	0	4	0	0	5
400 µg/ml	7.5%± 1.2%	200	5.5%±1.5%	0.08±0.03	6	0	9	0	0	15
800 µg/ml§	1.3%*±0.3%	0	-	-	-	-	-	-	-	-
CYCLOPHOSPHAMIDE + S-9										
4 µg/ml	4.2%*± 0.2%	200	39.0*±3.9%	0.65±0.13	58	14	56	1	0	129

• Doses expressed as total of BMS-186295/HCTZ. * Denotes statistically different from appropriate control ($p \leq 0.05$).
§ 800 µg/ml not evaluated, CHROM: chromosome, ABS: aberration, BKS: breaks, EXC: exchanges

3.5.10. SR 47436/HCTZ: *In Vivo* Micronucleus Test in Mice (Report #RS0042960523/03; Study #94646). Vol. 21

This GLP study was conducted by the department of Genetic Toxicology of Bristol-Myers Squibb Pharmaceutical Research Institute, Syracuse, NY between May and August 1994. The purpose of the study was to investigate the potential of SR 47436/HCTZ to produce clastogenic or aneugenic effects in mice (*in vivo*).

Methods

In a range finding non-GLP study (conducted on March 28, 1994), suspensions of SR 47436 (batch 93.06) and HCTZ (lot #48192) (in sodium carboxymethylcellulose, 1%) were administered sequentially to male and female CD-1 mice (Charles River) at single oral gavage doses (volume of 2 ml/kg) of 500/500, 1000/1000 or 2000/2000 mg/kg (5 mice/sex/dose). No treatment-related deaths occurred at any dose level. Treatment-related clinical signs observed were slight soiling in three male mice at 2000/2000 mg/kg. Analysis of peripheral blood samples obtained approximately 24 and 48 hours after the dose revealed no signs of bone-marrow toxicity, as measured by a reduction in polychromatic erythrocytes (PCEs). Based on this study, 2000/2000 mg/kg SR 47436/HCTZ was selected as the high dose for male and female mice for the definitive micronucleus study.

In the definitive study, groups of 10 male and 10 female mice were given, by oral gavage, suspensions of SR 47436/HCTZ at single doses of 500/500, 1000/1000 or 2000/2000 mg/kg. Two additional groups of male and female mice (10/sex/group) were dosed once with vehicle (2 ml/kg 1% CMC, negative control) orally or cyclophosphamide (25 mg/kg, positive control) intraperitoneally. The mice were approximately 9 weeks of age and weighed between 24 and 31 grams (females), or between 30.3 and 41.6 grams (males), when dosing was inititated. During the study, mice were housed individually in stainless steel cages. Approximately 24 and 48 hr after SR 47436/HCTZ, vehicle or cyclophosphamide administration, mice (5/sex/group/time period) were sacrificed. Bone marrow was isolated from both femurs and smears onto slides were prepared for evaluation. Frequency of micronucleated polychromatic erythrocytes (MN-PCE) for each sample was determined. The mean number of MN-PCE accepted for the negative control was 0.19% (±0.05%, range 0 to 0.49%). The percent of PCEs in the peripheral blood of mice is 3 to 4 % of total erythrocytes (mean 3.5%). The percent of PCEs in the bone-marrow of mice ranges from 45 to 60% with a mean percentage of approximately 50%. A toxicologically significant reduction in the PCE count in either peripheral blood or bone-marrow reflects potential cytotoxic effects on erythropoiesis.

Results

There were no deaths and no clinical signs of toxicity were observed in any SR 47436/HCTZ treated animals. There were no toxicologically significant decreases in PCEs in the low and high dose SR 47436/HCTZ treated mice. A slight elevation in MN-PCEs was observed in the mid-dose female group (1000/1000 mg/kg) sampled 48 hours after dosing . The difference between this value and the concurrent vehicle control was statistically significant ($P < 0.05$). However, the sponsor argues that the MN-PCE value (0.33%) did not exceed their laboratory's historical negative control data range (0.00 to 0.49%)

although it did exceed their historical control mean (0.19%). Since the response was not dose-dependent, the sponsor considers the difference incidental and not related to the administration of SR 47436/HCTZ. At doses of 500/500, 100/1000, and 2000/2000 mg/kg, the frequencies of MN-PCE in the bone-marrow ranged from 0.1% to 0.27% in males and from 0.13% to 0.33% in females. Cyclophosphamide, the positive control, was considered genotoxic as indicated by the large increase in MN-PCEs compared to the negative control ($p < 0.01$). Thus, the sponsor concludes that the SR 47436/HCTZ combination is not genotoxic when compared to negative controls in this assay.

4. OVERALL SUMMARY AND EVALUATION

Pharmacodynamics

Irbesartan (SR 47436) is a non-peptidic, orally-effective, potent and specific antagonist of angiotensin II, active at the AT_1 angiotensin II receptor. It was developed by Sanofi Recherche, France for the treatment of essential hypertension, congestive heart failure, and coronary heart disease. The applications under review (NDA 20,757 for irbesartan; NDA 20,758 for irbesartan/Hydrocholorothiazide (HCTZ)) are concerned with the hypertension indication only.

The mechanism of the interaction between irbesartan and the AT_1 receptor is complex: the antagonism was characterized as competitive antagonism in receptor binding studies, competitive unsurmountable antagonism in isolated aorta, and noncompetitive unsurmountable antagonism in decreasing b.p. responses to angiotensin II in pithed rat preparations. The activity of irbesartan is highly selective: the compound is inactive in binding studies with many other neurotransmitter receptors, ion channels, and ion transport mechanisms. The affinity of irbesartan for human AT_1 receptors from aortic smooth muscle membranes was compared with that of 12 of its metabolites. Three of those metabolites (SR90149A, M5 and M7) were as active as irbesartan.

Irbesartan(10 or 30 mg/kg, i.v.) *per se* was without a blood pressure lowering effect in normotensive rats. However, in angiotensin II-induced hypertension, both i.v. (0.1, 0.3, 1, or 3 mg/kg) and oral (0.3, 1, 3, 10, or 30 mg/kg) administration of irbesartan caused dose-dependent decreases in b.p. The effect was significant for both routes of administration from the dose of 0.3 mg/kg upwards. The onset of inhibition occurred within 15 min after oral dosing; the time to maximum inhibition ranged from 15 minutes (1 mg/kg) to 180 min (10 mg/kg). At the highest dose (30 mg/kg) the maximal inhibition occurred at 15 min postdose and it lasted at least until 180 min postdose. The test compound also inhibited the angiotensin II-induced increase in aldosterone secretion in a dose-dependent manner. In normotensive rats,irbesartan did not significantly reduce the pressor response to norepinephrine or vasopressin, suggesting selectivity and specificity in the action at angiotensin receptors. A comparison of effects of i.v. and intracerebroventricular (i.c.v.) administrations of irbesartan was made against pressor responses to angiotensin II administered i.v. or by i.c.v. injection in rats. Either i.c.v. (10 µg/kg) or i.v. (3 or 10 mg/kg) administration of irbesartan significantly and dose-dependently inhibited the pressor response to i.c.v. (0.3 µg/kg) administration of angiotensin II. In contrast, i.c.v. administration of irbesartan produced only a slight (13%, not significant) inhibition of the pressor response to i.v. (40 ng/kg) administered angiotensin II. The results suggest that irbesartan penetrates the brain to affect central angiotensin II receptors. Also, a ten times higher dose of irbesartan (10 mg/kg, i.v.) was needed to elicit a 60% inhibition of the pressor response to angiotensin II administered i.c.v.

The efficacy of irbesartan was evaluated in a few hypertension models. In 2 kidney, 1 clip (Goldblatt) renal hypertensive rats,irbesartan (10 mg/kg, p.o.) induced a marked and sustained hypotensive effect (>24 hr). The onset of hypotensive activity in this hypertension model was delayed by 60 min. In 2 kidney, 1 ligation renal hypertensive rats,irbesartan (1, 3, or 10 mg/kg, p.o.) produced a dose-dependent, progressive decline in mean arterial pressure, the onset more rapid (30 min) in rats given 10 mg/kg than in rats given 1 and 3 mg/kg irbesartan (150 and 120 min). At all three doses the peak activity occurred at 6 hr and the mean

arterial pressure was still below that of the control group (14%, P <0.05) at 24 hr postdose in those rats given 10 mg irbesartan/kg. The hypotensive effects were not accompanied by an increase in the heart rate in orally dosed animals. However, a significant increase in heart rate accompanied the fall in arterial pressure in intravenously dosed animals.

The antihypertensive effect of irbesartan (10 to 30 mg/kg, i.v.) in SHR, a low-renin hypertension model, was slight with the maximum effect ranging from 7 to 12%. This suggested that the portion of arterial blood pressure supported by the RAAS in SHR is less than that supported by the RAAS in the Goldblatt hypertensive rats and thus SHR is not a suitable model for the acute study of angiotensin II antagonists. Thus, the sponsor conducted a chronic experiment in which the effect of test substance could be studied on the early development of genetic hypertension (GH). SR 47436, chronically administered to the young SHR at the daily oral dose of 60 mg/kg from the age of 4 weeks to the age of 20 weeks, repressed the development of GH. On the last day of treatment, the arterial pressure of the treated animals was significantly lower than that of the control animals. After treatment interruption, the arterial pressure of the animals previously treated with irbesartan increased progressively but remained significantly lower than that of the control animals, at least until the age of 27 weeks, which demonstrates that the preventive effect of irbesartan against GH persisted after the end of its administration. The mechanism responsible for these preventative effects appears to be the interruption of the RAAS at a crucial time of GH development. It should be noted that the onset of hypotensive activity in hypertension models (both SHR and Goldblatt kidney) with oral administration of irbesartan varied, depending on the dose, from 30 (at high dose) to 120 (at low dose) min. In contrast, the onset of activity in normotensive animals (inhibition of angiotensin II-induced pressor responses) was 15 min and was independent of the dose administered. The reason for this is not clear. The antihypertensive effect of irbesartan was also investigated in the desoxycorticosterone acetate (DOCA)-salt hypertensive rat, another low-renin model of hypertension. In this study,irbesartan (30 mg/kg, p.o.) did not affect either mean blood pressure or heart rate. However, it increased both plasma renin activity and plasma angiotensin II levels. Thus, the results suggest that irbesartan blocks juxtaglomerular angiotensin II receptors and consequently induces a release of renin by interrupting negative feedback mechanisms.

Irbesartan (1, 3, 10 mg/kg, i.v.) caused a dose-dependent (18, 45 and 82%) decrease in the elevation of diastolic blood pressure induced by angiotensin II in conscious normotensive dogs. The onset of activity was short (30, 60, 120 min at doses of 10, 3, 1 mg/kg, respectively) and the effect lasted for less than 5 hr. No marked alterations in hemodynamic values attributed to irbesartan were noted following intravenous administration of 0.2 to 2 mg/kg irbesartan in anesthetized normotensive dogs. Also, hemodynamic, cardiac, and respiratory parameters were not significantly changed following oral administration of 30 and 60 mg/kg irbesartan. In sodium depleted dogs,irbesartan (10 mg/kg, p.o.) lowered b.p. with a tendency to increase heart rate, and increased plasma angiotensin II; plasma aldosterone tended to decrease.

In normotensive monkeys, both i.v. (1 mg/kg) and oral (1 and 3 mg/kg) administration of irbesartan caused inhibitory effects (maximal effects of 66 to 89%) on the angiotensin II-induced pressor response. The onset of hypotensive activity was 15 (i.v.) to 30 (oral) minutes and the maximal effect occurred 60 min postdosing. The duration of action, but not the intensity, was proportional to irbesartan dose. The effect decreased gradually and was still 20% at 5 hr suggesting a somewhat longer duration of action in normotensive monkeys compared to normotensive rats and dogs. In a 6-day repeat dose study,irbesartan (3

or 10 mg/kg, p.o.) produced a greater intensity of hypotensive activity after the third and the sixth dose than after the first dose (blood pressure not measured after 2nd, 4th or 5th dose) and the hypotensive activity was long lasting (persisting for more than 48 hr after the sixth dose). The hypotensive effects induced by irbesartan in the monkey were associated with changes of the RAAS, where plasma renin activity (PRA), active renin (AR) and angiotensin II increased, and aldosterone decreased. However, the changes were not proportional to irbesartan dose. After cessation of dosing (on 7th day), PRA and plasma concentrations of AR and angiotensin II returned to near pretreatment values at a slower rate than blood pressure. The sustained hypotension was without any rebound effect in spite of the activation of the RAAS. Similar observations were made in studies in sodium-depleted monkeys. The hypotensive effect of irbesartan in monkeys differs from the effect in rats and dogs with a more rapid onset of action, a higher intensity, and a longer duration of action in the primate. The effective dose ranges for irbesartan in rats, dogs and monkeys were: 1 to 30, 1 to 10 and 0.3 to 10 mg/kg, respectively, for oral dosing; and 0.1 to 10 and 0.1 to 3 mg/kg for intravenous dosing in rats and monkeys, respectively. The longer duration and the greater intensity of action of irbesartan in monkeys may be attributed to the unsurmountable (competitive or noncompetitive) nature of its antagonism of angiotensin II.

In other pharmacology studies,irbesartan (60-120 mg/kg, p.o.) had no marked activity in tests designed to assess potential activity on the central or autonomic nervous system in mice.

Drug Disposition (ADME)

Absorption

Absorption of irbesartan after oral administration was rapid in mice (T_{max} 0.5 to 1 hr), rats (T_{max} 2 to 4 hr), monkeys (T_{max} 1 to 4 hr) and humans (average T_{max} 1.5 hr) but slow in rabbits (T_{max} 8 hr). Absorption was almost complete in all these species. The bioavailability of irbesartan after a single (10 mg/kg) oral dose averaged 11% in female and 22% in male rats and ranged from 45 to 120% in male monkeys. Bioavailability was 60-80% in humans. Following a single i.v. or oral dose in mice, rats, rabbits and monkeys,irbesartan had a long terminal half-life (12-26 hr), a low plasma clearance, and a large distribution volume. These findings are consistent with the prolonged hypotensive activity observed with monkeys and, to a lesser extent, rats. In repeated daily oral dose studies, the exposure of mice, rats and monkeys to irbesartan increased with the dose but increases were less than dose proportional. Both plasma concentrations and AUC were notably higher in females than in males, especially at higher dosages. In rats and monkeys, plasma concentrations of irbesartan were similar on days 8 and 28/29 of a 4-week study, suggesting the rapid onset of steady-state conditions within 8 days of dosing. (See Tables 2.1.7.1 through 2.1.7.4). Table 4.1. summarizes the plasma toxicokinetic parameters of SR 47436 following repeated daily oral administration in animals and man.

Distribution

Irbesartan has little affinity for red blood cells (around 10% of whole blood radioactivity in humans, mice, rats, rabbits and monkeys). The binding of irbesartan to serum proteins in the rat, the monkey and the human was approximately 90% at pharmacologically active concentrations. It seemed saturable at concentrations higher than 50-100 mg/l. In the mouse and the rabbit, the binding to plasma proteins was low, 84.6 and 68.4%, respectively, and not saturable up to 150 mg/l in both species. In rats and male mice, absorption of radiolabeled irbesartan from the gastrointestinal tract (GIT) was rapid with peak blood and

tissue levels appearing 2 hours after oral dosing. Tissue distribution studies were done in mice and rats. Highest concentrations of radioactivity were found in the liver, GIT and kidneys. By 48 hours in rats and 96 hours in mice, the radioactivity had been essentially quantitatively eliminated with approximately 100% of the dose recovered in urine and feces.

TABLE 4.1
SUMMARY OF TOXICOKINETIC PARAMETERS FOR IRBESARTAN ACROSS SPECIES

Species	Study #	Dose (mg/kg/ day)	Study duration	Sample time	tmax		Cmax (mg/l)		$AUC_{0\text{-}24\ h}$ (mg.h/l)		Exposure multiple*	
					M	F	M	F	M	F	M	F
Mouse (CD-1)	TSA967	100	4-week	28 d	1	1	2	10.4	2.4	11.9	0.1	0.5
		300			2	0.5	25.2	18.5	48.1	48.0	2.3	2.1
		1000			1	2	38.1	47.1	76.1	122.4	3.7	5.2
	DDO505	150	13-week	13 wk	1	1	3.9	22.0	5.0	24.6	0.2	1.1
		500			1	1	13.7	38.0	20.0	93.7	1.0	4.0
	DDO522	1000	13-week	13 wk	2	1	28.3	61.3	104.8	136.5	5.1	5.8
		2000			1	1	46.5	92.5	226.7	269.1	11.0	11.5
Rat (Wistar)	TSA968	50	4-week	28 d	2	2	1.4	5.9	18.5	29.5	0.9	1.3
		500			2	2	22.8	75.8	71.3	255.8	3.5	10.9
		1000[a]			2	2	214.2			495.6		21.2
	DDO504	50	13-week	13 wk	2	1	1.5	2.8	22.6	23.6	1.1	1.0
		150			2	1	3.4	19.4	25.7	56.9	1.2	2.4
		500			2	2	20.6	258.8	60.5	476.4	2.9	20.2
Rat (S-D)	TXC949	250	6 month	26 wk	2	2	2.5	43.4	23.3	127.0	1.1	5.4
		500			2	2	5.8	283	41.2	26.8	2.0	12
		1000			2	2	58.6	88.3	198.0	226.0	9.2	9.7
Monkey	TSA898	250	1 month	33 d	3[b]		28.1[b]		107.7[b]		4.9[c]	
		500			2.5		57.1		329.4		15.0	
		1000			3.5		106.4		570.4		25.9	
	TXC841	10	6 month	190 d	2	2	0.8	0.8	7.0	6.9	0.3	0.3
		30			2	2	1.2	0.9	12.8	10.0	0.6	0.4
		90			2	2	2.5	2.1	26.1	21.0	1.3	0.9
Human[d]	CV13-1057	300 mg	4-week	29 d	2.3	1.3	3.7	4.1	20.6	23.4		

a: females only; b: no differences in the values between males and females; c: exposure ratio calculated based on the average AUC of male and female patients, i.e., 22 mg.h/l; d: in hypertensive patients

*AUC_{animal}/ AUC_{human}

Metabolism

Irbesartan was metabolized *in vivo* and *in vitro*. The proposed metabolic pathway of irbesartan and the distribution of metabolites across species is presented in Figure 2.3.13. The unchanged parent drug was the major circulating compound in the plasma of rats, rabbits, monkeys and humans (accounted for 49-91% of the radioactivity detected in the plasma); however, it was a minor component in the plasma of mice (12.5 and 2.6% at 30 and 5 min after dosing). In mice, the main identified compounds in plasma were an N-dealkyl derivative of the parent compound (metabolite F) and two isomers of monohydroxy-N-dealkyl irbesartan (metabolites G). These three metabolites were also present in the plasma of rats and the urine of

monkeys but not in any of the matrices in humans and rabbits. Several other quantitatively minor metabolites were present in the plasma of rats, two of which were identified as the N_2-glucuronide (metabolite E) and the imadazoline ring opened analog (metabolite J). These two metabolites were also found in the plasma of monkeys, and in the plasma, urine and feces of humans. A carboxylic acid metabolite (metabolite O), which accounted for about 9% of the plasma radioactivity in humans, was detected in the bile of male rats but was not detected in any of the matrices of other species. In rabbits, the major circulating metabolites were two different monohydroxylated metabolites resulting from hydroxylation on the cyclopentane ring (metabolites C).

The parent compound, detected as a minor component in the plasma of mice, was not excreted in mouse urine. It was extensively metabolized. The main identified compounds in mouse urine (27 to 38% of an administered dose) were four isomers of monohydroxy-N-dealkyl irbesartan (metabolites G) and one N-dealkyl-oxo derivative of irbesartan (metabolite R). These components were also present (in their free forms) in urine of male rats where, combined, represented most of the excreted radioactivity (8% of an administered dose). The metabolic pattern was different in female rats, with small amounts of parent drug (about 10% of excreted material), three monohydroxy irbesartan isomers (metabolites C), monohydroxy irbesartan glucuronide (metabolite D), and ring opened analogs of irbesartan (metabolites J, K and M). In rabbits, the main excreted compounds (approximately 22% of the administered dose) were identified as two different cyclopentane monohydroxy derivatives of irbesartan, N_2-glucuronide of parent drug (metabolite E), SR 49498 (metabolite J) and its monohydroxy derivatives, and several other mono- and di-hydroxy derivatives resulting from ω-1 oxidation on the butyl side chain and/or on the spirocyclopentane ring, and keto metabolites resulting from further oxidation of one monohydroxylated metabolite. The parent compound is little excreted in urine in both rabbits (10% at 0-8 hr and 0.6% at 24-48 hr) and monkeys (2, 15 and 14% on days 1, 7, and 14). Thirteen radioactive peaks were identified along with the unchanged parent compound in monkeys. The two prominent metabolites were SR 49498 (metabolite J) and SR 90150 (metabolite E), which accounted for 2.3 to 4.3% of the detected radioactivity. None of the above metabolites except for metabolites C and E (absent only in mice) were identified in urine or feces of human. A total of eight metabolites (metabolites B, C, E, H, O, N), accounting for 90% of total radioactivity administered, were identified in human urine and feces.

Metabolic profiles in feces were also investigated in rats and monkeys. In rats,irbesartan was detected as one of the main excreted compounds after days 1, 7 and 14 of administration (20, 51 and 54% of the detected radioactivity, respectively). In monkeys, free irbesartan accounted for 49 to 66% of the detected radioactivity. The presence of considerable amounts of intact parent drug in the feces was not interpreted as a consequence of incomplete absorption of the drug from the GIT since biliary excretion had been shown to be the main route of excretion in both rats and monkeys (see below). The presence of free irbesartan in feces may be a consequence of the hydrolysis of the N-glucuronide metabolite in the gut. Seven unconjugated metabolites (metabolites C, J and M) in fecal samples of rat and ten radioactive peaks in fecal samples of monkeys were identified.

Biliary metabolism was investigated in rats and monkeys. For both species, most of the radioactivity represented the N_2-glucuronide of irbesartan (metabolite E; 29-69% and 81-94% of the radioactivity excreted over 48 hours, respectively, in rats and monkeys). As percentage of the given dosages, N_2 glucuronide in bile amounted to at least 37-40% in rats and 45% in monkeys. Parent drug was excreted in

its free form in minor amounts, 0.5 to 2.9% and 1.3% of the administered dose in rats and monkeys, respectively. Other quantitatively minor compounds detected in bile of rats were di- and tri-hydroxy derivatives of irbesartan, mono- and di-hydroxy derivatives of SR 49498, SR 49498 glucuronide, glucuronides of monohydroxy derivatives of irbesartan and SR 49498. A few, but not all, of the above minor metabolites were also identified in the bile of monkeys. At least one metabolite (metabolite J) has been identified in human bile.

In vitro metabolism of irbesartan was investigated in primary cultures of hepatocytes and microsomal fractions from rat, dog, monkey, baboon and man, and using several genetically engineered microsomal fractions, each expressing a specific cytochrome P450 isozyme. Incubation of irbesartan with hepatocytes and microsomal fractions from all species indicated similar oxidative pathways with seven metabolites characterized in each species. The only difference was in the relative proportion of each metabolite across species. The main metabolites were monohydroxylated derivatives, oxidation occurring at different sites of the molecule. No di- (e.g., metabolites B, M and P) and tri- (metabolite A) hydroxylated derivatives were detected. N-dealkylation and N_2-glucuronidation pathways represented only minor metabolic processes. The rate of biotransformation in different species suggested that the metabolism of irbesartan in man (39 ± 17%) resembles most closely that in monkeys (40 ± 7%). Incubation of irbesartan with human hepatocytes in primary culture indicated that irbesartan is also subject to glucuronidation (irbesartan tetrazole N_2-glucuronide). The rate of conjugation was higher in monkeys than in man and slowest for the rat. From different *in vitro* model studies, using specific substrates or inhibitors of cytochrome P450 subfamilies and specific cytochrome P450 expressing systems, it was found that *CYP2C9* isozyme was the primary isozyme involved in the oxidation of irbesartan to its four monohydroxy derivatives, while *CYP3A4* slightly metabolized irbesartan to its monohydroxy metabolites. Additional *in vitro* studies conducted with human hepatocytes suggested that irbesartan is neither an inducer nor an inhibitor of cytochrome P450 1A and 3A gene subfamilies.

Excretion

The excretion of labeled irbesartan was rapid, with most of the radioactivity appearing in the urine within 24 hr in mice, rats and monkeys, and within 48 hr in rabbits. Radioactivity excreted in the urine, expressed as a percentage of the dose, was less than 10% in rats, 10-20% in monkeys, about 22% in rabbits and approximately 38% in mice. The latter species excreted similar amounts of radioactivity in urine and feces. The presence of considerable amounts of radioactivity in feces (>80% over 0-48 hr of dosing) in rats and monkeys is a consequence of a large biliary excretion (70 to 78% in rats and 45% in monkeys) rather than incomplete absorption. An enterohepatic recirculation has been documented in both rats and monkeys. In humans, the radioactive drug/metabolite is eliminated slowly in the urine and feces, 20% and 46%, respectively, by 168 hr after a single oral dose.

Toxicology

Acute Toxicity

Irbesartan was tested for acute toxic effects in mice and rats by the oral (2000 mg/kg), intraperitoneal (25, 200 and 2000 mg/kg) and intravenous (50 mg/kg) routes of administration. Oral or i.v. administration did not cause deaths in either species. On the other hand, all rats and mice given 2000 mg/kg intraperitoneally were found dead (no deaths at 200 mg/kg i.p.). The macroscopic examination performed on the animals

found dead or sacrificed two weeks after treatment did not reveal any gross lesions attibutable to irbesartan treatment. The acute oral toxicity of the irbesartan/HCTZ combination was determined in mice and rats at doses up to and including 2000/4000 mg/kg and 3000/500 mg/kg, respectively. There were no deaths and no gross lesions were seen during necropsy.

Chronic Toxicity

The potential for adverse effects following repeated administrations of irbesartan was evaluated in oral dosing studies of up to 12 months duration in monkeys and 24 months duration in rats and mice. Chronic administration studies with the irbesartan/hydrochlorothiazide combination were limited to 6 months duration.

The chronic toxicity of irbesartan was evaluated in monkeys at oral doses of up to 1000 mg/kg/day for one month and up to 500 mg/kg/day for one year. The 1000 mg/kg/day treatment (after 4 weeks) was associated with systemic exposures (AUCs of total irbesartan) that were, on average, 27-fold higher than those observed in humans at the maximum recommended daily dose of 300 mg. The following findings were associated with exposure to the drug: death at 1000 mg/kg/day, reduced body weight gain at 90 or more mg/kg/day, slight anemia at 30 or more mg/kg/day (as early as one month) and JG hyperplasia/ hypertrophy at all doses evaluated (as low as 10 mg/kg/day in a six month study). The kidney finding (JG hyperplasia/hypertrophy) is considered to represent an (unavoidable) adaptive response to the (desired) action of irbesartan on the RAS. Blood urea and creatinine were elevated at doses as low as 250 mg/kg/day administered for periods as short as one month. With the exception of JG hypertrophy/ hyperplasia, doses as high as 90 mg irbesartan/kg/day for one month or 20 mg irbesartan/kg/day for one year were well tolerated by monkeys.

The chronic toxicity of irbesartan was evaluated in rats at oral doses of up to 1000 mg/kg/day for six months and up to 500 mg/kg/day (1000 mg/kg/day in females) for 2 years. The 500 mg/kg/day treatment (after 4 weeks) was associated with systemic exposures (AUCs of total irbesartan) that were, on average, 3 (male)- and 11 (female)-fold higher than those observed in humans at the maximum recommended daily dose (300 mg). Notable findings were slight to moderate anemia, JG hyperplasia and increases in BUN and creatinine at doses as low as 250 mg/kg/day (as early as 6 months) and an increased incidence of perivascular inflammatory infiltrate and basophilic tubules in the cortex of the kidney at doses $\geq$500 mg/kg/day (only in the 24 month study). Increased mortality was observed for the animals (females) receiving 1000 mg/kg/day, after 78 weeks, in the 24 month study. Because of a concern over airborn contamination of control rats with irbesartan, identified in dose-rangefinding experiments, three control groups were included in the 24 month (carcinogenicity) study (one in the same room as the treated animals, one in a separate room, and a third which was separated from the treated groups after 14 weeks). The mean plasma irbesartan concentration in the control group housed with the treated animals was no more than 10% of the concentration in the low dosage group. Although the sponsor reported a statistically significant positive trend for uterine adenocarcinoma in this study, the CDER statistician, after correcting for multiple comparisons, found no significant trends or differences between control and high dose incidences of any tumor type. The incidence of uterine adenocarcinoma in the 500 and 1000 mg/kg/day groups of male rats, as well as in one of the male concurrent control groups, was above the historical control range of the laboratory at which the irbesartan study was conducted. The historical database for this laboratory, however, is quite small and the incidence observed with the above noted groups from the

irbesartan study was, with one exception, within another laboratory's larger historical database for the same strain of rat. The exception is the 500 mg/kg/day group with an incidence of about 11% (historical control incidence ranged from 2-8%). It is notable that whereas uterine carcinomas were observed in 3 of 54 control animals that were isolated from drug treated animals in week 14, there were no uterine carcinomas observed in 55 control animals that were housed in the same room as the treated animals for the full duration of the study. Although the 1000 mg/kg/day group had poorer survival than the lower dosage and control groups, 49% of those females survived to terminal sacrifice. Body weights were also lower in this group, primarily during the second year of the study. Significant reductions in body weight gain (21%) had been observed at the same dose in a preliminary 2-week repetitive dosing study. In view of the above, higher doses could not have been evaluated in female rats and the 1000 mg/kg/day dose may be considered to be above the MTD.

The chronic toxicity of irbesartan was evaluated in mice at oral doses of up to 2000 mg/kg/day for three months and up to 1000 mg/kg/day for up to two years. The 1000 mg/kg/day treatment (after four weeks) was associated with systemic exposures (AUCs of total irbesartan) that were, on average, 3 (male)- and 5 (female)-fold higher than those observed in humans at the maximum recommended daily dose of 300 mg. Treatment with 2000 mg/kg/day was associated with mortality and treatment with 1000 mg/kg/day was associated with basophilic and dilated cortical renal tubules, vacuolation of tubular epithelial cells, and slight hyperplasia of renal juxtaglomerular apparatus (the kidney pathology observed as early as 3 months). Whereas incidence of the pathology noted above was greater for females than males, only in males (1000 and 2000 mg/kg/day) were body weights significantly lower than control. In the two year study, survival was adversely affected at the highest dosage level in females and at the mid and high dose levels in males. Nonetheless, survival was greater than 50% after 78 weeks of treatment and at least 25% of males and 20% of females in each group survived to scheduled sacrifice at 104 weeks. Body weights of all treated female groups were consistently lower than those of the combined control group but the differences from control were not dose-dependent. Male body weight appeared to be unaffected by the drug. The sponsor reported a statistically significant positive trend for incidence of males with adrenal cortical adenoma (no adrenal carcinomas observed in treated mice). The incidence at the highest dose level was significantly higher than concurrent control, but only slightly above the laboratory historical control range. The sponsor also reported a positive trend for males with malignant and/or benign hepatocellular tumors (combined incidence analysis). The highest incidence was within the laboratory historical control range. The CDER statistician calculated a p value of 0.0113 for positive trend and 0.0122 for pairwise comparison of high dose and combined control group incidences of adrenal cortical adenoma. If this tumor is considered to be common to the CD-1 mouse, the above noted p values are above the cutoffs (0.005 for trend and 0.01 for pairwise comparison) used by CDER for concluding that the distribution of a tumor is drug-related. Although none of the 110 concurrent control mice presented with this tumor in the SR 47436 study, the historical control incidence provided by the same laboratory is 1.14% and a 1995 publication of Charles River Laboratories (which provides historical control data for adrenal tumors in CD-1 mice) reports a cortical adenoma incidence of 1.57%. As CDER statisticians define a common tumor as one seen at an incidence >1%, the adrenal cortical adenoma qualifies as a common tumor and the increased incidence observed in irbesartan treated groups is not considered to be related to the drug. A similar conclusion is reached when one looks at the p values resulting from the CDER statistician's analysis of group incidences of mice bearing carcinoma and/or adenoma of the adrenal cortex ($p=0.0293$ for trend and $p=0.0418$ for pairwise comparison of high dose and combined control groups). Our statistician's report also notes

borderline significance (p=0.0102) for a high dose *vs* control pairwise comparison for the increased incidence of irbesartan-treated male mice with hepatocellular adenomas and carcinomas when the two tumor types are considered together (both tumors common in the male CD-1 mouse). The trend p value was higher (0.0178) and not considered to be indicative of a drug-relationship. Unadjusted incidence at the high dose was 17/56. Low and intermediate dose groups had somewhat higher incidences (19/56 and 21/56, respectively). All of these incidences are within the upper limit of the laboratory historical control range (42.3%). It is notable that although all treated groups had higher hepatocellular tumor incidences than the combined concurrent control group, those differences were not much different than the difference between the two concurrent control groups (15/56 and 9/55). This reviewer does not consider the increase in liver tumors to be related to treatment with irbesartan.

In rats and monkeys, irbesartan/HCTZ was administered in a 1:1 ratio at oral doses of 10/10 and 90/90 mg/kg/day for 6 months. In order to detect possible enhancement of toxicity by the coadministration of irbesartan and HCTZ, separate groups of animals were treated with irbesartan or HCTZ alone at doses that corresponded to those used in the high dose combination, i.e., 90 mg/kg/day. There were no drug-related deaths. Anemia was observed in both species, predominantly in the high dose combination group and in animals given irbesartan alone. As with irbesartan alone, significant increases in blood urea nitrogen and creatinine were noted in irbesartan/HCTZ-treated high dose monkeys. Dose-related hyperplasia/ hypertrophy of the JG apparatus was seen in the kidneys of all groups of rats and monkeys treated with irbesartan. The severity (slight to moderate) was similar in animals treated with 90 mg irbesartan/kg alone and in combination with 90 mg HCTZ/kg. This finding was absent in rats and monkeys receiving HCTZ alone and in the control animals, and along with decreased heart weight is considered to be attributable to the pharmacological activity of irbesartan. Discoloration of the glandular stomach, which correlated with focal coagulative necrosis or ulceration of the mucosa, was noted in all groups of irbesartan-treated rats, but not monkeys, with a slightly higher incidence in the high dose combination group. Exposure to irbesartan was not affected by coadministration of HCTZ; however, exposure to HCTZ was about 60% greater when it was given in combination with irbesartan than when given alone. No evidence of accumulation was noted for either drug when given together or alone.

Irbesartan showed no evidence of phototoxicity or photoallergy when given orally to guinea pigs at a dose of 100 mg/kg.

Genotoxicity

The genotoxic potential of irbesartan was investigated in a microbial mutagenicity assay (*Salmonella typhimurium* tester strains), an *in vitro* unscheduled DNA synthesis assay, an *in vitro* Chinese hamster lung mutagenesis assay, an *in vitro* chromosomal aberration assay in human lymphocytes and an *in vivo* assay for clastogenic effects in mouse bone marrow (micronucleus test). Except for the *in vivo* micronucleus test and DNA repair assay, which was performed using rat hepatocytes with their own intrinsic metabolic capacity, a metabolizing system (S-9 mix derived from Aroclor pretreated rat liver) was used to compensate for the limited metabolizing function of bacteria or mammalian cells in culture. The genotoxic potential of the irbesartan/HCTZ combination (tested in a 1:1 weight ratio) was also determined in all the above assay systems except the unscheduled DNA synthesis assay. The above test battery failed to implicate irbesartan, alone or in combination with hydrochlorothiazide, as possessing mutagenic or

clastogenic activity.

NOTE: This review does not address the (rat and rabbit) reproductive toxicity studies conducted with irbesartan. The results and adequacy of those studies are addressed in a separate review by Dr. Sidney Stolzenberg.

5. LABELING

Those sections in the proposed labeling (2nd version, submission date April 23, 1997) that refer to preclinical studies covered by this review are considered acceptable with the following exception:

Under PRECAUTIONS *Carcinogenesis, Mutagenesis, Impairment of Fertility*, the sponsor's proposed carcinogenesis statement for irbesartan (line nos. 4 through 9 for NDA 20,757 and line nos. 5 through 10 for NDA 20,758) reads as follows:

> "No evidence of carcinogenicity was observed when irbesartan was administered at doses of up to 500/1000 mg/kg/day (males/females, respectively) in rats and 1000 mg/kg/day in mice for 2 years. These doses provided systemic exposures of 3.6 - 24.9 times (rats) and 3.8 - 6.2 times (mice) the exposures in humans receiving 300 mg daily."

Based on AUCs recorded after 4 weeks of daily dosing in toxicokinetic studies conducted in parallel with the carcinogenicity studies in mice (Study TSA967, RS0006950921/01) and rats (study TSA968, RS0006951214/01) and after 4 weeks of daily dosing in male and female hypertensive patients (protocol CV131-057), the animal/human exposure multiples need to be revised. [Note that whereas female rats and mice had appeciably higher plasma levels of irbesartan than male rats and mice (mean AUC 259% and 61% higher, respectively), there was little difference between human males and females (mean AUC 13% higher in females)]. It is recommended that the proposed paragraph be revised to read as follows:

> "No evidence of carcinogenicity was observed when irbesartan was administered at doses of up to 500/1000 mg/kg/day (males/females, respectively) in rats and 1000 mg/kg/day in mice for up to two years. For male and female rats, 500 mg/kg/day provided an average systemic exposure to irbesartan ($AUC_{0\text{-}24hr}$, bound plus unbound) about 3 and 11 times, respectively, the average systemic exposure in humans receiving the maximum recommended dose of 300 mg irbesartan/day, whereas 1000 mg/kg/day (adminstered to females only) provided an average systemic exposure about 21 times the average systemic exposure in humans receiving the maximum recommended human dose of 300 mg. For male and female mice, 1000 mg/kg/day provided an average systemic exposure about 3 and 5 times, respectively, the average systemic exposure in humans receiving the maximum recommended human dose of 300 mg."

6. RECOMMENDATION

These new drug applications for irbesartan and irbesartan/HCTZ are approvable with recommended changes in labeling.

G. Jagadeesh, Ph.D.

cc:
Original NDA 20,757 (Irbesartan); NDA 20,758 (Irbesartan/HCTZ)
HFD-110
HFD-110/CSO
HFD-110/G. Jagadeesh
HFD-110/S. Stolzenberg
HFD-024/J. DeGeorge
HFD-345/
Accepted by: 7-31-97
gj/7/28/97 /NDA20757.org

L. Borgstrom

JUL 30 1997

NDA 20-757 and NDA 20-758

REVIEW AND EVALUATION OF REPRODUCTIVE TOXICOLOGY DATA

Sidney J. Stolzenberg, Ph.D.
July 25, 1997

SUBMISSION DATE: 9/26/96
CENTER RECEIPT DATE: 9/26/96
REVIEWER RECEIPT DATE: 10/3/96

SPONSOR: Sanofi Winthrop, Inc.
Montpellier Cedex, France

AGENT: Bristol-Meyer Squibb
P. O. Box 4000
Princeton, NJ 08543-4000

NDA 20-757 Irbesartan Tablets

Pharmacological Class: Angiotensin II receptor antagonist

Indication: Hypertension

Formulation and Route of Administration: Each tablet contains 75, 150 or 300 mg of Irbesartan. Inactive ingredients in the tablet are lactose, microcrystalline cellulose, pre-gelatinized starch, croscarmellose sodium, poloxamer, silicon dioxide and magnesium stearate.

Dosage Regimen: The recommended initial dose is 150 mg once daily but for further reduction of blood pressure the dosage may be titrated to 300 mg once daily.

NDA 20-758 Irbesartan/Hydrochlorothiazide Tablets

Pharmacological Class: Angiotensin II receptor antagonist/diuretic drug combination

Indication: Hypertension

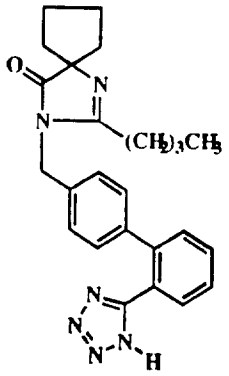
Formulation and Route of Administration: Each tablet contains 150 mg of Irbesartan and 12.5 mg of hydrochlorothiazide. Inactive ingredients include lactose monohydrate, microcrystalline cellulose, pre-gelatinized starch, croscarmellose sodium, ferric oxide red, ferric oxide yellow, silicon dioxide and magnesium stearate.

Dosage Regimen: The recommended initial dose is 150/12.5 mg once daily but for further reduction of blood pressure the dosage may be titrated to 300/25 mg once daily (two tablets).

RELATED INDs: Clinical trials for Irbesartan were conducted under B-M Squibb IND Clinical trials for Irbesartan/HCTZ were conducted under B-M Squibb IND

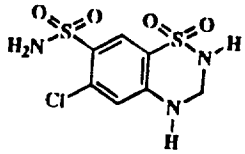
DRUG CHEMISTRY

Irbesartan
Code Names: SR 47436; BMS 186295; BMS-186295-01
Chemical Name: 2-butyl-3-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1,3-diazaspiro[4,4]non-1-en-4-one]
CAS Registry No: 138402-11-6
Chemical Structure:



Molecular Weight: 428.5

Hydrochlorothiazide
Code Names: None
Chemical Name: 2H-1,2,4-benzothiadiazine-7-sulfonamide,6-chloro-3,4-dihydro-1,1-dioxide
CAS Registry No: 58-93-5
Chemical Structure:



Molecular Weight: 297.73

TABLE OF CONTENTS

A. REPRODUCTIVE TOXICITY STUDIES

1. Fertility and General Reproduction Study of Irbesartan in Rats (Segment I).

Study No: SNF033

Performing Laboratory:

Sponsor: Sanofi Recherche
Montpellier Cedex, France

Date Study Initiated: June 14, 1993

Quality Assurance: A statement of conformance to GLPs is included.

Test Animals: CD Sprague Dawley rats (Charles River, UK), 36 males and 36 females per group; at commencement of treatment, males were 6 to 7 weeks old and weighed 168-228 g, females were 10-11 weeks old and weighed 200-246 g.

Procedure: SR 47436 (Lot no. 93-04), in 10% gum Arabic (w/v) vehicle, was administered once daily by oral gavage at doses of 0 (vehicle control), 50, 180 and 650 mg/kg/day. The dose volume was 10 ml/kg for each group. Males were dosed for 71 days before pairing with females of the same dosage groups, and throughout the mating period, until successful littering of F_0 females. Females were dosed for 15 days before pairing, throughout the mating and gestation periods, up to postpartum day (PPD) 24 and weaning of the pups. Twenty-three females in each group were randomly allocated for C-sectioning on GD 20, the remaining females were permitted to deliver their offspring and rear them to PPD 25.

Justification of Dosage: In a preliminary Segment I dose-range study (Study FER 230)) with 6 males and 6 females per group and doses of 0, 50, 180 and 650 mg/kg/day, no adverse effect was observed even at the highest dose and it was concluded that "a dose level of at least 650 mg/kg/day should be suitable as the highest dosage in the main study".

Observations and Measurements: All males and females were observed daily for general condition and mating; body weight and food consumption measurements for males were obtained weekly; female weights and food consumption measurements were more frequent obtained before mating and during pregnancy. Estrous cycles by vaginal smears were obtained before mating. C-sectioned dams (23 per group) were examined for number of corpora lutea in each ovary, number of implantation and resorption sites, number

of live fetuses, fetal and placental weights. Live fetuses were examined for determination of sex and external anomalies; half were examined for soft tissue abnormalities by free hand sectioning (Wilson technique); the remaining half were first internally examined by dissection and then cleared with KOH and stained with Alizarin-red (modification of Dawson's method) for detection of skeletal abnormalities. The remaining dams of each group were allowed to deliver; the litters were culled to 8 per dam (4/sex if possible) on PPD 4, and the offspring were weaned on PPD 25. Postnatal observations included determination of gestation length and body weights of dams and pups (PPD 1, then twice weekly to PPD 25). Dam and pup clinical signs, mortality and litter size were checked daily throughout lactation. Subsequent observations included timing of physical development of offspring (pinna unfolding, hair growth, testes descent, vaginal opening, tooth eruption and eye opening), auditory and visual function, activity (by means of infra-red light detectors in plastic cages), learning (water filled maze) and neuromuscular function (5 different tests). At 5 weeks of age, 26 male and 26 female offspring from each group were randomly selected (2/sex/litter if possible) for evaluation of sexual maturation and reproductive performance of F_1 progeny. F_2 male and female progeny were killed after completion of vaginal opening by females or at about 6 weeks of age, and were macroscopically examined.

Drug Related Effects

F_0 Parent Evaluation

Mortality: Three high dose dams were killed in extremis on GD 21 or 22 (at the time of expected parturition). Clinical signs and necropsy findings for those dams are shown in Table 1.

Other Effects: Mean body weights of treated males were lower than control (dose related for all 3 groups and statistically significant for mid and high dose) from weeks 1 to 15 (around 11% lower for high dose compared to control at week 15 of treatment). Food consumption was significantly decreased over the same time period mainly for high dose males. The mean body weights of treated females before mating were significantly lower than control by day 10 of treatment but were not dose related. The investigators claimed that the mean weight gain of the control group before pairing was high because of 5 acyclic females which showed high weight gain, and when the mean body weight gain was adjusted for these 5 females, there were no significant differences from control. There was, however, a statistically significant decrease in food intake by high dose females before mating, during both the first and second weeks of treatment.

During gestation, group mean body weights of treated females were

lower than control but there was no dose relationship. Similarly, group mean food intake of treated females was slightly lower than control, predominantly during the second half of pregnancy, but there was no dose relationship. Estrous cycles, mating performance and fertility were unaffected by treatment.

Fetal Evaluation: Mean fetal and placental weights at the high dose were significantly lower than control. The high dose group mean incidence of green-tinged amniotic sacs and amniotic fluid, and green-rimmed placentae exceeded concurrent control and background control ranges, but these findings were restricted to one litter and were not considered to be treatment related. In fetuses examined by free hand serial sectioning, treatment-related increases in litter incidences of unilateral and bilateral hydroureter, and unilateral and bilateral renal pelvic cavitation or absence of renal papilla were observed, which exceeded background control ranges in all 3 treated groups. In fetuses examined by dissection, treatment-related increases in litter incidences of unilateral and bilateral hydroureter, and unilateral and bilateral renal pelvic cavitation, which exceeded background control ranges in all 3 treated groups, were similarly observed. The investigators indicated that, at mid and high doses, there were dosage related increases in incidence of fetuses with subcutaneous edema (observed with free hand serial sectioning), which exceeded background control range.

Postnatal Evaluation:

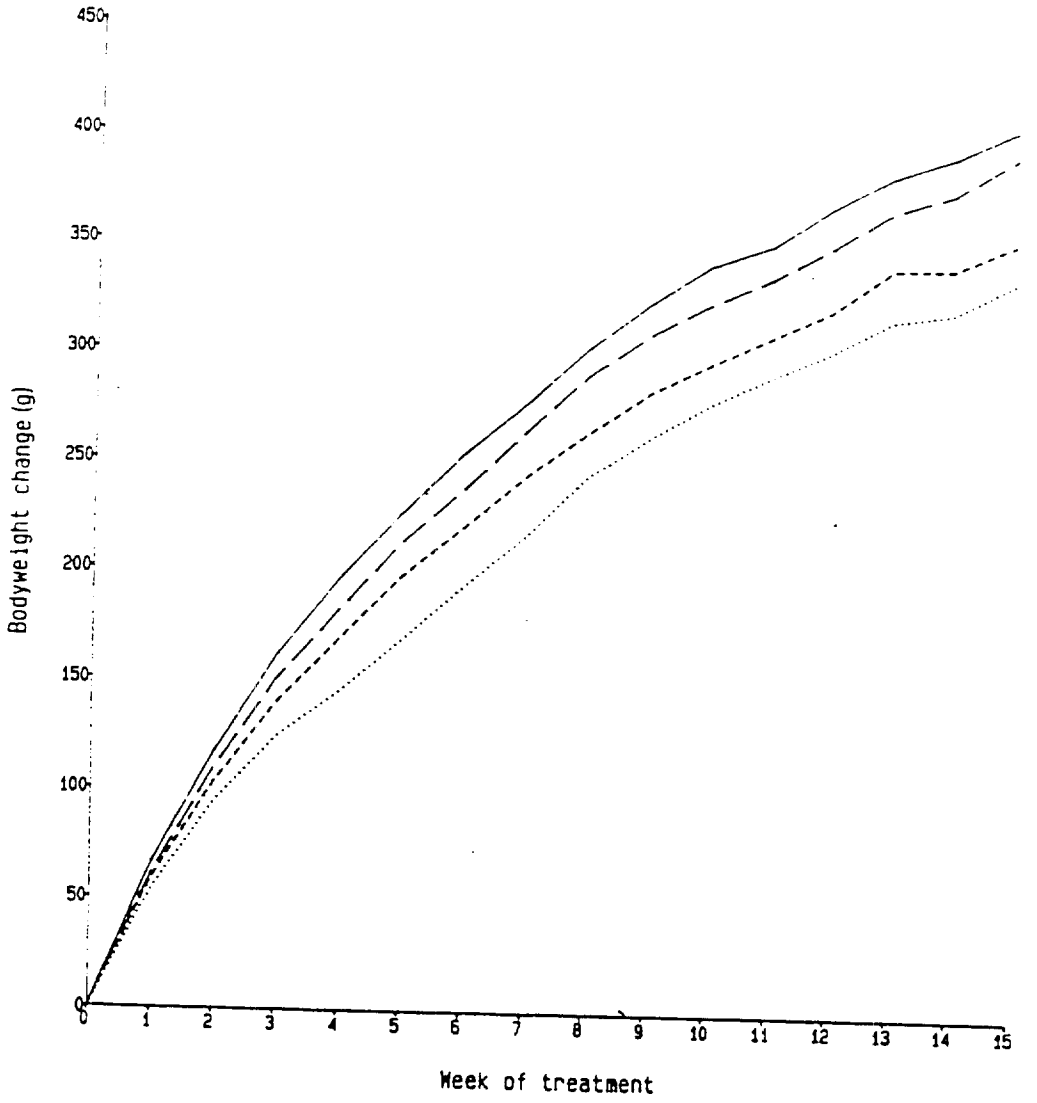
At high dose, the total live litter size on PPD 1 was slightly lower than concurrent control, but this was attributed to one litter which had only one live offspring on PPD 1. Offspring body weights on PPD 1 were unaffected by treatment of the dams. Although subsequent body weights of male and female offspring during lactation (all treated groups) were lower than control (significantly lower on PPD 25), the body weights were not dose related and, among offspring selected for post-weaning observation, there were no longer any treatment related differences in body weight.

At gross necropsy of pups that died, unselected pups on PPD 4 of culling or pups at 6 weeks of age, no findings that were treatment related were observed. The investigators concluded that the compound relate effects observed in fetuses (renal pelvic cavitation and hydroureter) were transient.

FIGURE 1

Bodyweight change of males (Fo)

Group 1 : Control
Group 2 : SR 47436 : 50 mg/kg/day
Group 3 : SR 47436 : 180 mg/kg/day
Group 4 : SR 47436 : 650 mg/kg/day

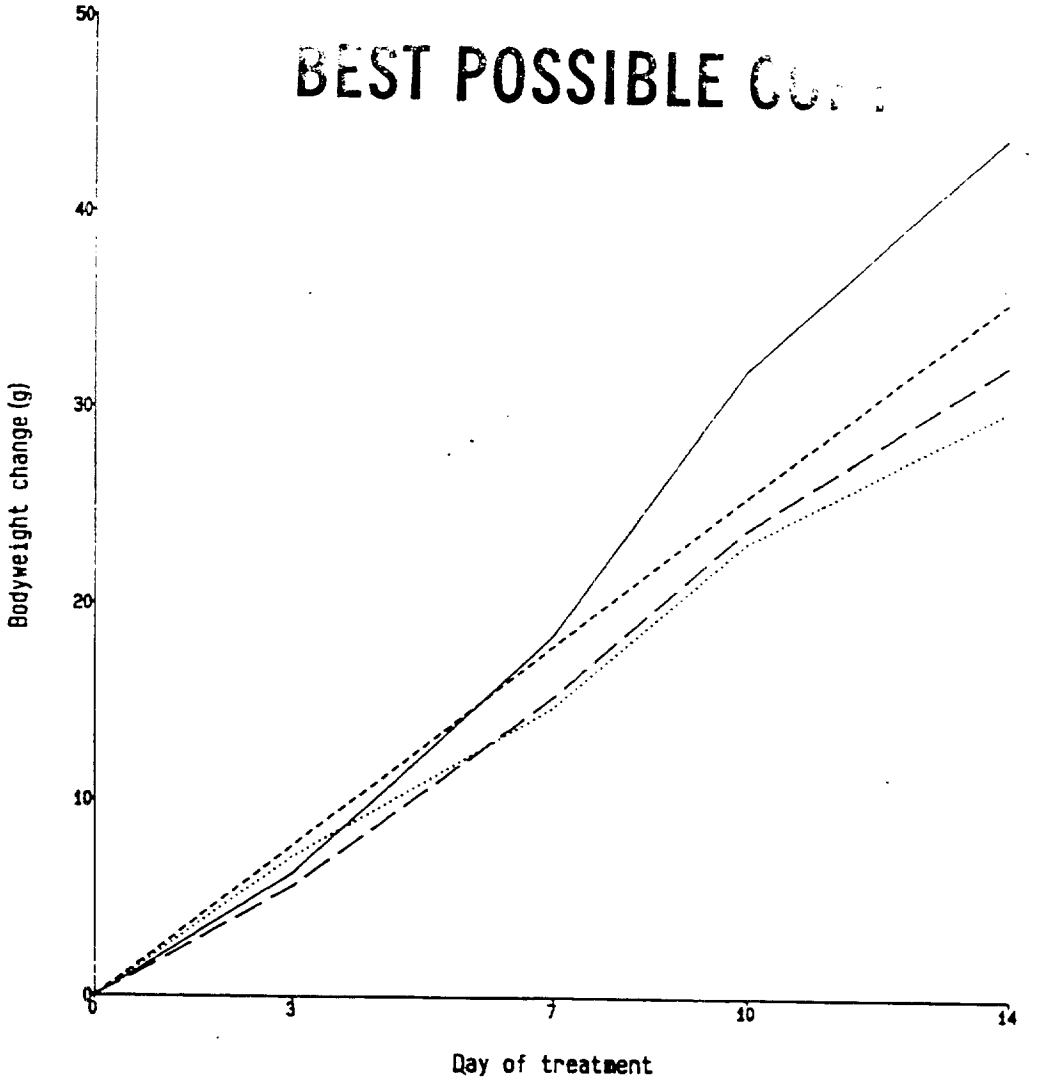


SNF0330M
15/06/94

FIGURE 2

Bodyweight change of females before pairing (Fp)

Group 1 : Control
Group 2 : SR 47436 : 50 mg/kg/day
Group 3 : SR 47436 : 180 mg/kg/day
Group 4 : SR 47436 : 650 mg/kg/day

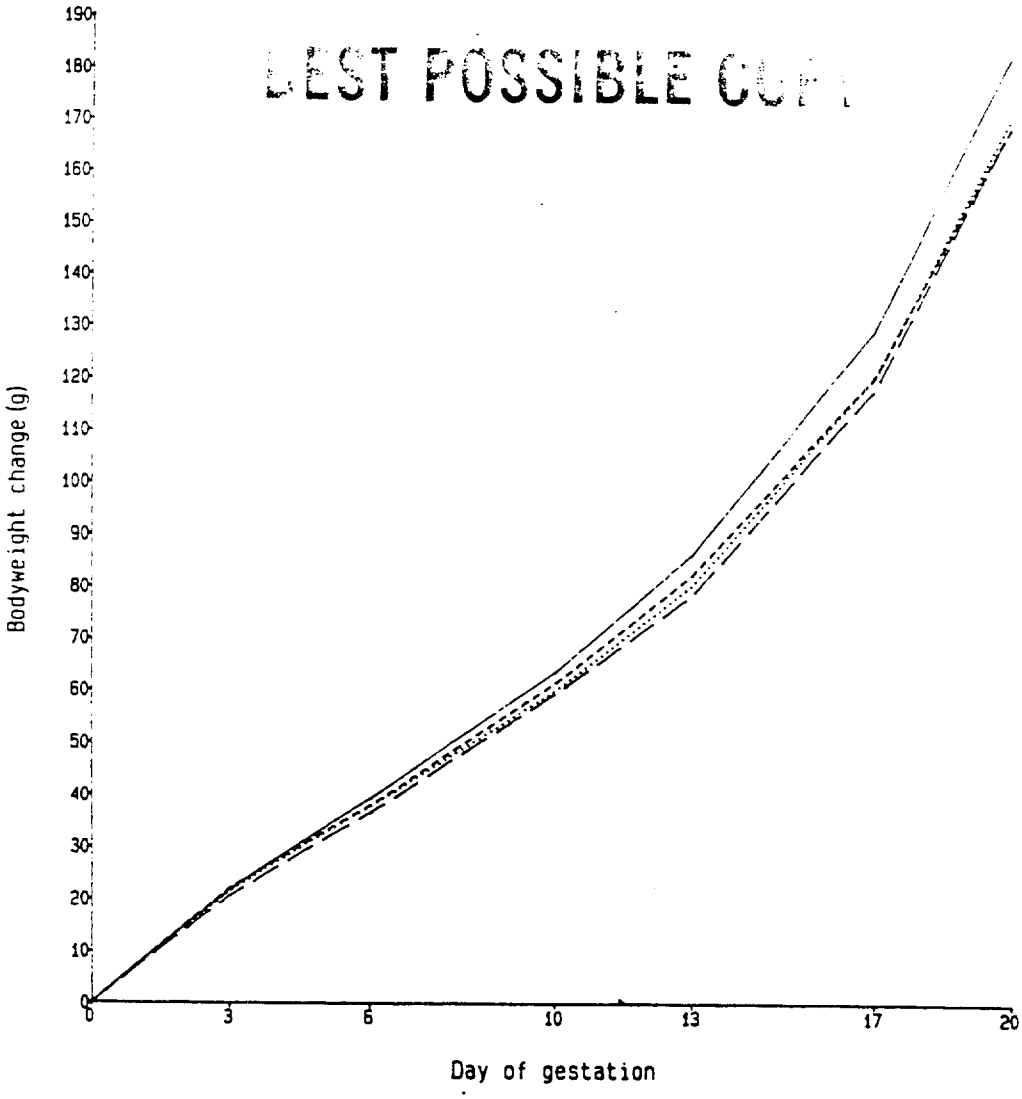


SNF0330F
26/10/93

FIGURE 3 (sponsor's figure 5)

Bodyweight change of females during gestation (Fo-F1)

Group 1 : Control
Group 2 : SR 47436 : 50 mg/kg/day
Group 3 : SR 47436 : 180 mg/kg/day
Group 4 : SR 47436 : 650 mg/kg/day

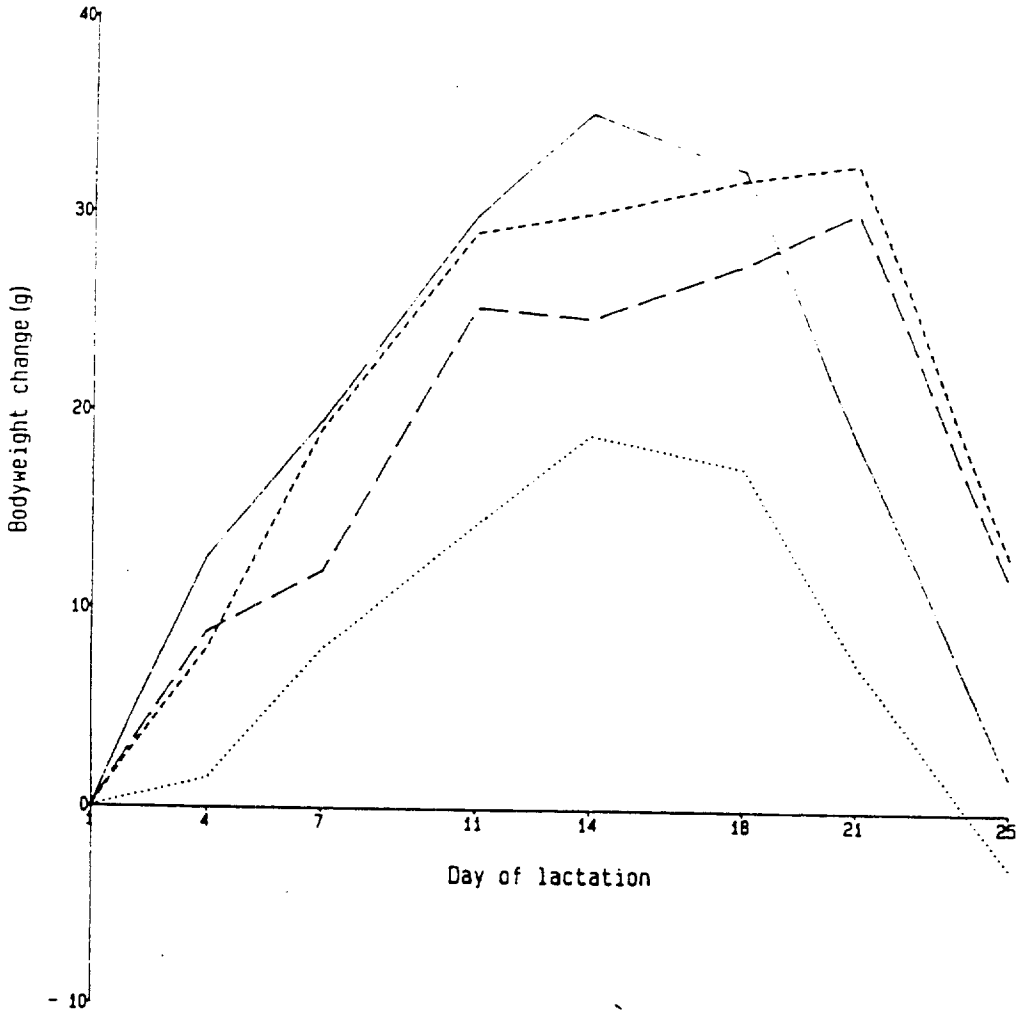


SNF0330G
14/06/94

FIGURE 4 (sponsor's figure 7)

Bodyweight change of females during lactation (Fo-F1)

Group 1 : Control
Group 2 : SR 47436 : 50 mg/kg/day
Group 3 : SR 47436 : 180 mg/kg/day
Group 4 : SR 47436 : 650 mg/kg/day

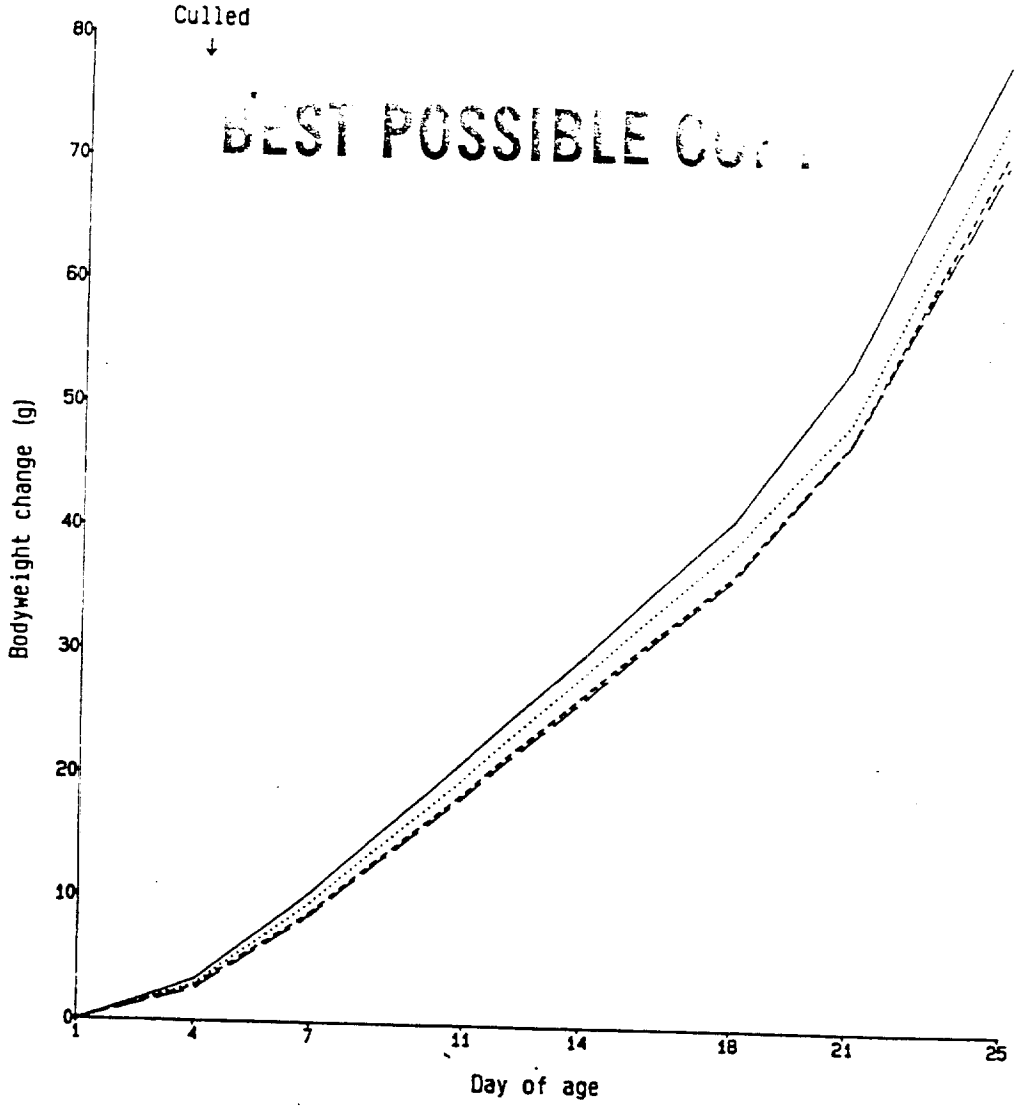


SNF0330L
14/06/94

FIGURE 5 (sponsor's figure 8)

Bodyweight change of male offspring (F1)

———— Group 1 : Control
— — — — Group 2 : SR 47436 : 50 mg/kg/day
---------- Group 3 : SR 47436 : 180 mg/kg/day
..... Group 4 : SR 47436 : 650 mg/kg/day

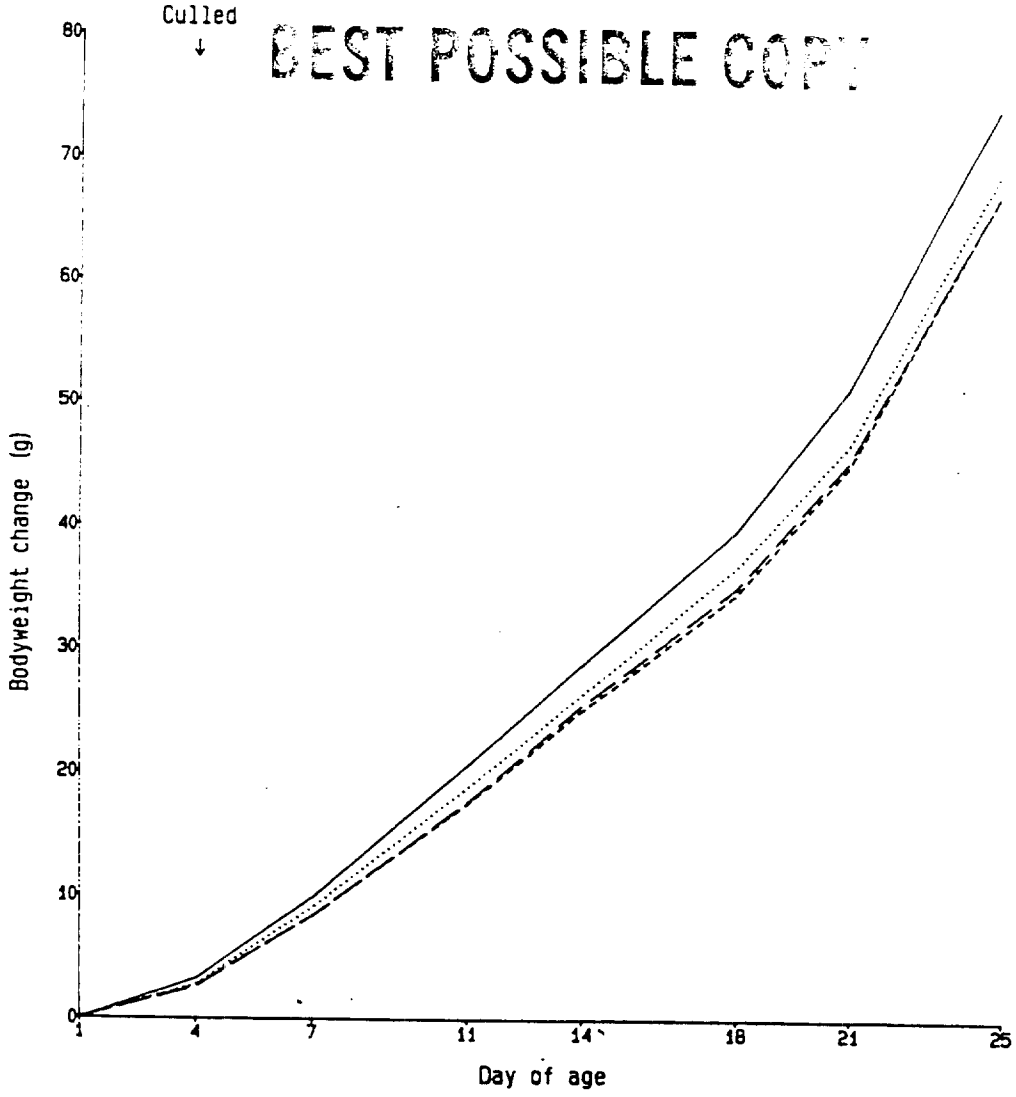


snf33a1

FIGURE 6 (sponsor's figure 9)

Bodyweight change of female offspring (F1)

Group 1 : Control
Group 2 : SR 47436 : 50 mg/kg/day
Group 3 : SR 47436 : 180 mg/kg/day
Group 4 : SR 47436 : 650 mg/kg/day



snf33f1

TABLE 1

Summary of mortality (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Animal number	History and circumstances of death	Summary of necropsy findings
4	1255	Killed *in extremis* on Day 21 of gestation. Brown staining on jaw, forelimbs, ventral abdomen and perigenital area. Brown discharge from anus, loose faeces, deep respiration, underactive, piloerection, pallor and pale eyes.	External: Red staining on muzzle, fur wet and matted around eyes, and fur wet, matted and stained yellow/brown around urinogenital area, hindlimbs and ventral abdomen. Internal: Sublingual and submandibular salivary glands pale yellow. Liver pale, with pale mottled areas on all lobes and the lobular pattern accentuated. Spleen pale and reduced. Numerous ulcerations on glandular mucosa of the stomach, caecal wall thickened. Entire gastro-intestinal tract contents fluid, and no faecal pellet formation in rectum. Female pregnant with 24 corpora lutea, 21 implantations, 1 early resorption, 1 dead foetus and 19 viable foetuses. Examination of foetuses: External: (All viable foetuses) One foetus was oedematous, and one foetus was small (2.75 g) with a small placenta (0.31 g). Internal: (Ten foetuses) All had bilateral hydroureter and renal cavitation.

TABLE 1 - continued

Summary of mortality (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Animal number	History and circumstances of death	Summary of necropsy findings
4	1258	Killed *in extremis* on Day 22 of gestation. Prolonged bleeding from the vagina (more than 7 hours), pallor and reduced body temperature.	External: Slight brown staining on muzzle, fur wet, matted and stained red around vagina. Internal: Liver slightly pale, with a pale area on the caudal surface of the median liver lobe. Spleen slightly reduced. Female pregnant with 15 corpora lutea, 15 implantations and 15 viable foetuses. Examination of foetuses: External: (All viable foetuses) One foetus had unilateral hindlimb malrotation. Internal: (Eight viable foetuses) Four foetuses had bilateral hydroureter and renal cavitation.

TABLE 1 - continued

Summary of mortality (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Animal number	History and circumstances of death	Summary of necropsy findings
4	1281	Killed *in extremis* on Day 21 of gestation. Pallor, piloerection, pale eyes, reduced body temperature, brown staining on lower jaw, forelimbs and paws, brown discharge from anus, loose faeces and weightloss.	External: Fur wet and matted around mouth, fur wet, matted and stained brown around anus and base of tail. Internal: Stomach contents fluid, contents of large and small intestines dark and fluid. Caecal contents reduced. No faecal pellet formation in rectum. Female pregnant with 20 corpora lutea, 19 implantations, three early resorptions and 16 viable foetuses. Examination of foetuses: External: (All viable foetuses) The majority of foetuses were small (mean weight 3.07 g) and placentae were small (mean weight 0.34 g). Internal: (Eight foetuses) Four had bilateral hydroureter, in addition two had bilateral renal cavitation.

TABLE 2

Group mean bodyweights (g) of males (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Week of treatment															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Mean	204	270	322	366	401	432	459	482	507	528	546	555	573	587	596	609
	SD	13	18	27	35	41	47	51	54	58	59	61	61	63	65	65	66
	n	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
2	Mean	206	267[a]	317	358	390	421	445	472	497	516	530	542	557	573	582	599
	SD	10	12	18	24	31	35	37	44	47	49	50	51	55	57	61	63
	n	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
3	Mean	205	264[b]	309[b]	346[b]	375[b]	404[b]	426[b]	450[b]	470[b]	488[b]	502[c]	514[b]	527[c]	546[b]	546[c]	558[c]
	SD	11	16	22	29	35	41	45	48	52	54	58	62	64	68	69	66
	n	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
4	Mean	204	258[c]	299[c]	329[c]	350[c]	374[c]	398[c]	422[c]	449[c]	467[c]	482[c]	495[c]	507[c]	521[c]	526[c]	540[c]
	SD	8	17	27	36	45	48	52	57	58	61	62	65	67	71	71	73
	n	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36

SD Standard deviation.
n Number of animals.
Weight gain from Week 0 significant when compared with Controls: a - $p<0.05$; b - $p<0.01$; c - $p<0.001$ (t-test following one-way analysis of variance).

TABLE 3

Group mean bodyweights (g) of females before pairing (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Day of treatment				
		0	3	7	10	14
1	Mean	229	235	248	261	273
	SD	9	10	12	14	19
	n	36	36	36	36	36
2	Mean	224	230	240	248[c]	257[c]
	SD	10	10	11	13	15
	n	36	36	36	36	36
3	Mean	224	232	242	250[b]	260[b]
	SD	9	9	11	11	12
	n	36	36	36	36	36
4	Mean	225	233	240	249[c]	256[c]
	SD	10	10	12	15	16
	n	36	36	36	36	36

SD Standard deviation.
n Number of animals.
Weight gain from Day 0 significant when compared with Controls:
b - $p<0.01$; c - $p<0.001$ (t-test following one-way analysis of variance).

TABLE 4

Group mean food consumption (g/rat/week) of males (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Week of treatment													
		1	2	3	4	5	6	7	8	9	10 Δ	12	13	14	15
1	Mean	179	192	193	185	189	194	193	196	194	194	184	200	189	186
	SD	12	7	8	7	10	10	11	10	11	13	17	17	11	11
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9
2	Mean	179	190	193	181	186	192	194	197	191	189	185	194	186	190
	SD	10	11	12	12	12	12	13	12	12	14	17	11	11	11
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9
3	Mean	176	186	188	180	182	187	192	187	186	189	186	192	176[b]	179
	SD	10	12	11	9	10	10	11	8	19	13	6	9	9	12
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9
4	Mean	171	179[b]	181[a]	170[b]	171[c]	184	186	186[a]	187	186	185	184[b]	177[a]	182
	SD	6	8	9	13	9	14	11	9	11	9	10	9	9	12
	n	9	9	9	9	9	9	9	9	9	9	9	9	9	9

Δ Animals paired: food consumption not recorded during Week 11.
SD Standard deviation.
n Number of cages (4 animals per cage).
Significant when compared with Controls: a - $p<0.05$; b - $p<0.01$; c - $p<0.001$ (t-test following one-way analysis of variance).

TABLE 5

Group mean food consumption
(g/rat/week) of females before pairing (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Week of treatment	
		1	2
1	Mean	127	138
	SD	7	9
	n	9	9
2	Mean	122	137
	SD	7	8
	n	9	9
3	Mean	124	133
	SD	8	7
	n	9	9
4	Mean	117[b]	122[c]
	SD	7	6
	n	9	9

SD Standard deviation.
n Number of cages (four animals per cage).
Significant when compared with Controls: b - $p<0.01$; c - $p<0.001$ (t-test following one-way analysis of variance).

TABLE 6

Oestrous cycles (F_0)

Group	: 1	2	3	4
Compound	: Control	----	SR 47436	----
Dosage (mg/kg/day)	: 0	50	180	650

Group	Number of animals		Regular 4 or 5 day cycles	Irregular[Δ] cycle	Acyclic[†]
1	36	n	26	5	5
		(%)	(72)	(14)	(14)
2	36	n	30	5	1
		(%)	(83)	(14)	(3)
3	36	n	34	2	0
		(%)	(94)	(6)	
4	36	n	32	4	0
		(%)	(89)	(11)	
Background control (23 studies)					
Mean (%)			93.1	4.2	2.6
Low (%)			72	0	0
High (%)			100	14	14

Δ At least one cycle of two, three or six to ten days.
† At least ten days without oestrus.
n Number of animals in category.

TABLE 7

Pre-coital interval (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Number of animals		Pre-coital interval (days)				
			1-4	5-8	9-12	13-16	17-21
1	36	n	28	4	3	0	1
		(%)	(78)	(11)	(8)		(3)
2	36	n	32	3	0	1	0
		(%)	(89)	(8)		(3)	
3	36	n	35	1	0	0	0
		(%)	(97)	(3)			
4	36	n	34	2	0	0	0
		(%)	(94)	(6)			

n Number of animals in category.

TABLE 8

Mating performance and fertility (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1 M	36	35	35	97	100	97
2 M	36	36	35	100	97	97
3 M	36	36	35	100	97	97
4 M	36	36	36	100	100	100
1 F	36	36	36	100	100	100
2 F	36	36	35	100	97	97
3 F	36	36	35	100	97	97
4 F	36	36	36	100	100	100

TABLE 9

Group mean bodyweights (g) of females during gestation (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Day of gestation						
		0	3	6	10	13	17	20
1	Mean	284	306	323	348	370	413	466
	SD	25	27	27	27	28	30	33
	n	36	36	36	36	36	36	36
2	Mean	265[f]	285	301	324	343	382[b]	433[b]
	SD	20	22	24	26	29	34	40
	n	35	35	35	35	35	35	35
3	Mean	264[f]	287	303	326	347	385[a]	433[b]
	SD	14	16	18	18	21	25	30
	n	35	35	35	35	35	35	35
4	Mean	264[f]	286	303	325	345	385[a]	435[a]
	SD	19	20	20	24	24	26	31
	n	36	36	36	36	36	36	36

SD Standard deviation.
n Number of pregnant animals.
Significantly different from Controls: f - $p<0.001$ (t-test following one-way analysis of variance).
Weight gain from Day 0 significant when compared with Controls:
a - $p<0.05$; b - $p<0.01$ (t-test following one-way analysis of variance).

TABLE 10

Group mean food consumption (g/rat/day) of females during gestation (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Days of gestation					
		0-2	3-5	6-9	10-12	13-16	17-19
1	Mean	26	29	31	32	32	32
	SD	3	3	3	3	3	3
	n	36	36	36	36	36	36
2	Mean	26	29	30	30[a]	30[a]	30[b]
	SD	4	4	4	4	4	4
	n	35	34	34	35	35	35
3	Mean	26	28	30	30	30[a]	30[a]
	SD	2	3	3	3	3	3
	n	35	35	35	35	35	35
4	Mean	25	27	29[b]	30[b]	31[a]	31[a]
	SD	2	3	3	3	3	3
	n	36	36	36	36	36	36

SD Standard deviation.
n Number of pregnant animals.
Significantly different from Controls: a - $p<0.05$; b - $p<0.01$ (t-test following one-way analysis of variance).

TABLE 11

Summary of observations at necropsy
of females on Day 20 of gestation (F_0)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4
Number of animals examined:	23	23[α]	23[α]	22
Number of animals with observations:	3	1	5	3
Observations: animals affected[ϕ]				
Brown staining on head	0	0	0	1
Hairloss/slight hairloss on body or forelimbs	1	1	1	1
Few punctate scabs on abdomen	1	0	0	0
Raised pale area on surface of spleen	1	0	0	0
Bilateral renal cavitation	0	0	1	0
Unilateral hydroureter	0	0	0	1
Bilateral hydroureter	0	0	1	0
Unilateral implantations	1	0	2	0
Dark green mucoid material in vagina and one uterine horn	0	0	1	0
Small amount of brown gelatinous material in cervix	0	0	1	0

ϕ One animal may have more than one observation.
α Includes one female which was not pregnant.

TABLE 12

Group mean litter data - females killed on Day 20 of gestation (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Number of pregnant animals		Corpora lutea count	Implant-ations	Viable young			Resorptions			Implantation loss (%)	
					M	F	Total	Early	Late	Total	Pre-	Post-
1	23	Mean	19.0	17.0	7.0	8.9	15.9	1.09	0.00	1.09	10.7	6.4
		SD	2.4	2.9	2.3	2.1	2.9	1.04	-	1.04		
2	22	Mean	17.5	16.6	8.4	7.7	16.1	0.50	0.00	0.50	5.2	3.0
		SD	2.6	2.3	2.1	2.5	2.4	0.71	-	0.71		
3	22	Mean	17.0	14.9	6.7	7.3	14.0	0.95	0.00	0.95	12.5	6.4
		SD	2.6	4.3	2.8	3.2	4.6	0.98	-	0.98		
4	22	Mean	17.6	16.0	7.8	7.5	15.3	0.73	0.00	0.73	9.5	4.5
		SD	2.0	2.4	2.2	2.1	2.3	0.85	-	0.85		
Background control (56 studies)												
Mean			18.0	16.5	7.8	7.7	15.5	0.94	0.03	0.96	8.7	5.9
Low			16.7	14.2	6.2	5.9	13.2	0.38	0.00	0.38	4.5	2.3
High			19.4	18.2	9.2	9.5	17.2	1.65	0.14	1.74	15.4	9.8

SD Standard deviation.

TABLE 13

Group mean foetal and placental weights (F.)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Foetal weight (g)			Placental weight (g)
		Male	Female	Overall	Overall
1	Mean	3.80	3.60	3.68	0.51
	SD	0.10	0.08	0.07	0.02
	n	23	23	23	23
2	Mean	3.72	3.46	3.60	0.49
	SD	0.08	0.10	0.07	0.02
	n	22	22	22	22
3	Mean	3.66	3.52	3.63	0.51
	SD	0.10	0.09	0.07	0.02
	n	21	22	22	22
4	Mean	3.60[a]	3.37[a]	3.49	0.48[a]
	SD	0.08	0.08	0.06	0.02
	n	22	22	22	22
Background control (56 studies)					
Mean		3.75	3.55	3.65	0.51
Low		3.50	3.37	3.45	0.48
High		3.93	3.75	3.84	0.56

SD Standard deviation.
n Number of litters.
Significant when compared with Controls: a - $p<0.05$ (weighed t-test following one-way analysis of variance).

TABLE 14

Summary of foetal observations at necropsy (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	366(23)	355(22)	307(22)	336(22)	16256 foetuses	56 studies
Number of male : female foetuses:	162:204	185:170	147:160	172:164		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Small foetus (less than 2.80 g)	3.0(4)	1.1(2)	0.3(1)	3.0(5)	1.45	
Large foetus (more than 4.10 g)	15.0(14)	6.8(6)	2.9(4)	8.3(6)	8.27	
Shiny foetus	1.4(1)	0.0(0)	0.3(1)	0.0(0)	0.33	
Pale foetus	0.5(2)	0.0(0)	0.0(0)	0.3(1)	0.10	
Squat foetus	0.0(0)	0.8(1)	0.0(0)	0.0(0)	0.23	
Domed head	0.0(0)	0.0(0)	0.3(1)	0.0(0)	0.10	
Abnormal foetus - craniorachishisis and unilateral ablepharon	0.0(0)	0.0(0)	0.0(0)	0.3(1)	#	
Unilateral macrophthalmia	0.3(1)	0.0(0)	0.0(0)	0.0(0)	#	
Unilateral anophthalmia	0.0(0)	0.0(0)	0.0(0)	0.3(1)	0.01	
Bilateral anophthalmia	0.0(0)	0.0(0)	0.3(1)	0.0(0)	0.01	
Upturned snout	0.0(0)	0.3(1)	0.3(1)	0.0(0)	0.06	
Pointed snout	0.0(0)	0.0(0)	0.0(0)	0.3(1)	0.04	
Oedema on one side of snout	0.0(0)	0.0(0)	0.0(0)	0.3(1)	#	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.

TABLE 14 - continued

Summary of foetal observations at necropsy (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	366(23)	355(22)	307(22)	336(22)	16256 foetuses	56 studies
Number of male : female foetuses:	162:204	185:170	147:160	172:164		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Lower jaw absent - small aperture at site of mouth	0.3(1)	0.0(0)	0.0(0)	0.0(0)	#	
Prominant blood vessel on one side of palate	0.0(0)	0.0(0)	0.7(2)	0.0(0)	0.01	
One hindlimb malrotated	0.0(0)	0.0(0)	0.3(1)	0.0(0)	0.01	
Unilateral forelimb flexure	0.0(0)	0.3(1)	0.0(0)	0.3(1)	0.02	
Bilateral forelimb flexure	0.3(1)	1.1(2)	0.0(0)	0.3(1)	0.20	
Small placenta (less than 0.35 g)	0.3(1)	1.4(4)	0.7(2)	3.0(4)	0.74	
Large placenta (more than 0.70 g)	1.6(4)	0.6(2)	1.0(3)	1.2(4)	1.70	
Swollen placenta	0.3(1)	0.0(0)	0.0(0)	0.0(0)	#	
Conjoined placenta	0.3(1)	0.0(0)	0.0(0)	0.0(0)	0.15	
Pale/pale area on placenta	0.3(1)	0.0(0)	0.3(1)	0.3(1)	0.03	
Green rimmed placenta	0.0(0)	0.0(0)	0.0(0)	2.7(1)	0.04	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.

TABLE 14 - continued

Summary of foetal observations at necropsy (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
External examination						
Number of foetuses (litters) examined:	366(23)	355(22)	307(22)	336(22)	16256 foetuses	56 studies
Number of male : female foetuses:	162:204	185:170	147:160	172:164		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Clotted blood around placenta	0.0(0)	0.0(0)	0.0(0)	2.7(1)	0.44	
Amniotic sac tinged green	0.0(0)	0.0(0)	0.0(0)	2.7(1)	0.01	
Amniotic fluid tinged green	0.0(0)	0.0(0)	0.0(0)	2.7(1)	0.06	
Amniotic fluid tinged red	0.0(0)	0.0(0)	0.0(0)	0.3(1)	0.01	

ϕ One foetus may have more than one observation.

TABLE 14 - continued

Summary of foetal observations at necropsy (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Internal examination[α]						
Number of foetuses (litters) examined:	187(23)	185(22)	160(22)	172(22)	7155 foetuses	42 studies
Number of male : female foetuses:	84:103	94:91	73:87	89:83		
Observations: % incidence[ϕ] (number of litters)					Mean	Study ranges
Median liver lobe small and pale	0.5(1)	0.0(0)	0.0(0)	0.0(0)	#	
Unilateral increased renal pelvic cavitation	2.7(4)	6.5(7)	7.5(7)	10.5(12)	0.63	
Bilateral increased renal pelvic cavitation	1.6(2)	6.5(5)	6.3(6)	12.8(11)	0.55	
Unilateral hydroureter	3.7(6)	7.6(7)	6.9(9)	14.0(14)	1.31	
Bilateral hydroureter	2.7(4)	10.3(10)	13.8(11)	14.0(14)	1.36	

α Foetuses examined internally prior to skeletal examination.
ϕ One foetus may have more than one observation.
No previous record in current background control data subset.

TABLE 15

Summary of foetal observations at free-hand serial sectioning (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	179(23)	170(22)	147(21)	164(22)	5267 foetuses	42 studies
Number of male : female foetuses:	78:101	91:79	74:73	83:81		
Observations: % incidence♦ (number of litters)					Mean	Study ranges
Head						
Intra-muscular nasal haemorrhage(s)	0.0(0)	0.6(1)	0.0(0)	1.2(1)	0.17	
Blood on tongue/in mouth/nasopharynx	0.6(1)	1.2(2)	1.4(2)	0.6(1)	1.01	
Unilateral slightly haemorrhagic vitreous humour	0.6(1)	0.6(1)	0.0(0)	0.0(0)	0.04	
Unilateral slight macrophthalmia	0.0(0)	0.6(1)	0.7(1)	0.6(1)	0.32	
Haemorrhage between one or more areas of brain and pia mater	0.0(0)	0.0(0)	0.0(0)	1.2(2)	0.80	

♦ One foetus may have more than one observation.

TABLE 15 - continued

Summary of foetal observations at free-hand serial sectioning (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	179(23)	170(22)	147(21)	164(22)	5267 foetuses	42 studies
Number of male : female foetuses:	78:101	91:79	74:73	83:81		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Thorax and abdomen						
Slight space between bodywall and organs	1.1(1)	0.0(0)	0.0(0)	0.6(1)	0.06	
Blood in trachea	2.8(3)	1.2(1)	0.7(1)	0.6(1)	0.47	
Blood filled thoracic lymph vessel(s)	0.6(1)	1.8(3)	0.0(0)	0.0(0)	0.55	
Origins of left carotid artery and left subclavian artery closer together on aortic arch	0.0(0)	0.6(1)	0.7(1)	0.0(0)	#	
Double aortic arch; kidneys slightly reduced	0.0(0)	0.0(0)	0.0(0)	0.6(1)	0.02	
Haemorrhagic pericardial fluid	11.2(9)	4.1(5)	0.0(0)	3.7(4)	1.01	
Slightly haemorrhagic pleural fluid	0.6(1)	0.0(0)	0.0(0)	0.0(0)	0.19	
Hepatic haemorrhage(s)	11.2(11)	17.6(11)	19.0(11)	18.9(16)	13.78	
Small additional liver lobe	16.8(16)	18.8(17)	13.6(13)	19.5(18)	18.91	
Localised internal abdominal haemorrhage	1.7(3)	1.2(2)	2.0(3)	3.0(3)	2.41	
Haemorrhagic peritoneal fluid	6.1(7)	1.2(2)	0.7(1)	3.0(2)	0.95	
Haemorrhagic abdomen	0.6(1)	0.0(0)	0.7(1)	0.6(1)	0.42	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.

TABLE 15 - continued

Summary of foetal observations at free-hand serial sectioning (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	179(23)	170(22)	147(21)	164(22)	5267 foetuses	42 studies
Number of male : female foetuses:	78:101	91:79	74:73	83:81		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Thorax and abdomen - continued						
Kidney and adrenal gland slightly displaced	1.1(2)	2.4(3)	0.0(0)	2.4(3)	1.06	
Unilateral absence of renal papilla and/or increased renal pelvic cavitation	0.0(0)	2.9(3)	5.4(6)	3.7(4)	0.61	
Bilateral absence of renal papilla	0.0(0)	0.0(0)	1.4(2)	0.6(1)	0.09	
Unilateral hydroureter	1.7(3)	6.5(7)	14.3(11)	11.6(13)	2.51	
Bilateral hydroureter	1.7(2)	3.5(5)	3.4(4)	9.1(8)	2.20	
Haemorrhagic fluid in renal pelvic region	0.0(0)	0.6(1)	1.4(1)	0.0(0)	#	
Ovary and uterine horn slightly displaced++	1.0(1)	0.0(0)	0.0(0)	0.0(0)	0.50	
Uterine horn slightly reduced++	0.0(0)	0.0(0)	1.4(1)	0.0(0)	0.11	
Testis(es) slightly displaced+	7.7(5)	11.0(7)	18.9(12)	12.0(10)	20.93	
Bladder slightly dilated	0.0(0)	0.6(1)	0.0(0)	0.0(0)	0.08	
Genital tubercle slightly elongated	2.2(2)	0.0(0)	0.0(0)	1.8(3)	0.40	
Threadlike tip to tail	0.0(0)	0.0(0)	0.0(0)	1.2(1)	0.08	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.
+/++ Percentage calculated on total number of male/female foetuses.

TABLE 15 - continued

Summary of foetal observations at free-hand serial sectioning (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetuses (litters) examined:	179(23)	170(22)	147(21)	164(22)	5267 foetuses	42 studies
Number of male : female foetuses:	78:101	91:79	74:73	83:81		
Observations: % incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Limbs and others						
Intra-muscular haemorrhage - hindlimb	1.1(2)	2.4(4)	8.2(7)	3.7(4)	5.90	
Subcutaneous haemorrhage(s):						
Nasal	1.1(2)	0.0(0)	0.0(0)	0.6(1)	0.53	
Cranial	0.0(0)	2.4(4)	0.0(0)	1.8(2)	2.77	
Jaw	1.1(1)	0.6(1)	0.7(1)	0.6(1)	2.70	
Submandibular	0.0(0)	0.0(0)	0.7(1)	0.0(0)	0.21	
Cervical	2.2(4)	0.0(0)	0.0(0)	2.4(4)	4.69	
Scapular	17.3(12)	13.5(9)	12.9(10)	12.2(12)	23.43	
Thoracic	2.8(5)	1.2(2)	1.4(2)	0.6(1)	5.28	
Fore/hindlimb(s)	4.5(7)	2.4(3)	6.1(7)	9.8(11)	15.04	
Abdominal	0.6(1)	0.6(1)	3.4(3)	2.4(4)	4.46	
Tail	0.0(0)	0.0(0)	0.0(0)	0.6(1)	0.23	
Subcutaneous oedema	4.5(4)	5.3(5)	11.6(7)	18.3(9)	1.54	
Abnormal foetus (see key to observations Appendix 12, Code U)	0.0(0)	0.0(0)	0.0(0)	0.6(1)	0.09	

ϕ One foetus may have more than one observation.

TABLE 16

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Head						
Small anterior fontanelle	0.5(1)	0.0(0)	0.0(0)	1.2(1)	1.96	
Medium anterior fontanelle	97.9(22)	99.5(22)	98.8(22)	98.8(22)	96.22	
Large antérior fontanelle	1.6(1)	0.5(1)	1.3(2)	0.0(0)	1.83	
Supra-occipital bone incompletely ossified	12.8(9)	14.6(16)	13.1(9)	4.1(5)	8.77	
Interparietal bone incompletely ossified	26.7(17)	22.7(18)	23.8(12)	12.2(17)	14.04	
Incomplete ossification of parietal bone	5.9(3)	3.8(6)	2.5(2)	0.0(0)	1.64	
Incomplete ossification of squamosal bone	6.4(5)	9.7(8)	6.9(4)	1.7(3)	1.66	
Incomplete ossification of frontal bone	1.1(2)	2.7(3)	2.5(3)	0.0(0)	0.44	
Discrete unossified area in frontal bone	0.0(0)	0.0(0)	0.6(1)	0.0(0)	0.10	
Incomplete ossification of palatine bones	0.0(0)	0.0(0)	0.6(1)	0.0(0)	0.10	
Incomplete ossification of basioccipital bone	0.5(1)	0.0(0)	0.6(1)	0.0(0)	#	
Craniopharyngeal canal enlarged	0.5(1)	0.0(0)	0.0(0)	0.0(0)	0.17	
Incomplete ossification of basisphenoid bone	1.1(1)	0.0(0)	1.3(2)	0.0(0)	4.43	
Presphenoid bone incompletely ossified or unossified	2.1(2)	0.5(1)	0.6(1)	0.0(0)	1.11	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.

TABLE 16 - continued

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Head - continued						
Plaque of bone between interparietal and parietal bones	0.0(0)	0.0(0)	0.0(0)	0.6(1)	0.21	
Frontal suture enlarged	0.5(1)	0.0(0)	0.0(0)	0.0(0)	0.16	
Fronto-nasal suture enlarged	4.3(3)	2.2(2)	1.9(3)	0.0(0)	0.90	
Incomplete ossification of jugal bone	0.0(0)	0.0(0)	0.6(1)	0.0(0)	0.26	
Incomplete ossification of maxillary bone	0.0(0)	0.5(1)	0.0(0)	0.0(0)	0.10	
Brachygnathia (see key to observations Appendix 13, Code M)	0.5(1)	0.0(0)	0.0(0)	0.0(0)	0.01	
Incomplete ossification of hyoid bone	4.3(6)	3.2(4)	4.4(4)	2.9(3)	6.24	
Hyoid bone unossified	11.8(11)	17.8(13)	10.6(9)	5.2(7)	13.35	
One or more incisors ossified	0.5(1)	0.5(1)	0.0(0)	0.6(1)	0.39	
Eye orbit small	0.0(0)	0.0(0)	0.6(1)	0.0(0)	0.03	
Domed head	0.0(0)	0.0(0)	0.0(0)	0.6(1)	0.09	

ϕ One foetus may have more than one observation.

TABLE 16 - continued

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Sternebrae and ribs						
Incomplete ossification of 1 sternebra	43.9(21)	25.4(20)	22.5(15)	19.8(13)	30.00	
Incomplete ossification of 2 sternebrae	38.5(22)	51.9(22)	45.6(20)	46.5(20)	45.78	
Incomplete ossification of 3 sternebrae	10.2(12)	16.2(15)	21.3(14)	22.1(11)	17.66	
Incomplete ossification of 4 sternebrae	2.7(5)	1.1(2)	5.0(7)	6.4(7)	3.41	
Incomplete ossification of 5 sternebrae	0.0(0)	0.0(0)	0.6(1)	0.6(1)	0.59	
Incomplete ossification of 6 sternebrae	0.5(1)	0.5(1)	0.6(1)	0.0(0)	0.57	
Ribs 13/13	92.0(23)	90.3(22)	89.4(22)	95.3(22)	86.88	
Ribs 13/14	7.0(10)	5.4(6)	7.5(8)	2.9(5)	7.81	
Ribs 14/14	1.1(2)	4.3(2)	3.1(3)	1.7(3)	5.25	
13th rib or ribs short	0.5(1)	0.0(0)	0.6(1)	0.6(1)	0.21	
Thickened ribs associated with short body and fore-limb flexure	0.0(0)	1.1(1)	0.0(0)	0.0(0)	0.36	
Cervical rib	0.0(0)	0.5(1)	0.0(0)	0.6(1)	0.20	

ϕ One foetus may have more than one observation.

TABLE 16 - continued

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Vertebrae						
Ossification of ventral arch of 1st cervical vertebra	1.1(2)	3.2(4)	5.6(6)	6.4(5)	8.88	
Incomplete ossification of cervical vertebral arches	0.0(0)	0.5(1)	0.0(0)	0.0(0)	0.14	
Ossification of the majority of cervical vertebral centra	0.5(1)	0.5(1)	0.6(1)	0.0(0)	1.14	
1st thoracic vertebral centrum unossified	2.7(3)	1.1(1)	1.9(3)	5.8(5)	1.03	
Incomplete ossification of one or more thoracic vertebral centra	3.7(6)	4.3(7)	1.9(2)	3.5(3)	10.72	
One or more thoracic vertebral centra bipartite	1.1(2)	0.5(1)	0.6(1)	0.0(0)	0.50	
Incomplete ossification of one or more lumbar vertebral centra	0.5(1)	0.5(1)	0.0(0)	0.6(1)	0.19	
Incomplete ossification of one or more lumbar vertebral arches	0.5(1)	0.0(0)	0.6(1)	0.0(0)	0.04	

ϕ One foetus may have more than one observation.

TABLE 16 - continued

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Vertebrae - continued						
Incomplete ossification of sacral vertebral arches	1.6(3)	0.5(1)	1.9(2)	0.0(0)	0.89	
25 pre-sacral vertebrae	0.5(1)	0.0(0)	0.0(0)	0.6(1)	0.07	
27 pre-sacral vertebrae	0.0(0)	0.5(1)	0.6(1)	0.0(0)	0.50	
Incomplete ossification of caudal vertebrae, less than 5 ossified	2.1(2)	1.6(3)	2.5(4)	7.6(7)	1.93	
Increased ossification of caudal vertebrae, more than 8 ossified	0.0(0)	0.0(0)	0.6(1)	0.0(0)	#	
Incomplete ossification of caudal vertebral arches	22.5(12)	27.6(19)	27.5(14)	24.4(16)	10.08	

ϕ One foetus may have more than one observation.
\# No previous record in current background control data subset.

TABLE 16 - continued

Summary of foetal observations at skeletal examination (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group:	1	2	3	4	Control data	
Number of foetus(s) (litters) examined:	187(23)	185(22)	160(22)	172(22)	7003 foetuses	42 studies
Observations: % foetal incidence$^{\phi}$ (number of litters)					Mean	Study ranges
Limbs and girdles						
Metacarpals/metatarsals 3/4	80.2(22)	81.1(22)	65.6(20)	79.7(22)	71.70	
Metacarpals/metatarsals 4/4	16.6(10)	17.8(11)	31.3(17)	20.3(12)	27.02	
Metacarpals and/or metatarsals incompletely ossified or unossified	3.7(5)	2.7(4)	3.1(3)	0.0(0)	2.04	
One or more phalangeal bones ossified	2.1(3)	1.6(2)	1.3(2)	0.0(0)	3.24	
One or both pubic bones incompletely ossified or unossified	5.3(7)	1.6(2)	2.5(3)	1.7(2)	4.36	
Ischial bones incompletely ossified or unossified	0.0(0)	0.5(1)	0.6(1)	0.0(0)	0.69	
Asymmetric pelvis, ilial bones associated with different sacral vertebrae	0.0(0)	0.5(1)	0.0(0)	0.6(1)	0.59	

ϕ One foetus may have more than one observation.

TABLE 17

Gestation length and gestation index (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group	Number of pregnant animals		Gestation length (days) 22	22½	23	23½	Number of live litters born	Gestation index (%)
1	13	n	3	6	3	1	13	100
		(%)	(23)	(46)	(23)	(8)		
2	13	n	1	11	1	0	13	100
		(%)	(8)	(85)	(8)			
3	13	n	2	8	1	2	13	100
		(%)	(15)	(62)	(8)	(15)		
4	14Δ	n	0	8	0	3	11	79
		(%)		(73)		(27)		
Background control (21 studies)								
Mean (%)			30.2	45.3	18.6	5.8		99.2
Low (%)			0	27	8	0		92
High (%)			62	77	38	23		100

n Number of animals in category.
Δ Percentage distribution calculated from 11 animals - three pregnant females killed *in extremis* at expected time of parturition.

TABLE 18

Group mean bodyweights (g) of females during lactation (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Day of lactation							
		1	4	7	11	14	18	21	25
1	Mean	344	357	364	374	379	377	363	346
	SD	16	18	23	23	18	27	25	21
	n	13	13	13	13	13	13	13	13
2	Mean	334	343	346	360	359	362	365	346
	SD	28	24	23	23	22	24	24	29
	n	13	13	13	13	13	13	13	13
3	Mean	333	341	353	362	363	365	366[a]	346
	SD	28	28	28	30	26	28	28	29
	n	13	13	13	13	13	13	13	13
4	Mean	365[d]	366	373	379	384	382	372	362
	SD	23	23	24	20	19	19	20	25
	n	11	11	11	11	11	11	11	11

SD Standard deviation.
n Number of animals.
Significant when compared with Controls: d - $p<0.05$ (t-test following one-way analysis of variance).
Weight gain from Day 1 of lactation significant when compared with Controls: a - $p<0.05$ (t-test following one-way analysis of variance).

TABLE 19

Group mean food consumption (g/rat/day) of females during lactation (F_0-F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Days of lactation						
		1-3	4-6	7-10	11-13	14-17 A	18-20 A	21-24 A
1	Mean	37	45	57	67	70	84	106
	SD	4	4	3	4	6	9	8
	n	13	13	13	13	13	13	13
2	Mean	33[a]	42	53	61	66	81	99
	SD	4	3	3	4	5	5	7
	n	13	13	13	13	13	13	13
3	Mean	31[b]	43	52	62	66	83	98
	SD	7	6	7	7	6	10	13
	n	13	13	13	13	13	13	13
4	Mean	32[a]	42	52	61	64	75	96
	SD	6	7	10	13	14	16	21
	n	11	11	11	11	11	11	11

A Includes diet consumed by offspring.
SD Standard deviation.
n Number of animals.
Significant when compared with Controls: a - $p<0.05$; b - $p<0.01$ (t-test following one-way analysis or variance).

TABLE 20 (sponsor's table 23)

Group mean bodyweights (g) of male offspring (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Day of age: Before culling		After culling						
		1	4	4	7	11	14	18	21	25
1	Mean	6.9	10.3	10.3	17.4	28.4	36.8	48.1	60.6	85.4
	SD	0.6	0.9	0.9	1.1	1.5	1.3	1.7	2.3	3.6
	n	13	13	13	13	13	13	13	13	13
2	Mean	6.4	9.0	9.0	15.1	25.0	32.8	42.9	53.9	76.8[c]
	SD	0.4	0.9	0.8	1.6	2.4	2.5	3.1	3.8	5.4
	n	13	13	13	13	13	13	13	13	13
3	Mean	6.6	9.4	9.5	15.5	25.5	33.4	43.4	54.2	78.0[b]
	SD	0.8	1.5	1.6	2.5	3.5	3.8	4.1	5.4	7.3
	n	13	13	13	13	13	13	13	13	13
4	Mean	6.9	9.9	10.0	16.6	27.0	35.3	46.0	56.1	80.8[a]
	SD	0.4	0.5	0.7	1.3	1.8	2.4	3.1	4.1	5.9
	n	10	10	10	10	10	10	10	10	10

SD Standard deviation.
n Number of litters.
Weight gain from Day 1 of age significant when compared with Controls:
a - $p<0.05$; b - $p<0.01$; c - $p<0.001$ (t-test following one-way analysis of variance).

TABLE 21 (sponsor's table 24)

Group mean bodyweights (g) of female offspring (F_1)

Group	:	1	2	3	4
Compound	:	Control	----	SR 47436	----
Dosage (mg/kg/day)	:	0	50	180	650

Group		Day of age: Before culling		After culling						
		1	4	4	7	11	14	18	21	25
1	Mean	6.5	9.8	9.8	16.4	27.1	35.3	46.1	57.5	80.3
	SD	0.6	0.9	0.8	1.2	1.4	1.5	2.2	2.5	2.8
	n	13	13	13	13	13	13	13	13	13
2	Mean	6.0	8.6	8.6	14.4	23.6	31.5	41.0	51.2	72.8[a]
	SD	0.5	1.0	1.0	1.6	2.3	2.6	2.9	3.6	4.4
	n	13	13	13	13	13	13	13	13	13
3	Mean	6.1	8.8	8.8	14.5	23.6	31.2	40.6	50.9	72.9[a]
	SD	0.8	1.4	1.4	2.2	2.7	3.3	3.8	5.4	7.5
	n	13	13	13	13	13	13	13	13	13
4	Mean	6.6	9.2	9.2	15.0	24.1	31.3	41.1	50.6	71.6[a]
	SD	0.8	0.6	0.6	2.1	4.3	5.7	7.4	9.0	12.5
	n	11	11	11	11	11	11	11	11	11

SD Standard deviation.
n Number of litters.
Weight gain from Day 1 of age significant when compared with Controls:
a - $p<0.05$ (t-test following one-way analysis of variance).