One year of inhalation treatment in rats at a level of 120 µg/kg/day and higher was associated with degeneration of seminiferous tubules that did not recover after an 8-week period without treatment. All other findings resolved after the 8-week period. This finding was not noted in other species tested.

CARCINOGENICITY

Dose range finding studies for carcinogenicity testing began in 1979. Carcinogenicity studies were conducted in rats and mice via drinking water in 1980 - 1982. Interpretation of the rat study was confounded by low survival. Additional histopathology was performed over the years to elucidate findings in the rat study, with a final amendment to the report dated 1989. Also in 1989, dietary range finding studies began in rats and mice. The rat dietary study was conducted from 1989 - 1991 and the mouse dietary study was conducted from 1990 - 1992.

The carcinogenicity studies where CGP 25827A was administered in the drinking water were reviewed prior to this submission and are not re-reviewed in this document. However the salient points from these studies necessary for the overall interpretation of the carcinogenic response to CGP 25827A have been considered during the course of this review. Individual reviews of the carcinogenicty studies where CGP 25827A was administered in the diet are presented below.

20. 24-Month Carcinogenicity Study in Rats

BACKGROUND INFORMATION

Study Title:	24-Month Carcinogenicity Study in Rats
Sponsor Study No.:	886178
Study Dates:	August 28, 1989 - September 5, 1991
Report Date:	March 26, 1992
Test Facility:	CIBA-GEIGY Limited Short/Long term Toxicology 4332 Stein, Switzerland (In-life testing) 4002 Basle, Switzerland (Analytical Laboratories and Histopathology)
GLP Status:	Compliant with OECD Guideline 451 and Japanese (EA 700, MHW 1039, MITI .1014)
NDA Volume:Page	56:1 and 57 - 58:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No.:	810589
Purity:	[redacted]

Control Article: Diet

Test System

Species/Strain: Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Housing: 5/cage
Age at Initiation: Approximately 5 weeks
Route: Diet
Duration of Exposure: 24 months
CAC Concurrence: No

Dosing

CGP 25827A was administered in the diet at doses of 0, 0.5, 2, 5, and 20 mg/kg/day. Doses were also selected based on the results of a previous carcinogenicity study (Sponsor Study No. 2069-104) where rats received 0, 0.125, 0.25 and 0.5 mg CGP 25827A/ml drinking water/day, yielding average daily doses of 0, 12, 25, and 51 mg/kg/day for males and 0, 18, 38, 76 mg/kg/day for females. CGP 25827A affected survival in all treated groups. A 28-day palatability study was also considered in dose selection; where animals consumed dietary levels of 0.43, 1.75, 4.46, and 18.1 mg CGP 25827A /kg/day and did not exhibit signs of systemic toxicity. A total of 75 rats/sex/group were assigned to treatment groups receiving CGP 25827A or control diet. Animals from each group were designated for "carcinogenicity study only;" "carcinogenicity and hematology;" or "carcinogenicity and drug level determinations." Dose levels and study groups of the rat dietary study are presented in the following table.

Dosing Information

Group	Carcinogenicity Only	Carcinogenicity and Hematology	Carcinogenicity and Drug Level Determinations in Blood and Urine	Target Dose
	No. Animals/sex	No. Animals/sex	No. Animals/sex	(mg/kg/day)
1	50	10	10	0
2	50	10	10	0.5
3	50	10	10	2
4	50	10	10	5
5	50	10	10	20

Evaluation Of Endpoints

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Daily
Clinical signs	Daily
Body weight	Weekly for Months 1 - 3 and monthly thereafter
Food consumption	Weekly for Months 1 - 3 and monthly thereafter
Water consumption	Monthly

Parameter	Frequency of Measurement
Ophthalmology	Prior to treatment and after 1 and 2 years (Control and Group 5 all intervals; Group 4 after 2 years only)
Hematology	Weeks 12, 26, 53, 78, and 105
Drug levels in Plasma	Weeks 5, 26, 53, 78, 105 (included in a separate report)
Drug levels in urine	Week 40 from individually housed rats (included in a separate report)
Prolactin levels	Months 1 and 3 (blood samples and vaginal smears) (included in a separate report)
Organ weights	Week 105 (brain, heart, liver, kidneys, adrenals, ovaries/testes, spleen, lung, pituitary)
Gross pathology	Week 105
Histopathology	Week 105

RESULTS

Mortality

There were no treatment effects on mortality. Adjusted survival was 43/70, 47/70, 50/70, 47/70, and 44/70 for Groups 1 - 5 males, respectively, and 42/70, 40/70 42/70, 43/70, and 33/70 in Groups 1 - 5 females, respectively.

Clinical Signs

There were no remarkable clinical signs.

Body Weight

Animals in treated groups gained weight more rapidly during the growth phase, resulting in higher than control mean body weight values for Weeks 1 - 20. As the rate of body weight gain decreased over time in all groups, mean body weight values for treated groups approached or became slightly less than controls.

Food Consumption

Animals in treated groups consistently consumed more diet than did animals in the control group. The efficiency of food utilization was lower for animals in the treated groups than in the controls.

Water Consumption

Animals in the treated groups consistently consumed slightly more water than did animals in the control group.

Ophthalmology

There were no treatment related ocular findings.

Hematology

There were no treatment related hematology findings.

Organ Weights

Organ weight data revealed effects of CGP 25827A on the liver (Group 5 males); heart (Group 2 -5 males and Group 3 females); lung (Group 2 - 5 males and females); testes (Group 4 and 5 males) ; and thymus (Group 5 males). Mean data for these organs are presented in the table at the end of this section.

Liver-to-body weight ratios for Group 5 males were significantly higher than control values.

Absolute heart weights and heart-to-body weight ratios were higher for all treated groups when compared to control values. Values were similar between all treated groups within each sex. Statistically significant differences from control for heart weights included the mean absolute heart weight for Group 3 females and heart-to-body weight ratios for Groups 2 - 5 males.

Lung weights were higher for treated animals when compared to controls, with statistically significant differences occurring for absolute weights in Groups 2 - 5 males and females and for lung-to-body weight ratios in Groups 2 - 5 males and 3 - 5 females.

Mean absolute testes weights for Groups 4 and 5 males were significantly lower than control values, however this was considered to be the effect of an unusually high weight for one of the control animals (no. 30 testes weight was 18.5g). Similarly, absolute thymus weights were lower than control for all treated groups of males, however this was attributed to the unusually high weight for one of the control animals (no. 9 thymus weight was 1885 mg). There were no significant differences from control when these outliers were excluded from analysis.

Notable Mean Absolute Organ Weights and Organ-to-Body Weight Ratios

		Males					Females				
Dose Group (mg/kg/day):		0	0.5	2	5	20	0	0.5	2	5	20
Liver (g)	Absolute	21.71	23.4	22.03	20.77	23.04	16.95	18.09	17.46	16.35	16.74
	Ratio	29.46	32.32	31.21	30.35	35.87*	34.20	34.62	35.26	33.20	33.55
Kidney (g)	Absolute	5.418	5.407	4.967	4.661	4.968	3.207	3.416	3.376	3.285	3.189
	Ratio	7.576	7.678	7.047*	6.894	7.956*	6.500	6.626	6.866	6.815	6.472
Heart (g)	Absolute	2.141	2.403	2.372	2.252	2.187	1.496	1.634	1.657*	1.620	1.556
	Ratio	2.945	3.333*	3.375*	3.309*	3.476*	3.029	3.153	3.352	3.356	3.146
Lung (g)	Absolute	2.534	3.075*	3.096*	2.974*	2.931*	1.969	2.213*	2.470*	2.302*	2.264*
	Ratio	3.495	4.266*	4.452*	4.361*	4.724*	4.043	4.571	5.045*	4.806*	4.624*

		Males					Females				
Dose Group (mg/kg/day):		0	0.5	2	5	20	0	0.5	2	5	20
Testes (g)	Absolute	4.819	4.193	4.095	3.780*	3.601*					
	Ratio	6.525	5.791	5.840	5.570	5.533					
Thymus (mg)	Absolute	250.2	173.2	158.3	146.4*	180.4	126.1	129.8	122.1	116.7	132.6
	Ratio	0.328	0.235	0.222	0.022	0.283	0.249	0.248	0.024	0.239	0.268

*Significantly different from control ($p \leq 0.05$).

Gross Pathology

Notable gross observations involved the male and female reproductive systems.

Gross findings involving the female reproductive system included ovarian changes described as "large/mass/cystic mass/nodule" at an incidence of 1/70, 0/70, 5/69, 8/70 and 8/69 in Groups 1 - 5, respectively. Ovarian cysts were observed at a higher incidence in all treated groups than controls (3/70, 15/70, 21/69, 28/70, and 26/69 in Groups 1 - 5, respectively).

Gross findings involving the male reproductive system included small seminal vesicles (6/70, 8/70, 7/69, and 1/70 in Groups 1 - 5, respectively) and small testes (7/70, 8/70, 9/70, 14/70, and 9/70 in Groups 1 - 5, respectively). Both findings were without microscopic correlate.

Histopathology

Treatment related neoplastic lesions were limited to the female reproductive system. Mesovarian leiomyoma was noted at an incidence of 0/70, 0/70, 1/69, 1/69, and 3/69 for Groups 1 - 5, respectively. The only other neoplastic finding occurred as a result of hyperplasia of ovarian granulosa/theca cells (also known as sex chord stromal cells) progressing to benign tumors at an incidence of 1/70, 5/70, 6/69, 6/69, and 8/69, for Groups 1 - 5, respectively. There were no significant differences from control in the incidence of malignant tumors. When the incidence of animals with proliferative granulosa/theca cell lesions was combined (i.e., hyperplasia, benign or malignant tumor), treatment related effects were statistically significant in all dose groups. Treatment related nonneoplastic findings in female reproductive organs consisted of an increased incidence of ovarian cysts in all treated groups and uterine polyps in Group 5. Remarkable microscopic findings in the female reproductive organs are summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Notable Microscopic Findings in Female Reproductive Organs

Dose (mg/kg/day):		0	.05	2	5	20
Ovary	N	70	70	69	69	69
Cysts	No.	7	24*	23*	34*	30*
	%	10	34	33	49	43
Granulosa/Theca cell (benign)	No.	1	5	6	6*	8
	%	1	7	9	9	12
Granulosa/Theca cell (hyperplasia)	No.	17	32	33*	31*	34*
	%	24	46	48	45	49
Granulosa/Theca cell (benign or malignant tumor)	No.	1	0	2	0	0
	%	1	0	3	0	0
Granulosa/Theca cell (hyperplasia, benign, or malignant tumor)	No.	18	36*	36*	33*	39*
	%	26	51	52	48	57
Mesovarian leiomyoma	No.	0	0	1	1	3
	%	0	0	1	1	4
Uterus	N	70	70	69	70	69
Polyps	No.	2	3	3	3	10*
	%	3	4	4	4	14

*Significantly different from control ($p \leq 0.05$).

The incidence of nonneoplastic lesions was increased in the thyroid of treated animals. C-cell proliferative lesions yielded significant differences from control in the incidence of hyperplasia (Groups 2 - 5 males and 4 - 5 females), but not for adenoma or carcinoma. When all C-cell proliferative lesions were combined (i.e., hyperplasia, adenoma, and carcinoma) significant differences from control were noted for Groups 3 - 5 males and 4 - 5 females. The incidence of thyroid C-cell proliferative lesions is summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence of Thyroid C-cell Proliferative Lesions

		Males					Females				
Dose Group (mg/kg/day):		0	0.5	2	5	20	0	0.5	2	5	20
Thyroid	N	67	70	68	67	69	69	67	66	67	70
C-cell hyperplasia	No.	24	35*	36*	40*	47*	17	15	17	30*	25
	%	36	50	53	60	68	25	22	26	45	36
C-cell adenoma	No.	7	3	7	6	11	4	7	14	3	9
	%	10	4	10	9	16	6	10	21	4	13
C-cell adenoma, carcinoma, or gangliocytoma	No.	8	3	8	7	11	4	8	14	3	10
	%	12	4	12	10	16	0	1	0	0	1
C-cell proliferative lesions (hyperplasia, adenoma, carcinoma, or gangliocytoma)	No.	32	37	40	42*	55*	20	22	29	31*	29
	%	48	53	59	63	80	29	33	44	46	41

*Significantly different from control ($p \leq 0.05$).

Several other nonneoplastic lesions were primarily remarkable due to increased severity when compared to control. These lesions involved the lung, heart, spleen, and Harderian

gland, and are summarized in the table at the end of this section. The presence of foam cells in the lung was increased in severity in Group 5 males and Groups 2 - 5 females. The incidence of this lesion was significantly higher than control for Groups 4 and 5 females. This finding correlates with the increased lung weights noted for treated animals. Myocardial fibrosis was noted in most of the control and treated rats, but with increased severity in the Group 4 - 5 males and Group 3 females. Additional microscopic findings attributable to treatment with CGP 25827A include in increased severity of splenic hemosiderosis in all groups of treated females and increased incidence of atrophy of the Harderian gland in all groups of treated males. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence and Severity of Notable Nonneoplastic Lesions

		Males					Females				
Dose Group (mg/kg/day):		0	0.5	2	5	20	0	0.5	2	5	20
Lung	N	70	70	69	70	70	70	69	68	70	70
Foam Cells	Total No.	24	27	23	25	33	15	23	24	30*	29*
	%	34	39	33	36	47	21	33	35	43	41
	Mild	20	23	19	21	26	10	9	5	3	8
	Moderate	4	4	4	4	5	5	8	13	17	11
	Severe	0	0	0	0	2	0	6	6	10	10
Heart	N	70	70	70	70	70	70	69	68	69	70
Myocardial Fibrosis	Total No.	69	65	65	70	68	45	48	55	47	52
	%	99	93	93	100	97	64	70	81	68	74
	Mild	46	41	37	35	29	26	22	21	23	27
	Moderate	23	24	28	35	37	19	26	33	24	21
	Severe	0	0	0	0	2	0	0	1	0	4
Spleen	N	70	70	69	69	70	70	70	69	70	70
Hemosiderosis	Total No.	35	39	41	40	35	54	54	61	63	55
	%	50	56	59	72	50	77	77	88	90	79
	Mild	20	24	24	16	14	24	14	11	18	13
	Moderate	14	14	17	23	20	24	25	26	30	27
	Severe	1	1	0	1	1	6	14	24	15	15
Harderian Gland	N	69	69	69	67	70	70	70	70	68	68
Atrophy	No.	30	41*	41*	50*	49*	50	58	60	52	58
	%	43	59	59	75	70	71	83	86	76	85
	Mild	23	27	30	38	35	17	22	13	13	11
	Moderate	7	14	10	12	14	31	31	39	33	39
	Severe	0	0	1	0	0	2	5	8	6	8

*Significantly different from control ($p \leq 0.05$).

CONCLUSION

Tumors observed in this study were limited to the female reproductive system and included leiomyomas in high dose females. There were no statistically significant differences in survival between treated and control groups.

Dietary treatment of rats with CGP 25827A at levels of 0, 0.5, 2, 5, and 20 mg/kg/day for two years did not yield any remarkable findings in the in-life or hematology parameters evaluated.

Evidence of a carcinogenic response was found in female reproductive tissues of animals from all treated groups. Specific types of tumors included mesovarian leiomyoma in the 20 mg/kg/day group and benign granulosa/theca cell (also know as sex chord stromal cells) in all treated groups.

Although not statistically significant, mesovarian leiomyoma is a known response to this class of drug. Other investigators have reported that early studies of several β_2 agonist failed to demonstrate mesovarian leiomyomas but later studies revealed "...that any β_2 andrenergic agonist would be expected to produce [this lesion] in rats if properly studied (adequate potency and bioavailability)..." (Sells and Gibson, 1987). The incidence of this lesion in the high dose group supports the validity of the study in that adequate doses and bioavailability of CGP 25827A were present in the rats of this study to ascertain the carcinogenic potential of the test compound for this finding (Kelly et. al., 1993; Jack et. al., 1983).

The increased incidence of benign granulosa/theca cell tumors is considered to be the endpoint of a hyperplastic response as it did not progress to malignancy. Other treatment-related effects noted in female reproductive tissues included ovarian cysts (observed grossly and microscopically) in all treated groups and uterine polyps in the 20 mg/kg/day dose group.

Nonneoplastic lesions were observed in the endocrine, respiratory and cardiovascular systems of animals from all treated groups. Endocrine system changes consisted of an increased incidence of thyroid C-cell hyperplasia. Respiratory system changes consisted of an increased severity of foam cells in the lungs of all treated groups of females, and increased incidence of foam cells in the 5 and 20 mg/kg/day dose group females. Cardiovascular changes consisted of increased severity of myocardial fibrosis in the 5 and 20 mg/kg/day dose group males. Harderian gland atrophy was also increased in incidence and severity in the 5 and 20 mg/kg/day dose group males, and in incidence in the 20 mg/kg/day dose group females.

In conclusion, the carcinogenic response of rats to CGP 25827A is consistent with that reported in the literature for other β_2-adrenoceptor stimulants. A no observable effect level of 2 mg/kg/day was identified for the benign granulosa/theca cell tumors.

21. 24-Month Carcinogenicity Study in Mice

BACKGROUND INFORMATION

Study Title:	24-Month Carcinogenicity Study in Mice
Sponsor Study No.:	906172
Study Dates:	September 24, 1990 - October 27, 1992
Report Date:	May 13, 1993
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division 4002 Basle, Switzerland
GLP Status:	Compliant with OECD Guideline 451 and Japanese (EA 700, MHW 1039, MITI 1014)
NDA Volume:Page	49:1 and 50 - 52:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No.:	000290
Purity:	———
Control Article:	Diet

Test System

Species/Strain:	Albino mice, Tif:MAGf(SPF), MAG x NIH hybrid
Housing:	Individual
Age at Initiation:	Approximately 7 weeks
Route:	Diet
Duration of Exposure:	24 months
CAC Concurrence:	No

Dosing

The results of a 3-month study where mice were dosed at 40, 140, 400, and 1400 mg/kg/day were considered in dose selection. Findings noted at the lowest dose tested included increased heart weights, decreased glucose levels and increased urea levels when compared to controls. In addition, kidney weights were increased relative to control at the 140 mg/kg/day level and above.

A total of 85 mice/sex/group were assigned to treatment groups receiving CGP 25827A at levels of 0, 2, 5, 20 and 50 mg/kg/day. Animals from each group of this carcinogenicity study were designated for "carcinogenicity study only;" "carcinogenicity and

hematology;" or "carcinogenicity and blood level determinations" as illustrated in the following table.

Group	Dosing Information: Carcinogenicity only: No. Animals/sex	Carcinogenicity and Hematology: No. Animals/sex	Carcinogenicity and Plasma drug levels: No. Animals/sex	Target Dose (mg/kg/day)
1	50	10	25	0
2	50	10	25	2
3	50	10	25	5
4	50	10	25	20
5	50	10	25	50

Evaluation Of Endpoints

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly for Months 1 - 3 and monthly thereafter
Food consumption	Weekly for Months 1 - 3 and monthly thereafter
Hematology	Weeks 26, 53, 78, and 105
Plasma Levels	Weeks 5, 26, 53, 78, 105
Organ Weights	Week 105 (brain, heart, liver, kidneys, adrenals, ovaries/testes, spleen, lung)
Gross pathology	Week 105
Histopathology	Week 105

RESULTS

Mortality

There were no effects on survival that would preclude meaningful interpretation of tumor data or would indicate a frank treatment-related effect on mortality. For the males, survival in the treated groups was consistently higher than the control group but without a dose response relationship. Survival in Group 3 (5 mg/kg/day) males was significantly higher than in the control group. Survival in the females was similar between groups, with slightly but not statistically significant decreased survival in Group 5 (50 mg/kg/day) compared to the control value.

The following table is a summary of overall mortality and adjusted survival. Overall mortality is indicated by the number of animals that were found dead or sacrificed *in extremis* over the total number exposed/sex/group. Adjusted survival is the number of animals alive over the total number at risk, excluding animals sacrificed according to schedule at Weeks 5, 26, 53, and 78.

Overall Mortality and Adjusted Survival at Termination

Dose Group (mg/kg/day)	Overall Mortality No. (%)		Adjusted Survival No. (%)	
	Males	Females	Males	Females
0	40/85 (47)	33/85 (39)	27/67 (40)	31/66 (47)
2	32/85 (38)	36/85 (42)	33/63 (52)	31/66 (47)
5	19/85 (22)*	35/85 (41)	48/67 (71)	32/67 (48)
20	28/85 (33)	34/85 (40)	37/64 (58)	32/66 (48)
50	28/85 (33)	46/85 (54)	38/66 (58)	20/66 (30)

*Significantly different from control, $p \leq 0.05$.

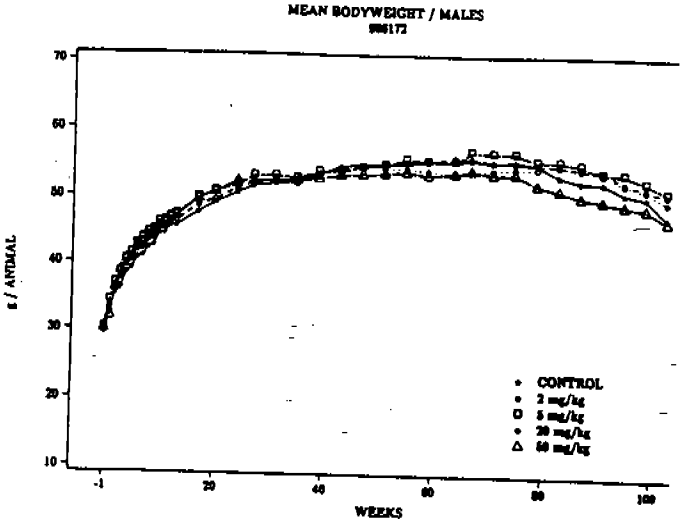
Clinical Signs

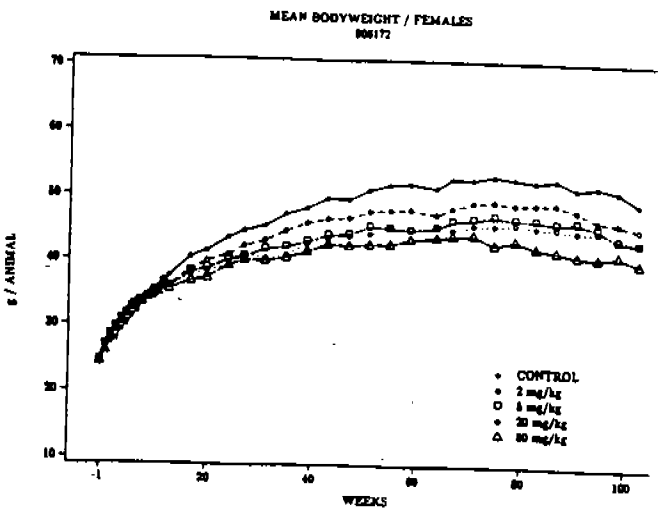
The incidence of palpable masses and swellings (females) was slightly higher in the 20 and 50 mg/kg/day dose groups when compared with controls. The number of animals exhibiting palpable masses was 1, 6, 9, 5, and 11 for Groups 1 - 5 males, respectively, and 7, 8, 13, 9, and 17 for Groups 1 - 5 females, respectively. The number of animals exhibiting swellings was 14, 11, 9, 11, and 18 for Groups 1 - 5 males, respectively, and 0, 2, 9, 15 and 13 for Groups 1 - 5 females, respectively. These findings generally correlated with enlarged livers revealed during necropsy observations. There were no other remarkable clinical signs.

Body Weight

Mean body weight and body weight gain were similar between groups during the initial growth phase of the animals (weeks 1 - 13). Thereafter, body weight values for males did not reveal a definitive compound effect, but a dose-related decrease was revealed for the females, with statistical significance achieved for Groups 2 - 5 for at least weeks 24 - 79, and for Groups 3 - 5 thereafter. The magnitude of the difference between female treated groups and controls was always less than 10% in Group 2, with percent of control values at the end of the study at 92, 87, 88, and 81, for Group 2 - 5 females, respectively. The low magnitude of the change in the Group 2 females supports a no effect level of 2 mg/kg/day for body weight effects of CGP 25827A. The lack of a difference in body weight *gain* during the growth span of the mice does not support the achievement of a maximum tolerated dose using this parameter.

Mean body weight values for the duration of the study are depicted in the following graphs.





Food Consumption

Animals in the treated groups consistently ate more than animals in the control group, achieving statistically significant differences from control in all groups of treated males and the 20 and 50 mg/kg/day group of treated females for at least 15 of the 36 measured food consumption intervals. Food consumption ratios, reported as g food/kg body weight/day, were consistently higher for treated groups of females when compared to controls, in an apparent dose response relationship throughout the study.

Hematology

There were no remarkable differences from control in hematology parameters evaluated.

Plasma Levels

Mean plasma concentrations of CGP 25827A for mice in this study were reported in a separate study —— 43/1993, v. 66, p. 166). Using ———— the limit of detection was estimated to be approximately ———— Plasma concentration levels of CGP 25827A were not detected in the 2 mg/kg/day group, below quantifiable limits in the 5 mg/kg/day group, and averaged 3.2 and 6.3 nmol/L in the 20 and 50 mg/kg/day dose groups respectively.

Organ Weights

Treatment related effects on organ weights were noted in the heart (Group 2, 4, and 5 females), liver (Group 5 males and 4 - 5 females), spleen (Group 4 - 5 females), and testes Group 3 - 5 males).

Mean absolute heart weights and/or heart-to-body weight ratios were higher for treated females than control females as early as Week 5 and remained higher than controls throughout the 26, 53, 78 and 105 sacrifice intervals. Although a clear dose response relationship was not demonstrated, statistical significance was often achieved for females in Groups 2, 4, and 5. The increase in Group 3 females was statistically significant for absolute heart weight at the Week 26 interval and heart-to-body weight ratio at the week 105 interval, but was not consistently higher than control values at other sacrifice intervals. For males, the effect on heart weight and/or heart-to-body weight ratios was only evident at Week 26 in Groups 3, 4, and 5 and Week 53 in Group 3. Statistically significant differences from control were not observed for males at Weeks 78 and 105.

Liver weights were increased in group 5 males and Group 4 and 5 females at the week 105 interval. This finding was consistent with masses and swellings observed antemortem.

Spleen weights and or spleen-to-body weight ratios for females were higher than control values as early as Week 5 for Groups 3 - 5 and persisted through all other intervals for Groups 4 and 5. Statistical significance was noted in Group 3 at Week 5 and in Groups 4 and 5 at Weeks 5, 26, 53, and 78. This effect was not consistently demonstrated in male mice.

Absolute testes weights or testes-to-body weight ratios were significantly lower than control values for Groups 3, 4, and 5 at Week 105 only.

Gross Pathology

Gross findings attributable to treatment with CGP 25827A involved the liver, testes, and uterus. Liver masses and small testes in treated animals were consistent with effects observed in organ weight data. In addition, uterine masses were grossly visible in more treated than control animals. The incidence of these findings summarized in the table below includes all animals at the terminal sacrifice for the liver and testes findings. As the uterine masses were primarily observed in animals that died on test, the numbers indicated include all scheduled and unscheduled deaths for this finding.

Incidence (%) of Treatment-Related Gross Findings

	Males					Females				
mg/kg/day:	0	2	5	20	50	0	2	5	20	50
Liver - mass	64	85	61	94	100	35	37	31	53	60
Testes - small	0	6	9	21	41					
Uterus - mass						7	12	11	18	21

Histopathology

Notable microscopic findings involved the liver (Groups 2 - 5), heart (Groups 5), female genital tract/uterus (Group 2 - 5), and testes (Groups 5). The incidence of these findings is summarized in the table presented at the end of this section.

Hepatocellular hypertrophy and/or benign hepatoma was noted primarily in animals from treated groups, however these findings occurred in the absence of a dose response relationship and without statistical significance. The relatively low incidence of these lesions in Groups 4 and 5 at the terminal sacrifice can be attributed to the increased incidence of neoplastic lesions in these groups. At study termination, hepatocellular carcinoma was noted in 24, 33, 28, 36, and 57% of males in Groups 1 - 5, respectively and 3, 10, 6, 25 and 30% of females in Groups 1 - 5, respectively. The incidence of animals with liver tumors (hepatoma and carcinoma combined) was 33, 37, 47, 48 and 48 for Groups 1 - 5 males, respectively, and 13, 17, 22, 31 and 22% for Groups 1 - 5 females, respectively.

Effects on smooth muscle were evidenced by microscopic findings in the heart of Group 4 and 5 males and genital tract for all groups of treated females. The incidence of myocardial fibrosis was 8, 8, 6, 10, and 22% for Groups 1 - 5 males, respectively. The incidence of smooth muscle tumors (including leiomyoma and leiomyosarcoma) in the uterus and female genital tract was 5, 19, 19, 20 and 26% for Groups 1 - 5, respectively.

Testicular tubular atrophy was most severe in high dose males. Concomitant findings include the absence of sperm in the epididymus and impaction of semineferous tubules in Groups 3, 4, or 5 males. The incidence and severity of testicular tubular atrophy is summarized in the following table. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Incidence and Severity of Testicular Tubular Atrophy

Dose (mg/kg/day)	Mild	Moderate	Severe	Total
0	44	9	2	55
2	36	12	6	54
5	39	14	9	62
25	26	18	7	51
50	18	11	22*	51

*Significantly different from control, $p \leq 0.05$.

The following table is a summary of the incidence of the treatment-related histopathology lesions. Intervals refer to all animals sacrificed at study termination (Term) or all animals exposed to CGP 25827A (Total). Sample sizes for each of these intervals are presented at the end of the table. The number (No.) indicates the number of animals with a given lesion; multiple occurrences of a given lesion in any one animal are only counted once. The percent (%) indicates the incidence at the indicated interval. Statistically significant

differences from control are based on the total number of animals exposed to CGP 25827A without regard to duration of exposure. The numbers in the following table were obtained from statistical analyses provided by the Sponsor.

Summary Incidence of Treatment-Related Microscopic Findings

	Interval		Males					Females				
Dose (mg/kg/day):			0	2	5	20	50	0	2	5	20	50
Liver												
Hypertrophy	Term	No.	6	17	23	20	10	3	17	14	11	2
		%	24	52	50	61	27	10	57	44	34	10
	Total	No.	19	43*	38*	38*	27	12	27*	26*	18	14
		%	22	52	45	45	32	14	32	31	21	16
Benign Hepatoma	Term	No.	8	9	14	14	8	7	6	9	12	8
		%	32	27	30	42	22	23	20	28	38	40
	Total	No.	24	24	31	33	25	11	12	17	19	14
		%	28	29	36	39	29	13	14	20	22	16
Carcinoma	Term	No.	6	11	13	12	21	1	3	2	8	6
		%	24	33	28	36	57	3	10	6	25	30
	Total	No.	10	12	19	17	26*	2	5	3	11*	7*
		%	12	14	22	20	31	2	6	4	13	8
All Hepatocellular Tumors	Total	No.	28	31	40	40	41	11	14	19	26*	19*
		%	33	37	47	48	48	13	17	22	31	22
Heart												
Myocardial Fibrosis	Term	No.	1	4	3	4	13	1	0	1	0	0
		%	4	12	7	12	35	3	0	3	0	0
	Total	No.	7	7	5	8	19	1	1	1	0	0
		%	8	8	6	10	22	1	1	1	0	0
Testes												
Tubular Atrophy	Term	No.	24	29	43	29	29					
		%	96	88	93	88	78					
	Total	No.	55	54	62	51	51					
		%	65	65	73	61	60					
Absence of Sperm in Epididymus	Total	No.	2	7	12	7	24					
		%	2	8	14	8	28					
Semineferous Tubule Impaction	Total	No.	2	6	3	10	9					
		%	2	7	4	12	11					
Genital Tract												
Uterine Leiomyoma	Term	No.						3	4	6	7	4
		%						10	13	19	22	20
	Total	No.						4	13*	13*	14*	17*
		%						5	12	15	16	19
Leiomyosarcoma	Total	No.						0	3	3	3	5
		%						0	4	4	4	6
Smooth Muscle Tumor	Total	No.						4	16*	16*	17*	22*
		%						5	19	19	20	26

Interval	Males					Females				
Dose (mg/kg/day):	0	2	5	20	50	0	2	5	20	50
Term n	25	33	46	33	37	31	30	32	32	20
Total n	85	83	85	84	85	85	84	85	85	85

* Significantly different from control $p \leq 0.05$.

CONCLUSION

While there may have been a minimal effect on survival in the high dose females, the incidence of mortality is not of a magnitude that yields a statistically significant difference from control. Further, the onset of death was the same in the control and high dose group (Week 33). Although a dose response relationship in body weight was demonstrated in the females, there was no appreciable difference in body weight gain. Therefore the study failed to reach the MTD based on survival or body weight.

The Sponsor provided two separate analyses of survival and used one to support the claim that the MTD was exceeded for high dose females. The analysis used to support this conclusion would not generally be acceptable for analysis of carcinogenicity data. As stated in the methods, 85 animals/sex/group were originally assigned to the study, each subgroup was listed as part of the carcinogenicity study and was included in gross and microscopic analyses. Only the first 50 animals/sex/group were included in statistical analysis that indicated a significant effect on survival in the high dose females. Several animals dying late in the study were disregarded in this analysis as they were not included in the first 50/sex assigned to the group. In a separate analysis, all 85 animals/sex/group were included as appropriate and results indicated that the effect on survival in the high dose females was not statistically significant ($p = 0.07$). All animals at risk should be included in the denominator for survival analysis. The Sponsor reported 14/50 (28%) survival in the first 50 animals per sex assigned to the high dose group. When animals scheduled for interim sacrifice are excluded from the original 85 animals assigned to the high dose female group, the actual number at risk is 66. Thus a more accurate reflection of survival for the high dose females is 20/66 (30%) compared with 31/66 (47%) in the controls.

Body weight and body weight gain values for treated groups were similar to control values during the growth phase of the animals (weeks 1 - 13). Thereafter body weight values were lower for the 5, 20, and 50 mg/kg/day dose group females when compared to controls. The magnitude of the difference was consistently greater than 10% of the control value.

Gross and microscopic evaluation of tissues revealed treatment-related effects on the smooth muscle (heart and female genital tract), liver, spleen, and testes. The totality of gross findings, organ weight data, and microscopic findings yielded consistent interpretation of the treatment-related effects for each organ system. Neoplastic findings included hepatocarcinoma in high dose males and smooth muscle tumors (leiomyoma and/or leiomyosarcoma) in the uterus or female genital tract in all groups of treated

females. The continuum of the liver lesions described as hypertrophy, benign hepatoma, and carcinoma is consistent with that reported in the literature (Goodman et al. 1991), and increased in severity as doses increased. The smooth muscle tumors of the female reproductive organs were noted in all dose groups. Other investigators have reported identical findings with other β_2 agonists (Gibson et al. 1987, Sells and Gibson, 1987).

The incidence of myocardial fibrosis was significantly higher than control values for males in the 20 and 50 mg/kg/day dose groups. The no observable effect level (NOEL) for this finding was 5 mg/kg/day in mice.

The severity of testicular tubular atrophy was significantly greater in 50 mg/kg/day dose males when compared to controls. Concomitant findings were described as: the absence of sperm in the epididymus and impaction of semineferous tubules in the 5, 20, and 50 mg/kg/day dose groups. Overall, the NOEL for male reproductive organs is 2 mg/kg/day.

In conclusion, this study was adequately designed and with sufficient survival to ascertain the carcinogenic potential of CGP 25827A when administered to mice at levels of 0, 2, 5, 20, and 50 for two years.

Overall Interpretation and Evaluation of Carcinogenicity Studies

The formation of leiomyomas in the female genital tract is a known response of rodents to treatment with high doses of beta agonists. The presence of mesovarian leiomyomas in both rat studies and the mouse dietary study indicates that adequate potency, bioavailability, and strain sensitivity was achieved to ascertain the carcinogenic potential of CGP 25827A (Sells and Gibson, 1987). This response has been demonstrated to be a pharmacodynamic response of beta agonists and has been prevented in mice and rats by simultaneous treatment with propanolol (a beta blocker) (Gibson et al., 1987). It was noted in rats at 15 (drinking water) and 20 (dietary) mg/kg/day.

The hyperplasia and benign granulosa/theca cell tumor formation is considered to be a pharmacodynamic effect of CGP 25827A. Beta agonists increase intracellular cAMP levels and thereby increase steroidogenesis in steroid-producing cells and could stimulate theca cells in the ovary (Aguado et al. 1982; Marsh 1975). Beta stimulation has also been shown to increase the sensitivity of theca cells to physiological gonodatropin (Dyer and Erickson, 1985).

The increased incidence of hepatic tumors observed in the mouse dietary study differs significantly from control for the 50 mg/kg/day group males and the 20 mg/kg/day group females.

Although the incidence of thyroid C-cell tumors (64 mg/kg/day males) and mammary adenomas (32 mg/kg/day females) were increased in rat drinking water study, the incidence was within historical ranges of the performing laboratory.

Adrenal subcapsular adenoma and carcinomas were observed in the mouse drinking water study in the 267 mg/kg/day dose males. This tumor type appears to be the endpoint of a hyperplastic response to the drug.

In conclusion, there was no evidence that the responses observed in the carcinogenicity studies were the result of a direct interaction with DNA. Instead, it appears that the responses are exaggerated pharmacodynamic effects typical of high dose exposure to beta agonists.

REPRODUCTIVE TOXICOLOGY

Formoterol fumarate did not have any adverse effect on reproductive or developmental indices under the conditions of study. However it was shown to elicit peri- and postnatal toxic effects in rats at levels of 6 mg/kg and above.

Reviews of individual studies are presented below.

22. Reproduction test of Formoterol fumarate (BD 40A) Fertility Study in Rats

BACKGROUND INFORMATION

Study Title:	Reproduction test of Formoterol fumarate (BD 40A) Fertility Study in Rats
Sponsor Study No.:	[redacted]-D-4-1
Laboratory Study No.:	Not applicable
Study Dates:	Prior to 1977. Exact date not stated.
Report Date:	Not reported.
Test Facility:	[redacted]
GLP Status:	Study pre-dates GLP.
NDA Volume:Page	60:1

METHODS

Test Article:	Formoterol fumarate (BD 40A)
Batch No:	Not stated
Purity:	Not stated
Control Article:	0.5% aqueous solution of methyl cellulose
Purity:	Not stated
Species/Strain:	Slc:SD Rats
Route:	Oral gavage

Twenty adult male rats per group were dosed once daily for 9 weeks prior to the mating period. Females were dosed beginning 2 weeks prior to mating and continued through Day 7 of gestation. Control animals were treated according to this same schedule with

0.5% methyl cellulose solution. Daily doses were 0, 0.2, 30, and 60 mg/kg and were administered at a volume of 5 ml/kg for each group. The F_0 females were killed on gestation day 20 and the F_1 generation was weighed and examined for the incidence of skeletal and visceral malformations and variations and reproductive parameters.

RESULTS

In the F_0 generation, females dosed at the 0.2 mg/kg and above had fluctuations in body weight and food consumption. Signs of toxicity at higher dose groups included decreased liver and testes weights in the 6 mg/kg males; decreased spontaneous activity, pleural congestion, swelling and increased parotid and submaxillary glands, wet abdomens, decreased kidney weight and nasal mucosal hemorrhage in 30 mg/kg males and above; and increase heart and lung weight in females in the 30 mg/kg group.

There were no effects on pregnancy rates, number of corpora lutea, number of implantation sites, or number of viable fetuses between treated and control groups. There were no effects on the F_1 generation.

CONCLUSION

No effects on pregnancy or fertility were observed in this study at doses that were associated with maternal or paternal toxicity. There were also no effects on fetuses.

23. Reproductive Study in Rats

BACKGROUND INFORMATION

Study Title:	Reproductive Study in Rats
Sponsor Study No.:	820741
Laboratory Study No.:	Not applicable
Study Start Date:	June 16, 1982
Report Date:	February 28, 1983 with Amendments to July 4, 1985
Test Facility:	CIBA-GEIGY- Limited Experimental Toxicology 4332 Stein, Switzerland
GLP Status:	Compliant
NDA Volume:Page	60:218

METHODS

Test Article:	CGP 25827A-E
Batch No:	Lot L10 (milled)
Purity:	Not stated
Control Article:	Distilled water
Purity:	Not stated

Species/Strain: Tif:RAIf (SPF) Rats
Route: Oral gavage

Thirty adult male rats per group were dosed once daily for 60 days prior to mating and during the 12-day mating period. Thirty adult females per group were dosed at least 2 weeks prior to mating and continued throughout gestation. Half of the females were also treated throughout lactation. Control animals were treated according to this same schedule with distilled water. Daily doses were 0, 0.3, 1, and 3 mg/kg, and were administered at a volume of 10 ml/kg for each group.

Half of the females were necropsied at Day 21 of presumed gestation. The other half were allowed to deliver and were necropsied after weaning (postpartum Day 28). The progeny (F_1 generation) of these females were not treated and allowed to breed within their group to produce an F_2 generation.

Effects on fertility of the F_0 and F_1 generations, fetuses from the F_1 generation, and 21-day old rats from F_2 generation were evaluated.

RESULTS

Signs of toxicity were observed in animals treated at the 0.3 mg/kg level and above and consisted of slight increases in female body weight gain and food consumption, most notably during lactation and isolated incidences of altered testicular morphology. Food consumption in the 3.0 mg/kg group F_0 females was lower than control values during lactation. This was associated with an increased mortality among suckling pups in this group.

There were no differences between treated and control groups in fertility indices. There were no remarkable effects on pregnancy rates, number of corpora lutea, number of implantation sites, or number of viable fetuses between treated and control groups.

Effects on the fetuses in the F_1 generation of the treated groups occurred within historical control ranges and were within a 99% confidence limits of the vehicle control. These consisted of delayed ossification of the calcanie in the all treated groups, decreased body weight gain and "activity index" in the 1 mg/kg group, and increased suckling mortality in the 3 mg/kg group. In addition, spontaneous death was noted for all pups in one litter each in the 0.3 and 3 mg/kg groups, but again, overall incidence was within historical control ranges.

There was no effect on the untreated animals from the F_1 generation selected for mating or on their progeny in the F_2 generation.

CONCLUSION

Treatment of the F_0 generation with CGP 25827A-E did not have any effect on mating performance, fertility, pregnancy rate or duration of gestation in either the F_0 or F_1 generations. There was no evidence of structural abnormalities in either the F_1 or F_2 offspring that could be attributed to treatment.

24. Reproduction test of Formoterol fumarate (BD 40A) Teratological Study in Rats

BACKGROUND INFORMATION

Study Title:	Reproduction test of Formoterol fumarate (BD 40A) Teratological Study in Rats
Sponsor Study No.:	D-4-2
Laboratory Study No.:	Not stated
Study Dates:	September 1986 – May 1977
Report Date:	Not stated
Test Facility:	[redacted]
GLP Status:	Not compliant. Performed prior to establishment of GLPs.
NDA Volume:Page	61:1

METHODS

Test Article:	CGP 25827A
Batch No:	Lot 1
Purity:	Not stated
Control Article:	0.5% methylcellulose
Purity:	Not stated
Species/Strain:	Sprague Dawley/Slc Rats
Route:	Oral gavage

CGP 25827 was administered at daily doses of 0, 0.2, 6, or 60 mg/kg from gestation days 7 through 17. Approximately two-thirds of the F_0 dams/group were killed on gestation day 20 and the remaining third were allowed to give birth and raise their progeny through weaning on day 21. These offspring were evaluated for postnatal development. Selected F_1 offspring were raised until sexual maturity paired for mating.

Embryotoxicity, fetotoxicity, and teratology parameters were evaluated.

RESULTS

Slight increases in F_0 maternal body weight gain and delayed ossification in F_1 pups were observed in all treated groups. In the 6 and 60 mg/kg dose groups, absolute heart weight

and heart-to-terminal body weight ratios were higher than control values and control F_1 fetal body weights were lower than control values. In the 60 mg/kg dose group, liver, kidney, spleen and ovary absolute and/or relative weights were higher than control values in the F_0-treated females.

There were no treatment-related effects on any reproductive parameters fetal sex ratios, or the incidence of fetal skeletal or visceral malformations or variations in treated groups. Fetotoxicity was observed in the 6 and 60 mg/kg dose groups. Delayed ossification was observed in minor bone structures in all F_1 fetuses from all dose levels. Effects on the F_1 offspring are summarized in the following table.

Effects of BD40A on F_1 Offspring

Dose (mg/kg):	0	0.2	6	60
At Birth				
No. Dams	10	10	10	10
Duration of Pregnancy (mean (SD) days)	21.5 (.53)	21.8 (.42)	21.8 (.42)	21.1 (.32)
No. of Implant Sites				
Total	150	125	154	145
Mean (SD)	15.0 (.67)	15.2 (1.55)	15.4 (1.51)	14.5 (1.78)
Live Pups				
Total	139	136	140	136
Mean (SD)	13.9 (1.10)	13.6 (1.51)	14.0 (1.63)	13.6 (2.46)
Mean Delivery Ratio (%)	94.7	89.8	90.9	95.3
Sex Ratio (male/female)	60/70	60/76	64/76	60/76
Mean (SD) weight of Newborns				
Male	5.7 (0.73)	6.1 (0.36)*	6.2 (0.52)*	5.9 (0.20)
Female	5.3 (0.44)	5.7 (0.35)*	5.9 (0.31)**	5.7 (0.16)*
Total No. of Stillborn	3	0	0	3
Total No. of Pups that Died within 4 Days After Birth	3	1	0	9
Until Weaning				
Day 4 Survival Before Adjustment	136	135	140	127
Day 4				
Male	50	49	47	49
Female	50	51	53	50
Day 7				
Male	50	49	45	49
Female	50	50	53	50
Day 14				
Male	50	48	45	47
Female	49	50	52	50

Dose (mg/kg):	0	0.2	6	60
Day 21				
Male	50	48	45	47
Female	49	50	52	50
Survival Ratio (%)				
Male	100	98.0	96.0	96.0
Female	98.0	98.0	98.3	100

*, ** Statistically Significant p<.05 or .01.

There were no effects on fertility or reproductive performance of either the F_0 or F_1 generations. No remarkable effects were noted in the F_2 generation.

CONCLUSION

There was no evidence of embryotoxic or teratogenic effects in rats treated with CGP 25827A under the conditions of this study. Treatment of the F_0 generation with CGP 25827A did not have any effect on mating performance, fertility, pregnancy rate or duration of gestation in either the F_0 or F_1 generations. Fetotoxicity was evidenced by lower-than-control fetal weights in the 6 and 60 mg/kg F_1 groups. There was no evidence of structural abnormalities in either the F_1 or F_2 offspring that could be attributed to treatment.

25. Reproduction test of Formoterol fumarate (BD 40A) Teratological Study in Rabbits

BACKGROUND INFORMATION

Study Title: Reproduction test of Formoterol fumarate (BD 40A) Teratological Study in Rabbits
Sponsor Study No.: D-4-3
Laboratory Study No.: Not stated
Study Dates: Not stated however test material was obtained June 1976
Report Date: Not stated
Test Facility: [redacted]
GLP Status: Not compliant. Performed prior to establishment of GLPs.
NDA Volume:Page 62:1

METHODS

Test Article: (BD 40A)
Batch No: Lot 1
Purity: Not stated

Control Article:	0.5% methylcellulose
Purity:	Not stated
Species/Strain:	Japanese Albino Rabbits
Route:	Oral gavage

BD 40A was administered at daily doses of 0, 0.2, 60, or 500 mg/kg from gestation days 6 through 18. The dose volume was 5 ml/kg, based on the body weight at the beginning of gestation. Cesarean sections were performed on 10 dams from each group on Day 29 of pregnancy. These fetuses were evaluated for viability, external abnormalities, and then visceral and skeletal abnormalities.

RESULTS

No obvious effects of treatment were noted in the F_0 or F_1 generations from the group treated at the 0.2 mg/kg dose level. At the 60 mg/kg dose level, food consumption for dams in the F_0 generation was consistently lower than control. This was the only finding in the dams treated at the 60 mg/kg dose level. At the 500 mg/kg dose level, myocardial fibrosis was noted in 1/10 animals in the F_0 dams. Neonatal mortality was noted in the F_1 generation (75.4% survival compared to 97.6% survival in the control group) and is believed to be the result of a lower number of implantation sites (mean = 9.0, 8.9, 8.9 and 7.3 for Groups 1 - 4, respectively) and fewer live fetuses (mean = 8.7, 7.8, 8.3, and 6.1 for Groups 1 - 4, respectively) in this high dose group. There were no treatment-related effects on reproductive indices, fetal body weights, or teratogenic indices.

CONCLUSION

Food consumption was lower than control values for F_0 animals treated at the 60 mg/kg dose level but no effects on the F_1 generation were noted at this level. A decrease in neonatal survival was noted within 24 hours postpartum at the 500 mg/kg dose level when compared to controls. This study supports a no observable effect level (NOEL) of 60 mg/kg for rabbit fetuses under the conditions of this assay.

26. Reproduction test of Formoterol fumarate (BD 40A) Peri- and Post-natal Study in Rats

BACKGROUND INFORMATION

Study Title:	Reproduction test of Formoterol fumarate (BD 40A) Peri- and Post-natal Study in Rats
Sponsor Study No.:	D-4-4
Laboratory Study No.:	Not stated
Study Dates:	April - November, 1977
Report Date:	Not stated
Test Facility:	

GLP Status: Not compliant. Performed prior to establishment of GLPs.
NDA Volume:Page 62:178

METHODS

Test Article: (BD 40A)
Batch No: Lot 1
Purity: Not stated
Control Article: 0.5% methylcellulose
Purity: Not stated
Species/Strain: Slc: SD rats
Route: Oral gavage

Twenty rats per group were administered BD 40A orally at levels of 0.2, 6, and 30 mg/kg. The dose volume was 5 ml/kg. Pregnant F_0 generation females were administered the test material on gestation days 17 - 21. Animals of the F_1 generation were not dosed but could have been exposed through their mother's milk and F_2 animals were naïve.

Parameters evaluated include pregnancy rates, gestation duration, and delivery rates in the F_0 and F_1 generation. Offspring in the F_1 and F_2 generations were examined for visceral or skeletal malformations or variations.

RESULTS

There were no effects on pregnancy rates, gestation duration or delivery rates in the F_0 and F_1 generation. There were no treatment related findings in visceral or skeletal malformations or variations offspring in the F_1 and F_2 generations.

Male pups in the F_1 generation had low birth weight in groups treated at 6 mg/kg and above and female birth weights were low for the 30 mg/kg group. Neonatal mortality was observed in the 6 mg/kg and above F_1 generation.

Effects of BD40A on F_1 Offspring

Dose (mg/kg):	0	0.2	6	30
At Birth				
No. Dams	20	20	20	20
Duration of Pregnancy (mean (SD) days)	21.5 (.51)	21.3 (.44)	21.3 (.47)	21.5 (.61)
No. of Implant Sites				
Total	309	311	291	310
Mean (SD)	15.5 (1.96)	15.6 (1.67)	14.6 (2.04)	15.5 (1.91)
Live Pups				