The Sponsor also addressed binding, potency, and selectivity of the RR and SS enantiomers of Fomoterol, but the data are inconclusive since complete separation of the constituent enantiomers was not demonstrated.

The pharmacodynamic effects of Formoterol fumarate were consistent with those that would be expected of a highly selective beta-2-andrenoceptor agonist.

SAFETY PHARMACOLOGY

Information included in this section was summarized from reports provided by the Sponsor and published literature.

Cardiovascular system effects

Formoterol fumarate is associated with increased heart rate, maximum dp/dt, pulmonary artery and capillary pressure, coronary blood flow, cardiac output and myocardial oxygen consumption (Sponsor Study No. 10/91, January 29, 1991). Decreased peripheral, pulmonary and coronary resistance were also observed in this same study. The selectivity of beta-adrenoceptor agonists for the beta-2-adrenoceptor has been demonstrated for Formoterol fumarate, although there is also a cardiovascular response to the drug. Generally, beta-2-adrenoceptor mediated effects include relaxation of airway smooth muscle, although some cardiovascular activity has also been noted. Beta-1-adrenoceptor mediated effects include changes in contractility or beating rate of myocardial preparations *in vitro*.

Formoterol has been shown to have high selectivity for the beta-2-adrenoceptor subtype. Studies have demonstrated that formoterol displaces labeled beta-1-adrenoceptors selectively labeled with a beta-adrenoceptor antagonist (^{3}H-CGP 12177) (Lemoine, et al. 1992a; Lemoine, et al. 1992b; Kaumann, et al. 1985; and Lemoine et al., 1985). Homologous down regulation of beta-2 but not beta-1-adrenoceptors has been demonstrated after 14 days of treatment with high doses of formoterol (Kompta et al., 1995 and Kompta et al. 1994).

Although bronchoselectivity has been demonstrated in studies with Fomoterol fumarate, there exists a subdominant population of functionally coupled beta-2-adrenoceptors in the myocardium of animals and humans. Based on curve fitting of concentration-response data obtained in guinea pig studies, Formoterol fumarate appears to act principally on beta-2-adrenoceptors in the right atrium to induce increased contraction rate while acting with much lower affinity at beta-1-adrenoceptors in the left atrium to mediate some degree of increased contraction force. This effect coupled with an apparent reflex tachycardia secondary to a beta-2-adrenoceptor mediated vasodilatation and hypotension are likely responsible for the changes observed in the cardiovascular system after treatment with Formoterol fumarate.

Immune system effects

Fomoterol has inhibitory effects on a number of inflammatory cells and processes because beta-2-adrenoceptors are widely distributed in effector cells and responding tissues. It is a potent inhibitor of anaphylactic degranulation of mast cells and basophils, resulting in suppression of mediator release from immunologically sensitized tissues. Fomoterol inhibits both active and passive anaphylaxis *in vivo*.

Formoterol reduces eosinophil adhesion to venules and the Sponsor postulates that this may be the mechanism by which formoterol attenuates eosinophilic lung inflammation in experimental animals. Formoterol attenuates eosinophilic activation directly, however little effect was demonstrated in human alveolar macrophage superoxide production.

Gastrointestinal effects

Formoterol decreases gastric acid secretion and decreases gastric, duodenal and ileal motility, but without significant effect on gastric transit time.

PHARMACOKINETICS AND TOXICOKINETICS:

The absorption, distribution, metabolism, and elimination properties of Formoterol fumarate were studied in rats, mice, and dogs using radiolabeled and non-radiolabeled analytical methods. Analytical methods evolved over time to quantify unchanged Formoterol fumarate in plasma and urine collected from animals. Results were obtained using [redacted]

Pharmacokinetic Parameters

Mean pharmacokinetic values were obtained from a variety of species and time points. At this writing, some of the data are unclear and the Sponsor is requested to clarify the data. The AUC data play a critical role in the evaluation of the safety of Formoterol fumarate. The Sponsor noted in their submission that there were difficulties with the method(s) used to evaluate plasma levels in animals studies. The values provided are much higher than would reasonably be expected in a drug of this type and class. In addition, we note that the Cmax identified in the mouse dietary carcinogenicity study is 6.3 nmol/l for a 50 mg/kg/day dose, far below the number provided in the mouse drinking water study (AUC = 4300 nmol/ml) and used for comparison to humans.

Absorption

In the rat, oral absorption was measured using radio-labeled Formoterol fumarate after a single dose of 50 µg/kg (——F-1-4-1). ^{3}H-Formoterol fumarate was primarily absorbed from the small intestine. Enterohepatic circulation was also demonstrated in

this study by administering bile collected from animals dosed with ^{3}H-Formoterol fumarate to naïve animals. In this case, 69% of the administered dose was absorbed.

Distribution

The distribution of radio-labeled Formoterol fumarate was studied using both [redacted] in mice (DM 1/1991) and rats [redacted] -F-1-4-1). These studies indicate the accumulation of Formoterol fumarate does not occur at a level or a rate that would pose a safety concern.

Following a *single oral dose* of 0.5 or 5 mg ^{3}H-Formoterol fumarate/kg in 3 rats/group, concentrations of the test material were found in various tissues and organs within 30 minutes of dosing ([redacted] -F-1-4-1). Concentrations were highest in the kidney, liver, lung, plasma, and whole blood. Concentrations of ^{3}H-Formoterol fumarate increased in various tissues for the first 6 hours and decreased dramatically by the sample collection at 24 hours.

Following *repeated oral administration* of 0.05 mg ^{3}H-Formoterol fumarate/kg/day for 21 days in rats, the concentrations of radioactivity in tissues gradually increased until Day 14 and remained constant thereafter (Sasaki, et al. 1983).

Protein Binding

The binding of Formoterol fumarate to plasma protein was assessed *in vitro* using rat, dog, and human plasma ([redacted] F-1-1-6), and *in vivo* in dogs after oral dosing with 0.1 mg Formoterol fumarate/kg. *In vitro* binding was 50 to 65% for all species and independent of the concentration tested (0.1 - 100 ng/ml). *In vivo* binding in the dog ranged from 44 to 60% and was constant over the 10-hour post-dose sample collection period. Formoterol fumarate primarily bound to albumin with only negligible amounts bound to α-1-acid glycoprotein or γ-globulin [redacted] R 41/1991).

Metabolism

Data from rat and dog studies demonstrate that Formoterol fumarate is metabolized extensively after oral administration before reaching systemic circulation (Sasaki et al. 1983). The primary metabolite is a direct phenolic O-glucuronide (Ia) and can be found in mice, rats, dogs, and humans. Other metabolites also occur via glucoronidation. The following diagram illustrates the biotransformation pathway of Formoterol fumarate. A table comparing the metabolic profiles of several species is also presented.

Species Comparison of the Metabolic Profiles of Formoterol fumarate

Test Model	Major Metabolite (% of dose)	Other Metabolites	Reference
	In vitro Hepatocytes		
Rat	Ia[1]	IIa and IIb[2]	DM(EU) 28/1996
Guinea Pig	Ia		DM(EU) 28/1996
Dog	Ia		DM(EU) 28/1996
Marmoset	Ia	Ib[3]	DM(EU) 28/1996
	In vivo Models		
Mouse (oral)	Ia (~70% in urine)		DM 1/1991
Rat (oral)	Ia (95% in bile) I[4] (5% in urine)		Sasaki et al. 1982
Dog (oral)	Ia (~80% in bile) I (~20% in urine)		Sasaki et al. 1982

[1] Direct phenolic O-glucuronide.
[2] Phenolic glucuronides of the O-desmethyl metabolite.
[3] Direct aliphatic O-glucuronide.
[4] Unchanged Formoterol fumarate.

Human (oral)	I (6% in urine) Ia (24% in urine) Ib (5% in urine) II (<1% in urine) IIa + IIb (15% in urine)	B 46/87

Transpulmonary transport and metabolism

In vitro models using perfused rat lung and radio-labeled Formoterol fumarate indicate that the drug is rapidly transported to the blood from the airway and is not metabolized by the lung (93/02/C/2R).

Elimination

Elimination of Formoterol fumarate and its metabolites is rapid and complete. It occurs to a great extent within the first 24 hours following dosing and is complete within 4 to 5 days (Sasaki et al. 1982, — F-1-4-5, — F-1-4-3, DM 1/1991, DM 2/1991). In rats and dogs, excretion occurs through hepatic metabolism and most of the radio-labeled Formoterol fumarate is recovered from the bile and/or feces (DM 2/1991, — F-1-4-3). In mice, renal excretion is evidenced by a relatively high percentage of radio-labeled Formoterol fumarate recovered in urine (DM 1/1991). With repeated dosing in dogs, plasma levels of Formoterol fumarate and urinary excretion concentrations increase during the first 2 days after the initiation of dosing and remain constant thereafter (— F-1-4-7). Results of excretion studies in rats, dogs, and mice are summarized in the following table.

Mean Cumulative Excretion of Radio-labeled Formoterol fumarate

Dose	% in Bile	% in Urine	% in Feces	% Total	Reference
		Rats			
5 mg/kg	72.11	21.12	6.37	99.60	DM 2/1991
20 mg/kg	75.22	21.08	3.57	99.87	
50 mg/kg	70.76	19.96	5.00	94.45	
		Dogs			
10 µg/kg	-	36.8	52.1	88.9	— F-1-4-3
100 µg/kg	-	36.9	52.0	88.9	
		Mice			
6 mg/kg	-	64.22	33.6	97.82	DM 1/1991
60 mg/kg	-	75.88	23.80	99.68	

Conclusions Regarding Pharmacokinetics and Toxicokinetics

Formoterol fumarate is absorbed after first pass glucoronidation. Oral absorption and bioavailability vary between species as a result of the extent of first pass metabolism and

enterohepatic circulation. In rats, glucuronide metabolites are excreted in bile and Formoterol fumarate is then re-absorbed in the small intestine. Plasma protein binding was similar between rat, dog, and human plasma and ranged from 50 to 65%. Biotransformation products occur as a result of O-glucoronidation, with direct phenolic glucuronide as a primary metabolite in mice, rats, dogs, and humans. Elimination of Formoterol fumarate appears to be rapid and complete within 4 to 5 days of dosing. In rats, most radio-labeled Formoterol fumarate is recovered in the bile and feces. In mice, renal excretion is evidenced by relatively high levels of radio-labeled Formoterol fumarate recovered in urine.

TOXICOLOGY

A comprehensive data base of single dose and repeat dose toxicology studies were submitted and reviewed to support the safety of Formoterol fumarate. Studies by the oral route were performed in rats, mice, and dogs. Studies by the inhalation route using the dry powder formulation were performed in rats and dogs for up to one year.

The studies were, for the most part, adequately designed and performed in accordance with Good Laboratory Practice standards, except where otherwise noted in the individual reviews of each study. Findings of toxicity were consistent with the pharmacologic action of beta-2-adrenoceptor agonists.

Individual review of the studies in presented followed by an overall summary of the studies.

Acute Toxicity

The acute toxicity of Formoterol fumarate was studied in mice, rats and dogs by oral, intravenous (i.v.), intraperitoneal (i.p.), subcutaneous (sc.) and inhalation routes of administration. These studies are summarized in the following table.

Acute Toxicity of Formoterol fumarate

Species	Route	Dose (mg/kg - oral, i.v., i.p., sc.) (mg/l - inhalation)	Findings	Reference
Mouse	Oral	0, 3850, 5000, 6500, 8450, 11000, 143000(♀)	LD_{50} :6696 -♂ and 8308 -♀	D-1-1
Rat	Oral	0, 593 (♂), 889 (♂), 1330 (♂), 2000, 3000, 4500, 6750, 10100 (♀)	LD_{50} :3125 -♂ and 5583 -♀	D-1-1
Juvenile Rat	Oral	7-day old 0, 680, 810, 970 1170, 1400, 1680, 2020	7-day old LD_{50} :1120 -♂ and 1260 -♀	D-1-3
		22-day old 5000 (♀), 6000, 7200, 8600, 10400, 12400	22-day old LD_{50} :7990 -♂ and 7480 -♀	

Species	Route	Dose (mg/kg - oral, i.v., i.p., sc.) (mg/l - inhalation)	Findings	Reference
Chinese Hamster	Oral	10, 100, 300, 600, 1000	LD_{50} :322 (♂/♀)	825339
Dog	Oral	Escalating doses in 2 dogs/sex: .0001, .001, .01, .1, 1, 3, 10, 30, 100, 3000	.001 - ↑ heart rate .1 - ventricular extrasystol; ↑ SGOT 1 - ↑ ALK PHOS; salivation; crawling; runny nose 10 - ↑ SGPT; emesis 3000 - death (1/2 ♂) Necropsy: myocardial necrosis and hemorrhage; lung congestion and bronchopneumonia; yellow liver with fatty degeneration.	— D-1-2
Rat	Inhalation	.3, .81, .94, 1.82, 5.7	LC_{50} : 1.35	846402
Rat	Inhalation	.05, 1, 5	LC_{50} : 4.8	841012
Rat	Inhalation	.5, 1, 5	LC_{50} : 6.07	841011
Dog	Inhalation	0, 6.7, 14.3, 37.3, 101.5	No mortality; ↑ heart rate	906238
Dog	Inhalation	12 µg/dog (aerosol metered dose)	↑ heart rate; ↑ contraction force	855190
Dog	Inhalation	12 µg/dog fresh and outdated batches	No difference between batches. ↑ heart rate; ↑contraction force; focal necrosis and mineralization in papillary heart muscle	886174
Dog	Inhalation	2.4 mg/dog (metered dose)	None remarkable.	896160
Rat	Inhalation	3.5	LC_{50} > 3.5 (no mortality)	896183
Rat	Inhalation	3.91, 3.78, 4.52	LC_{50} > 4.52 (no mortality)	926108
Mouse	Intravenous	0, 53, 61, 70.1, 80.6, 92.7, 106.6	LD_{50} : 71.8 (♂), 70.9 (♀)	— D-1-1
Mouse	Intraperitoneal	141, 169, 203, 244, 293, 351	LD_{50} : 238 (♂), 206 (♀)	— D-1-1
Mouse	Subcutaneous	424 (♂), 508, 610, 732, 878, 1054	LD_{50} : 642 (♂), 6665 (♀)	— D-1-1
Rat	Intravenous	70.1, 80.6, 92.7, 106.6, 122.6 141	LD_{50} : 97.8 (♂), 100.8(♀)	— D-1-1
Rat	Intraperitoneal	118 (♂), 141, 169, 203, 244, 293, 351 (♀)	LD_{50} : 172 (♂), 207 (♀)	— D-1-1
Rat	Subcutaneous	555, 722, 938, 1220, 1586, 2062 (♀)	LD_{50} : 997 (♂), 1097 (♀)	— D-1-1

Repeat Dose Oral Toxicity Studies

Repeat dose toxicity studies were performed in rats, mice and dogs. These studies are reviewed below.

Rats

The subchronic toxicity of Formoterol fumarate in rats was studied by the oral route via dietary and drinking water administration and by the inhalation route. The oral studies were conducted up to a duration of 3 months and were considered in selecting doses for the carcinogenicity studies (presented later in this review). The inhalation studies were conducted up to a duration of 1 year.

Findings were consistent between routes of administration and included effects on the heart and reproductive organ systems. In rats dosed up to 3 months in the drinking water

at levels of 10 mg/kg/day and above, myocardial fibrosis, increased uterine weights, and decreased testes, epididymides, seminal vesicles, and prostate weights were observed. Body weight and food consumption were higher for all treated groups when compared to controls, also without regard to route of administration.

After 3 months of inhalation treatment at levels of 0.34 mg/kg/day and above, increases were observed in heart weight, red cell parameters, and body weight and food consumption. The kidney and liver weights were higher than control for rats treated at 0.442 mg/kg/day. One year of inhalation treatment 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 recovered after the 8-week period and are described in the individual review summaries presented below.

Individual reviews of the oral and inhalation repeat dose toxicity studies are presented below. Each study review is organized by background information, methods, results, and conclusions.

1. 28-Day Palatability Study in Rats

BACKGROUND INFORMATION

Study Title:	28-Day Palatability Study in Rats
Sponsor Study No.:	896027
Study Dates:	April 4 - May 2, 1989
Report Date:	September 22, 1989
Test Facility:	CIBA-GEIGY Limited Experimental Toxicology 4332 Stein Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	19:1

METHODS

Test Material	
Test Article:	CGP 25827A
Batch No:	810187
Purity:	Not stated
Control Article:	Diet
Test System	
Species/Strain:	Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route:	Diet
Housing:	Individual
Duration of Exposure:	28 Days

Dosing Information

Five rats/sex were assigned to treatment groups receiving 0, 5, 20, 50, and 200 ppm of CGP 25827A in the diet. These doses correspond to 0, 0.4, 1.7, 4, and 18 mg/kg/day. The purpose of this study was to evaluate the palatability of CGP 25827A in diet and to provide information regarding drug levels in plasma and microscopic changes in the heart.

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Plasma Level Determinations	At termination (reported separately)
Gross pathology	At termination
Histopathology	At termination (heart only)

RESULTS

Antemortem Observations

There were no remarkable observations in mortality, clinical signs, body weight, or food consumption data.

Gross Pathology

There were no remarkable gross observations.

Histopathology

There was no microscopic evidence of cardiotoxicity, including myocardial fibrosis in rats treated with CGP 25827A in the diet at levels up to 18 mg/kg/day for 28 days.

CONCLUSION

No signs of toxicity were noted in rats treated with CGP 25827A in the diet at levels up to 18 mg/kg/day for 28 days. Additionally, CGP 25827A appeared to be palatable to rats in the diet at the tested levels.

2. 3-Month Range Finding Study in Rats (Administration in Food)

BACKGROUND INFORMATION

Study Title:	3-Month Range Finding Study in Rats (Administration in Food)
Sponsor Study No.:	906205 (Supplement to 886178, 24-month study)
Study Dates:	June 26 - September 25, 1990
Report Date:	January 8, 1992
Test Facility:	CIBA-GEIGY Limited Short/Long-term Toxicology 4332 Stein Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page	22:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No:	810589
Purity:	———
Control Article:	Diet

Test System

Species/Strain:	Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route:	Diet
Housing:	5/cage
Duration of Exposure:	3 months

Dosing Information

Ten rats/sex were assigned to treatment groups receiving CGP 25827A at levels of 0, 0.5, 2, 5 and 20 mg/kg/day in the diet. The purpose of this study was to supplement the dietary carcinogenicity study in rats with pharmacokinetic data generated from the same species, strain and test material used in the previous study. Five rats/sex/group were used for each sample collection interval. Pharmacokinetic data were presented in a separate report (B68/1991).

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily

Parameter	Frequency of Measurement
Body weight	Weekly
Food consumption	Weekly
Drug Level Determinations	Reported separately
• Plasma -	• Weeks 5 and 14
• Urine -	• Weeks 3 and 11
Gross pathology	Weeks 5 and 14

RESULTS

Mortality

There were no remarkable observations in mortality.

Clinical Signs

There were no remarkable observations in clinical signs.

Body Weight

Mean absolute body weight and body weight gain values were higher in treated groups when compared to controls throughout the study.

Food Consumption

Food consumption values were consistently higher for all treated groups when compared to controls. Food consumption ratios, calculated as g food consumed/body weight/day, were not different between control and treated groups.

Gross Pathology

There were no remarkable gross observations.

CONCLUSION

Minimal toxicity information was collected in this study as the purpose was to evaluate plasma levels of CGP 25827A, when administered to rats in the diet at levels of 0, 0.5, 2, 5 and 20 mg/kg/day. This study was conducted as a supplement to a carcinogenicity study in rats (No. 886178) . Pharmacokinetic data were presented in a separate report (No. —— 6/1992).

Body weight values were higher for all treated groups when compared to controls.

3. 3-Month Oral (via drinking water) Toxicity Study in Rats

BACKGROUND INFORMATION

Study Title:	3-Month Oral (via drinking water) Toxicity Study in Rats
Sponsor Study No.:	90-6174
Study Dates:	August 24 - December 3, 1990
Report Date:	September 22, 1989
Test Facility:	CIBA-GEIGY Limited Preclinical Safety, Section of Experimental Toxicology Basel, Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page	20:193 and 21:1

METHODS

Test Material

Test Article:	CGP 25827A
Batch No:	400190
Purity:	[redacted]
Stability:	7 days under study conditions
Control Article:	Water

Test System

Species/Strain:	Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route:	Drinking water
Housing:	Individual
Duration of Exposure:	3 months

Dosing Information

Ten rats/sex were assigned to treatment groups receiving 0, 0.125, 0.25, and 0.5 mg/ml of CGP 25827A in the drinking water. Overall mean test article intake calculated from water consumption averaged 0, 10, 19, and 43 mg/kg/day for Group 1 - 4 males, respectively, and 0, 13, 27 and 40 mg/kg/day for Group 1 - 4 females, respectively. Five rats/sex/group were designated as satellite animals and received a single gavage dose of CGP 25827A at levels of 0, 12.5, 25, and 50 mg/kg for Groups 2 - 5, respectively.

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Daily
Food consumption	Twice weekly
Water consumption	Daily
Hearing test	Pretest, Weeks 6 and 14
Ophthalmology	Pretest, Weeks 6 and 13
Hematology	Pretest, Weeks 5, 9, and 14
Serum chemistry	Pretest, Weeks 5, 9, and 14
Urinalysis	Weeks 1, 4, 8 and 13
Organ weights	At termination (kidneys, liver, spleen, testes, epididymides, seminal vesicles, heart, mandibular gland, thymus, prostate, brain, axillary lymph nodes, lungs, pituitary, adrenals, thyroid, ovaries, uterus)
Gross pathology	At termination
Histopathology	At termination

Satellite animals were included in the evaluation of toxicity and were also used for determinations of drug levels in plasma. Results of plasma analyses were presented in a separate report.

Histopathology evaluations included an evaluation of mitotic activity in thyroid C cells using immunohistochemical staining using [redacted] label.

RESULTS

Clinical Signs

Swellings were noted in the axillary, mandibular, and inguinal regions of all treated animals. These findings were not noted in any control animal.

Body Weight

Mean absolute body weight and body weight gain values were higher in treated groups when compared to controls during the first half of the study. After Week 6, the rate of body weight gain was lower for the high dose males when compared to controls. Mean body weight values remained consistently higher for treated groups when compared to controls throughout the study.

Food Consumption

Food consumption values for animals in all treated groups were consistently higher than control values.

Water Consumption

No remarkable findings were noted for water consumption.

Hearing Test

No remarkable findings were noted for hearing tests.

Ophthalmology

No remarkable findings were noted for ophthalmology.

Hematology

Thrombocyte counts were generally lower for animals in all treated groups when compared to control groups. However coagulation factors were not affected.

Serum Chemistry

No consistent differences from control or dose-response relationships were revealed in analysis of serum chemistry parameters.

Urinalysis

No remarkable findings were noted in urinalysis.

Organ Weights

Epididymides, seminal vesicle, and prostrate weights of males in the high dose group were slightly less than those in control group. Mean uterine weights were slightly higher for all females in treated groups when compared to controls. No microscopic correlates were observed for the noted organ weight changes.

Gross Pathology

An increase in skeletal muscle mass was noted for animals in all treated groups. Although not stated, this finding appears to refer to an increase in *volume* as it correlates with higher-than-control body weight values for treated animals.

Histopathology

Microscopic findings were revealed in the heart and thigh muscle.

In the heart, the incidence of granulation tissue and fibrosis was 0/10, 1/10, 2/10, and 0/10 for Groups 1 - 4 males, respectively and 0/10, 0/10, 0/10 and 1/10 for Groups 1 - 4 females, respectively.

Hypertrophy was observed in the thigh muscle of all treated rats and no control rats. Mononuclear focal infiltrates and single cell necrosis were also observed in most of the treated animals and none of the controls.

No increase in mitotic activity was revealed in the analysis of thyroid C cells.

CONCLUSION

CGP 25827A, when administered to rats in the drinking water at levels averaging 0, 10, 19, and 43 mg/kg/day for Group 1 - 4 males, respectively, and 0, 13, 27 and 40 mg/kg/day for Group 1 - 4 females, respectively, resulted in microscopic changes in the heart (all dose groups) and thigh musculature (all dose groups). Weights of male reproductive organs (epididymides, seminal vesicles, and prostate) were lower in treated than control. Uterine weights were slightly increased for all treated groups when compared to controls. Body weight and food consumption values were higher for all treated groups when compared to controls. A no observable effect level (NOEL) was not identified for these findings.

No increase in mitotic activity was revealed in thyroid C cells.

4. 3-Month Oral (via drinking water) Pharmacokinetic Study in Rats

BACKGROUND INFORMATION

Study Title:	3-Month Oral (via drinking water) Pharmacokinetic Study in Rats
Sponsor Study No.:	90-6052
Study Dates:	June 10 - September 16, 1991
Report Date:	May 11, 1992
Test Facility:	CIBA-GEIGY Limited Pharmaceuticals Division Basel, Switzerland
GLP Status:	Compliant with GLP Switzerland, Procedures and Principles, March 1986
NDA Volume:Page	21:349

METHODS

Test Material

Test Article:	CGP 25827A
Batch No:	400190
Purity:	———

Stability:	7 days under study conditions
Control Article:	Solvent to CGP 25827A and acidified tap water
Test System	
Species/Strain:	Albino rats, Tif:RAIf(SPF), RII/1 x RII/2 hybrid
Route:	Drinking water followed by a gavage dose after 90 days
Housing:	Individual
Duration of Exposure:	3 months

Dosing Information

Five rats/sex were assigned to treatment groups receiving 0, 0.125, 0.25, and 0.5 mg/ml of CGP 25827A in the drinking water for three months. Overall mean test article intake calculated from water consumption averaged 0, 9, 21, and 44 mg/kg/day for Group 1 - 4 males, respectively, and 0, 11, 25 and 47 mg/kg/day for Group 1 - 4 females, respectively. All animals and received a single gavage dose of CGP 25827A at levels of 0, 12.5, 25, and 50 mg/kg for Groups 1 - 4, respectively, following the 3-month treatment period.

Observations

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Body weight	Pretest and three times per week
Water consumption	Daily

Blood samples were taken for analysis of CGP 25827A levels in plasma and were presented in a separate report ([redacted] 16/1992).

RESULTS

Mortality

No mortality was noted.

Body Weight

Mean absolute body weight and body weight gain values were higher in treated groups when compared to controls during the first 2 weeks of study. After Week 2, the rate of body weight gain was similar between treated and control groups but mean body weight values remained consistently higher for treated groups when compared to controls throughout the study.

Water Consumption

No remarkable findings were noted for water consumption.

CONCLUSION

Minimal toxicity information was collected in this study as the purpose was to evaluate plasma levels of CGP 25827A, when administered to rats in the drinking water at levels of 0, 12.5, 25 and mg/kg/day. This study was conducted in an attempt to clarify inconsistencies in pharmacokinetic data from a previous 3-month study in rats (90-6174). Pharmacokinetic data were presented in a separate report (——— 6/1992).

Body weight values were higher for all treated groups when compared to controls.

5. Preliminary Inhalation Tolerance Study in Rats

Study Title:	Preliminary Inhalation Tolerance Study in Rats
Sponsor Study No.:	90-6223
Laboratory Study No.:	650361
Study Dates:	October 9 - 19, 1990
Report Date:	March 7, 1991
Test Facility:	———
GLP Status:	Compliant
NDA Volume:Page	33:275

METHODS

Test Article:	CGP 25827A
Batch No:	14/909/1
Purity:	———
Control Article:	Aerosol fluorocarbon propellants
Purity:	Not stated
Species/Strain:	Sprague-Dawley Rats
Route:	Nose Only Inhalation
Exposure Conditions:	Aluminum cylindrical chamber with apparatus to actuate aerosol cans to achieve target concentrations.
Duration of Exposure:	Group 1-- single dose; Groups 2, 3, 4 -- 7 days
Dose Levels:	Group 3 – 0.85 mg/kg/day; Group 4 – 5.52 mg/kg/day

Dosing Information

Group	No. Animals per sex	No. of Cans x Actuations/min.	Chamber Conc. (mg/l)	Nominal Conc. (mg/l)	Mass Mean % of Particles with <6 µm Diameter
1 (High)[a]	5	6 x 6	0.13	0.043	96.4
2 (Control)[b]	5	6 x 6	.012	0	93.8
3 (Low)	5	3 x 2	.002	0.007	96.6
4 (High)	5	6 x 6	0.13	0.043	96.0

[a] Single dose group.

[b] Values reported represent placebo excipients only.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Daily
Food consumption	Weekly
Clinical Pathology	Day 8
Gross pathology (including organ weights)	Study termination: (after Day 1 for Group 1 and Day 7 for Groups 2 - 4)

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	Two accidental deaths were attributed to blood sampling: one Group 2 male and one Group 4 female.
Clinical signs	There were no remarkable clinical signs.
Body weight	Body weight gain values were higher in both groups of treated animals when compared to controls.
Food consumption	There were no effects on food consumption.
Clinical Pathology	The following hematology and clinical chemistry parameters were significantly different from control: • Hemoglobin concentration - ↓ Group 3 ♂♀ and Group 4 ♀; • White blood cell count - ↓ Group 4 ♀; • Blood urea nitrogen - ↓ Group 3 - 4 ♂♀; • Creatinine - ↓ Group 4 ♀ • Total bilirubin - ↓ Group 4 ♀ • Lactic dehydrogenase ↑ Group 3 ♂ and Group 4 ♂♀.
Gross pathology (including organ weights)	Lung weights were higher for Group 4 females when compared to control. There was no gross correlate for this finding or any other remarkable gross observation.

CONCLUSION

In absence of histopathology, the interpretation of the increased lung weight in high dose females and changes in clinical pathology of treated animals is uncertain. The results in this study were used to select doses for a 3 month study in rats (90-6224).

6. Preliminary Inhalation Feasibility Study in Rats (MDPI Formulation)

BACKGROUND INFORMATION

Study Title: Preliminary Inhalation Feasibility Study in Rats [redacted] Formulation)
Sponsor Study No.: 93-6078
Laboratory Study No.: 653006
Study Dates: April 14, 1993 - May 22, 1993
Report Date: June 24, 1994
Test Facility: [redacted]
GLP Status: Compliant
NDA Volume:Page 35:1

METHODS

Test Article: CGP 25827A Dry Powder Formulation (Foradil)
Batch No: 1066/1
Purity: [redacted]
Vehicle: Lactose at a ratio of 1:69 (CGP 25827A:lactose)
Purity: NA
Species/Strain: Sprague Dawley Rat
Route: Nose Only Inhalation
Exposure Conditions: Rotating brush generator with monitored air flow, temperature, and humidity.
Duration of Exposure: 5 days

Dosing Information

Group	No. Animals per sex	Dose (mg/kg/day)	Gravimetric Conc. (µg/l air)	Exposure	Mean % of Particles <3 µm Diameter
1	5	3.19	158	10 min. on 20 min.off for 250 min.	50.7
2	5	-	-	1 h on 1 day	-
3	5	0.52	95	1 h on 1 day	56.1
4	5	0.69	51	1 h/day for 5 days	57.2

The purpose of this study was to demonstrate that the described dosing apparatus would yield similar systemic exposure to a different system. Doses were selected based on previous studies with CGP 25827A. Exposure could not be demonstrated in Group 2 animals so the experiment was repeated in Group 3 animals and dosing was extended to a 5-day interval in Group 4 animals.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Clinical signs	During dosing and 1 hour post dose during treatment and daily for 14 days after the last treatment.
Body weight	Prior to treatment and at least weekly intervals thereafter.
Urine for Proof of Absorption	After the single exposure (Groups 1 and 3) and on Day 5 (Group 4)
Blood for Proof of Absorption	After the single exposure (Groups 1 and 3)

RESULTS

There were no remarkable findings in the parameters measured. Urine and blood levels of CGP 25827A were not included in this report.

CONCLUSION

This study was to examine the feasibility of the dosing apparatus with the dry powder formulation of CGP 25827A. Target concentrations as measured by [redacted] concentration and particle size were achieved for Groups 1, 3, and 4. Definitive evidence of exposure (i.e., blood and urine levels of CGP 25827A) was not included in the final report.

7. 28-Day Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation (1/69) in Rats

BACKGROUND INFORMATION

Study Title: 28-Day Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation (1/69) in Rats
Sponsor Study No.: 926111
Laboratory Study No.: 323605
Study Dates: July 28, 1992 - August 28, 1992
Report Date: April 7, 1993
Test Facility: [redacted]
GLP Status: Compliant
NDA Volume:Page 38:1

METHODS

Test Article: CGP 25827A (Foradil) Dry Powder Formulation (1/69)
Batch No: 1066/1 and 1066/2
Purity: [redacted]
Control Article: Lactose [redacted] 100 Mesh
Purity: U.S.P.
Species/Strain: Wistar rats, Han-Ibm., outbred, SPF-quality
Route: Nose Only Pulsed Inhalation
Exposure Conditions: [redacted] aerosol generator [redacted] [redacted] discharged through a [redacted] This method was selected to achieve the required test article concentrations with a mass median aerodynamic diameter of 3 µm or less.
Duration of Exposure: 29 days

Dosing Information

Group	No. Animals per sex	Exposure Duration (min.)	Dose Levels (mg/kg/day)	Target Conc. (mg/l air)	Conc. (µg/l air)	Mean % of Particles <3 µm Diameter
1 (Air Control)	15	250	-	0	-	-
2 (Lactose)	15	90	-	1.7	1.64	-
3 (Low)	15	10	0.156	1.7	1.73	1.197
4 (Mid)	15	30	0.504	1.7	1.81	1.290
5 (High)	15	90	1.153	1.7	1.69	0.970

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Water Consumption	Weekly
Ophthalmoscopy	Prior to treatment and at the end of the study
Urine for Proof of Absorption	During the first and last weeks of exposure
Plasma for Proof of Absorption	At the end of the third week of treatment
Clinical Pathology	At the end of Week 4
Gross pathology (including organ weights)	At the end of Week 4
Histopathology (adrenal, heart, lungs, liver, 4 levels of the nasal cavity, spleen, testes, trachea, ovaries, and mandibular lymph nodes)	At study termination for control and high dose groups; gross lesions and mandibular lymph nodes were examined from all groups.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	A statistically significant increase in body weight gain was noted in all groups of treated males when compared to controls. Mean body weights and body weight gain values were significantly higher for all groups of treated females when compared to controls.
Food consumption	Treated males from Group 5 and females from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	Glucose levels for all treated groups were significantly lower than control values. The response was dose-related in females. Statistically significant differences from control values were reported for various other parameters in hematology and clinical chemistry determination. These changes could not be definitively attributed to treatment with CGP 25827A because they did not occur in a dose related response, were inconsistent between air- and lactose- control comparisons, and were within historical control ranges.
Gross pathology	
Organ Weights	Heart weights (and/or corresponding ratios) were significantly higher for males and females in all dose groups when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (1.15 mg/kg/day). Notably, there were no adverse effects on the respiratory system.

8. Subchronic (90-Day) Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation

BACKGROUND INFORMATION

Study Title: Subchronic (90-Day) Repeated Dose Pulsed Inhalation Toxicity Study with CGP 25827A Dry Powder Formulation
Sponsor Study No.: 90-6145
Laboratory Study No.: 248580
Study Dates: April 2 - July 4, 1990
Report Date: June 3, 1991
Test Facility: [redacted]

GLP Status: Compliant
NDA Volume:Page 35:1

METHODS

Test Article: CGP 25827A Dry Powder Formulation
Batch No: 14/704/80
Purity: [redacted]
Control Article: Lactose 150 MESH [redacted]
Purity: Not stated
Species/Strain: Albino Rat, Tif:RAIf (SPF)
Route: Nose Only Pulsed Inhalation
Exposure Conditions: [redacted] aerosol generator [redacted] [redacted] discharged through a [redacted] This method was selected to minimize particle size-related selection within the formulation between the excipient and the active ingredient.
Duration of Exposure: 91 - 94 days

Dosing Information

Group	No. Animals per sex	Dose (µg/kg/day)		Target Conc. (mg/l air)	Active Ingredient Conc. (µg/l air)	Mean % of Particles <3 µm Diameter
		Males	Females			
1 (Air Control)	15	-	-	0	-	-
2 (Lactose)	15	-	-	1.0	-	66.2
3 (Low)	15	2.5	3.7	0.1	0.11	84.7
4 (Mid)	15	8.1	12.2	0.3	0.36	78.9
5 (High)	15	26.1	39.2	1.0	1.16	58.8

Doses were selected based on previous studies with CGP 25827A. The high dose with the highest technically feasible dose using the dosing apparatus described.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Daily
Body weight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Prior to treatment and at the end of the study
Urine for Proof of Absorption	After the first exposure and 1 and 3 months
Clinical Pathology	Weeks 6 and 13
Gross pathology (including organ weights)	After 91days of treatment
Histopathology (complete tissue list including 4 levels of the nasal cavity)	At study termination for control and high dose groups; gross lesions and mandibular lymph nodes were examined from all groups.

RESULTS

Results are summarized in the following table.

Parameter	Remarkable Findings
Mortality	No remarkable findings.
Clinical signs	No remarkable findings.
Body weight	A dose related increase in body weight gain was noted in all groups of treated males, with statistical significance for Group 5. Mean body weights for Group 4 females and Groups 4 and 5 males were consistently higher than control values.
Food consumption	Treated animals from all groups consumed larger quantities of food when compared to controls.
Ophthalmoscopy	No remarkable findings.
Clinical Pathology	The following parameters were significantly different from control: • Glucose - Weeks 6 and 13, ↓ Group 3 - 5 ♂; • Aspartate aminotransferase - Week 6 and 13, ↑ Group 5 ♂; • Alanine aminotransferase - Week 6, ↑ Group 5 ♀; Week 13, ↑ Groups 5 ♂♀.
Gross pathology	
Organ Weights	Heart weights were significantly higher for males and females in Group 5 when compared to controls.
Necropsy	No remarkable findings.
Histopathology	No remarkable findings.

CONCLUSION

The increased body weight and heart weight noted in this study is consistent with that noted in other toxicity studies with this drug. The Sponsor concluded that CGP 25827A was well tolerated up to the highest inhaled dose (26.1 and 39.2 µg/kg/day for males and females, respectively). Notably, there were no adverse effects on the respiratory system.

9. 3 Month Inhalation Toxicity Study in Rats

BACKGROUND INFORMATION

Study Title: 3 Month Inhalation Toxicity Study in Rats
Sponsor Study No.: 90-6224
Laboratory Study No.: 650361
Study Dates: October 12, 1990 - February 20, 1991
Report Date: January 30, 1992
Test Facility: [redacted]

GLP Status: Compliant
NDA Volume:Page 34:1

METHODS

Test Article: CGP 25827A
Batch No: 14/909/1
Purity: ———
Control Article: Aerosol fluorocarbon propellants
Purity: Not stated
Species/Strain: Sprague-Dawley Rats
Route: Nose Only Inhalation
Exposure Conditions: Aluminum cylindrical chamber with apparatus to actuate aerosol cans to achieve target concentrations.
Duration of Exposure: 3 months

Dosing Information

Group	No. Animals per sex	Dose mg/kg/day	No. of Cans x Actuations/min x h/day	Chamber Conc. (µg/l)	Nominal Conc. (µg/l)	Mass Mean % of Particles with <6 µm Diameter
1 (Control) [a]	10	0	6 x 6 x 4 h	0	0	94.3
2 (Low)	10	0.034	3 x 2 x 1 h	2.0	7.2	92.1
3 (Mid)	10	0.136	3 x 4 x 2 h	4.0	14.4	94.2
4 (High)	10	0.442	6 x 6 x 4 h	6.7	43.2	94.2

[a] Values reported represent placebo excipients only.

The low dose was selected as a small multiple of the anticipated human dose. The high dose was the highest dose that could be achieved using the dosing apparatus for this study.

Toxicity was assessed by evaluating the following parameters:

Parameter	Frequency of Measurement
Mortality	Twice daily
Clinical signs	Twice daily
Body weight	Weekly
Food consumption	Weekly
Ophthalmoscopy	Prior to treatment and During Weeks 6 and 13
Clinical Pathology	Weeks 7 and 13
Gross pathology (including organ weights)	After 91days of treatment
Histopathology (complete tissue list including anterior and posterior nasal cavities, larynx, anterior and posterior trachea, and lung sections through bronchioles)	At study termination for control and high dose groups