Dose (mg/kg):	0	0.2	6	30
Total	275	288	253	230
Mean (SD)	13.8 (2.10)	14.4 (1.79)	12.7 (3.44)	11.5 (4.77)
Mean Delivery Ratio (%)	90.7	93.6	94.2	88.5
Sex Ratio (male/female)	127/148	146/142	133/120	120/110
Mean (SD) weight of Newborns				
Male	6.3 (0.43)	6.0 (0.42)*	6.0 (0.49)*	5.5 (0.54)**
Female	5.9 (0.48)	5.7 (0.39)	5.8 (0.42)	5.3 (0.49)**
Total No. of Stillborn	5	3	21**	47**
Total No. of Pups that Died within 4 Days After Birth	6	3	54**	95**
Until Weaning				
Day 4 Survival Before Adjustment	269	285	199	135
Day 4				
Male	97	99	77	62
Female	101	99	82	60
Day 7				
Male	96	99	72	59
Female	101	98	81	55
Day 14				
Male	94	92	59	45
Female	101	98	67	37
Day 21				
Male	94	92	58	43
Female	100	98	64	37
Survival Ratio (%)				
Male	96.8	93.0	74.7*	61.6**
Female	99.0	99.0	79.2*	53.8**

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

CONCLUSION

The Sponsor concluded that the maximum no effect level for pups and dams is between 0.2 and 6 mg/kg under the conditions of this study. There were no effects on reproductive indices.

27. Reproduction test of Formoterol fumarate (BD 40A) Foster Nursing Study in Rats

BACKGROUND INFORMATION

Study Title: Reproduction test of Formoterol fumarate (BD 40A) Foster Nursing Study in Rats

Sponsor Study No.: —— D-4-5
Laboratory Study No.: Not stated
Study Dates: October 1976 - January 1977
Report Date: July 1982
Test Facility: [redacted]

GLP Status: Not compliant. Performed prior to establishment of GLPs.
NDA Volume:Page 63:1

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

This foster nursing study was conducted to elucidate some of the findings in a previous Segment III study. CGP 25827A was administered to 2 groups (S_1 and S_2) of 15 pregnant rats at a dose of 6 mg/kg and a volume of 5 ml/kg. Two additional groups (C_1 and C_2) of rats received the vehicle under an identical dosing regimen. Dosing occurred on gestation days 17 - 21. Upon delivery cross fostering groups were established as follows: C_1 dams fostered pups from C_2; C_2 dams fostered pups from S_1; S_1 dams fostered pups from S_2; and S_2 dams fostered pups from C_1.

RESULTS

No obvious effect on dams or offspring were observed when control groups cross fostered. Dams treated with CGP 25827A had increased body weight during lactation. Offspring of dams treated with CGP 25827A had increased stillbirth rate, an increased neonatal death rate, both prior to and after cross fostering, and a decreased birth weight regardless of whether they were raised by treated or control dams. Treated offspring raised by control dams recovered in body weight gain until they were comparable to control pups by day 14 postpartum. An inhibition of body weight gain after day 14 was observed in control offspring raised by treated dams.

Effects of BD40A on F_1 Offspring After Cross Fostering

Dose Group:	Control (C1)	Control (C2)	6 mg/kg (S1)	6 mg/kg (S2)
Alive Day 0 After Cross Fostering	205	188	202	213
No. of pups that died within 4 days after birth (%)	6 (2.9)	76 (40.4)**	66 (32.7) **	6 (2.8)
No. of Survivors Until Day 35 After Birth				
Day 4 Survival Before Adjustment				
Total	199	112	136	207
Male	95	59	66	109
Female	104	53	70	98
Day 4				
Male	73	51	61	76
Female	77	52	64	74
Day 7				
Male	73	47	61	74
Female	76	50	62	73
Day 14				
Male	70	40	49	70
Female	75	44	52	71
Day 21				
Male	70	39	48	69
Female	75	44	52	71
Day 28				
Male	30	19	25	29
Female	30	20	25	30
Day 21				
Male	30	19	25	29
Female	30	20	25	30
Survival Ratio (%)				
Male	95.9	76.5**	78.7**	90.8
Female	97.4	84.6**	81.3**	95.9

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

CONCLUSION

These results indicate that CGP 25827A elicits peri- and postnatal toxic effects in rats under the conditions of this study.

Summary of Reproductive Toxicology Studies

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. Several mechanisms of this toxicity have been proposed by the Sponsor, but none have been shown definitively. It appears that since only pups exposes *in utero* were affected in the cross fostering study that pup mortality is not a consequence of exposure through lactation or of suppressed milk production by treated dams. Rather, the observed peri- and postnatal mortality is likely attributed to *in utero* exposure of the fetuses when the dams are treated with CGP 25827A at levels of 6 mg/kg and above.

GENETIC TOXICOLOGY

Formoterol fumarate was tested in an extensive battery of genotoxicity and mutagenicity assays and was consistently found to be negative. The following assays were conducted.

In vitro assays:

- mutagenicity in microorganisms
- reversion in bacteria
- *salmonella*/mammalian-microsome mutagenicity
- V79 Chinese hamster point mutation test
- unscheduled DNA synthesis repair in rat hepatocytes
- unscheduled DNA synthesis repair in human fibroblasts
- transformation assay in mammalian fibroblasts
- chromosome analysis of CHO cells

In vivo assays:

- mouse micronucleus test
- rat micronucleus test
- chromosome analysis in somatic cells of Chinese hamsters.

Details of each of the tests are included in this review.

28. Mutagenicity Tests of Formoterol fumarate (BD 40A) in Microorganisms

BACKGROUND INFORMATION

Study Title:	Mutagenicity Tests of Formoterol fumarate (BD 40A) in Microorganisms
Sponsor Study No.:	D-7-1
Study Dates:	Not stated
Report Date:	October 27, 1981
Test Facility:	

[redacted]

GLP Status: Non-compliant; deficiencies identified
NDA Volume:Page 63:75

METHODS

Test Materials
Test Articles: Formoterol fumarate (BD 40A)
Decomposition product (BD 177)
Batch No: Not stated
Vehicle Control: Dimethylsulfoxide (DMSO)
Positive Control: Nitrofurazone; 0.1% Phenobarbital
Negative Control: Kanamycin sulfate

Test System
Assay System: Regenerative test – M45 strain (recombinant deficient) and H17 *bacillus subtilit*
Reversion test – *Salmonella typhimurium* strains TA98, TA100, TZ1537, TA1535, TA1537 and TA1538
Metabolic Activation System: Rat liver S9

Exposure
Definitive Dose: Doses were selected based on cytotoxic levels achieved in range finding studies. Regenerative test – 6 doses of BD 40A ranging from 8 - 4000 µg/disk; 3 doses of BD 177 ranging from 8 to 200 µg/disk
Reversion test – 3 doses of BD 40A ranging from 40 - 1000 µg/plate; 3 doses of BD 177 ranging from 40 to 1000 µg/plate both in the presence and absence of S9
Incubation Conditions: Regenerative test – 16 - 18 hr. at 37°C
Reversion test – 2 days at 37°C
Replicates: Three

Observations
Parameters Measured: Regenerative test – cell growth inhibition
Reversion test – number of revertant his^+ colonies

RESULTS

BD 40A or BD 177 did inhibit cell growth under the conditions tested. No evidence of mutagenicity was revealed in the presence or absence of S9 metabolic activation.

CONCLUSION

Both BD 40A and BD 177 are non-mutagenic under the described conditions.

29. Mutagenicity Tests of Formoterol fumarate (BD 40A):Reversion of Formoterol fumarate (BD 40A)

BACKGROUND INFORMATION

Study Title:	Mutagenicity Tests of Formoterol fumarate (BD 40A):Reversion of Formoterol fumarate (BD 40A)
Sponsor Study No.:	— D-7-2 (82809)
Study Dates:	August 9, 1982 - June 10, 1983
Report Date:	July 18, 1983
Test Facility:	[redacted]
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	63:102

METHODS

Test Materials

Test Articles:	Formoterol fumarate (BD 40A) Amine derivative (A-1)
Batch No:	L-9
Purity:	[redacted]
Vehicle Control:	Dimethylsulfoxide (DMSO)
Positive Controls:	N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) - 2 µg/plate N-methyl-N'-nitro-N- nitrosoguanidine (MNNG) - 5 µg/plate Sodium azide - 1 µg or 0.5 µg/plate 2-nitrofluorene (2-NF) - 2 µg or 1 µg/plate 9-aminoacridine (9-AA) - 80 µg/plate 2-aminoanthracene (2-ANTH) - 40 µg or 10 µg/plate Benzo-[a]-pyrene (BP) - 5 µg Dimethylnitrosamine (DMN) - 0.1 ml/plate

Test System

Assay System: Various strains of *S. typhimurium* and *E. Coli*

Metabolic Activation System: Rat liver S9

Exposure

Definitive Dose: Definitive doses were selected using data from cytotoxicity screens. The highest non-toxic concentration was used as the maximum dose.
- 8 doses of BD 404A ranging from 0.156 - 20 mg
- 5 doses of A1 ranging from 0.01 - 3 mg

Incubation Conditions: 2 days at 37°C

Replicates: At least two

Observations

Parameters Measured: Number of his^+ (*S. typhimurium*) or trp^+ (*E. Coli*) revertant colonies

RESULTS

The number revertant colonies in the presence of BD 40A or the breakdown product A1 was similar to those in the vehicle control group in all bacterial strains tested, both in the presence and the absence of metabolic activation by S9 fraction.

CONCLUSION

Both BD 40A and A1 are non-mutagenic under the described conditions.

30. Salmonella/Mammalian Microsome Mutagenicity Test

BACKGROUND INFORMATION

Study Title: Salmonella/Mammalian Microsome Mutagenicity Test

Sponsor Study No.: 841042

Study Dates: November 13, 1984 - February 7, 1985

Report Date: March 22, 1985

Test Facility: CIBA-GEIGY Limited, Pharmaceuticals Division
Experimental Pathology Laboratories
Basle, Switzerland

GLP Status: Compliant with 21 CFR 58

NDA Volume:Page 63:131

METHODS

Test Materials

Test Articles: CGP 25827A

Batch No: 810284
Purity: Not stated
Vehicle Control: Methanol (toxicity: 0.08 - 5000 µg/0.1ml; mutagenicity: 20 - 5000 µg/0.1ml)
Positive Control: Listed below

Test System
Assay System: Various strains of *Salmonella typhimurium* (listed below)
Metabolic Activation System: Rat liver S9

Exposure
Definitive Dose: Definitive doses were selected using data from cytotoxicity screens. The highest non-toxic concentration was used as the maximum dose. Five doses ranging from 20 - 5000 µg/0.1ml were used.
Incubation Conditions: 48 hours at 37°C
Replicates: Three

Observations
Parameters Measured: Number of his^+ revertant colonies

Positive Control Materials for Each Tester Strain

Without S9 Activation		With S9 Activation	
Strain	Positive Control	Strain	Positive Control
TA 98	daunorubicin-HCl (buffered)	TA 98	2-aminoanthracene
TA 100	4-nitroquinoline-N-oxide	TA 100	2-aminoanthracene
TA 102	mitomycin-C	TA 102	2-aminoanthracene
TA 1535	sodium azide	TA 1535	cyclophosphamide
TA 1537	aminoacrinide HCl monohydrate	TA 1537	2-aminoanthracene

RESULTS

The number revertant colonies in the presence of CGP 25827A was not different from negative controls.

CONCLUSION

CGP 25827A is non-mutagenic under the described conditions.

31. V79 Chinese Hamster Point Mutation Test

BACKGROUND INFORMATION

Study Title:	V79 Chinese Hamster Point Mutation Test
Sponsor Study No.:	841043
Study Dates:	November 13, 1984 - February 7, 1985
Report Date:	September 19, 1985 with September 1, 1987 amendment
Test Facility:	CIBA-GEIGY Limited Protection of Health and Environment Experimental Pathology Basle, Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	63:148

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810284
Purity:	Not stated
Negative Control:	Test medium
Positive Control:	dimethylnitrosamine (DMN)

Test System

Assay System:	Various strains of *Salmonella typhimurium* (listed below)
Metabolic Activation System:	Rat liver S9

Exposure

Definitive Dose:	Definitive doses were selected using data from cytotoxicity screens. The highest non-toxic concentration was used as the maximum dose. Eight doses ranging from 25.0 µg/ml - 1.0 mg/ml with microsomal activation and 8 doses ranging from 2.0 µg/ml - 80 µg/ml without activation were used.
Incubation Conditions:	48 hours at 37°C
Replicates:	Three

Observations

Parameters Measured:	Number of colonies resistant to 6-thioguanine (6-TG) or ouabain (OUA) in treated and control groups.

RESULTS

The number revertant colonies in the presence of CGP 25827A was not different from negative controls.

CONCLUSION

The described test is appropriately specific and sensitive to detect forward mutations including point mutations, frame-shift, and deletions. CGP 25827A is non-mutagenic under the described conditions.

32. [redacted] DNA Repair Test on Rat Hepatocytes

BACKGROUND INFORMATION

Study Title:	[redacted] DNA Repair Test on Rat Hepatocytes
Sponsor Study No.:	841039
Study Dates:	January 10 - March 6, 1985
Report Date:	June 5, 1987
Test Facility:	CIBA-GEIGY Limited Protection of Health and Environment Experimental Pathology [redacted] Basle, Switzerland
GLP Status:	Not stated
NDA Volume:Page	63:170

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810284
Purity:	[redacted]
Negative Control:	Test medium
Positive Control:	dimethylnitrosamine (DMN)

Test System

Assay System:	Rat hepatocytes

Exposure

Definitive Dose:	Definitive doses were selected using data from cytotoxicity screens. The highest concentration for the definitive study was selected based on sufficient numbers of cells adhering to the coverslip; at least 25% cell viability; and a corresponding percentage of cell in good morphologic condition. Five doses ranging from .20 - 25 µg/ml were used.

Incubation Conditions: 6 days after washing
Replicates: Four

Observations
Parameters Measured: Number silver grains per nucleus upon ——— analysis.

RESULTS

There was no difference between treated and negative control groups in the number of silver grains per nucleus.

CONCLUSION

The described test is appropriately specific and sensitive to detect unscheduled DNA-synthesis as a consequence of DNA damage. CGP 25827A is non-mutagenic under the described conditions.

33. ——— DNA Repair Test on Human Fibroblasts

BACKGROUND INFORMATION

Study Title: ——— DNA Repair Test on Human Fibroblasts
Sponsor Study No.: 841041
Study Dates: January 10 - March 7, 1985
Report Date: June 5, 1987
Test Facility: CIBA-GEIGY Limited
Protection of Health and Environment
Experimental Pathology ———
Basle, Switzerland
GLP Status: Not stated
NDA Volume:Page 63:183

METHODS

Test Materials
Test Articles: CGP 25827A
Batch No: 810284
Purity: Not stated
Negative Control: Test medium
Positive Control: 4-nitroquinoline-N-oxide4 (NQO)

Test System
Assay System: Human fibroblasts

Exposure

Definitive Dose: Definitive doses were selected using data from cytotoxicity screens. The highest concentration for the definitive study was selected based on sufficient numbers of cells adhering to the coverslip; at least 25% cell viability; and a corresponding percentage of cell in good morphologic condition. Four doses ranging from 3.2 - 400 µg/ml were used.

Incubation Conditions: 6 hours after washing

Replicates: Not stated

Observations

Parameters Measured: Number silver grains per nucleus upon [redacted] analysis.

RESULTS

There was no difference between treated and negative control groups in the number of silver grains per nucleus.

CONCLUSION

The described test is appropriately specific and sensitive to detect unscheduled DNA-synthesis as a consequence of DNA damage. CGP 25827A is non-mutagenic under the described conditions.

34. Transformation/Liver Microsome Test (*In vitro* test for transformation-inducing properties in mammalian fibroblasts)

BACKGROUND INFORMATION

Study Title:	Transformation/Liver Microsome Test (*In vitro* test for transformation-inducing properties in mammalian fibroblasts)
Sponsor Study No.:	841044
Study Dates:	September 25, 1986 - July 7, 1986
Report Date:	October 10, 1986
Test Facility:	CIBA-GEIGY Limited Toxicology II Basle, Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	63:195

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810284
Purity:	Not stated
Negative Control:	Solvent and Untreated test medium
Positive Control:	Non-activated - methylcholanthrene (1.5 and 3.0 µg/ml) Activated - 2-acetylaminoflurene (50 and 100 µg/ml)
Metabolic Activation System:	Rat liver S9

Test System

Assay System:	Mouse embryo fibroblasts (BALB/3T3)

Exposure

Definitive Dose:	Definitive doses were selected using data from cytotoxicity screens. The highest concentration for the definitive study was selected as that which caused a 50% reduction in colony-forming ability when compared to the negative control. Five doses ranging from 1.313 - 21 µg/ml were used without activation and five doses ranging from 12.5 - 200 µg/ml were use with activation.
Incubation Conditions:	72 hours without activation and 24 hours with activation
Replicates:	Fifteen

Observations

Parameters Measured:	Number of transformed cells.

RESULTS

There was no difference between treated and negative control groups in the number of transformed cells.

CONCLUSION

The described test is appropriately specific and sensitive to detect morphologic changes due to transformation of mammalian cells induced by chemical substances. CGP 25827A did not induce cell transformations under the described conditions.

35. Chromosome Studies of Somatic Cells of Chinese Hamster

BACKGROUND INFORMATION

Study Title:	Chromosome Studies of Somatic Cells of Chinese Hamster
Sponsor Study No.:	841040

Study Dates:	September 25, 1986 - July 7, 1986
Report Date:	August 8, 1985
Test Facility:	CIBA-GEIGY Limited Protection of Health and Environment Experimental Pathology ——— Basle, Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	63:231

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810284
Purity:	———
Negative Control:	Sodium carboxymethylcellulose (CMC)
Positive Control:	Cyclophosphamide (intraperitoneal route)

Test System

Assay System:	Somatic cells harvested from the bone marrow of treated Chinese hamsters.

Exposure

Definitive Dose:	Definitive doses were selected using data from tolerability screens. The highest dose for the definitive study was selected as that which produced no deaths. Animals were dosed by oral gavage at levels of 80.5, 161, and 322 mg/kg.
Number of Animals:	4 animals/sex/treatment group and 6 animals/sex/control group

Observations

Parameters Measured:	Morphologic evaluation of bone marrow cells for chromosome aberrations manifest as: 1.) breaks, exchanges, deletion, or fragmentation; 2.) gaps or decay; or 3.) numerical aberrations.

RESULTS

No specific aberrations were noted in chromosomes harvested from animals treated with CGP 25827A.

CONCLUSION

The described test is appropriately specific and sensitive to detect *in vivo* mutagenic effects (i.e., structural chromosomal aberrations) on somatic cells harvested from treated animals. CGP 25827A was non-mutagenic under the described conditions.

36. Mutagenicity Testes of Formoterol fumarate (BD 40A): Micronucleus test of Formoterol fumarate (BD 40A) in Mice

BACKGROUND INFORMATION

Study Title:	Mutagenicity Testes of Formoterol fumarate (BD 40A): Micronucleus test of Formoterol fumarate (BD 40A) in Mice
Sponsor Study No.:	D-7-3 (Amendment No. 82806)
Study Dates:	May 24, 1982 - June 4, 1982
Report Date:	July 13, 1983
Test Facility:	
GLP Status:	Non-compliant; deficiencies identified
NDA Volume:Page	63:244

METHODS

Test Materials

Test Articles:	Formoterol fumarate (BD 40A)
Batch No:	L-9
Purity:	
Negative Control:	0.5% aqueous methylcellulose and untreated animals
Positive Control:	Cyclophosphamide (intraperitoneal route)

Test System

Assay System:	Somatic cells harvested from the bone marrow of treated male Crj:CD-1 mice.

Exposure

Definitive Dose:	Definitive doses were selected using data from previous acute oral toxicity tests. The highest dose for the definitive study was equivalent to the LD_5. Animals received 2 doses by oral gavage at levels of 200, 400 and 800 mg/kg.
Number of Animals:	5 animals/ group

Observations

Parameters Measured:	Morphologic evaluation of bone marrow cells for the incidence and appearance of polychromatic erythrocytes (PCEs).

RESULTS

There was no increased incidence of micronucleus cells per 1000 PCEs from animals treated with CGP 25827A when compared to negative controls.

CONCLUSION

The described test is appropriately specific and sensitive to detect *in vivo* effects (i.e., chromosome aberrations) in somatic cells harvested from treated animals. CGP 25827A did not induce clastogenic or mutagenic effects under the described conditions.

37. CGP 25827A: Micronucleus Test, Rat *In vivo* Study

BACKGROUND INFORMATION

Study Title:	CGP 25827A: Micronucleus Test, Rat *In vivo* Study
Sponsor Study No.:	896251
Study Dates:	May 28, 1990 - September 5, 1990
Report Date:	October 31, 1990
Test Facility:	CIBA-GEIGY Limited Basel, Switzerland
GLP Status:	Compliant with 21 CFR 58
NDA Volume:Page	63:266

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810187
Purity:	——
Negative Control:	Carboxymethylcellusole (CMC) 0.5%
Positive Control:	Cyclophosphamide (intraperitoneal route)

Test System

Assay System:	Somatic cells harvested from the bone marrow of treated male Crj:CD-1 mice.

Exposure

Definitive Dose:	Definitive doses were selected based on a ratio of the human dose. The high dose corresponds to approximately 25,000 times a human oral dose of 240 µg and 62,500 times a human inhaled dose of 96 µg. (This exceeds the anticipated human inhaled dose of —— requested I the current application.) Dose levels and sacrifice intervals are presented below.
Number of Animals:	5 animals/group

Observations

Parameters Measured:	Morphologic evaluation of bone marrow cells for the incidence and appearance of polychromatic erythrocytes (PCEs).

Dose Levels and Sacrifice Intervals for Parts 1 and 2 of Study

Part 1		Part 2	
Dose (mg/kg)	Sacrifice Interval (hours)	Dose (mg/kg)	Sacrifice Interval (hours)
100	16	25	24
100	24	50	24
100	48	100	24

RESULTS

There was no increased incidence of micronucleus cells per 1000 PCEs from animals treated with CGP 25827A when compared to negative controls.

CONCLUSION

The described test is appropriately specific and sensitive to detect *in vivo* effects (i.e., chromosome aberrations) in somatic cells harvested from treated animals. CGP 25827A did not induce clastogenic or mutagenic effects under the described conditions.

38. Chromosome Studies on Chinese Hamster Ovary Cell Line CCL 61 *In vitro*

BACKGROUND INFORMATION

Study Title:	Chromosome Studies on Chinese Hamster Ovary Cell Line CCL 61 *In vitro*
Sponsor Study No.:	896209
Study Dates:	February 12, 1990 - June 18, 1990
Report Date:	November 8, 1990
Test Facility:	CIBA-GEIGY Limited Toxicology II Basle, Switzerland
GLP Status:	Compliant 21 CFR 58
NDA Volume:Page	63:297

METHODS

Test Materials

Test Articles:	CGP 25827A
Batch No:	810187
Purity:	[redacted]
Vehicle Control:	Dimethylsulfoxide (DMSO)
Positive Control:	Mitomycin-C; Cyclophosphamide

Test System

Assay System: Chinese hamster ovary (CHO) cells (CCL 61 cell line)
Metabolic Activation System: Rat liver S9

Observations

Parameters Measured: Morphologic examination for chromosome aberrations

Exposure Conditions

Experiment	Treatment	CGP 25827A (µg/ml)	S9	Recovery
Original				
#1	18 hr	46.88, 93.75, 187.5	without	0 hr
#1	3 hr	375.0, 751.0, 1500.0	with	15 hr
Confirmatory				
#1 & #2	18 hr	46.88, 93.75, 187.5	without	0 hr
#1 & #2	3 hr	375.0, 751.0, 1500.0	with	15 hr
#3	42 hr	46.88, 93.75, 187.5	without	0 hr
#4	3 hr	187.5, 375.0, 750.0	with	39 hr

RESULTS

In all experiments with CGP 25827A, chromosome aberrations in the treated groups were similar in incidence to those in the negative control group.

CONCLUSION

CGP 25827A is non-mutagenic under the described conditions.

Summary of Genetic Toxicology Studies

Formoterol fumarate is not mutagenic and not genotoxic as shown by an the extensive battery of test described. This provides further evidence that the carcinogenic responses observed in the studies previously reviewed are not of a genotoxic mechanism.

SPECIAL TOXICOLOGY STUDIES

The Sponsor included the several special studies in the submission and these are summarized in the table below.

Special Toxicology Studies

Study Type	Methods	Findings	Ref.
Antigenicity in Mice	i.p. injection with measurement of anti-IgE antibodies.	No anti-CGP 25827A IgE antibodies were found.	D-6-2
Skin Sensitization in Guinea	sensitization and challenge	No dermal	840421

Study Type	Methods	Findings	Ref.
pigs	phases	sensitization was noted	
5-day IV Local Tolerability in Rabbits	5 daily i.v. injections in ear vein with microscopic follow-up	slight to moderate irritation at injection site; not different from control vehicle	855125
Skin Irritation in Rabbits	5-day exposure under patch	minimal to slight irritation on depilated skin; resolution within 24 hr	835278

Summary Special Toxicology Studies

The special toxicology studies are generally unremarkable.

OVERALL SUMMARY AND EVALUATION

Introduction

An NDA was submitted to support the safety and efficacy of a new drug, formoterol fumarate (Foradil™). The drug is indicated for the prevention and maintenance treatment of bronchoconstriction in patients years of age and older with reversible obstructive airways disease, including patients with symptoms of nocturnal asthma, and for the prevention of exercise-induced bronchospasm. Formoterol fumarate will be administered twice daily via an Aeorliser™ oral inhalation device. The maximum daily dose is mcg. This document is a review of the preclinical pharmacology and toxicology data submitted to support the safety of the drug for the proposed use in humans.

[ ] Fomoterol fumarate belongs in the beta-2-andrenoceptor agonist pharmacologic class.

Fomoterol fumarate provides therapeutic benefit by relieving and preventing bronchocontstriction by relaxing airway smooth muscle via specific interaction with beta-2-adrenoceptors. The efficacy of fomorterol at beta-2-adrenoceptors has been measured in both functional airway smooth muscle relaxation and biochemical second messanger assays where cAMP were determined. High levels of agonism were demonstrated using conditions of induced tone or the presence of high levels of cholinergic agonists. Treatment with formoterol or other beta-2-andrenoceptor agonists is associated with reassertion relaxation, suggesting that it is functionally retained in or near the beta-2-adrenoceptor despite extensive washing of *in vitro* airway smooth muscle preparations.

The onset of action of formoterol appears to be comparable to that of albuterol, yet the duration of action appeared consistently greater than that of isoprotenerol or albuterol. The onset of action was reported to be 1.7 ± 0.3 minutes for formoterol, 0.8 ±0.2 minutes for albuterol, and 17.6 ± 5.0 minutes for salmeterol when administered to guinea pig isolated trachea. (Jeppson et al., 1989). The duration of action of formoterol was in excess of 6 hours in isolated human bronchus (Advenier et al, 1991).

Bronchoselectivity was demonstrated in studies with Fomoterol fumarate, however increases in both the force and rate of the myocardium were observed after administration of high doses to animals. The Sponsor reports that these changes are likely attributed to: "(i) a subdominant beta-2-adrenoceptor pop reflex compensation for decreased peripheral resistance caused by beta-2-adrenoceptor mediated relaxation of arterial smooth muscle and (ii) direct stimulation of a subdominant beta-2-andrenoceptor population coupled to ionotrophy and chronotrophy in the heart."

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 selective beta-2-andrenoceptor agonist.

Scope of Evaluation

A comprehensive data base of studies was submitted and reviewed to support the safety of Formoterol fumarate, including:

- genotoxicity studies (a total of 11 studies);
- acute, subchronic, and chronic toxicity studies in mice, rats and/or dogs via oral and inhalation routes of administration (a total of 44 studies were considered in this review and 13 studies were considered in previous reviews);
- reproduction and teratology studies covering all phases (a total of 5);
- absorption, metabolism, distribution, and excretion studies (a total of 52 studies);
- four carcinogenicity studies (two each in mice and rats).

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. Formoterol fumarate was not mutagenic or teratogenic in the assays submitted.

Safety Evaluation

A safety concern with Formoterol fumarate, as with any known beta agonist, is the potential for cardiotoxicity. This effect was demonstrated in the animal studies and manifests as increased heart rate and force, reddening of the mouth and ventral surface and myocardial degeneration in dogs treated by the inhalation route at levels as low as 3 µg/kg/day. The clinical signs were observed at the onset of dosing and the myocardial fibrosis was evident within one month (926074). At an inhaled level of 15.16 µg/kg/day for one year, the clinical sign was evident but the myocardial fibrosis was not (936116). The AUC for dogs dosed orally at 0.1 mg/kg is 101.6 nmol·h/l, which is 191 times the expected maximum human daily dose AUC.

In rodents, effects on the heart were evidenced as increases in heart weight and myocardial fibrosis. Increased heart weights were observed in rats dosed by the inhalation route as early as 6 months at a level as low as 30 µg/kg day. However these rats did not show myocardial fibrosis. In fact, myocardial fibrosis was not noted after inhalation treatment until after one year and only in males at a dose of 400 µg/kg/day (or 0.4 mg/kg/day).

Clinical Relevance of Safety Issues

The cardiotoxicity of beta agonists appears to be a consequence of their pharmacologic activity. The effect on the heart is first seen as an increased heart rate, which could be a consequence of a direct interaction with beta receptors in the heart, but is most probably the result of reflex tachycardia, secondary to beta-2-mediated vasodilation and hypotension. Once this effect reaches excessive levels, ischemic changes occur since the oxygen supply can no longer be maintained. This results in focal necrosis and subsequent fibrosis in the anaerobic regions. The papillary muscle of the left ventricle appears to be particularly sensitive. If the effects on the heart result from pharmacologic activity of the agents it follows that their potency as cardiotoxins will be related to their potency as beta agonists. This was confirmed in a direct experimental comparison in dogs in which formoterol was found to be 10 times more active than ——— at inducing increased heart rate and cardiac lesions, which is keeping with the potency of these two compounds as beta agonist. If excessive tachycardia is a prerequisite for cardiotoxicity, no effects are to be expected at doses which do not increase the heart rate. Clinical experience in humans shows that doses below 72 µg/day do not effect heart rate. Thus, the proposed — µg/day maximum human dose appears to be within an acceptable safety margin for cardiotoxicity.

Other Clinically Relevant Issues

No other clinically relevant issues were identified for Formoterol fumarate. Toxicity manifests as exaggerated pharmacodynamic effects of beta agonists. The effects

observed with Formoterol fumarate were consistent with those described in the literature for other beta agonists.

Conclusions

Taken together, the data submitted supports the safety of Formoterol fumarate under the proposed conditions of use. Adequate safety margins appear to exist for inhalation exposure, although additional data is being sought to make valid plasma level comparisons between animals and humans.

Formoterol fumarate was not genotoxic or mutagenic in any of the assays submitted. The following table summarizes major findings and the dose levels at which they occur.

Summary of Notable Findings and Dose Ratios to Adult Humans on a mg/m^2 Basis

Assay	Species	Route	Dose (mg/kg)	Dose (mg/m^2)	Dose Ratio for Adults	Effect
1-Year Chronic	Dog	Oral	.01	0.2	6	Myocardial fibrosis
1-Year Chronic	Dog	Inhale	0.015	0.3	8	NOAEL
1-Year Chronic	Rat	Inhale	0.030	0.18	5	NOAEL
1-Year Chronic	Rat	Inhale	0.120	0.72	20	Degeneration of seminiferous tubules
10Year Chronic	Rat	Inhale	0.4	2.4	70	Myocardial fibrosis
Reproduction and Developmental Toxicology	Rat	Oral	6	36	1000	Stillbirth and neonatal death
Carcinogenicity	Rat	Water	15	90	2500	Ovarian leiomyoma
Carcinogenicity	Rat	Diet	20	60	3400	Ovarian leiomyoma
Carcinogenicity	Mouse	Diet	2	6	170	Ovarian leiomyoma + leiomyosarcoma
Carcinogenicity	Mouse	Diet	20	60	1700	Hepatocellular carcinoma
Carcinogenicity	Mouse	Diet	50	150	4200	Testicular tubular atrophy
Carcinogenicity	Mouse	Water	267	801	22,000	Adrenal subcapsular adenoma + carcinoma

Language to be used in Letter to Sponsor

The language reported under the Recommendations section below can be used in communication with the Sponsor.

RECOMMENDATIONS

1. It was noted during the course of the review of the dietary carcinogenicity studies that the incidence of some findings in the statistical analysis does not exactly match that reported in the summary incidence tables. For example, in the mouse dietary study,

the incidence of benign hepatoma reported in the incidence table (v.49 p. 176) differs from that presented in the statistical analysis (v.49, p. 428), as illustrated in the following table.

Incidence of Benign Hepatoma in the Incidence Table and Statistical Analysis

	Reference	
Group	v.49 p.176	v.49 p. 428
0	18/85	24/85
2	19/85	24/85
5	21/85	31/85
20	24/84	33/84
50	15/85	25/85

The Sponsor should recheck their submission and clarify the discrepancies.

2. 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.

   The values from the dietary study seem more realistic based on the dose and thus we are concerned with the claimed exceeding large dose multiples between humans and animals. Specifically, we are concerned with reported exposure data (AUC, Cmax, etc.) associated with the carcinogenicity studies, reproductive and development studies, and the chronic toxicity studies. The Sponsor should provide realistic exposure information for these studies, or provide an explanation of why such data are not attainable. We note that in humans levels as low as an AUC of 1.33 nmol·h/l based on an inhaled dose of 120 µg, is measurable.

3. The Sponsor should report the assumed deposition factor for the inhalation studies included in this submission.

/S/ 5/28/98
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/S/ 5.28.98
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cc HFD-570/Division File
HFD-570/H. Sheevers
HFD-570/P. Jani
HFD-570/T. Zoetis

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