

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	b(4)
Sponsor Study No.:	-D-4-1	
Laboratory Study No.:	Not applicable	
Study Dates:	Prior to 1977. Exact date not stated.	
Report Date:	Not reported.	
Test Facility:	_____	b(4)
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 F₀ females were killed on gestation day 20 and the F₁ generation was weighed and examined for the incidence of skeletal and visceral malformations and variations and reproductive parameters.

RESULTS

In the F₀ 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 F₁ 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 GU 2.1 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 (F₁ generation) of these females were not treated and allowed to breed within their group to produce an F₂ generation.

Effects on fertility of the F₀ and F₁ generations, fetuses from the F₁ generation, and 21-day old rats from F₂ 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 F₀ 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 F₁ 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 calcaneus 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 F₁ generation selected for mating or on their progeny in the F₂ generation.

CONCLUSION

Treatment of the F₀ generation with CGP 25827A-E did not have any effect on mating performance, fertility, pregnancy rate or duration of gestation in either the F₀ or F₁ generations. There was no evidence of structural abnormalities in either the F₁ or F₂ 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 b(4)
Laboratory Study No.:	Not stated
Study Dates:	September 1986 – May 1977
Report Date:	Not stated
Test Facility:	_____ b(4)
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 F₀ 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 F₁ offspring were raised until sexual maturity paired for mating.

Embryotoxicity, fetotoxicity, and teratology parameters were evaluated.

RESULTS

Slight increases in F₀ maternal body weight gain and delayed ossification in F₁ 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 F₁ 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 F₀-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 F₁ fetuses from all dose levels. Effects on the F₁ offspring are summarized in the following table.

		Effects of BD40A on F₁ Offspring			
		Dose (mg/kg):			
		0	0.2	6	60
<i>At Birth</i>					
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
<i>Until Weaning</i>					
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 **b(4)**

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: _____ **b(4)**

GLP Status: Not compliant. Performed prior to establishment of GLPs.

NDA Volume:Page 62:1

METHODS

Test Article: (BD 40A)

Batch No: Lot I

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 F₀ or F₁ 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 F₀ 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 F₀ dams. Neonatal mortality was noted in the F₁ 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 F₀ animals treated at the 60 mg/kg dose level but no effects on the F₁ 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 **b(4)**
Laboratory Study No.: Not stated
Study Dates: April - November, 1977
Report Date: Not stated
Test Facility: _____ **b(4)**

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 F₀ generation females were administered the test material on gestation days 17 - 21. Animals of the F₁ generation were not dosed but could have been exposed through their mother's milk and F₂ animals were naïve.

Parameters evaluated include pregnancy rates, gestation duration, and delivery rates in the F₀ and F₁ generation. Offspring in the F₁ and F₂ 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 F₀ and F₁ generation. There were no treatment related findings in visceral or skeletal malformations or variations offspring in the F₁ and F₂ generations.

Male pups in the F₁ 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 F₁ generation.

Effects of BD40A on F₁ Offspring

	Dose (mg/kg):	0	0.2	6	30
<i>At Birth</i>					
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					
	Total	275	288	253	230
	Mean (SD)	13.8 (2.10)	14.4 (1.79)	12.7 (3.44)	11.5 (4.77)

	Dose (mg/kg):	0	0.2	6	30
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**
<i>Until Weaning</i>					
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 **b(4)**

Laboratory Study No.: Not stated

Study Dates: October 1976 - January 1977

Report Date: July 1982
 Test Facility: _____

b(4)

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 (S₁ and S₂) of 15 pregnant rats at a dose of 6 mg/kg and a volume of 5 ml/kg. Two additional groups (C₁ and C₂) 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: C₁ dams fostered pups from C₂; C₂ dams fostered pups from S₁; S₁ dams fostered pups from S₂; and S₂ dams fostered pups from C₁.

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 F₁ 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)
<i>No. of Survivors Until Day 35 After Birth</i>				

Dose Group:		Control (C1)	Control (C2)	6 mg/kg (S1)	6 mg/kg (S2)
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 exposed *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	b(4)
Sponsor Study No.:	— D-7-1	b(4)
Study Dates:	Not stated	
Report Date:	October 27, 1981	
Test Facility:	_____	b(4)

b(4)

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 subtilis*
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) **b(4)**

Study Dates: August 9, 1982 - June 10, 1983

Report Date: July 18, 1983

Test Facility: _____

_____ **b(4)**

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) **b(4)**

Batch No: L-9

Purity: _____

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

Strain	Without S9 Activation	With S9 Activation	
	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

b(4)

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. DNA Repair Test on Rat Hepatocytes

b(4)

BACKGROUND INFORMATION

Study Title: _____ ; 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 (_____)
Basle, Switzerland
GLP Status: Not stated
NDA Volume:Page 63:170

b(4)

b(4)

METHODS*Test Materials*

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

b(4)

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.

b(4)

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

b(4)

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

b(4)

b(4)

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 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 (<i>In vitro</i> 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-acetylaminofluorene (50 and 100 µg/ml)
Metabolic Activation System:	Rat liver S9

Test System

Assay System:	Mouse embryo fibroblasts (BALB/3T3)
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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 (—) **b(4)**
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: _____

b(4)

GLP Status: Non-compliant; deficiencies identified

NDA Volume:Page 63:244

METHODS**Test Materials**

Test Articles: Formoterol fumarate (BD 40A)
Batch No: L-9 **b(4)**
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 LD₅. 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 **b(4)**
 Purity: _____
 Negative Control: Carboxymethylcellulose (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 48 µg requested in 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

Dose (mg/kg)	Part 1		Part 2	
	Dose (mg/kg)	Sacrifice Interval (hours)	Dose (mg/kg)	Sacrifice Interval (hours)
100	100	16	25	24
100	100	24	50	24
100	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 **b(4)**

Batch No: 810187

Purity: _____

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 pigs	sensitization and challenge phases	No dermal sensitization was noted	840421
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 6 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 Aeoliser™ oral inhalation device. The maximum daily dose is 48 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-adrenoceptor agonist pharmacologic class.

Fomoterol fumarate provides therapeutic benefit by relieving and preventing bronchoconstriction by relaxing airway smooth muscle via specific interaction with beta-2-adrenoceptors. The efficacy of fomoterol at beta-2-adrenoceptors has been measured in both functional airway smooth muscle relaxation and biochemical second messenger 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 fomoterol or other beta-2-adrenoceptor 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 fomoterol appears to be comparable to that of albuterol, yet the duration of action appeared consistently greater than that of isoproterenol or albuterol. The onset of action was reported to be 1.7 ± 0.3 minutes for fomoterol, 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 fomoterol 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-adrenoceptor population coupled to ionotrophy and chronotrophy in the heart."

b(4).

The Sponsor also addressed binding, potency, and selectivity of the RR and SS enantiomers of Formoterol, 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-adrenoceptor 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 $\mu\text{g}/\text{kg}/\text{day}$. 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 $\mu\text{g}/\text{kg}/\text{day}$. 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 $\mu\text{g}/\text{kg}/\text{day}$ (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 48 µg/day maximum human dose appears to be within an acceptable safety margin for cardiotoxicity.

b(4)

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/m² 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	Rat	Oral	6	36	1000	Stillbirth and neonatal death

Assay	Species	Route	Dose (mg/kg)	Dose (mg/m ²)	Dose Ratio for Adults	Effect
Developmental Toxicology						
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

Group	Reference	
	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 C_{max} 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, C_{max}, 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.

Tracey Zoetis, M.S.
Pharmacology/Toxicology Reviewer

Hilary Sheevers, Ph.D.
Team Leader

Original NDA 20,831

cc HFD-570/Division File
HFD-570/H. Sheevers
HFD-570/P. Jani
HFD-570/T. Zoetis

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Appendix 2

Review #01 of IND ~~_____~~ dated May 7, 2002

16 Page(s) Withheld

 Trade Secret / Confidential (b4)

 Draft Labeling (b4)

 Draft Labeling (b5)

 Deliberative Process (b5)

Withheld Track Number: Pharm/Tox- 4

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Timothy Robison

5/7/02 02:36:45 PM

PHARMACOLOGIST

Comment to sponsor for conducting a 2-week bridging toxicology
study in rats.

Robin Huff

5/8/02 04:55:28 PM

PHARMACOLOGIST

I concur.

Appendix 3

Review #01 of IND 68,782 dated January 2, 2004

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 68,782

Review number: #01

Sequence number/date/type of submission:

#000/December 15, 2003/Initial Submission

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Dey, L.P.
2751 Napa Valley Corporate Drive
Napa, CA 94558

Manufacturer and supplier for drug substance: See IND 64,525

Reviewer name: Timothy W. Robison, Ph.D.

Division name: Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: January 2, 2004

Drug:

Trade name:

Generic name (list alphabetically): Formoterol fumarate dihydrate

Code name:

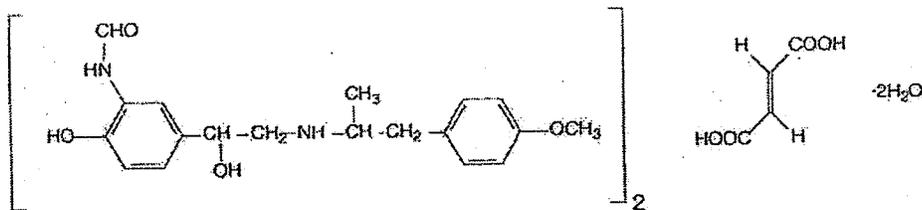
Chemical name: \pm 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl] formanilide fumarate dihydrate

CAS registry number:

Mole file number:

Molecular formula/molecular weight: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$ / 840.92

Structure:



Relevant INDs/NDA/DMFs:

IND:

NDA 20-831 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

NDA 21-279 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

Drug class: β_2 -Adrenergic Agonist

Indication: Chronic obstructive pulmonary disease (COPD)

Clinical formulation: See IND _____

b(4)

Route of administration: Inhalation (using a nebulizer)

b(4)

Proposed clinical protocol: Under IND _____, the sponsor initiated clinical trials, DL-052, DL-056, and DL-057. The results from these studies will present safety information for formoterol inhalation solution and establish the dose to be used in the pivotal trial. The pivotal trial is a randomized, 12-week, double-blind, parallel-group, placebo- and active-controlled design to evaluate the efficacy and safety of formoterol inhalation solution (XX mg) in COPD patients. This will be followed by an optional 40-week open label safety extension. Approximately 625 COPD patients will be enrolled in this pivotal trial.

DL-052: A randomized, double-blind, double-dummy, placebo-controlled, 5-way crossover trial to compare dose responses between single doses of Formoterol inhalation solution (42 and 84 µg) and Foradil® Aerolizer® (12 and 24 µg) in COPD patients (male and female ≥50 years old). There will be a minimum of 2 to 7 days between each treatment. This trial consists of 35 patients. Formoterol inhalation solution administered at single doses of 42 and 84 µg produced comparable effects as compared to Foradil® Aerolizer® at 12 µg. Dey presented this information at an EOP2 meeting with the Division on May 13, 2003. Based upon discussions with Division, Dey initiated DL-056 and DL-057.

DL-056: A randomized, open-label, 4-way crossover trial to evaluate and compare the pharmacokinetics of formoterol inhalation solution (10, 20, 244 µg) and Foradil® Aerolizer® (12 µg) in COPD patients (male and female ≥50 years old). There will be a minimum of 5 days between each treatment. This trial consists of 16 patients.

DL-057: A randomized, double-blind, placebo-controlled, 7-way crossover, dose-finding study to evaluate doses of formoterol inhalation solution (2.5, 5, 10, 20, and 40 µg) and identify the lowest dose that is comparable to Foradil® Aerolizer® at 12 µg in COPD patients (male and female ≥50 years old). There will be a minimum of 3 to 8 days between each treatment. This trial consists of 49 patients.

Previous clinical experience: Foradil® (formoterol fumarate) Aerolizer™, manufactured by Novartis Pharmaceuticals Corporation, is an approved drug product for the treatment of asthma and chronic obstructive pulmonary disease.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: The sponsor opened IND _____ for formoterol fumarate inhalation solution on _____. This investigational drug product was proposed for treatment of _____ and chronic obstructive pulmonary disease (COPD) as well as prevention of _____. The sponsor has opened the present application for the administrative purpose of transferring COPD patients receiving formoterol inhalation solution to a separate IND.

b(4)

Studies reviewed within this submission: None.

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PHARMACOLOGY/TOXICOLOGY REVIEW

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

The sponsor is relying upon preclinical and clinical experience with Foradil® Aerolizer™ (NDA 20-831 and NDA 21-279) to support their clinical trials with formoterol fumarate inhalation solution.

Reviews of preclinical toxicology studies submitted to NDA 20-831, which can be obtained through the Freedom of Information Act, were referenced to evaluate the safety of the sponsor's proposed clinical trials (see Review #01 of IND ~~_____~~). Based upon inhalation toxicology studies with rats in which formoterol was administered as a dry powder formulation for periods ranging from 1 to 12 months, target organs of toxicity were the testes, spleen, salivary gland, nasal cavity, lung, and heart. From a preclinical standpoint, inhaled clinical doses of 42 and 84 µg/day are supported for periods up to 28 days (see table below). The highest clinical doses supported for periods up to 28 days are ≤11.5 mg/kg/day (i.e., ≤575 µg/50 kg). The highest clinical dose supported for periods >28 days is 42 µg/day (21 µg BID), although, the safety margin (i.e., 3.6) is less than that normally desired (i.e., approximately ≥10).

b(4)

A NOAEL was not established in the 4-week inhalation toxicology study with dogs, although, clinical development of formoterol was allowed to proceed despite adverse findings. Toxic effects in dogs were attributed primarily to increased heart rate. In a clinical setting, heart rate can be monitored.

Safety margins for clinical doses of formoterol fumarate inhalation solution in adult subjects.

Species	Study Duration	Doses (Deposited Dose) µg/kg/day	NOAEL (Deposited Dose) µg/kg/day	Safety margins for clinical doses of formoterol fumarate inhalation solution	
				42µg/50kg = 0.84	84µg/50kg = 1.68
Rat	28-days Dry powder	16, 50, & 115.3	115.3	137.3	68.6
	3-months Dry powder	M: 0.25, 0.8, & 2.6 F: 0.4, 1.2, & 3.9	2.6/3.9	3.1/4.6	1.5/2.3
	6/12-months Dry powder	3, 12, & 40	3	3.6	1.8
Dog	4-Weeks Dry powder	0.6, 2.8, & 11	None ^a	-	-

a. A NOAEL was not established in the 4-week inhalation toxicology study with formoterol in dogs based upon findings of increased heart rate and myocardial fibrosis at all doses.

Proposed clinical doses of formoterol fumarate inhalation solution in COPD patients in current trials appeared to be supported by available preclinical data.

Recommendations: From a preclinical standpoint, the proposed clinical trials (in progress) appear reasonably safe to proceed.

For clinical trials with durations >28 days, the highest clinical dose, supported by preclinical data, is 42 µg/day (21 µg BID).

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Joseph Sun, Ph.D.

Non-Concurrence - _____
(see memo attached)

cc: list:
IND 68,782 Division File, HFD-570
GreenA, HFD-570
AnthraciteR, HFD-570
SunC, HFD-570
RobisonT, HFD-570

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Timothy Robison
1/2/04 02:58:28 PM
PHARMACOLOGIST

Joseph Sun
1/2/04 04:27:40 PM
PHARMACOLOGIST
I concur.

Appendix 4

Review #02 of IND 68,782 dated May 13, 2004

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 68,782

Review number: #02

Sequence number/date/type of submission: #003/April 21, 2004/Amendment

Information to sponsor: Yes (X) No ()

Sponsor and/or agent: Dey, L.P.
2751 Napa Valley Corporate Drive
Napa, CA 94558

Manufacturer and supplier for drug substance: See IND

Reviewer name: Timothy W. Robison, Ph.D.

Division name: Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: May 13, 2004

Drug:

Trade name:

Generic name (list alphabetically): Formoterol fumarate dihydrate

Code name:

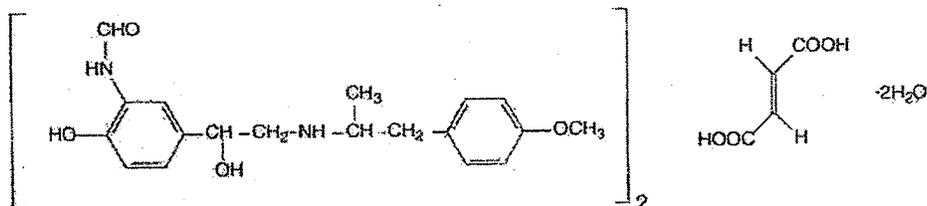
Chemical name: \pm 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl] formanilide fumarate dihydrate

CAS registry number:

Mole file number:

Molecular formula/molecular weight: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$ / 840.92

Structure:



Relevant INDs/NDAs/DMFs:

IND

NDA 20-831 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

NDA 21-279 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

Drug class: β_2 -Adrenergic Agonist

Indication: Chronic obstructive pulmonary disease (COPD)

Clinical formulation: See IND

b(4)

b(4)

Route of administration: Inhalation (using a nebulizer)

Proposed clinical protocol:

A 12-Week Double-Blind, Parallel-Group, Placebo- and Active-Controlled Trial to Evaluate the Efficacy and Safety of Formoterol Fumarate Inhalation Solution 20 µg in the Treatment of Patients with Chronic Obstructive Pulmonary Disease (COPD), followed by a 40-Week Open-Label Safety Extension.

The sponsor has proposed a 12-week double-blind, parallel group, placebo- and active-controlled trial to evaluate the efficacy and safety of formoterol fumarate inhalation solution in COPD patients (male and female subjects >40 years of age). Subjects will receive one of three treatments for 12 weeks as shown in the table below. Approximately 690 COPD patients will be randomized in a 2: 2: 1 ratio as follows: 276 patients to the formoterol fumarate inhalation solution 20 µg BID group, 276 patient to the Foradil Aerolizer 12 µg BID group, and 138 patients to the placebo group. After completing the 12-week double-blind period, patients will enter a 40-week open-label extension period and receive either formoterol fumarate inhalation solution 20 µg BID or Foradil Aerolizer 12 µg BID. Accounting for patient attrition, safety data for formoterol fumarate inhalation solution will be available for approximately 300 patients treated for 6 months and 100 patients treated for 12 months. The primary efficacy variable will be the standardized absolute AUC_{0-12hr} for FEV_1 measured over a period of 12 hr following the morning dose of study medication at week 12.

Treatment	Dose
Placebo Aerolizer + Formoterol Fumarate Inhalation Solution	20 µg Formoterol fumarate twice daily
Foradil Aerolizer + Placebo Inhalation Solution	12 µg Formoterol fumarate twice daily
Placebo Aerolizer + Placebo Inhalation Solution	Twice daily

Women of childbearing potential must have a negative serum pregnancy test at screening. These women must agree to avoid becoming pregnant for the duration of the 1-year study by using adequate contraception at study entry and throughout the trial.

Previous clinical experience: Foradil® (formoterol fumarate) Aerolizer™, manufactured by Novartis Pharmaceuticals Corporation, is an approved drug product for the treatment of asthma and chronic obstructive pulmonary disease.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and drug history: The sponsor opened IND _____ for formoterol fumarate inhalation solution on _____. This investigational drug product was proposed for treatment of _____ and chronic obstructive pulmonary disease (COPD) as well as prevention of _____. The sponsor has opened the present application for the administrative purpose of transferring COPD patients receiving formoterol inhalation solution to a separate IND.

b(4)

The following comment was communicated to the sponsor after the review of the initial submission for IND _____, (see Review #01 dated _____).

The proposed drug product involves a change from the approved dry powder capsule (i.e., Foradil® Aerolizer™) to an inhalation solution. This may lead to differences in product performance; therefore, conduct a 2-week inhalation bridging toxicology study to determine if there are differences in toxicity profile between Foradil® and the formoterol fumarate inhalation solution. Given that primary concerns are with local toxicity, this study should be conducted in rats, since it is possible to administer greater inhaled doses of formoterol fumarate to rats than dogs. The study report should be submitted to the Division prior to the start of the 12-week repeat dose trials.

In the present submission, the sponsor provided a draft final report of the 14-day inhalation study with rats.

Studies reviewed within this submission:

1. 4-day inhalation toxicology study with rats.
2. 14-day inhalation toxicology study with rats.

Studies not reviewed within this submission: None.

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PHARMACOLOGY/TOXICOLOGY REVIEW

IV. GENERAL TOXICOLOGY:

Rat

Study title: Maximum Tolerated Dose-Range-Finding Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate in Rats (Draft Final Report).

Key study findings:

▶ A 4-day inhalation toxicology study, male rats were exposed to formoterol fumarate inhalation solution at total doses of 166, 499, and 1673 µg/kg/day or formoterol fumarate dry powder at total doses of 522 and 1692 µg/kg/day. Deposited doses of formoterol fumarate inhalation solution were 11.6, 34.9, and 117.1 µg/kg/day, respectively. Deposited doses of formoterol fumarate dry powder were 36.5 and 118.4 µg/kg/day, respectively.

▶ There were no treatment-related clinical signs, effects on body weight gain, serum Tropinin T levels, or target organs of toxicity. There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and formoterol fumarate dry powder.

▶ Dose selections for formoterol fumarate inhalation solution and formoterol fumarate dry powder were considered adequate. The high dose of formoterol fumarate inhalation solution was limited by the aqueous solubility of formoterol fumarate. The high dose of formoterol fumarate dry powder was approximately equivalent to the high dose used with innovator product.

Study no: — Study Number N102782

Volume #, and page #: Volume 1, Pages 326-378

Conducting laboratory and location: _____

Date of study initiation: October 16, 2003 (In-life exposures began on November 17, 2003).

GLP compliance: Draft Final Report with no signatures.

QA report: yes () no (X)

Drug, lot #, radiolabel, and % purity: Formoterol fumarate (Lot # 0112011) by nebulization and Formoterol fumarate dihydrate powder (Lot #0112011).

Formulation/vehicle: The vehicle for formoterol fumarate inhalation solution (FFIS) was a solution of sterile water for injection (USP), sodium chloride (USP), sodium citrate dihydrate (USP), and citric acid monohydrate (USP).

Methods (unique aspects): A 4-day inhalation toxicology study was conducted with male rats in order to identify a maximum tolerated dose of nebulized formoterol

fumarate and to compare any toxic effects with neat formoterol fumarate dihydrate powder.

Dosing:

Species/strain: Male Sprague-Dawley (— CD[®] (SD) IGS BR) rats were obtained from

b(4)

#/sex/group or time point (main study): 5 male rats/group

Satellite groups used for toxicokinetics or recovery: There were 3 male rats/group for the Air-Control and Vehicle-Control Groups and 9 male rats/group for Formoterol fumarate-treated groups.

Age: Rats were approximately 7-8 weeks old at the start of treatment.

Weight: Mean body weights for control and treatment groups ranged from 229.3 to 236.0 g on the first day of treatment.

Doses in administered units: The liquid aerosol generation and delivery system was constructed to administer formoterol fumarate at target doses of 150, 450, and 1500 µg/kg using the maximum practical test article solution concentration of 1.2 mg/mL (e.g., the maximum solubility of formoterol fumarate was 1.5 mg/mL). A target formoterol fumarate aerosol concentration of 25 µg/L was utilized to expose animals for 12, 36, and 120 min/day, respectively. Aerosols for liquid test article systems were generated using a pair of Pari LC Plus nebulizers.

A separate dry powder generation and delivery system was utilized to administer formoterol fumarate dihydrate powder at target doses of 450 and 1500 µg/kg. A target formoterol fumarate aerosol concentration of 25 µg/L was utilized to expose animals for 36 and 120 min/day, respectively. The dose of 1500 µg/kg represents the reported high dose of formoterol dry powder with the innovator product. The aerosol generation/delivery system for the dry powder exposure system was composed of a linear powder feeder, a dry powder disperser, and a plenum.

Separate vehicle and air control systems were utilized to expose animals for 120 min/day. Aerosol for the vehicle system was generated using a Pari LC Plus nebulizer.

Aerosol concentrations were evaluated by sampling the atmospheres at the breathing zones of animals using 25 mm Glass fiber filters. The net gain of the dry filter mass was divided by the total volume of air sampled, yielding the total mass aerosol concentration. Subsets of filters were analyzed by chemical specific methods for formoterol fumarate mass content. The formoterol fumarate mass content was divided by the total volume of air sampled yielding the formoterol fumarate aerosol concentration. During in-life exposures, atmosphere samples for evaluation of aerosol concentrations were collected at least once every 30 min from the reference port for vehicle, liquid, and dry powder groups. One filter sample per 30 min was collected during air-control exposures. In addition to the filter analyses, the dry powder system was monitored with

b(4)

— Aerosol monitoring system.

Total mass and Formoterol Fumarate Inhaled Dose Estimations (Core Toxicology Animals).

Dose Group	BW, g	Min Volume L/min	Exposure Duration min	Inhaled Volume L	Total mass Aerosol Conc. µg/L	FF Aerosol Conc. µg/L	Inhaled mass µg/kg	Inhaled FF µg/kg	Deposited FF ^b µg/kg
1. Air-Control	238	0.127	120	15.3	0.0	0.0	0.0	0.0	0.0
2. Vehicle-Control ^a	236	0.126	120	15.1	285	0.0	18300	0.0	0.0
3. Low Dose - Liquid	228	0.123	12	1.5	297	25.6	1900	166	11.6
4. Mid Dose - Liquid	226	0.122	36	4.4	297	25.6	5800	499	34.9
5. High Dose - Liquid	222	0.121	120	14.5	298	25.6	19500	1673	117.1
6. Low Dose - Dry Powder	225	0.122	36	4.4	25	26.7	500	522	36.5
7. High Dose - Dry Powder	227	0.123	120	14.7	29	26.1	1900	1692	118.4

- a. Target vehicle aerosol concentration for the vehicle approximate the vehicle mass in the high dose group.
- b. A deposition factor of 7% was used to calculate deposited doses.

Total mass and Formoterol Fumarate Inhaled Dose Estimations (Toxicokinetic animals).

Dose Group	BW, g	Min Volume L/min	Exposure Duration min	Inhaled Volume L	Total mass Aerosol Conc. µg/L	FF Aerosol Conc. µg/L	Inhaled mass µg/kg	Inhaled FF µg/kg	Deposited FF ^a µg/kg
1. Air-Control	247	0.131	120	15.7	0.0	0.0	0.0	0	0
2. Vehicle-Control	250	0.132	120	15.8	285	0.0	18100	0	0
3. Low Dose - Liquid	254	0.133	12	1.6	297	25.6	1900	162	11.3
4. Mid Dose - Liquid	255	0.134	36	4.8	297	25.6	5600	484	33.9
5. High Dose - Liquid	255	0.134	120	16.1	298	25.6	18800	1617	113.2
6. Low Dose - Dry Powder	250	0.132	36	4.8	25	26.7	500	508	35.6
7. High Dose - Dry Powder	258	0.135	120	16.2	29	26.1	1800	1639	114.7

- a. Target vehicle aerosol concentration for the vehicle approximate the vehicle mass in the high dose group.
- b. A deposition factor of 7% was used to calculate deposited doses.

Formoterol fumarate dose estimates:

1. Calculated minute volume (mL/min) = 2.1 x body weight (g)^{0.75}

2. Inhaled volume (L/day) = Calculate minute volume (L/min) x Exposure duration (min/day)
3. Inhaled formoterol fumarate (mg/day) = mean aerosol concentration (mg/L) x accumulate inhaled volume (L/day)
4. Inhaled formoterol fumarate dose (mg/kg/day) = inhaled formoterol fumarate (mg/day)/BW (kg)

Aerosol particle size distributions for the liquid and vehicle dose groups were measured using an _____ . Aerosol particle size distributions for dry powder dose groups were measured using a _____ . The relationship in the stage masses was used to evaluate the aerosol particle distribution by calculating the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). All impactor stage filters (except air- and vehicle-control samples) were analyzed by chemical specific methods for formoterol fumarate mass content. Particle size distributions were characterized with both total gravimetric and chemical data whenever possible.

b(4)

Particle size distribution

Exposure system	Gravimetric		Chemical	
	MMAD (µm)	GSD	MMAD (µm)	GSD
Vehicle				
Liquid Dose				
Dry Powder				

b(4)

Route, form, volume, and infusion rate: Nose-only inhalation exposure. Separate exposure systems were utilized for air-control, vehicle, liquid exposures, and dry powder groups. Exposure tubes containing animals were fastened to inhalation chambers with the nose portion of the tube protruding through a gasket into the chamber.

Observations and times:

Clinical signs: Clinical observations were conducted at least once daily approximately 1-3 hr after each exposure throughout the exposure phase of the study.

Body weights: Body weights were measured on days 1 and 4. Body weights were also measured at necropsy (day 5) for core toxicology study animals.

Food consumption: Not measured.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Not performed.

Clinical chemistry: Blood samples for measurement of serum Troponin T levels were collected on day 5.

Urinalysis: Not performed.

Gross pathology: Rats in core toxicology study groups were sacrificed and submitted to necropsy examinations. Collection of tissues was limited to gross lesions, esophagus, heart, larynx (3 levels), lungs with bronchi, nasal cavities and turbinates, tongue, and trachea.

Organs weighed: Not performed.

Histopathology: Histopathological examination of tissues was limited to the larynx, trachea, lung, and heart.

Toxicokinetics: Blood samples for measurement of plasma formoterol levels were collected on day 4 at 0.083, 0.5, 1, 3, 5, and 24 hr after dosing. Three rats/group/time point were used. Two blood samples were obtained from each rat. Rats were sacrificed and discarded without examination after collection of the second blood sample.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs.

Body weights: There were no treatment-related effects on body weight gain during the 4-day treatment period.

Body weight gains for male rats in vehicle-control, low dose FFIS (formoterol fumarate inhalation solution), mid dose FFIS, high dose FFIS, low dose FFD (formoterol fumarate dry powder), and high dose FFD groups were 101.2, 141.1, 131.9, 105, 119.4, and 105.5% of the air-control, respectively. Body weight gains were slightly increased for rats in the low and mid dose formoterol fumarate inhalation solution groups and rats in the low dose formoterol fumarate dry powder group, possibly due to shorter inhalation exposure times and pharmacological effects of the β_2 agonist, formoterol.

Clinical chemistry: The sponsor reported that serum Troponin T levels were below the limit of detection (0.01 ng/mL) for all animals except #702 in the high dose formoterol dry powder group with a level of 0.053 ng/mL. The sponsor contended that this value might be spurious since there were no histopathological findings of cardiomyopathy for this animal. No data was provided for independent verification. Given that cardiomyopathy was observed in several animals from control and treatment groups (see Histopathology), Troponin T may be a poor biomarker of heart damage.

Gross pathology: There were no treatment-related gross pathological findings.

Histopathology: Histopathological examination of tissues was limited to the larynx, trachea, lung, and heart. There were no target organs of toxicity in this limited examination. Cardiomyopathy was observed in all groups and graded as minimal for all animals except #705 in high dose formoterol fumarate dry powder group, which was graded as mild. Cardiomyopathy was subacute to chronic in all rats and was judged to be a normal background finding in this rat strain. For one animal in the high dose formoterol fumarate dry powder group, there were findings of a minimal neutrophilic laryngeal inflammatory lesion. Given the low incidence of this finding, a relationship to treatment was unclear. There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and formoterol fumarate dry powder.

Histopathological findings

Organ/Tissue	Air-Control	Vehicle-Control	Formoterol fumarate inhalation solution			Formoterol fumarate dry powder	
Heart -cardiomyopathy	4/5	3/5	2/5	1/5	5/6	3/5	4/5
Larynx -inflammation	0/5	0/5	0/5	0/5	0/6	0/5	1/5

Toxicokinetics: Detection of plasma formoterol levels were sufficiently complete in Group 5 (high dose formoterol fumarate inhalation solution group) and 7 (high dose formoterol fumarate dry powder group) to permit toxicokinetic evaluation. For other groups, plasma concentrations of formoterol at most time points were below the limit of quantitation (BLOQ) and apparently did not permit toxicokinetic evaluation.

The only data provided in the report were plasma drug concentrations at 5 min after dosing. $C_{5 \text{ min}}$ values were approximately dose proportional for formoterol fumarate inhalation solution and dry powder groups.

Plasma formoterol concentrations at 5 min after dosing.

Group	Mean Observed $C_{5 \text{ min}}$, ng/mL
1. Air-Control	BLOQ
2. Vehicle-Control	BLOQ
3. Low Dose-Formoterol fumarate inhalation solution	0.82
4. Mid Dose-Formoterol fumarate inhalation solution	2.2
5. High Dose-Formoterol fumarate inhalation solution	5.6
6. Low Dose-Formoterol fumarate dry powder	3.8
7. High Dose-Formoterol fumarate dry powder	8.4

Study title: 14-Day Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate Dihydrate in Rats (Draft Final Report).

Key study findings:

► In a 14-day inhalation toxicology study, 5 rats/sex/group were exposed to formoterol fumarate inhalation solution (FFIS) at total doses of 195, 584, and 1966 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder (FFD) at a total dose of 1966 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of FFIS were 13.7, 40.9, and 137.7 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The deposited dose of FFD was 121.5 $\mu\text{g}/\text{kg}/\text{day}$.

► There were no treatment-related effects on clinical signs, hematology, or clinical chemistry parameters. Body weight gain and food consumption were increased for formoterol treatment groups, which is an expected effect of B_2 agonists.

► Absolute and relative heart weights were increased for males in the mid dose FFIS, high dose FFIS, and high dose FFD groups and females in the high dose FFIS and high dose FFD groups.

► Histopathological evaluation of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates. These organs and tissues were previously identified as target organs in inhalation toxicology studies conducted with formoterol in rats.

► For the heart, cardiomyopathy was observed was observed for 1 of 5 male rats in the high dose FFIS group. Myocardial fibrosis was previously reported in inhalation toxicology studies conducted with formoterol in rats.

► There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and formoterol fumarate dry powder.

Study no: / _____ Study Number N102783
Volume #, and page #: Volume 1, Pages 379-452
Conducting laboratory and location: _____

b(4)

Date of study initiation: December 16, 2003 (In-life exposures began on January 12, 2004)

GLP compliance: No. Draft Final Report with no signatures. This study report did not contain toxicokinetic data.

QA report: yes () no (X)

Drug, lot #, radiolabel, and % purity: Formoterol fumarate was supplied by Dey L.P. The test article was received as a dry powder identified as Formoterol Fumarate Dihydrate (_____), which was utilized for solution formulation with the vehicle and the dry powder for inhalation aerosol formulation. The lot number 0112011.

Formulation/vehicle: The vehicle was sterile water for injection (USP), sodium chloride (USP), sodium citrate dihydrate (USP), and citric acid monohydrate (USP).

Methods (unique aspects): In a 14-day inhalation toxicology study with 5 rats/sex/group, the comparative toxicity of nebulized formoterol fumarate solution to formoterol fumarate dihydrate powder was examined when administered to rats by nose-only inhalation.

Dosing:

Species/strain: Sprague-Dawley — CD® (SD) IGS BR) rats were obtained from _____

b(4)

#/sex/group or time point (main study): 5 rats/sex/group

Satellite groups used for toxicokinetics or recovery: There were 3 rats/sex/group for the Air-Control and Vehicle-Control Groups and 6 rats/sex/group for Formoterol fumarate-treated groups.

Age: Animals were 7-8 weeks old at the start of treatment.

Weight: Mean body weights for male and female groups at the start of treatment were 227.7-232.2 g and 167.4-170.0 g, respectively.

Doses in administered units: The liquid aerosol generation and delivery system was constructed to administer formoterol fumarate at target doses of 150, 450, and 1500 µg/kg using the maximum practical test article solution concentration of 1.2 mg/mL (e.g., the maximum solubility of formoterol fumarate was 1.5 mg/mL). A target formoterol fumarate aerosol concentration of 25 µg/L was utilized to expose animals for 12, 36,

and 120 min/day, respectively. Aerosols for liquid test article systems were generated using a pair of Pari LC Plus nebulizers.

A separate dry powder generation and delivery system was utilized to administer formoterol fumarate dihydrate powder at a target dose of 1500 µg/kg. A target formoterol fumarate aerosol concentration of 25 µg/L was utilized to expose animals for 120 min/day. The dose of 1500 µg/kg represents the reported high dose of formoterol dry powder with the innovator product. The aerosol generation/delivery system for the dry powder exposure system was composed of a linear powder feeder, a dry powder disperser, and a plenum.

Separate vehicle and air control systems were utilized to expose animals for 120 min/day. Aerosol for the vehicle system was generated using a Pari LC Plus nebulizer.

Aerosol concentrations were evaluated by sampling the atmospheres at the breathing zones of animals using 25 mm Glass fiber filters. The net gain of the dry filter mass was divided by the total volume of air sampled, yielding the total mass aerosol concentration. Subsets of filters were analyzed by chemical specific methods for formoterol fumarate mass content. The formoterol fumarate mass content was divided by the total volume of air sampled yielding the formoterol fumarate aerosol concentration. During in-life exposures, atmosphere samples for evaluation of aerosol concentrations were collected at least once every 30 min from the reference port for vehicle, liquid, and dry powder groups. One filter sample per 30 min was collected during air-control exposures. In addition to the filter analyses, the dry powder system was monitored with (), Aerosol monitoring system. b(4)

Total mass and Formoterol Fumarate Inhaled Dose Estimations (Core Toxicology Animals; Males/Females).

Dose Group	BW, g	Min Volume L/min	Exposure Duration min	Inhaled Volume L	Total mass Aerosol Conc. µg/L	FF Aerosol Conc. µg/L	Inhaled mass µg/kg	Inhaled FF µg/kg	Deposited FF ^b µg/kg
1. Air-Control	261	0.136	120	16	0.0	0.0	0.0	0.0	0.0
	187	0.106	120	13	0.0		0.0	0.0	0.0
2. Vehicle-Control ^a	262	0.137	120	16	265	0.0	16600	0	0.0
	188	0.107	120	13	265	0.0	18000	0	0.0
3. Low Dose - Liquid	269	0.140	12	2	362	30	2300	187	13.1
	193	0.109	12	1	362	30	2500	203	14.2
4. Mid Dose - Liquid	270	0.140	36	5	361	30	6700	559	39.1
	192	0.108	36	4	361	30	7300	609	42.6
5. High Dose - Liquid	267	0.139	120	17	364	30.2	22700	1887	132.1
	193	0.109	120	13	364	30.2	24600	2045	143.2
6. High Dose - Dry Powder	268	0.139	120	17	27	26.8	1700	1668	116.8
	197	0.110	120	13	27	26.8	1800	1801	126.1

- a. Target vehicle aerosol concentration for the vehicle approximate the vehicle mass in the high dose group.
- b. A deposition factor of 7% was used to calculate deposited doses.

Total mass and Formoterol Fumarate Inhaled Dose Estimations (Toxicokinetic animals; Males/Females).

Dose Group	BW, g	Min Volume L/min	Exposure Duration min	Inhaled Volume L	Total mass Aerosol Conc. µg/L	FF Aerosol Conc. µg/L	Inhaled mass µg/kg	Inhaled FF µg/kg	Deposited FF ^a µg/kg
1. Air-Control	261	0.136	120	16	0.0	0.0	0.0	0.0	0.0
	187	0.106	120	13	0.0	0.0	0.0	0.0	0.0
2. Vehicle-Control	262	0.137	120	16	268	0.0	16800	0.0	0.0
	188	0.107	120	13	268	0.0	18200	0.0	0.0
3. Low Dose - Liquid	269	0.140	12	2	325	27.0	2000	168	11.8
	193	0.109	12	1	325	27.0	2200	182	12.7
4. Mid Dose - Liquid	270	0.140	36	5	360	29.9	6700	558	39.1
	192	0.108	36	4	360	29.9	7300	608	42.6
5. High Dose - Liquid	267	0.139	120	17	363	30.1	22600	1877	131.4
	193	0.109	120	13	363	30.1	24500	2035	142.5
6. High Dose - Dry Powder	268	0.139	120	17	27	26.9	1700	1679	117.5
	197	0.110	120	13	27	26.9	1800	1813	126.9

- a. Target vehicle aerosol concentration for the vehicle approximate the vehicle mass in the high dose group.
- b. A deposition factor of 7% was used to calculate deposited doses.

Formoterol fumarate dose estimates:

1. Calculated minute volume (mL/min) = 2.1 x body weight (g)^{0.75}
2. Inhaled volume (L/day) = Calculate minute volume (L/min) x Exposure duration (min/day)
3. Inhaled formoterol fumarate (mg/day) = mean aerosol concentration (mg/L) x accumulate inhaled volume (L/day)
4. Inhaled formoterol fumarate dose (mg/kg/day) = inhaled formoterol fumarate (mg/day)/BW (kg)

Aerosol particle size distributions for the liquid and vehicle dose groups were measured using an _____ . Aerosol particle size distributions for dry powder dose groups were measured using a _____

The relationship in the stage masses was used to evaluate the aerosol particle distribution by calculating the mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). All impactor stage filters (except air- and vehicle-control samples) were analyzed by chemical specific methods for formoterol fumarate mass content. Particle size distributions were characterized with both total gravimetric and chemical data whenever possible.

Particle size distribution

Exposure system	Gravimetric		Chemical	
	MMAD (µm)	GSD	MMAD (µm)	GSD
Vehicle				
Liquid Dose				
Dry Powder				

b(4)

b(4)

Route, form, volume, and infusion rate: Nose-only inhalation exposure. Separate exposure systems were utilized for air-control, vehicle, liquid exposures, and dry powder groups. Exposure tubes containing animals were fastened to inhalation chambers with the nose portion of the tube protruding through a gasket into the chamber.

Observations and times:

Clinical signs: Animals were observed twice daily for moribundity/mortality during the study. Observations for clinical signs of toxicity were conducted 1-3 hr after each exposure throughout the treatment phase of the study.

Body weights: Body weights were measured on days 1, 3, 6, 9, and 14. Fasted body weights were measured at necropsy on day 15.

Food consumption: Food consumption was measured on days 1, 3, 6, 9, and 14.

Ophthalmoscopy: Not performed.

EKG: Not performed.

Hematology: Blood samples for measurement of hematology parameters were collected on day 15.

Clinical chemistry: Blood samples for measurement of clinical chemistry parameters were collected on day 15.

Urinalysis: Not performed.

Gross pathology: Rats were sacrificed on day 15 and submitted to necropsy examination.

Organs weighed: Absolute and relative organ weights were determined for adrenal glands, brain, heart, kidneys, liver, lung, testes, and thymus.

Histopathology: Histopathological examination of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates.

Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected on day 14 at 5, 30, 60, and 90 min post-exposure. Toxicokinetic data was not provided in the Draft Final Report.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical signs. One female in the mid dose FFIS group was observed to hyperactive from days 5 to 7.

Body weights: Body weight gains were increased for formoterol fumarate-treated groups, which was attributed to the pharmacological action of this β_2 agonist.

Body weight gains for the male rats in the vehicle control, low dose FFIS (formoterol fumarate inhalation solution), mid dose FFIS, high dose FFIS, and FFD (formoterol fumarate dry powder) groups were 113.7, 138, 141.9, 137.5, and 132.3% of the air-control, respectively. Body weight gains for the female rats in the vehicle control, low dose FFIS, mid dose FFIS, high dose FFIS, and FFD groups were 109.3, 140.1, 138.1, 139.1, and 145.4% of the air-control, respectively.

Food consumption: Food consumption was slightly increased in formoterol treatment groups. This slight effect might be attributed to the pharmacological action of formoterol.

Food consumption (g/day), mean of days 3, 6, 9, and 14.

Group	Males	Females
Air-control	21.425	16.275
Vehicle-control	22.125 (103.3%)	16.425 (100.9%)
Low dose, FFIS	22.45 (104.8%)	17.425 (107.1%)
Mid dose, FFIS	23.35 (109%)	17.175 (105.5%)
High dose, FFIS	22.30 (104.1%)	17.425 (107.1%)
High dose, FFD	23.125 (107.9%)	18.05 (110.9%)

Hematology: There were some minor changes of reticulocyte percentage and platelet counts that appeared to have little or no toxicological significance.

Reticulocyte percentage for male rats in the low dose FFIS, mid dose FFIS, high dose FFIS, and high dose FFD groups were increased to 125, 137.5, 137.5, and 125% of the air-control (2.4%), respectively. Platelet counts for female rats in the low dose FFIS, mid dose FFIS, high dose FFIS, and high dose FFD groups were increased to 94.7, 88.9, 87.2, and 85.6% of the control ($1217 \times 10^3/\mu\text{L}$), respectively.

Clinical chemistry: There were some minor changes of clinical chemistry parameters (i.e., ALT, ALP, AST, and BUN) that appeared to have little or no toxicological significance.

ALT activities were slightly elevated for males in the high dose FFIS and high dose FFD groups and all female treatment groups. AST activities were slightly elevated for females in the high dose FFIS and high dose FFD groups. AST activities were elevated for females in the high dose FFIS and high dose FFD groups.

Clinical chemistry parameters

Tissue/Organ	Males						Females					
	AC	VC	L D	MD	HD	DP	AC	VC	LD	MD	HD	DP
Alanine transferase, U/L	38	39	40	42	45 (118%)	46 (121%)	29	29	35 (121%)	35 (121%)	36 (124%)	41 (141%)
Alkaline phosphatase, U/L							11 .8	140 (119%)	139 (118%)	121 (103%)	149 (126%)	156* (132%)
Aspartate aminotransferase, U/L							73	76	75	74	90 (123%)	84 (115%)
BUN mg/dL							14	16 (114%)	17 (121%)	19* (136%)	19* (136%)	19* (136%)

AC = Air-Control; VC = Vehicle-Control, LD = Low Dose Formoterol fumarate inhalation solution, MD = Mid Dose Formoterol fumarate inhalation solution, HD = High Dose Formoterol fumarate inhalation solution, and DP = Dry Powder.

Organ weights: Several differences in organ weights between controls and treatment groups were observed; however, there were no corresponding histopathological changes. Absolute and relative heart weights were increased for males in the mid dose

FFIS, high dose FFIS, and high dose FFD groups and females in the high dose FFIS and high dose FFD groups. Absolute and relative kidney weights were decreased for females in the high dose FFD group. Relative lung weights were decreased for females in the high dose FFD group. Relative liver weights were decreased for females in the high dose FFD group. Relative thymus weights were decreased for female treatment groups.

Organ weights

	Males						Females					
	AC	VC	LD	MD	HD	DP	AC	VC	LD	MD	HD	DP
Heart g	1.191	1.108	1.240 (104%)	1.311 (110%)	1.389 (117%)	1.384 (116%)	0.900	0.831	0.894	0.915 (102%)	0.958 (106%)	0.981 (109%)
Heart %BW	0.434	0.408	0.406	0.425	0.457 (105)	0.471 (109%)						
Heart %Br.W	65.00	58.79	68.08	70.01	74.59 (115%)	77.15 (119%)	52.27	49.39	50.63	51.67	54.36 (104%)	56.38 (108%)
Kidneys g							1.552	1.528	1.519	1.570	1.507	1.374 (89%)
Kidneys %BW	0.822	0.827	0.694* (84%)	0.735* (89%)	0.696* (85%)	0.712* (86%)	0.800	0.762 (95%)	0.731 (91%)	0.747 (93%)	0.730 (91%)	0.668* (84%)
Lung g	1.722	1.628	1.982 (115)	2.080 (121%)	2.056 (119%)	1.749						
Lung %BW							0.817	0.674	0.710	0.705	0.728	0.647 (79%)
Lung %Br.W							92.78	79.95 (86%)	84.25 (91%)	83.79 (90%)	85.64 (92%)	76.98 (83%)
Liver g							6.119	6.091	6.010	5.813	6.052	5.249 (86%)
Liver %BW	3.212	3.063	2.762 (86%)	2.877 (90%)	2.621* (82%)	2.686 (84%)	3.139	3.022	2.892	2.772	2.919	2.536* (81%)
Liver %Br.W							354.26	362.20	340.62	328.92	343.23	302.06 (85%)
Thymus g	0.499	0.468	0.547 (110%)	0.557 (112%)	0.557 (112%)	0.497						
Thymus %BW							0.253	0.239	0.213 (84%)	0.237 (94%)	0.221 (87%)	0.222 (88%)

AC = Air-Control; VC = Vehicle-Control, LD = Low Dose Formoterol fumarate inhalation solution, MD = Mid Dose Formoterol fumarate inhalation solution, HD = High Dose Formoterol fumarate inhalation solution, and DP = Dry Powder.

Gross pathology: A pale splenic nodule was observed in animal #551 (female 1500 µg/kg/day nebulized FFIS). Histopathological evaluation determined that it was an area of granulomatous inflammation, of uncertain etiology.

Histopathology: Histopathological evaluation of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates. These organs and tissues were identified as target organs in previous toxicology studies with formoterol in rats that can be obtained through the FOI Act.

For the heart, cardiomyopathy was observed for 1 of 5 male rats in the high dose FFIS group. Myocardial fibrosis has been previously reported in inhalation toxicology studies with formoterol in rats that can be obtained through the FOI Act. The findings of cardiomyopathy with FFIS appear to be similar to findings with formoterol dry powder. The incidence and occurrence of cardiomyopathy in the present study was significantly different from that reported in the 4-day inhalation toxicology study, where it was observed in all control and treatment groups.

For the lung, interstitial inflammation was observed for 1 male in the high dose FFD group and 1 female each in the low and mid dose FFIS groups. Histiocytosis was observed for 1 female in each of the mid and high dose FFIS groups. Previous inhalation toxicology studies with formoterol in rats identified findings in the lungs of increased severity of large foamy macrophages around the bronchioles. The findings in the lungs in the present study may be spontaneous in nature and have no relation to treatment with formoterol.

For the spleen, atypical lymphocyte hyperplasia was observed for 1 male in the high dose FFIS group and pyogranulomatous inflammation was observed for 1 female in the high dose FFIS group. Previous inhalation toxicology studies with formoterol in rats identified findings in the spleen of increased incidence and/or severity of extramedullary hematopoiesis. The findings in the spleen in the present study may be spontaneous in nature and have no relation to treatment with formoterol.

There were no findings in the nose, salivary gland, testis, and trachea.

There were no apparent differences in toxic effects between formoterol fumarate inhalation solution and formoterol fumarate dry powder.

Histopathological findings (n = 5 rats/sex/group).

Organ/Tissue	Sex	AC	VC	LD	MD	HD	DP	
Heart -lymphocytic infiltrate	M	0	1	0	0	1	1	
	F	0	0	0	0	1	1	
	-cardiomyopathy	M	0	0	0	0	1	0
		F	0	0	0	0	0	0
Lung -interstitial inflammation	M	0	0	0	0	0	1	
	F	0	0	1	1	0	0	
	-histiocytosis	M	0	0	0	0	0	0
		F	0	0	0	1	1	0
Spleen -lymphocyte, hyperplasia, atypical	M	0	0	0	0	1	0	
	F	0	0	0	0	0	0	
	-inflammation, pyogranulomatous	M	0	0	0	0	0	0
		F	0	0	0	0	1	0

AC = Air-Control; VC = Vehicle-Control, LD = Low Dose Formoterol fumarate inhalation solution, MD = Mid Dose Formoterol fumarate inhalation solution, HD = High Dose Formoterol fumarate inhalation solution, and DP = Dry Powder.

Toxicokinetics: Toxicokinetic data was not provided in the present submission; however, the data shown below was provided in an e-mail from the sponsor dated April 2, 2004. From this preliminary toxicokinetic data, it appears that formoterol and desformoterol constituted approximately 75-80% and 20-25% of the total exposure, respectively, following treatment with either formoterol fumarate inhalation solution or formoterol fumarate dry powder. Exposure to formoterol and desformoterol appeared to

be approximately 2-fold higher with formoterol fumarate dry powder as compared to formoterol fumarate inhalation solution. There appeared to be no sex-related differences in exposure to formoterol or desformoterol.

Plasma Concentration of Formoterol and the Metabolite, Desformoterol, in Rats of the Formoterol Fumarate Solution and Formoterol Fumarate Dry Powder High-Dose Groups.

Time (min)	Formoterol-Solution (ng/mL)		Desformoterol Solution (ng/mL)	
	Male	Female	Male	Female
5	27.7	23.7	8.8	6
15	18.4	23.2	5.9	5.5
30	15.1	13.1	5.4	4.3
60	8.8	10.4	3.5	2.6
90	7.7	10.6	3.1	1.9
Time (min)	Formoterol-Dry Powder (ng/mL)		Desformoterol Dry Powder (ng/mL)	
	Male	Female	Male	Female
5	74.9	60.5	19	13.2
15	56.5	56.2	17.8	10.9
30	42	34.8	10.8	9.1
60	24.2	21.9	8.7	8
90	15.4	11.5	5.4	5.8

Samples were collected on Day 14 following the last exposure in the high dose Formoterol Solution and Formoterol Dry Powder groups. The inhaled doses in males and females were respectively, 1887/2045 and 1668/1801 $\mu\text{g}/\text{kg}/\text{day}$.

Toxicology summary:

A 4-day inhalation toxicology study, male rats were exposed to formoterol fumarate inhalation solution at total doses of 166, 499, and 1673 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder at total doses of 522 and 1692 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of formoterol fumarate inhalation solution were 11.6, 34.9, and 117.1 $\mu\text{g}/\text{kg}/\text{day}$, respectively. Deposited doses of formoterol fumarate dry powder were 36.5 and 118.4 $\mu\text{g}/\text{kg}/\text{day}$, respectively. There were no treatment-related clinical signs, effects on body weight gain, serum Tropinin T levels, or target organs of toxicity. Doses selections for formoterol fumarate inhalation solution and formoterol fumarate dry powder were considered adequate. The high dose of formoterol fumarate inhalation solution was limited by the aqueous solubility of formoterol fumarate. The high dose of formoterol fumarate dry powder was approximately equivalent to the high dose used with innovator product.

In a 14-day inhalation toxicology study, 5 rats/sex/group were exposed to formoterol fumarate inhalation solution (FFIS) at total doses of 195, 584, and 1966 $\mu\text{g}/\text{kg}/\text{day}$ or formoterol fumarate dry powder (FFD) at a total dose of 1966 $\mu\text{g}/\text{kg}/\text{day}$. Deposited doses of FFIS were 13.7, 40.9, and 137.7 $\mu\text{g}/\text{kg}/\text{day}$, respectively. The deposited dose of FFD was 121.5 $\mu\text{g}/\text{kg}/\text{day}$. There were no treatment-related effects on clinical signs, hematology, or clinical chemistry parameters. Body weight gain and food consumption were increased for formoterol treatment groups, which is an expected effect of B_2 agonists. Absolute and relative heart weights were increased for males in the mid dose FFIS, high dose FFIS, and high dose FFD groups and females in the high dose FFIS and high dose FFD groups. Histopathological evaluation of tissues was limited to the

larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates. These organs and tissues were previously identified as target organs in inhalation toxicology studies conducted with formoterol in rats. For the heart, cardiomyopathy was observed for 1 of 5 male rats in the high dose FFIS group. Myocardial fibrosis was previously reported in inhalation toxicology studies conducted with formoterol in rats

Toxicology conclusions: Toxic effects of FFIS were comparable to those for FFD in both the 4- and 14-day inhalation toxicology studies with rats. There were no differences in product performance between FFIS and FFD.

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Histopathology Inventory for IND # 68,782

Study	4-day inhalation ^a	14-day inhalation ^b
Species	Rat	Rat
Adrenals		X*
Aorta		X
Bone Marrow smear		
Bone (femur)		
Brain		X*
Cecum		X
Cervix		
Colon		X
Duodenum		X
Epididymis		X
Esophagus	X	X
Eye		X
Fallopian tube		
Gall bladder		
Gross lesions	X	X
Harderian gland		X
Heart	X	X*
Ileum		X
Injection site		
Jejunum		X
Kidneys		X*
Lachrymal gland		
Larynx	X (3 levels)	X (3 levels)
Liver		X*
Lungs	X	X*
Lymph nodes, cervical		
Lymph nodes mandibular		
Lymph nodes, mesenteric		X
Lymph nodes, tracheobronchial		X
Mammary Gland		X
Nasal cavity	X	X
Optic nerves		X
Ovaries		X
Pancreas		X
Parathyroid		X
Peripheral nerve		
Pharynx		
Pituitary		X

Prostate		X
Rectum		
Salivary gland		X
Sciatic nerve		X
Seminal vesicles		X
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen		X
Sternum		X (with marrow)
Stifle joint		X
Stomach		X
Testes		X*
Thymus		X*
Thyroid		X
Tongue	X	X
Trachea	X	X
Urinary bladder		X
Uterus		X
Vagina		X
Zymbal gland		
Standard List		

X, histopathology performed

*, organ weight obtained

a. Histopathological examination of tissues was limited to the larynx, trachea, lung, and heart.

b. Histopathological examination of tissues was limited to the larynx, trachea, lung, heart, testes, spleen, salivary glands, and nasal cavity and turbinates.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

The sponsor is relying upon preclinical and clinical experience with Foradil® to support their clinical trials. The sponsor's drug product is an inhalation solution administered by nebulizer, while Foradil® is a dry powder capsule administered with an Aerolizer™. The sponsor's proposed drug product involves a change from the approved dry powder capsule to an inhalation solution. This may lead to differences in product performance. A 2-week inhalation bridging toxicology study is needed to determine that there are no differences in the toxicity profile between Foradil® and formoterol fumarate inhalation solution (Regulatory Toxicology and Pharmacology 25: 189-193, 1997). In the present submission, the sponsor provided this 2-week inhalation bridging toxicology study.

In a 14-day inhalation toxicology study, 5 rats/sex/group were exposed to formoterol fumarate inhalation solution (FFIS) at three different doses. A single dose of formoterol fumarate dry powder (FFD) was included as a comparator at a level approximately equivalent to the high dose of FFIS. There were no significant differences in toxic effects between FFIS and FFD. Thus, the product performance of FFIS appears to be equal to or less than FFD.

Reviews of preclinical toxicology studies that can be obtained through the Freedom of Information Act were referenced to evaluate the safety of the sponsor's proposed clinical trials (see Review #01 of IND —). Based upon inhalation toxicology studies with rats in which formoterol was administered as a dry powder formulation for periods ranging from 1 to 12 months, target organs of toxicity were the testes, spleen, salivary gland, nasal cavity, lung, and heart. From a preclinical standpoint, the inhaled clinical dose of 40 µg/day (20 µg BID) for Phase III clinical trials is supported for chronic administration (see table below), although, the safety margin (i.e., 3.75) is less than that normally desired (i.e., approximately ≥10). A NOAEL was not established in the 4-week inhalation toxicology study with dogs, although, clinical development of formoterol was allowed to proceed despite adverse findings. Toxic effects in dogs were attributed primarily to increased heart rate. In a clinical setting, heart rate can be monitored.

b(4)

Safety margins for the clinical dose of formoterol fumarate inhalation solution at 40 µg/day (20 µg BID) in adult subjects.

Species	Study Duration	Doses (Deposited Dose) µg/kg/day	NOAEL (Deposited Dose) µg/kg/day	Safety margins for clinical doses of formoterol fumarate inhalation solution
				40µg/50kg = 0.8
Rat	28-days Dry powder	16, 50, & 115.3	115.3	144
	3-months Dry powder	M: 0.25, 0.8, & 2.6 F: 0.4, 1.2, & 3.9	2.6/3.9	3.25/4.9
	6/12-months Dry powder	3, 12, & 40	3	3.75
Dog	4-Weeks Dry powder	0.6, 2.8, & 11	None ^a	-

a. A NOAEL was not established in the 4-week inhalation toxicology study with formoterol in dogs based upon findings of increased heart rate and myocardial fibrosis at all doses.

The proposed clinical dose of formoterol fumarate inhalation solution at 40 µg/day (20 µg BID) in COPD patients for Phase III clinical trials appears to be supported by available preclinical data.

Recommendation:

The product performance of formoterol fumarate inhalation solution appears to be equal to or less than formoterol fumarate dry powder.

The proposed clinical dose of formoterol fumarate inhalation solution at 40 µg/day (20 µg BID) in COPD patients for Phase III clinical trials appears to be supported by available preclinical data.

Draft Letter:

Final reports of the 4-day inhalation toxicology study with rats (Study Number N102782) and 14-day inhalation toxicology study with rats (Study Number N102783) should be provided within 120 days of submission of the draft final reports. These reports should include individual animal line listings for all parameters that were assessed.

b(4)

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Joseph Sun, Ph.D.

Non-Concurrence - _____
(see memo attached)

cc: list:

- IND 68,782 Division File, HFD-570
- GreenA, HFD-570
- GunkelJ, HFD-570
- SunC, HFD-570
- RobisonT, HFD-570

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Timothy Robison
5/13/04 10:01:35 AM
PHARMACOLOGIST

Joseph Sun
5/19/04 12:24:35 PM
PHARMACOLOGIST
I concur.

Appendix 5

Review #03 of IND 68,782 dated January 25, 2005

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 68,782

Review number: #03

Sequence number/date/type of submission: #007/October 5, 2004/Amendment
#014/January 12, 2005/Amendment

Information to sponsor: Yes (X) No ()

Sponsor and/or agent Dey, L.P.
2751 Napa Valley Corporate Drive
Napa, CA 94558

Manufacturer and supplier for drug substance: See IND 64,525

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.

Division name: Pulmonary and Allergy Drug Products

HFD #: 570

Review completion date: January 25, 2005

Drug:

Generic name (list alphabetically): Formoterol fumarate dihydrate

Chemical name: \pm 2-hydroxy-5-[(1RS)-1-hydroxy-2-[[[(1RS)-2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl] formanilide fumarate dihydrate

Molecular formula/molecular weight: $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4 \cdot 2H_2O$ / 840.92

Relevant INDs/NDAs/DMFs:

IND ~~_____~~ (Dey, Formoterol fumarate inhalation solution)

NDA 20-831 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

NDA 21-279 (Foradil[®] Aerolizer[™], Novartis Pharmaceuticals Corporation)

b(4)

Drug class: β_2 -Adrenergic Agonist

Indication: Chronic obstructive pulmonary disease (COPD)

b(4)

Clinical formulation: See IND ~~_____~~

Route of administration: Inhalation (using a nebulizer)

Proposed clinical protocol: See previous reviews.

Previous clinical experience: See previous reviews.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

1. Draft Study Protocol: 90-Day Inhalation Toxicity Study in Rats to Qualify Desformyl Formoterol in a Formoterol Drug Product.
2. Maximum Tolerated Dose-Range-Finding Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate in Rats (Final Report).
3. 14-Day Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate Dihydrate in Rats (Final Report).

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Studies not reviewed within this submission: None.

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2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

During the EOP2 meeting with the sponsor on April 2, 2004, the Division discussed requirements for the qualification of the degradant, desformyl formoterol (desformoterol). Earlier in drug development, the Division had communicated that desformoterol might be qualified by demonstrating that it is a major systemic metabolite from the 14-day inhalation toxicity study with formoterol fumarate in rats. From the preliminary toxicokinetic data of this study, it appeared that desformoterol constituted approximately 20-25% of the total exposure. In a reconsideration of this issue, the Division was concerned that demonstrating desformoterol as a major systemic metabolite does not address issues regarding its potential local toxicity in the lung given concerns for the intended treatment population and the susceptibility of the respiratory airways to injury.

The sponsor was offered two options for the qualification of this degradant:

1. Desformoterol can be formed by nonenzymatic metabolism. It is suggested that in vitro metabolism of formoterol in rat bronchoalveolar lavage fluid at 37°C be assessed over a 1-hr period. A range of formoterol concentrations should be examined (e.g., at least 3 concentrations of formoterol). Human bronchoalveolar lavage fluid would also be acceptable. If substantial in vitro generation of desformoterol can be demonstrated, further study would not appear to be needed.
2. However, if there is only minor or negligible in vitro generation of desformoterol, the sponsor will need to conduct a 90-day inhalation toxicology study with desformoterol in the rat since the drug product will be administered on a chronic basis.

In Amendment #014, the sponsor provided a protocol for a 90-day inhalation toxicology study with desformoterol in rats for Division comments. They plan to initiate this study in February or March of 2005.

2.6.6.3 Repeat-dose toxicity

Study title: Draft Study Protocol: 90-Day Inhalation Toxicity Study in Rats to Qualify Desformyl Formoterol in a Formoterol Drug Product.

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Proposed Study Protocol: The sponsor has submitted a draft protocol to qualify desformyl formoterol (desformoterol), a degradant in Formoterol fumarate inhalation solution, through a 90-day, nose-only inhalation study with rats. The study will consist of 4 groups. Two test article dose groups will receive an aerosol of degraded formoterol drug product solution containing approximately 4.5% (0.45 µg/mL) or 7% (0.7 µg/mL) desformoterol generated using nebulizers. An additional two groups will serve as the

air- and vehicle-controls. Control and treatment groups are shown in the table below. Test atmospheres (aerosol) will be generated using nebulizers and delivered to rats by a nose-only exposure system. Separate exposure systems will be constructed for the liquid test system, vehicle, and air exposures. Each exposure group will be exposed daily to the assigned aerosol concentration up to 3 hr exposure duration for 90 consecutive days. Satisfactory achievement of total aerosol mass or test article concentrations will be documented for each exposure system through gravimetric and/or chemical analyses. Appropriate temporal stability and spatial uniformity will be characterized for each exposure system except the air control through gravimetric measurement for total mass and chemical measurement for the test article/mass. Particle size distribution measurements of the vehicle and test article will be conducted using cascade impactors. Gravimetric and analytical results will be used to determine the amount of total mass (gravimetric) and test article (analytical) on each stage of the impactor.

Treatment groups

Group	Exposure duration (min)	FFIS Conc. ($\mu\text{g}/\text{mL}$)	DF Conc. % of API	FF Aerosol Conc. ($\mu\text{g}/\text{mL}$)	FF Dose $\mu\text{g}/\text{kg}$	DF Dose $\mu\text{g}/\text{kg}$	rats/sex/group	
							Toxicity	TK
Air-Control	180	0	0	0	0	0	10	3
Vehicle-Control	180	0	0	0	0	0	10	3
Low Dose	180	10	4.5%	0.42	37.8	1.7	10	9
High Dose	180	10	7%	0.42	37.8	2.6	10	9

Abbreviations: FFIS = Formoterol fumarate inhalation solution; DF = Desformoterol; and FF = Formoterol fumarate

The study will include clinical observations, measurements of body weight and food consumption, toxicokinetic analysis on days 45 and 90, and clinical pathology measurements on days 45 and 90. Necropsy examinations will be conducted on toxicity or toxicokinetic animals found dead or sacrificed in a moribund condition during the treatment period. On day 91, all surviving core toxicology rats will be sacrificed and submitted to necropsy examination. Toxicokinetic animals will be sacrificed and discarded without examination. Organs and tissues will be collected and fixed in 10% neutral-buffered formalin. Organ weights will be measured for the adrenal glands, brain, heart, kidneys, liver, lung, testes, and thymus. Histopathology will be limited to examination of the larynx, trachea, lung, heart, nasal cavity, and turbinates.

The sponsor has estimated that desformoterol concentrations of 0.45 and 0.7 $\mu\text{g}/\text{mL}$ should provide deposited airway multiples of approximately 4 and 6 times the clinical dose, respectively, assuming 100% deposition in humans and 10% deposition in rats.

Evaluation: In accordance with the EOP2 meeting on April 2, 2004, the sponsor has elected to conduct a 90-day inhalation toxicology study to qualify the degradant,

desformoterol. This study is designed to assess the local toxicity of desformoterol in the respiratory tract. In the 14-day inhalation bridging toxicology study with formoterol in rats, desformoterol was measured as a systemic metabolite. Thus, desformoterol is qualified with regard to systemic toxicity. Therefore, limitation of histopathological examinations of tissues and organs to the larynx, trachea, lung, heart, nasal cavity, and turbinates is acceptable. During the EOP2 meeting, the Division recommended that 3 concentrations of desformoterol be tested to characterize the toxicity of this compound; however, 2 concentrations are acceptable assuming that an adequate safety margin for the clinical dose is established. This study will utilize desformoterol spiked into formoterol rather than isolated desformoterol (preferable), which is in accordance with ICH Q3A and Q3B. The NOAEL in this study with rats should establish a minimum 10-fold safety margin. The two doses of desformoterol in the proposed study provide only a 4 to 6-fold safety margin. Thus, it is recommended that the sponsor increase the doses of desformoterol in the proposed study or add additional dose(s) with higher levels of desformoterol.

Study title: Maximum Tolerated Dose-Range-Finding Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate in Rats (Final Report).

Key study findings:

- Inhalation exposure of rats to either formoterol fumarate inhalation solution or formoterol fumarate dry powder led to significant systemic exposures to desformoterol.

Study no.: _____ Study Number N102782

Volume #, and page #: Amendment #007, Volume 1, Pages 1-168

Conducting laboratory and location: _____

b(4)

Date of study initiation: October 16, 2003 (In-life exposures began on November 17, 2003).

GLP compliance: This study was not monitored for compliance with GLP regulations.

QA report: yes () no (X)

Methods: A 4-day inhalation toxicology study was conducted with male rats in order to identify a maximum tolerated dose of nebulized formoterol fumarate and to compare any toxic effects with neat formoterol fumarate dihydrate powder. The sponsor provided toxicokinetic data that was not available in the draft final report. There were no other significant differences between the draft final report and final report. The final report contained line listings for individual animals and the Troponin T data was verified.

Observation and Times:

Toxicokinetics: Blood samples for measurement of plasma formoterol and desformoterol concentrations were collected on day 4 at 0.083, 0.5, 1, 3, 5, and 24 hr after dosing. Three rats/group/time point were used. Two blood samples were obtained from each

rat. Rats were sacrificed and discarded without examination after collection of the second blood sample. A LC/MS/MS method was used for the detection of formoterol and desformoterol.

Results:

Toxicokinetics:

Toxicokinetic parameters were assessed for formoterol and desformoterol. Formoterol and desformoterol were detected in plasma samples from air-control and vehicle-control groups that were collected at 5 min after dosing. Later time points were not evaluated. No explanation was provided for these findings.

For low and mid dose formoterol fumarate inhalation solution (FFIS) groups, systemic exposures were confined to desformoterol as the parent compound, formoterol, was not detected. For treatment groups that received either high dose formoterol fumarate inhalation solution, low dose formoterol fumarate dry powder (FFDP), or high dose formoterol fumarate dry powder, systemic exposures to desformoterol constituted 92.1, 88.7, and 95.4% of the total exposure to formoterol and desformoterol combined, respectively. The data suggests that desformoterol is a metabolite of formoterol and the majority of formoterol is metabolized to desformoterol and other metabolites not examined in the present study.

For the treatment groups that received formoterol fumarate inhalation solution, C_{max} and AUC values for desformoterol increased with elevating dose of formoterol. The increase in AUC value for desformoterol from the low to mid dose appeared to be dose proportional; however, the increase in AUC value for desformoterol from the mid to high dose appeared to be significantly less than dose proportional. Increases of C_{max} were slightly less than dose proportional. For treatment groups that received formoterol fumarate dry powder, C_{max} and AUC values for desformoterol increased with elevating dose; however, these increases were slightly less than dose proportional.

Exposures to formoterol and desformoterol was relatively comparable between the low dose FFIS, high dose FFIS, and low dose FFDP groups.

Toxicokinetic parameters for formoterol fumarate

Treatment Group	C_{max} , ng/mL	Half-life, hr	AUC _{1hr} , ng·hr/mL	AUC _∞ , ng·hr/mL
1. Air-Control	0.759	NA	NA	NA
2. Vehicle-Control	0.531	NA	NA	NA
3. Low Dose-Formoterol fumarate inhalation solution	1.62	NA	ND	ND
4. Mid Dose-Formoterol fumarate inhalation solution	2.19	NA	ND	ND
5. High Dose-Formoterol fumarate inhalation solution	7.15	0.4	8.41	9.3
6. Low Dose-Formoterol fumarate dry powder	9.85	0.3	10.4	10.7
7. High Dose-Formoterol fumarate dry powder	7.51	0.4	7.89	8.6

Toxicokinetic parameters for desformyl formoterol

Treatment Group	C _{max} , ng/mL	Early phase Half- life, hr	Late Phase Half- life, hr	AUC _{last} ng·hr/mL	AUC _∞ ng·hr/mL
1. Air-Control	2.50	NA	NA	NA	NA
2. Vehicle-Control	1.85	NA	NA	NA	NA
3. Low Dose-Formoterol fumarate inhalation solution	6.23	1.2	ND	14.2	24.4
4. Mid Dose-Formoterol fumarate inhalation solution	11.8	0.7	26.2	49.7	81.8
5. High Dose-Formoterol fumarate inhalation solution	30.4	0.6	19.9	77.1	108
6. Low Dose-Formoterol fumarate dry powder	22.8	1.1	9.6	66.2	83.8
7. High Dose-Formoterol fumarate dry powder	55.9	0.5	9.7	162	179

Study title: 14-Day Inhalation Study of Nebulized Formoterol Fumarate Inhalation Solution Compared to Neat Formoterol Fumarate Dihydrate in Rats (Final Report).

Key study findings:

- Inhalation exposure of rats to either formoterol fumarate inhalation solution or formoterol fumarate dry powder led to significant systemic exposures to desformoterol.

Study no: Study Number N102783

Volume #, and page #: Amendment #007, Volume 1, Pages 175-482

Conducting laboratory and location: _____

b(4)

Date of study initiation: December 16, 2003 (In-life exposures began on January 12, 2004)

GLP compliance: Yes.

QA report: yes (X) no ()

Methods (unique aspects): In a 14-day inhalation toxicology study with 5 rats/sex/group, the comparative toxicity of nebulized formoterol fumarate solution to formoterol fumarate dihydrate powder was examined when administered to rats by nose-only inhalation. The sponsor provided toxicokinetic data that was not available in the draft final report. There were no other significant differences between the draft final report and final report.

Observation and Times:

Toxicokinetics: Blood samples for measurement of plasma formoterol and desformoterol concentrations were collected on day 14 at 5, 30, 60, and 90 min post-exposure. A LC/MS/MS method was used for determination of plasma formoterol and desformoterol concentrations.

Results:

Toxicokinetics: For treatment groups that received Formoterol fumarate inhalation solution (FFIS), C_{max} and AUC values for formoterol and desformoterol increased in an approximate dose proportional manner. For the treatment group that received Formoterol fumarate dry powder (FFD), systemic exposures to formoterol and desformoterol were higher than those observed for treatment groups that received FFIS. For treatment groups that received FFIS or FFD, systemic exposures to formoterol were significantly higher than exposures to desformoterol. This contrasts to the 4-day maximum tolerated dose study where exposures to desformoterol were significantly higher than exposures to formoterol. Systemic exposures to desformoterol in the present study ranged from 16.5 to 29.1% of total exposures (i.e., formoterol + desformoterol).

Toxicokinetic parameters for formoterol fumarate

Treatment Group	C_{max} , ng/mL		Half-life, min		AUC _{90 min} , ng min/mL		AUC _∞ , ng min/mL	
	M	F	M	F	M	F	M	F
Air control	BLOQ	BLOQ	ND	ND	ND	ND	ND	ND
Vehicle control	BLOQ	BLOQ	ND	ND	ND	ND	ND	ND
Low Dose, FFIS	2.43	BLOQ	65	ND	124	ND	171	ND
Mid Dose, FFIS	12.3	8.97	42	96	588	529	785	999
High Dose, FFIS	22.8	20.4	64	66	1030	1160	1680	2020
High Dose, FFD	60.9	49.4	44	39	3050	2540	3890	3050

Abbreviations: BLOQ = Below Limit of Quantitation; FFIS = Formoterol fumarate Inhalation solution; and FFD = Formoterol fumarate dry powder.

Toxicokinetic parameters for desformoterol

Treatment Group	C_{max} , ng/mL		Half-life, min		AUC _{90 min} , ng min/mL		AUC _∞ , ng min/mL	
	M	F	M	F	M	F	M	F
Air control	BLOQ	BLOQ	ND	ND	ND	ND	ND	ND
Vehicle control	BLOQ	BLOQ	ND	ND	ND	ND	ND	ND
Low Dose, FFIS	0.857	0.740	ND	ND	ND	ND	ND	ND
Mid Dose, FFIS	1.82	1.29	76	142	93.8	80.0	175	198
High Dose, FFIS	4.81	3.94	158	86	229	238	690	493
High Dose, FFD	9.55	7.88	69	68	663	459	973	731

Abbreviations: BLOQ = Below Limit of Quantitation; FFIS = Formoterol fumarate Inhalation solution; and FFD = Formoterol fumarate dry powder.

2.6.6.9 Discussion and Conclusions

In the 14-day inhalation bridging toxicology study with formoterol in rats, desformoterol was observed to be a significant systemic metabolite. This bridging study along with toxicology studies conducted with formoterol available through FOI qualifies desformoterol with regard to systemic toxicity. To qualify desformoterol with regard to local toxicity, the sponsor has provided a protocol for a 90-day inhalation toxicology with rats for Division comment. This study will use two doses of desformoterol spiked in formoterol that provide a 4- to 6-fold safety margin for the proposed clinical dose. The NOAEL identified in this study with rats should establish a minimum 10-fold safety

margin. Thus, it is recommended that the sponsor increase the doses of desformoterol in the proposed study or add additional dose(s) with higher levels of desformoterol.

OVERALL conclusions and recommendations

Summary:

Desformoterol is a degradant in the drug product, formoterol fumarate inhalation solution. It is expected that levels will be significantly in excess of the 1.0% qualification threshold (ICH Q3B) (i.e., the sponsor has estimated a level of approximately — 40 µg Formoterol/day x — Desformoterol = — µg DF or — µg DF/kg for a 50 kg individual). A 14-day bridging inhalation toxicology study with formoterol in rats established that desformoterol was a systemic metabolite. ICH Q3B states that "degradation products that are significant metabolites, present in animal and/or human studies, would not need further qualification." However, there are Division concerns that desformoterol has not been qualified with regard to local toxicity in the lung. In Amendment #014, the sponsor provided a protocol for a proposed 90-day inhalation toxicology study with rats to qualify desformoterol. This study will use two doses of desformoterol spiked in formoterol that provide a 4- to 6-fold safety margin for the proposed clinical dose. It is noted that the NOAEL identified in this study with rats should establish a minimum 10-fold safety margin. Thus, it is recommended that the sponsor increase the doses of desformoterol in the proposed study or add additional dose(s) with higher levels of desformoterol.

b(4)

Recommendations: The following comments should be communicated to the sponsor.

Draft Letter:

We have reviewed the contents of Amendment #014 dated January 12, 2005 and have the following comments.

With regard to the proposed 90-day inhalation toxicology study with rats to qualify desformoterol at —, the clinical dose of formoterol fumarate inhalation solution, the NOAEL identified in this study should establish at least a 10-fold safety margin. Thus, it is recommended that you increase the doses of desformoterol in the proposed study or add additional dose(s) with higher levels of desformoterol.

b(4)

Reviewer signature: _____
Timothy W. Robison, Ph.D.

Supervisor signature: Concurrence - _____
Joseph Sun, Ph.D.

Non-Concurrence - _____
(see memo attached)

cc: list:

IND 68,782 Division File, HFD-570

GreenA, HFD-570

GunkelJ, HFD-570

KimC, HFD-570

SunC, HFD-570

RobisonT, HFD-570

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/s/

Timothy Robison
1/25/05 09:47:25 AM
PHARMACOLOGIST

Joseph Sun
1/25/05 05:27:05 PM
PHARMACOLOGIST
I concur.

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/s/

Timothy Robison
2/20/2007 03:26:46 PM
PHARMACOLOGIST

Joseph Sun
2/20/2007 03:34:11 PM
PHARMACOLOGIST
I concur.

NDA Pharmacology Fileability Check List

NDA No: 22-007

Date of submission: June 28, 2006

Date of Fileability meeting: August 22, 2006

Information to Sponsor Yes () No (X)

Date of check list: August 22, 2006

(1) On its face, is the Pharm/Tox section of the NDA organized in a manner to allow substantive review? Yes (X) No () NA ()

(2) On its face, is the Pharm/Tox section of the NDA legible for review? Yes (X) No () NA ()

(3) Are final reports of all required and requested preclinical studies submitted in this NDA? Yes (X) No () NA ()

	Yes	No	NA
Pharmacology*	(X)	()	()
ADME*	(X)	()	()
Toxicology* (duration, route of administration and species specified)			
acute	(X)	()	()
subchronic and chronic studies	(X)	()	()
reproductive studies	(X)	()	()
carcinogenicity studies	(X)	()	()
mutagenicity studies	(X)	()	()
special studies (Impurity)	(X)	()	()
others	(X)	()	()

*This is a 505(b)(2) NDA. The application cross references NDA 20-831 and NDA 21-279 for pharmacology, ADME, and toxicology of formoterol fumarate. The sponsor conducted a 14-day inhalation toxicology studies with rats to bridge their formoterol fumarate inhalation solution drug product to the approved dry powder formulation. The sponsor also conducted a 90-day inhalation toxicology with rats to qualify the degradant, desformoterol, found in the drug product.

(4) If the formulation to be marketed is different from the formulation used in the toxicology studies, is repeating or bridging the studies necessary? Yes (X) No () NA ()

If no, state why not?

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/s/

Timothy Robison
8/24/2006 12:02:39 PM
PHARMACOLOGIST

Joseph Sun
8/24/2006 04:55:39 PM
PHARMACOLOGIST
I concur.