Table 51. LD_{50} of Formoterol (mg/kg)

Route	Inhalation	IV	IP	SC	PO
Mice	18	100	190	1050	3130
Rats	>24	72	225	655	6700

Repeated dose toxicity: Repeated exposures of formoterol (up to 1 year) in rats and dogs indicate that the heart is the target organ of toxicity across species and regardless the route of administration. The effect on male reproductive system occurs at higher doses.

Cardiac toxicity: Changes in the heart after repeated exposure are both functional and phathological: tachycardia, changes in EKG, cardiac hypertrophy and cardiac necrosis/fibrosis. Tachycardia in dogs occurs at doses as low as 0.4 µg/kg/day and 2 mg/kg/day for the inhalation and oral administration, respectively. Tachycardia can also be associated with epitopic arrhythmia. Severe cardiac effect from large doses may even result in deaths. Table 52 lists major histological changes in these studies.

Table 52. Cardiotoxicity of Formoterol in Rats and Dogs

Rat					Dog				
Duration, route	Dose (ug/kg)	AUC ratio	Cardiac lesions Male	Female	Duration, route	Dose (ug/kg)	AUC ratio	Cardiac lesions Male	Female
3 mo. Inh. T2433	0	-	1/10	1/10	1 mo. Inh. T3120	0	-	0/3	0/3
	9	69	0/10	0/10		0.1	1	0/3	1/3
	22	155	0/10	0/10		0.4	5.4	1/3	0/3
	90	287	2/10	0/10		2.1	40	3/3	2/3
3 mo. PO young T3136	0	-	0/10	0/10	1 mo. PO T2579	0	-	0/3	1/3
	200	6	0/10	0/10		2	4	0/3	0/3
	800	17	1/10	0/10		15	24	1/3	2/3
	3000	48	1/10	2/10		100	216	2/3	2/3
6 mo. Inh. T2860	0	-	0/20	0/20	12 mo. Inh. T3077	0	-	0/5	0/5
	2.3	17	0/20	0/20		0.7	2	1/5	0/5
	12	79	2/20	0/20		8.6	18	1/5	1/5
	72	225	5/20	1/20		92	219	3/5	0/5
6 mo. PO Yoshida, 1986	0	NE*	4/12	4/12	12 mo. PO Yoshida 1986	1	NE	0/3	0/3
	3		5/12	4/11		10		0/3	0/3
	30		6/12	4/12		100		1/3	1/3
	300		6/12	5/13		1000		3/3	3/3
	3000		9/12	4/12					
	12000		9/11	6/12					

* NE = not examined. -- = No apparent NOAEL value was established.

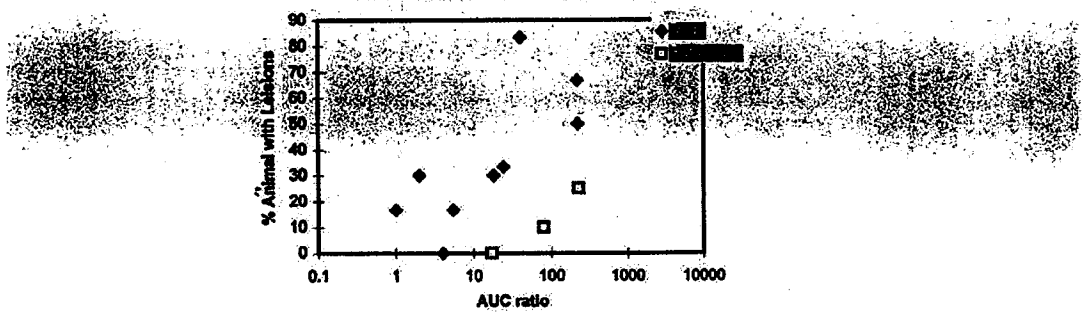
AUC ratio was extracted from submission N010 (S3), p 4 - 5, submission of 10-26-94. The AUCs in humans after 24 ug formoterol were 0.17, 0.22, 0.25 and 0.31 nmol.h/L for 4, 6, 8 and 24 hour intervals, respectively. The Cmax in humans was 0.064 nmol/L. Thus, An AUC ratio of 10 would mean that the animal AUC was 10 times the

human AUC value.

Apparently, the cardiotoxicity of formoterol defines the NOAEL values of formoterol. Of the three studies in dogs, only the one-month oral study (2579) showed a NOAEL values of 2 µg/kg/day while the other two studies (an 1-month inhalation and a 12-month oral; T3120 T3077) failed to establish any NOAEL values. However, the NOAEL value for the 6-month inhalation rat study was 2.3 µg/kg/day. Yoshida *et. al.* (1983) reported occurrence of cardiac fibrosis in 6-month studies at oral doses of as low as 10 µg/kg/day and 30 µg/kg/day in dogs and rats, respectively. Considering the bioavailability of the drug (10 - 15%), Yoshida's results are very similar to that of the sponsor. Yoshida et al also showed that the cardiac lesions are apparent even after a recovery period of 10 weeks in rats. Altogether, data consistently indicate that minimal or no margin of safety exists for the proposed clinical dose.

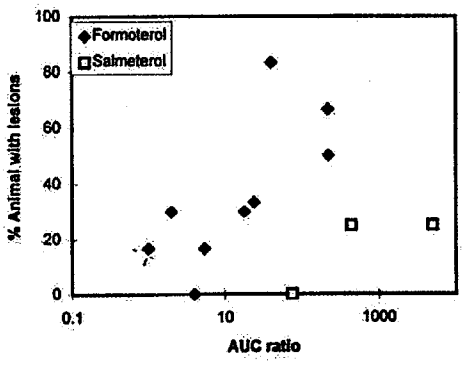
Further evaluation was conducted to explore the dose-response relationship of the cardiac lesions. Incidences (expressed as percentage of animals bearing the response) of cardiac lesions as a function of plasma formoterol concentration in the repeated exposure studies are presented in Fig. 2. The AUC ratios are derived by dividing AUC in animals with AUC of clinical dose in humans. Data in dogs are complied from several repeated studies (T3120, T2579 and T3077). The rat data was from a 6-month inhalation study (T2860). Cardiac lesions are clearly dose-related in both rats and dogs. Furthermore, dogs are more susceptible than rats. Again, no or limited margin of safety existed for the clinical dose.

Fig. 2. Cardiac lesions of formoterol in animals



The lack of safety margin of formoterol prompted a question of whether it is true for the whole class of beta agonists. Beta agonists have been extensively used for the treatment of asthma. Cardiotoxicity is also observed with other beta agonists in preclinical studies. Effort was made to apply the same technique of analysis. Unfortunately, high quality AUC data are lacking for most bronchodilators, especially albuterol that is the most frequently used drug. Therefore, a comparison was conducted between formoterol and salmeterol, the approved long-acting beta-agonist. The Fig. 3 shows their cardiotoxicity as a function of plasma formoterol concentrations. The salmeterol data were from a 12-month dog study (oral plus inhalation exposure).

Fig. 3. Cardiac Lesions of Formoterol and Salmeterol



It is obvious that formoterol is much more cardiotoxic than salmeterol (sponsored by GlaxoWellcome) at the clinical doses. In the salmeterol study, the low, mid and high dose groups dogs were given salmeterol 0.15, 0.5 and 2.0 mg/kg/day orally and 0.04, 0.08 and 0.16 mg/kg/day by inhalation, respectively. AUC values for these groups were 42, 250 and 2857 ng.h/L. They corresponded to 73, 440 and 5000 times of the expected human AUC at therapeutic doses (0.57 ng.h/L). Cardiac lesions were seen in the mid (2/8) and high dose (2/8) groups only. A large of margin of safety (at least 73) exists for salmeterol. On the other hand, existence of none or minimal margin of safety for formoterol suggests that cardiotoxicity of the drug should be of a safety concern.

The safety concern about formoterol prompted consultations with Drs. Virgil Whitehurst, Hilary Sheevers, and Raymond Anthracite. Dr. Whitehurst is the pharmacologist with expertise in beta agonists and the original reviewer for this application in the Division. Dr. Raymond Anthracite is the Medical Reviewer for this IND. According to these reviewers, higher incidences of deaths were noticed clinically with the formoterol trials, compared to other clinical asthma trials with other agents. A causal effect relationship for cardiac lesions was lacking. Most deaths were either asthma-related or with unknown causes.

Formoterol is also under development by other company —— Clinical trials for this IND are almost completed and its NDA is expected to reach the Agency soon. A series of toxicity studies of formoterol were also submitted by its sponsor and have been reviewed by the Division. Unfortunately, most of these studies were conducted in Japan in the 1970's. Pharmacokinetic information was incomplete and thus, direct comparison was impossible. Few inhalation studies were submitted recently and need to be reviewed. Final evaluations should be made after reviewing the data from all sources.

Comment: The sponsor argued that the cardiac lesions (males: 0/3-C, 1/3-LD, 0/3-MD, 3/3-HD; female: 0/3-C, 1/3-LD, 0/3-MD and 2/3-HD) in the two low dose levels (0.1 and 0.4

µg/kg/day) of the one-month toxicity study in dogs (T3120) was spontaneous. However, none of the control animals in the experiment showed the cardiac lesions. To verify this, incidence of cardiac lesions between the control dogs in the entire toxicology program and all low dose groups (AUC ratio ≤ 5.4) were compared (Table 53). The incidences of the cardiac lesions in the treated group (10.7%) was twice the control group (4.5%). This, along with the pharmacokinetic analysis (fig. 3) strongly suggested that the cardiac lesions in the low dose groups be treatment-related.

Table 53. Cardiac Lesions in the low dose groups (AUC ratio < 5)

	Dog# with lesion	Dog# without lesion	Total#	% w/ lesion
Control	1	21	22	4.5
Treatment	3	25	28	10.7
Total	4	46	50	8.0

Reproductive toxicity: The effect of formoterol on reproductive system was evaluated in rats and rabbits. Formoterol is not teratogenic at oral doses of up to 3.0 mg/kg (rats), 3.5 mg/kg/day (rabbits), and at inhalation dose of up to 91 µg/kg/day in rats, respectively. At a higher oral dose (60 mg/kg/day, T3014), slight increases in the incidences of asymmetric bipartite sternbrae, anomalous cervicothoracic artery and a slight decrease in pup weights were apparent in rabbits. The increases in the incidences of rib abnormalities was reported previously (Sato *et. al.*, 1984).

Several studies (T2579, T3136, T3015, T2860 and T3137) also showed that formoterol affected the male reproductive system: the testicular atrophy, decreased male fertility, decreased weight and/or size of the testis, and/or scrotal hyperemia in both rats and dogs. Findings in the male reproductive system along with plasma drug concentration/AUCs are listed in Table 54. (Scrotal hyperemia is not included.):

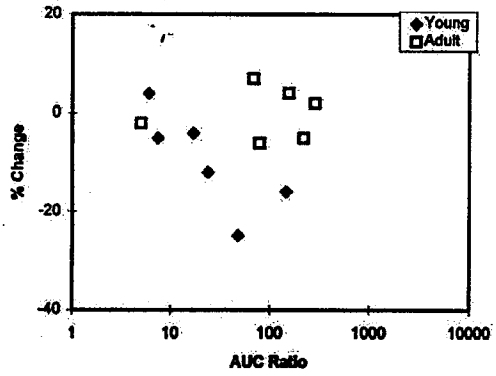
Table 54. Reproductive Effects of Formoterol in Male Animals

Study duration	Dose [µg/kg/day]	AUC ratio@	Findings in male reproductive system	Testis atrophy	Study No.
Dog,	0	--		0/3	T3120
1 month	0.1	1	No significant findings (NSF)	1/3	
inhalation	0.4	5.4		1/3	
	2.1	40		0/3	
Dogs	0	--		0/3	T2579
1 month	2	4	NSF	0/3	
oral	15	24	NSF	0/3	
	100	216	↓TW (19%), ↓PW (50%), ↓ EW (19%)	0/3	
Rats	0	--		0/10	T2433
3 months	9	69	NSF, ↑ TW (7%)	NE*	
inhalation	22	155	↑ TW (4%)	NE	
	90	287	↑ TW (2%)	0/10	
Mice	0	-		1/12	T2578
3 months	100	3	NSF	0/12	
oral	1000	23		1/12	
	10000	160		0/12	
Rats, young	0	--		0/6	T3137
3 months	2.6	7.4	↓ Testicular weight (TW, 5 %)	0/6	
inhalation	13	24	↓TW (12%)	1/6	
	67	145	↓TW (16%)	0/6	
Rats, young	0	--		1/10	T3136
3 months	200	6	↓TW (4%)	4/10	
oral	800	17	↓TW (7%), ↓ Testicular size (TS, 1/10)	1/10	
	3000	48	↓TW (25%), ↓ TS (3/10)	7/10	
Rats	0	-		0/20	T2860
6 months	2.3	17	↓ Prostate weight (PW, 8%), ↓ TW (2%)	0/20	
inhalation	12	79	↓PW (7%), ↓ TW (6%)	1/20	
	72	225	↓PW (10%) ↓TW (5%)	0/20	
Rats	0	--			T3105
6 months	200	5	NSF		
inhalation	300	79	↓ Fertility (1/16)		
Seg. I fert.	15000	1935	↓ Fertility (6/16)		
Dogs	0	--		0/5	T3077
12 months	0.7	2	NSF	0/5	
oral	8.6	18		1/5	
	92	219		0/5	

* NE = Not examined, EW = epididymides weight, PW = prostate weight, TW = testes weight, NSF = No significant findings. @ AUC ratio was extracted from submission N010 (S3), p 4 - 5, submission of 10-26-94. The AUCs in humans after 24 ug formoterol were 0.17, 0.22, 0.25 and 0.31 nmol.h/L for 4, 6, 8 and 24 hour intervals, respectively. The Cmax was 0.064 nmol/L. Thus, An AUC ratio of 10 would mean that the animal AUC was 10 tiems the human AUC value.

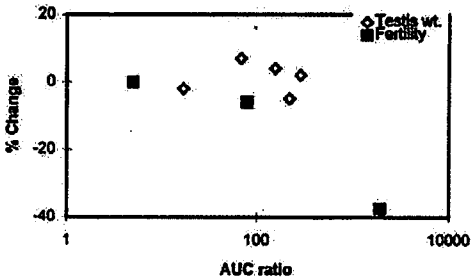
The effect of formoterol on the male reproductive system occurs predominantly in rats. One 3-month oral study (T3136) showed that formoterol induced testicular atrophy in young rats at dose as low as 0.2 mg/kg/day. Decreased testicular weight and /or size were also apparent in young rats (T3136, T3137). Decreased adult male fertility occurred at a higher dose (15 mg/kg/day, T3105). To further explore this relationship, testicular weight as a function of plasma formoterol concentration is plotted in Fig. 4.

Fig. 4. Testis weight vs plasma formoterol conc. in rats



A good correlation exists for the plasma concentration and testicular size in the young animals but absent in the adult rats. The decrease in testis size, along with testicular atrophy, strongly suggest that male reproductive system is also the target organ of formoterol toxicity. Further evaluation was conducted to explore the relationship between the testis weight and fertility and the plasma concentration in adult rats (Fig. 5):

Fig. 5. Changes in Testis Weight & Fertility in Adult Rats



Decreased male fertility occurred at rather higher plasma concentrations (AUC ratios of 79 or more). A study in dogs also showed that decreased prostate size at an AUC ratio of 216. The mechanism of this effect of formoterol on male reproductive system is not clear. Nonetheless, increased temperature of the epididymides (due to hyperemia) and the decreased libido could be contributing factors. The increase in testicular temperature may decrease viability of the sperm. The decrease sperm viability can be the cause of decreased fertility. It is well known the prolonged use of beta agonist may decrease the libido which may affect mating performance.

Overall, formoterol is less likely to cause adverse effect on the reproductive system at therapeutic doses in adults. However, cautions should be taken when pediatric patients are concerned and the effect of formoterol in young patients should be reevaluated. Formoterol did not affect delivery and post natal development in rats at inhalation doses of 3.4 up to µg/kg/day.

Toxicity to other system: Changes in other organs after repeated formoterol exposure are less prominent. Prolonged administration of formoterol can result in a decrease blood glucose levels regardless the route of administration (T 2433, T2860, T3077, T3136). This decrease in blood glucose levels occurred in both sexes and became more pronounced as the time progress. Slight increase in blood urea levels and in hematological parameters (hematocrit, hemoglobin concentration and erythrocyte and leukocyte numbers) were observed in several studies but most values were in the normal reference range. They may be of minor toxicological significance. Three studies (T2433, T2860, T3136) showed slight decreases in absolute brain weight in the formoterol treated groups. Dose-response relationship was occasionally seen. Toxicological significance of this equivocal results is unknown.

Genotoxicity of formoterol was tested in *Salmonella typhirium* TA 1535, TA1537, TA1538, TA98, TA100; in L5178Y mouse lymphoma cell thymidine kinase forward mutation assay; in the *in vivo* rat micronucleus assay and human lymphocytes chromosome aberrations assay. Formoterol at high concentrations (4300 mg/plate) was weakly mutagenic in Salmonella strain T1538 in the presence of S9 liver fraction. The drug was not mutagenic or clastogenic in any of the other assays.

A preliminary review of the carcinogenicity data in mice and rats indicated that leiomyoma was associated with the formoterol treatment in rats. This is not surprising because leiomyoma has been recognized as class effect for beta agonists and and is considered irrelevant to humans (Kelly et al., *J Amer Col Toxicol, 1993;12:13-21*). There were no other apparent treatment-related tumor findings. Final review and evaluation of the formoterol carcinogenicity will be carried out in consultation with the statistician reviewers.

Formoterol clinical formulations were not hemolytic to human blood, nor did it induce protein flocculation when mixed with human serum *in vitro*. The drug was slightly irritant when given subcutaneously.

Conclusion

Preclinical safety evaluation of formoterol as a bronchodilator has been completed except for the carcinogenicity studies. The heart is the major target tissue of formoterol toxicity. Changes in the heart include tachycardia, alterations in EKG, and cardiac necrosis/fibrosis. The effect of the male reproductive system occurs at higher doses in rats. These changes included decreased male fertility in adults, and decreased testicular sizes and/or testicular atrophy in young male rats. Formoterol is not teratogenic at inhalation doses of up to 91 µg/kg/day during pregnancy in rats. It causes late embryonic death and asymmetric bipartite steamebrae at dose of 60 mg/kg/day in rabbits. The drug apparently does not affect post-natal development in rats. The drug is weakly mutagenic in *Salmonella* strain T1538 in the presence of the S9-activation system and causes leiomyoma in rats and mice. However, these tumor findings in the rodents are believed to be irrelevant to humans.

There are safety concerns about cardiotoxicity of the drug in the ongoing trials. Prolonged treatment with formoterol can result in cardiac fibrosis in animals and this cardiac lesion is not readily reversible. The NOAEL for cardiotoxicity in 6-month inhalation exposure is 2.3 µg/kg/day in rats. No NOAEL values was clearly established in dogs. Pharmacokinetic analysis indicates that cardiac lesions in dogs occurs at AUC equals the human AUC from clinical therapeutic doses. Therefore, minimal margin of safety exists for formoterol.

Beta agonists have been known to be cardiotoxic in animals. They, nonetheless, have been used extensively under physician's guidance. For example, salmeterol that is another long acting beta 2 agonist and is also cardiotoxic is relatively safety in the asthma indication. Cardiotoxicity of salmeterol in dogs, however, occurs at AUCs 73 times or higher clinical doses. In another word, a safety factor of more than 73 exists for salmeterol. While safe use of other beta 2 agonists for asthma have been established clinically, minimal or no safety margin exists for formoterol. The lack of safety margin of formoterol raises serious concerns about safety of the drug clinically. Therefore, cardiac effects of the drug in the on-going clinical trials should be monitored more closely and final evaluation should be based on a thorough evaluation of the correlation between preclinical and clinical data. I informed Dr. Ray Anthracites cardiotoxicity of formoterol in preclinical studies.

Formoterol is also under development by another sponsor. Preclinical toxicology studies were also conducted by its sponsor and data from these studies are available to the Agency (IND Nos. 34342, 43720, 47013). Review of these data would be helpful for the understanding and evaluation of safety of formoterol.

Recommendation:

1. A clinical evaluation of the cardiotoxcity of formoterol is highly recommended.

2. Use of formoterol should be cautioned against in male pediatric patients. The drug may

inhibit the development of the male reproductive system, although its cardiac toxicity may not be primary concern in this population. The sponsor was asked to provide additional information on the effect on the male reproductive system (via telecon on Sept. 13, 1996). Final recommendation will be made upon a review of any new data.

3. Formoterol should be cautioned against in patients taking MAO inhibitors, phosphodiesteratse inhibitors and some xanthine products due to drug-drug interaction.

4. Final safety of formoterol should be based on thorough review of the clinical data and the preclinical data related to this drug.

Luqi Pei 3/5/97
Luqi Pei, Ph.D.
Pharmacologist/Toxicologist

Draft review completed on 11/1/96.
Revised by Dr. Sheevers on 11/15/96 and 2/12/97, 3/4/97

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HFD-570/Drs. Pei/ Anthracite/Dr. Sheevers/ Himmel/Leak
N:|IND — pharm\96-04-24.rev

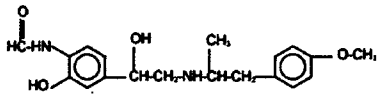
Timothy W. Robison 3/5/97

Appendix 5: Review of IND — dated December 2, 1997

Appears This Way
On Original

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 2

IND No.	
Serial Nos., Contents and Dates of Submission:	N029 (IT), Correspondence to telephone conversations in September and October, 1996. These telecons addressed spontaneous cardiac lesions in dogs, and the effect of formoterol on male fertility and reproductive system in juvenile rats. Submitted on 20-JUN-97
Information to be conveyed to Sponsor:	Yes ( ), No (X).
Reviewer:	Luqi Pei, Ph.D. (HFD-570)
Date of Review Completed:	December 2, 1997
Sponsor:	Astra Merck Inc.
Drug Name:	*Generic:* Formoterol fumarate dihydrate *Brand:* *Code:* D 2522
Structure	
Class:	β_2-agonist
Indication:	Asthma
Route of Administration:	Oral inhalation
Proposed Clinical Dose:	6, 12, 24 μg, bid or 0.25, 0.5, and 1.0 μg/kg/day

Documents reviewed in the IND.

1. Correspondence (comments) to inquiry about effect of formoterol on male fertility and reproductive system in young rats. P 1.
2. Incidence of spontaneous cardiac lesions in dogs. P 158.

Background:

Chronic administration of formoterol at high doses causes cardiotoxicity in rats and dogs (Pharmacology and toxicology review of Luqi Pei on March 5, 1997). It may also affect the

fertility in adult male rats and the development of reproductive system in male juvenile rats. Such effects, however, are much less clear at low doses. These observations raised potential concerns about safety of the drug in the clinical trials. To address the issue, conversations were held between the reviewer and the sponsor in September and October in 1997 (9/13/96, 9/16/96, and 10/31/96). The purposes of these conversations were to request additional information. The sponsor was asked to: 1) further evaluate effect of formoterol on the male fertility and development of reproductive system of the juvenile rats, 2) provide the incidence of spontaneous cardiac lesions in dogs.

REVIEW:

I. Effect on male fertility.

High dose formoterol affects on male fertility (Table 1). In a Segment I fertility study (T3015), formoterol at an oral dose of 15 mg/kg/day decreased the pregnancy rate (↓ 22%) in females. When the same males were paired later with 2 other untreated females, a decrease in the pregnancy rate was again observed in these untreated females that paired with the high dose males. Also, most of the non-pregnant females lacked any evidence of mating. These results indicate that the decrease in fertility reflects formoterol's effect on the males. The methods used in this study are briefly described in the following paragraph. Detailed information can be found in the pharmacology and toxicology review of Luqi Pei, Ph.D. on March 5, 1997.

Table 1. Mating & Performance in the Oral Segment I Fertility Study in Male Rats

	both ♂ & ♀ treated				Treated ♂ only	
Dose (mg/kg/day)	0	0.2	3.0	15	0	15
AUC ratio (animal/human)	-	5	79	1935	-	1935
Failed to induce preg. in both paired ♀	0/16	0/16	1/16	1/16	0/16	0/16
Induce preg. in only 1 /2 ♀	0/16	0/16	0/16	6/16	1/16	6/16

Sprague-Dawley rats (32♀ + 16 ♂/dose) were given by gavage formoterol at 0, 0.2, 3 and 15 mg/kg/day. The respective treatment duration were:

Females: 2 weeks before mating, through mating to gestation day 19;
Males: 9 weeks prior to mating to the time of the sacrifice in the males (total treatment duration of 25 weeks).

One half of the dams were sacrificed on day 20 and their fetus were delivered by C-section for fetal examinations. The other half of the dams were allowed to give birth and sacrificed on day 21 post partum.

Decreases in pregnancy rate was observed in the high dose group (Table 1). No significant effect on the pregnancy rate was observed in the low and mid dose groups. These observations

suggest that the mid dose do not adversely affect male fertility.

To further evaluate this observation, the treatment to the high dose and control males were continued. Later, these males were paired with another 2 untreated females. Fertility parameters were monitored. These males were sacrificed at the end of treatment. Total treatment duration in the males was 6 months. The high dose male groups showed similar findings.

The sponsor reanalyzed the results of the fertility section of the Seg. I study in rats (T3015). No new information was revealed. Sperm analysis was not conducted in the study.

The sponsor also performed analysis of the combined incidences of pregnancy in the control and high dose groups. During the experiment, each male was paired with a total of 4 females (two females each mating period). As Table 2 shows, all the control males were fertile while half of the high dose males failed to impregnate at least one of the paired four females. This failure to impregnate both the treated and untreated females suggested that formoterol may affect fertility in the males, not the females. Histological changes in the testis, however, were lacking in the treated males. Unfortunately, sperm analysis was not conducted in the study. Nonetheless, these observations are not surprising because longer and chronic administration of other beta-agonists at high doses have been shown to cause testicular atrophy and empty epididymis in rats (Pharma/tox review of Lawrence Sancilio, Ph.D. on July 9, 1992 in NDA 20236). Moreover, other beta-agonists have been known to decrease libido in man.

Table 2. Incidence of pregnancy in females paired with formoterol treated males

Pregnancy rate (incidence/paired)	Dose (mg/kg/day)	
	0	15
100 % pregnant (4/ 4)	15	8
75 % pregnant (3 /4)	1	4
50 % pregnant (2 /4)	-	3
25 % pregnant (3 /4)	-	1
Total	16	16

The above data indicated that formoterol decreased male fertility at 15 mg/kg/day (PO), approximately 2000 times the human daily inhalation dose based on AUC. The drug did not affect male fertility at doses up to 3 mg/kg/day, approximately 40 times the human daily dose based on AUC. In addition, data in the literature show no evidence of impaired male fertility at a lethal dose (30 mg/kg/day, PO) in rats (Sato and Kaneko, 1984). Metabolic pathways of formoterol appear to be similar between the rats and humans. It appears that a safety margin of 40 exists between the NOAEL in rats and the expected clinical exposure based on AUC. This large safety margin renders the impairment of male fertility by formoterol a less significant safety concern at present.

II. Effect on reproductive system of the juvenile male rats

Three 3-month repeat dose toxicity studies (2 oral and 1 inhalation) were conducted in

juvenile male Sprague-Dawley rats (16 days old at the beginning of treatment). Testicular effects of formoterol in these studies are summarized in Table 3. The original oral studies (T3136) showed increases in the incidences of testicular atrophy in the low and high dose groups. This prompted a repeat study to confirm the previous findings. The repeat study did not show any remarkable effect; dose levels and systemic exposures of these two studies were similar. The inhalation study conducted later did not reveal such a finding either.

Table 3. Testicular effect of formoterol in 3-month toxicity studies in young rats

Route	Duration	Dose	Cmax	Testicular effect		Ratio (rat/human)	
		(mg/kg/day)	(nmol/L)	Atrophy	weight Δ@	by Cmax	by AUC#
Oral	Original	0	-	1/10	-	-	-
(T3136)		0.2	≥ 0.35	4/10	↓4 %	≥ 3.3	n.c.
		0.8	0.85	1/10	↓11 %	8.1	17
		3.0	3.1	7/10	↓25 %$	29	60
Oral	Repeat	0	-	1/12	0	-	-
(T3160)		0.03	*	1/12	0	n.c.	n.c.
		0.2	*	5/12	↓1 %	n.c.	n.c.
		0.8	1.0	0/12	↑1 %	9.8	15
		3.0	3.8	1/12	↓7 %	37	56
Inhalation		0	-	0/6	-		
(T3137)		0.03	1.9	0/6	↓5 %	18	15
		0.16	10	1/6	↓7 %	97	82
		0.78	40	0/6	↓16 %	380	291

* Below the low limit of quantitation.
n.c. = not calculated
\# AUC ratio in this report was high than previous reported. For example, The AUC ratio of the high dose in the inhalation study was reported to be 145 [N010(S3), p4-5]. In this report, the ratio for the same study and dose was 291.
$ Statistically different from the control ($P < 0.05$).
@ Absolute testicular weight.

The sponsor interpreted the testicular atrophy in formoterol-treated juvenile rats as an equivocal finding and of no relevance in clinical setting. The argument was based on:

1) the incidences of testicular atrophy in the first study was not dose-related (1/10-C, 4/10-LD, 1/10-MD, 7/10 HD).
2) Testicular atrophy in the original oral study was not reproducible.
3) The inhalation study that achieved much higher systemic exposure of the drug did not reveal any testicular abnormalities.
4) The intended clinical use of formoterol was through inhalation. Thus, these oral studies are not relevant in safety evaluation of the inhaled formoterol.

Most arguments have their merits and are reasonable. However, arguments 1 and 4 may be questionable. The argument 1 was based on two observations: 1) the mid dose groups in the original study showed the incidences of testicular atrophy similar to the control group (1/10-C vs 1/10-MD; see Table 3); thus, the observation (4/10) in the low dose group should be regarded as spontaneous. 2) The repeat study only showed similar incidences testicular atrophy (5/12) in the mid dose group. No such effect was observed in the two higher dose groups; the mid and the

highest dose levels in these two studies were identical. These observations suggest that the testicular atrophy be a rather variable parameter and its spontaneous incidence rate could be about 40%. Although the high dose group of the original study showed an increase in the incidences of the testicular atrophy, the repeat study failed to reproduce this effect. According to the sponsor the lack of reproducibility and variable nature of the testicular atrophy render the observation in the high dose a non-treatment-related effect.

The sponsor's arguments appear to be logical. However, all the increases in the incidences of testicular atrophy occurred in the treated group. Furthermore, 40% and 70% of the animals in the low and high dose groups (compared to none in the controls) in the original study showed testicular atrophy. This response appears to be dose-related. In addition, such a high incidence of occurrence (70%) in the high dose group (vs 10% in controls) indicates that it is a significant finding and cannot be easily dismissed as a non-treatment-related effect without a good justification. The reasons for the lack of dose-response relationship between the low and mid dose groups, however, is unknown at present. Neither was the rather high incidences of testicular atrophy in the low groups. Thus, these data, along with the analysis of testicular weight, strongly suggest that formoterol may affect development of the male reproductive system in the juvenile rats.

Organ weight alterations are usually sensitive and quantitative indicators of toxicity. Damage to the testes may be detected as a weight change only at doses higher that those required to produce significant effects in other measurements of the gonadal status (Zenick et al, 1994). Formoterol was found to decrease testicular weights in the treated rats, especially at the high doses, in all three studies (Table 3). The reduction in testicular size, along with histological changes, strongly suggest that formoterol may affect development of male reproductive system in juvenile rats. Clinical significance of this finding is still unclear at present, but as previously discussed, a segment I fertility study (T3015) shows that high dose formoterol decreases male fertility at high doses. NOAEL values, however, were established in these studies and they ranged from 0.16 mg/kg/day (IH) and 0.8 mg/kg/day (PO).

The forth argument appears to be flawed. The effect of formoterol on male reproductive system is the result of the systemic exposure, not local exposure. The inhalation and the oral studies bear the same weight in the safety evaluation of formoterol toxicity on the reproductive system given the same AUC levels.

The NOAEL values in juvenile rats are approximately 15 (PO) and 80 (IH) times the expected daily dose in children, on an AUC basis. Thus, a sufficient margin of safety appears to exist. Data support safety of formoterol in clinical trials in children.

III. Spontaneous cardiac lesions in the control dogs.

Formoterol causes a dose-related increase in the incidence and severity of cardiac lesions (fibrosis and/or necrosis) in dogs (Table 4). The increase in the incidence and severity of the lesions in the low dose animals is not clear. Discussions are necessary to explore whether these findings were a spontaneous or drug-related effect. This was addressed by comparing the

observed lesions with the concurrent and historic data.

Table 4. Incidences of Cardiac Lesions in the Formoterol Toxicity Studies

Dose (µg/kg/day)	AUC ratio	Inhalation		Oral		Pooled data	
		T3006 5 days	T3120 1 month	T2579 1 month	T3077 12 month	Incidence	%
0	-	0/2	0/6	2/6*	0/10	2/24	8.0
0.5	-	0/2					
0.5	1		1/6				
0.7	2				2/10	4/32	12.5
2	4			0/6			
3	-	0/2					
3	5.4		1/6				
9	18				3/10		
15	-	2/2				12/24	50.0
15	24			2/6			
15	40		5/6				
90	219				5/10	9/16	56.3
100	216			4/6			

* Laboratory historic reference incidence: 4/92 (4.3 %).

Spontaneous cardiac fibrosis occurs in dogs, but the incidences appear to be low. Astra Safety Assessment, a testing laboratory of the sponsor, conducted 15 studies (not including formoterol studies) in dogs during the period of 1991 - 1995. Incidences of spontaneous cardiac lesions (cardiac necrosis and/or fibrosis) in these 15 studies was 4/92 (4%). According to their pathologist, this is a good estimator of the true background.

Concurrent controls of the four formoterol studies also showed cardiac lesions. Pooled data showed that their average incidence rates of cardiac lesions was 8%, twice of the historic reference value (4 %). More close examination revealed that this high incidence was due to a one-month oral studies (T2579), which possesses an incidence rate of 33% (2/6). The other three studies did not show any evidence of cardiac lesions (0/18), an incidence rate comparable to the reference. When all control animals in the lab were pooled together, the incidence of cardiac lesions was 5.2 %, also comparable to the reference data. Thus, an estimate of the spontaneous incidence of cardiac lesions appears to be approximately 5%.

The incidence rate of the cardiac lesions in the concurrent control group of study T2579 is 33%. This rate is significantly (6 times) higher than the spontaneous rate of 5%. Using the spontaneous rate of 33%, the sponsor argued that the incidence of cardiac lesions in the low dose groups of other studies be considered as spontaneous because these incidences (1/6 to 2/10) were lower. Also, the sponsor stated that the intended clinical use of the drug is inhalation, rendering the oral studies less relevant in safety evaluation of the inhaled formoterol. Emphasis should be placed to the inhalation studies based on the "weight of evidence approach".

To better analyze the effect in the low dose group, incidences of cardiac lesions in the low dose groups were pooled. The low dose groups were defined as having an AUC ratio of 1 - 5.4 (animal AUC/human AUC). These groups have an overall cardiac lesion incidence of 13%

(4/20), about 2.5 times background levels of 5% (4/116). Thus, these cardiac lesions in the low dose groups are most likely a treatment-related effect. Also, two studies (T3120 and T3077) failed to establish any NOAEL values. For the reasons pointed out previously, the low dose in the one-month oral study (T2579) cannot be used as a NOAEL value. This "weight-of-evidence" approach suggests that formoterol could at least partially contribute to the cardiac lesion in the low dose groups. It also suggests that a very small safety factor exists for the proposed clinical doses. To address this concern, inhalation studies with longer exposure duration should be conducted prior to these large scale and long duration phase 3 clinical trials

According to the Annual Report of 1996 (vol. 11.1, p 2, N021, 05-APR-96), the sponsor is not conducting any clinical trails in the US at present. No annual report has been received this year.

Luqi Pei, Ph.D.
Pharmacologist/Toxicologist

References

Sato and Kaneko, (1984). Reproductive Study of formoterol (3): Fertility study in rats. *falia pharmacol japon* , 27:257-265.

Zenick H., et al, (1994). Assessment of male reproductive toxicity. In Principles and Methods of Toxicology. ed. AW Hayes. Raven Press, New YorK. p 937-988.

Draft review completed on 11/13/97.
Revised by Dr. Hilary Sheevers on 11/16/97

Ori: IND
HFD-570/Division File
HFD-570/Drs. Pei/ Anthracite/Dr. Sheevers/ Himmel/Leak
N:|IND pharm\96-04-24.rev

Appendix 6: Reviews of IND [redacted] dated March 2, 1998 and April 6, 1998

Appears This Way
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CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) REPORT AND FDA-CDER RODENT CARCINOGENICITY DATABASE FACTSHEET

IND:		**Date:**	March 2, 1998
Drug Code:	D 2522	**CAS No.:**	

Division(S):	HFD-570
Drug Name(S):	Formoterol fumarate dihydrate
Sponsor:	Astra Marck Inc.
Laboratory:	Laboratory Safety Assess., AB Astra, Sweden
P/T Reviewer(S):	Luqi Pei, Ph.D.
P/T Review Date:	April 6, 1998
Carcinogenicity Study Report Date:	Nov. 2, 94 (rats); Sept. 8, 1995 (mice)
Therapeutic Category:	Bronchodilator
Pharmacological/Chemical Classification:	Beta 2 adrenergic agonist
Prior FDA Dose Concurrence:	None

MUTAGENIC/GENOTOXIC:

Formoterol is weakly positive in *S. typhimurium* strain TA 1538 at a high concentration (+S9 at 4300 µg/plate); Negative in *S. typhimurium* strains TA 1535, 1537, TA 98, TA 100, mouse lymphoma assay (L5178 cell line), human lymphocyte chromosomal aberration assay *in vitro*, and mouse micronucleus assay *in vivo*.

RAT CARCINOGENICITY STUDY :

Rat Study Duration (Weeks):	104 weeks
Study Starting Date:	April 25, 1991
Study Ending Date:	May 27, 1993
Rat Strain:	Sprague-Dawley
Route:	Inhalation (nose-only)
Dosing Comments:	30 min. exposure/day

No. Rats in group: 50/sex/group (C1, C2, LD, MD, HD)

Rat Dose Levels (µg/kg/day)*

Rat Low Dose:	0.0019	Rat Middle Dose:	0.009
Rat High Dose:	0.058		

* Theoretical total body burden based on particle size distribution.

Basis for Doses Selected:	MTD, AUC ratio (50x humans).
Prior FDA Concurrence:	None.
Rat Carcinogenicity:	Weakly positive, O & U leiomyomas in F; negative in males.

RAT TUMOR FINDINGS:

A non-statistically significant increase in incidences of leiomyomas (2/50-HD vs 0/100-C) in the uterus and mesovarium was observed in the high dose females rats. Although not statistically significant, this tumor rate was much higher than the historical background (4/7748) in this laboratory. No leiomyoma was observed in the mid and low dose groups.

RAT STUDY COMMENTS:

Leiomyoma, a rare tumor in rats, is known to be associated with beta agonist administration in this species (Kelly *et al., L Amer Col Toxicol,* 1993;12:13-21). Formoterol is a beta agonist. The (combined) incidence of leiomyomas (4%) in the high dose group was much higher than the historic background from this laboratory (0.05%). In addition, formoterol caused a dose-related and statistically significant increase in the incidences of leiomyomas in mice. Thus, formoterol should be considered as at least weakly positive, if not positive, in its carcinogenicity in rats.

MOUSE CARCINOGENICITY STUDY:

Mouse Study Duration (Weeks):	104 weeks
Study Starting Date:	April 30, 1992
Study Ending Date:	May 20, 1994

Mouse Strain:	Swiss
Route:	oral gavage
Dosing Comments:	daily dosing
No. Mouse in group:	60/sex/group (C1, C2, LD, MD, HD)

Mouse Dose Levels (mg/kg/day)

Mouse Low Dose:	0.1	Mouse Middle Dose:	0.5
Mouse High Dose:	2.5		

Basis for Doses Selected (MTD; AUC ratio):	AUC ratio (65x human exposure).
Prior FDA Concurrence:	None.
Mouse Carcinogenicity):	Positive: U (females); negative: males

MOUSE TUMOR FINDINGS:

A dose-related and statistically significant increase in the incidences of uteral leiomyomas (trend test p = 0.0002) was observed in female mice. The distribution of the tumor incidences and the associated P values of the Fisher's Exact test are presented in the Table 1. Tumor incidences were significantly increased in the mid and high dose groups when compared to the control 1 and the pooled control values (P < 0.0009); however, they may not be statistically significant (P = 0.017) when compared with the control 2 which had a tumor rate of 6.7%. Current guidelines consider the tumor incidence of ≥ 1% as a common tumor and a critical value of 0.005 applies. The pooled tumor rate in the control groups was 3.3%.

Table 1. Incidences and Statistical Analysis of Uteral Leiomyomas in Female Mice

Group	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5
Leiomyoma:					
incidence	0/60	4/60	7/60	11/60	13/60
rate (%)	0	6.7	11.6	18.3	21.7
Fisher's exact test value					
Treated vs C1	-		0.0065	0.0003	0.0001
Treated vs C2			0.264	0.0476	0.0169
Treat vs mean of C1 & C2			0.036	0.0009	0.0001

MOUSE STUDY COMMENTS:

The dose-related and statistically significant increase in the incidence of leiomyomas was found in the treated females. This indicates that the leiomyoma is a treatment-related effect. Also, all treated groups possessed higher tumor incidences than the highest background level. Furthermore, leiomyoma is known to be associated with beta 2 agonists in mice (*Gibson et al., Toxicol Pathol,* 1987;15(4) 457-467). Formoterol is a beta agonist. Thus, it is appropriate to conclude that formoterol is carcinogenic in mice.

Question for discussion:

Should we consider the non-statistically significant increase in leiomyomas in rats as drug-related effect and included in the labeling? (This tumor is commonly seen in this class of drug in species).

Hilary Sheevers, Ph.D.
Pharmacologist/Toxicologist Team Leader

Luqi Pei, DV.M.D. Ph.D.
Pharmacologist/Toxicologist Reviewer

ABBREVIATIONS FOR TUMOR SITE IDENTIFICATION*

Aden =	Adenoma
Carc =	carcinoma
LU =	lung
O =	ovary
SK =	skin
U =	uterus

* R. W. Tennant and J. Ashby, Mutation Research, 257 (1991), 209-27.

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original, Review No. 3

IND No. —

Serial Nos., Contents and Dates of Submission:

018	Inhalation carcinogenicity study in rats	1/16/96
020	Oral carcinogenicity study in mice	01/06/96

Information to be conveyed to Sponsor: Yes ( ), No (X).

Reviewer: Luqi Pei, Ph.D. (HFD-570)

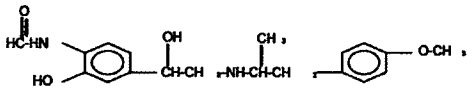
Date of Review Completed April 6, 1998

Sponsor: Astra Merck Inc.

Drug Name: *Generic Name:* Formoterol fumarate dihydrate
Code Name: D 2522

Chemical Names: (R*,R*)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1- ethylethyl]amino]ethyl]-phenyl]formamide, (E)-2-butendioate (2:1), dihydrate

Structure:



Formula and Molecular Weight: $(C_{19}H_{26}N_2O_4)_2 \cdot C_4H_4O_4 \cdot H_2O$, MW=840.9

Class: B_2-agonist

Indication: Asthma

Route of Administration: Oral inhalation

Proposed Clinical Dose: 6, 12, 24 µg, bid or 0.25, 0.5, and 1.0 µg/kg/day.

Formulation:	Use	µg/actuation	% w/w
Lactose	Excipient	600	
Formoterol	bronchodilator	6, 12	

Documents reviewed in the IND.

1. 2-year oral carcinogenicity study in mice. submission of 1/16/96, vol. 10.1, p 45.
2. 2-year inhalation carcinogenicity study in rats. submission of 1/11/95, vol. 5.2 - 5.8, p 1.

REVIEW:

1. 2-year inhalation carcinogenicity study in rats (submission of 1/16/96, vol. 5.2 - 5.8, p1

Testing lab:	Laboratory of Safety Assessment, AB Astra, Sweden
Study number:	90106
Study dates:	4/25/91 - 5/27/93, Report date - 11/2/94
GLP:	Yes
Dose:	0 (C), 0 (C), 1.9 (L), 9.0 (M), 58 (H) µg/kg/day
Batch No.	100/91

Methods:

Three groups of Sprague-Dawley rats (50/sex/dose) were exposed nose-only to 1.9, 9.0, and 58 µg/kg/day of formoterol in lactose for 2-years to study its carcinogenic potential. The daily exposure duration was 30 minutes. The estimated MMAD was [redacted]. The respective air mass concentrations, the formoterol concentration, the estimated total body burden, and the lung deposition of formoterol in the treated groups are summarized in Table 1.

Table 1. Dose estimates of the Carcinogenicity Study in Rats

Groups	Mean aerosol mass conc. (µg/L air)	Mean formoterol concentration (µg/L air)	Mean total body burden* (µg/kg)	Mean lung deposition* (µg/kg)
I	0	0	0	0
II	14	0.3	1.9	0.43
III	65	1.4	9.0	2.0
IV	385	8.5	58	11
V	363	0	0	0

* Accumulative and theoretical number based on the measured particle size distribution. The deposition factors for total body and lung depositions were 0.45 and 0.09 respectively.

These animals were 9 weeks old at the start of the dosing. All surviving animals were sacrificed at weeks 104 to 106 of dosing. Necropsy and histological examinations were conducted in all terminally and pre-terminally sacrificed (or dead) animals. Plasma drug levels was determined in satellite animals (n = 1/sex/time point) on day 8 and 176. The following parameters were monitored:

Clinical signs:	1/month $_{\text{year 1}}$, 1/ 2 wk $_{\text{year 2}}$
Body weight:	Weekly $_{\text{week 0 -13}}$, 1/ 2 wk $_{\text{week 14 - 3}}$, 1/ 4 wk $_{\text{week 3- - 106}}$,
Food consumption:	Weekly $_{\text{week 0 - 13}}$, 1/ month $_{\text{week 14 - 106}}$
Heart rate:	Pre and during exposure on weeks 4, 13, 25, 36, 48, 63, 80, 87 and 103
Ophthalmology:	pretest, years 1 and 2
Clinical pathology:	hematology $_{\text{months 13, 19, 24 and 26}}$, blood chemistry $_{\text{month 24}}$
Plasma drug level:	Days 7, 345, 533 at hours 0.25, 0.5, 0.75, 1, 2, 4, 8 and 24
Pathology:	
Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, testes, prostate, spleen, thymus, uterus
Necropsy:	Preterminal and terminal sacrifice
Histopathology:	Specimens from all rats and relevant gross changes of the following organs: Adrenals, aorta, bone and marrow, brain, bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, ileum, jejunum, heart, kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes, mammary glands, nasal cavity and sinuses, optic nerves, ovaries, pancreas, pharynx, pituitary, prostate, rectum, salivary glands, skeletal muscle, spinal cord, stomach, spleen, testes, treacha (interim analysis), thymus, thyroid and parathyroids, urinary bladder, uterus, vagina

Comment: No dosing was performed on 5 holidays (days 40, 225, 404, 591 and 599). Failure of dosing on these days may not significantly affect the out come of the study.

Results:

Mortality: Fifty-nine percent (296/500) of animal died pre-terminally. There was no apparent differences in mortalities between treated and control groups (Table 2 and Figure 1).

Table 2. Mortality during the carcinogenicity study in rats

Group	I	V	II	III	IV
Dose (µg/kg/day)	0	0	1.9	9.0	58
Incidence					
Male	29	26	28	34	32
Female	33	35	33	31	30
Percentage					
Male	58	52	56	68	64
Female	66	70	66	62	60

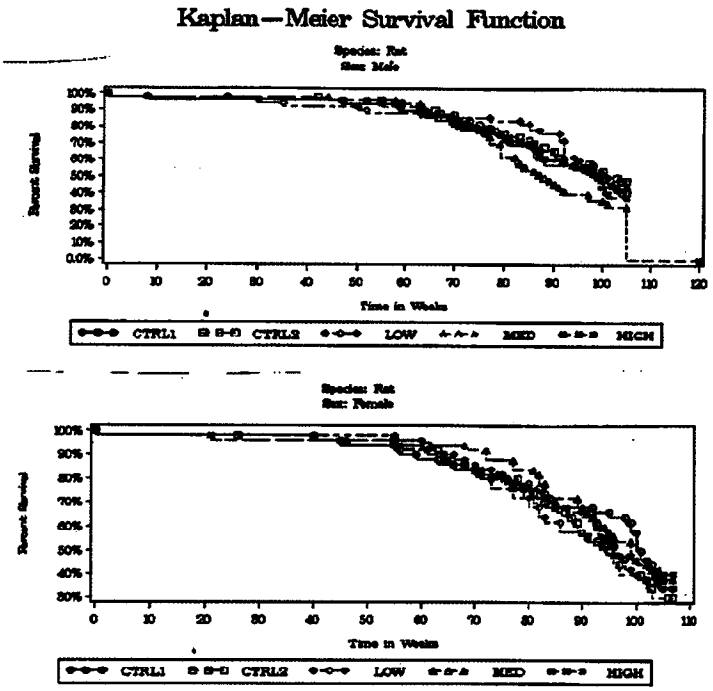


Fig 1. Mortality as a function of time in the 2-year carcinogenicity study in rats. Upper panel: males and lower panel: females. Dose levels are 0, 1.9, 9.0, 58, 0 µg/kg/day for groups 1, 2, 3, 4, and 5, respectively. There were 50 animals in each group.

Clinical signs: No treatment-related effects were observed.
Palpable mass: No treatment-related effect were seen (Table 3).

Table 3. Incidences of palpable mass during the carcinogenicity study in rats

Group	I	V	II	III	IV
Dose (µg/kg/day)	0	0	1.9	9.0	58
Week 52					
Male	7	7	5	4	4
Female	6	4	4	6	7
Week 103					
Male	31	30	22	25	26
Female	39	33	36	38	37
1st week of appearance					
Male	14	34	38	34	30
Female	26	43	30	26	30

Body weight: Figure 2 and Table 4 present changes in actual and relative body weight throughout the study. Mean body weight decreased in all treated groups starting at week 30 and persisted thought the dosing period. A dose-related decrease in body weight was most apparent in males at week 75 when their body weights peaked. The respective decreases were 9, 10 and 12% for the low, mid and high dose groups, compared to the mean of the control groups.

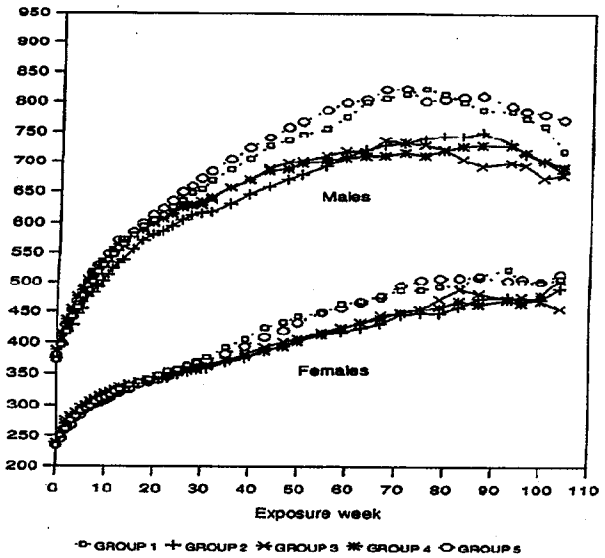


Fig 2. Mean body weight as a function of time in the 2-year carcinogenicity study in rats. Dose levels are 0, 1.9, 9.0, 58 µg/kg/day and 0 for groups 1, 2, 3, 4 and 5, respectively.

Table 4. Mean Body Weight in the 2-year Carcinogenicity Study in Rats

	Male					Female				
Group	I	V	II	III	IV	I	V	II	III	IV
Dose (µg/kg/day)	0	0	1.9	9.0	58	0	0	1.9	9.0	58
Absolute (g)										
Week 0	371	378	374	375	388	234	235	239	239	238
Week 55	756	787	694	711	703	451	452	417	420	416
Week 75	823	802	740	729	712	488	505	451	457	457
Week 104	732	786	697	692	705	508	515	490	460	507
Week 106	-	-	-	-	-	531	543	513	470	523
Relative to controls										
(%), Week 0	-	-	-	-	↑3.7	-	-	↑1.9	↑1.9	↑1.4
Week 55	-	-	↓10	↓7.8	↓8.9	-	-	↓7.6	↓7.0	↓7.9
Week 75	-	-	↓9.2	↓10.3	↓12.4	-	-	↓9.2	↓8.0	↓8.0
Week 104	-	-	↓8.2	↓8.8	↓7.2	-	-	↓4.2	↓10.1	↓0.8
Week 106	-	-	-	-	-	-	-	↓4.5	↓12.5	↓2.6

Food consumption (weekly): The high dose group showed an increase in food consumption throughout dosing while the mid dose animals showed an increase in food consumption only at early phase of the dosing (Fig. 3). No difference was noticed in the low dose group compared to the controls.

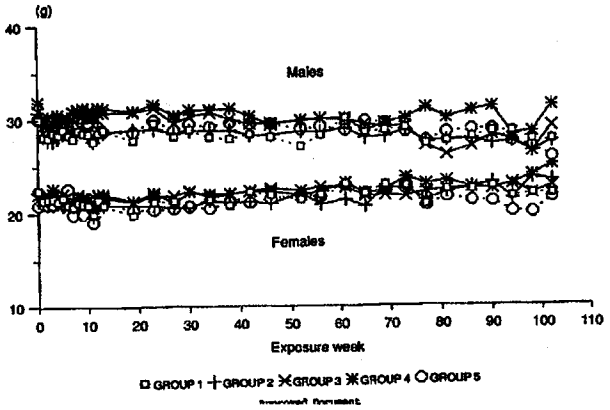


Fig 3. Mean Food consumption as a function of time in the 2-year carcinogenicity study in rats. Dose levels are 0, 1.9, 9.0, 58, 0 µg/kg/day for groups 1, 2, 3, 4 and 5, respectively.

Water consumption: Trend in water consumption was similar to the food consumption.

Ophthalmology: No treatment-related effects were observed.

EKG: Dose-related increases in heart rates were seen in all treated groups (Table 5). This increase in heart rates lasted more than 30 minutes. However, tolerance tended to develop during the second year, especially in the high dose animals. Also, the high dose group showed an increased incidences of premature contraction (4-C, 1-LD, 3-MD and 11-HD).

Table 5. Changes* in Mean Heart Rates in the 2-year Carcinogenicity Study in Rats (n = 3/ ponit)

	Male				Female			
Dose (µg/kg/day)	0	1.9	9.0	58	0	1.9	9.0	58
Week 4	- 2.8	8.0	27.0	30.7	- 1.1	11.6	10.9	30.0
Week 26	- 3.5	11.7	30.9	34.1	- 5.3	8.5	18.9	23.5
Week 50	- 2.3	4.1	25.1	33.7	- 3.8	7.1	11.9	37.2
Week 63	- 8.5	0.3	18.0	22.1	1.9	5.6	13.8	32.2
Week 87	- 2.7	9.7	23.8	14.5	- 9.0	2.0	9.4	21.6
Week 103	0.3	3.7	20.9	13.3	1.3	3.2	4.6	18.9

* Compared to baseline.

Clinical chemistry (weeks 5, 13 & 24):

Hemotology: No toxicologically significant effects were observed. At the end of the study, the high dose animals showed a moderate decrease in mean platelet numbers (14 - 24%).

Blood chemistry: Decreases (18% - 28%) in mean plasma glucose levels were seen in high dose group of both sexes; Occasional and minor disturbances in serum mineral concentrations were seen in the mid and high dose groups.

Organ weights: Dose-related increases in heart (absolute and relative) and lung (relative only) weights were seen in both mid and high dose groups (Table 6). Changes in other organs are minimal or unnoticed.

Table 6. Organ weights in the 2-year inhalation carcinogenicity study of formoterol in rats (terminal sac.)

	Male				Female			
Groups	I, V#	II	III	IV	I, V#	II	III	IV
Dose(mg/kg/day)	0	1.9	9.0	58	0	1.9	9.0	58
Animals #	45	22	16	18	32	2.81		
Body weight (g)	729	672	666	680	502	482	464	491
Brain (g)	2.35	2.36	2.28	2.33	2.14	2.12	2.12	2.15
% body weight	0.34	0.36	0.35	0.35	0.45	0.46	0.47	0.46
Heart (g)	1.94	2.01	2.17	2.18*	1.44	1.45	1.50	1.65**
% body weight	0.28	0.31	0.33**	0.33*	0.29	0.31	0.33*	0.35**
Kidney (g)	4.33	4.40	4.03	4.20	2.81	2.78	2.69	2.96
% body weight	0.63	0.67	0.62	0.62*	0.58	0.59	0.59	0.64
Lung (g)	2.19	2.37	2.48*	2.48**	1.67	1.72	1.73	1.82
% body weight	0.32	0.36	0.39**	0.37**	0.34	0.37	0.38	0.39
Testes/ovary (g)	3.60	3.51	3.38	3.41	0.09	0.09	0.08	0.08
% body weight	0.50	0.53	0.53	0.51	0.020	0.018	0.018	0.019

Pooled controls (Groups I and V), * $P < 0.05$, ** $P < 0.01$.

Pathology:

Necropsy: There were slight increases in the incidences of gray and white foci in the lung and eczematous skin in both sexes and ovary cysts in the females (Table 7).

Table 7. Gross Pathology in a 2-Year Inhalation Carcinogenicity Study of Formoterol

	Male					Female				
Dose (µg/kg/day)	0	0	1.9	9.0	58	0	0	1.9	9.0	58
N	50	50	50	50	50	50	50	50	50	50
Lung, gray & white foci	1	3	2	4	0	3	6	2	4	9
Kidney, pale	1	3	2	0	3	0	0	0	0	1
Ovary, cysts	-	-	-	-	-	6	8	7	9	14
Skin, subcutis, eczematous	5	13	12	14	19	8	5	7	9	13

Histopathology:

Non-neoplastic findings: Noticeable microscopic findings (Table 8) included myocardionecrosis/ fibrosis in the heart, remnant of the pituitary adenoma in the brain (female), and Leidig cell hyperplasia in the testes (male).

Table 8. Non-neoplastic lesions in a 2-yrear Inhalation carcinogenicity Study of Formoterol

	Male					Female				
Group	I	V	II	III	IV	I	V	II	III	IV
Dose (µg/kg/day)	0	0	1.9	9.0	58	0	0	1.9	9.0	58
Terminal & preterminal sac.										
Heart, myonecrosis/fibrosis	2/40	7/39	5/42	12/35	7/42	5/37	6/37	2/36	3/41	9/36
Brain, pituit. adeno. remnant	0/50	1/50	0/50	0/50	0/50	0/50	0/50	2/50	4/50	5/50
Testis, Leydig cell hyperplas.	1/50	1/50	1/50	0/50	4/50	-	-	-	-	-
Preterminal sac/death										
Brain, pituit./ adeno. remnant						0/34	0/33	0/33	3/31	4/30

Neoplastic findings (Table 9): The high dose females showed increases in the incidence (2) of Leiomyomas of the ovary and uterus (one each), and an increase in the incidences of lung

metastatic carcinoma (3). The origin of these lung tumors were the mammary carcinoma (2) and the thyroid gland (1). Also, all three rats died pre-terminally.

Table 9. Neoplastic Lesions (Total) in a 2-Year Inhalation Carcinogenicity Study of Formoterol in Rats

	Male					Female				
Group	I	V	II	III	IV	I	V	II	III	IV
Dose (µg/kg/day)	0	0	1.9	9.0	58	0	0	1.9	9.0	58
N	50	50	50	50	50	50	50	50	50	50
Tumor bearing animals (%)	96	96	88	82	82	98	100	98	98	98
Brain/ astrocytoma	1	1			1			2		1
granular cell tumor		1			1					
metastatic carcinoma		2		1			2	1	2	
mixed glioma		1								
schwannoma					1					
Heart/ hemangioma etc. (total)		1			2					
metastatic sarcoma					1					
Liver/ adenoma/hepatocyte	2	2			2	2		1		
carcinoma/hepatocyte	2	4			1					1
metastatic sarcoma					1					
Lung/ metastatic carcinoma	2	2	2	1	1	1		1	2	3
metastatic sarcoma	1	2	2							1
tumor/bronchoalveolar	1									
Trachea/pharynx, met sarcoma				1		1				
Pancreas/ carcinoma/ islet c.	2									
adenoma/ acinar	2	1			1		1			
adenoma/ islet cell	5	6	6	3	2	4		2		2
Kidney, metastatic carcinoma		1								
mesenchymal tumor	1						1			
Testes/ Leydig celltumor	1	1	4		2	-	-	-	-	-
mesothelioma			1			-	-	-	-	-
Pituitary G/ carcinoma/aden.	29	29	29	29	23	44	43	45	42	33
Thyroid G/ adenoma	9	8	6	8	7	5	3	2	8	5
carcinoma/C-cell		1	1	1		1	1			1
Parathyriod G/ adenoma			1		1					1
Mammary G/ fibroadenoma	1/1	1/1	2/2	-	1/1	27	27	28	28	29
adenocarcinoma	0/1	0/1	1/2	-	0/1	15	17	14	15	17
Ovary/ Tumor/granulose cell	-	-	-	-	-	1	1			2
mesovarian leiomyoma	-	-	-	-	-					1
Uterus/ leiomyoma	-	-	-	-	-					1
adenocarcinoma	-	-	-	-	-	1				
Skin/subcutis/all tumors	21	23	15	19	18	9	2	2	3	7

Plasma drug levels: Drug concentration was determined on days 8 and 176 (n=1/sex/time point/dose). The limit of quantitation was 0.20 nmol/l when 2.0 ml plasma was used. Plasma levels were below the LOQ and could not be determined for most time in the low dose animals. Therefore, reliable AUC values were not available. Table summarizes plasma levels 15 minutes after dosing.

Table 10. Plasma Formoterol levels in the 2-year Inhalation carcinogenicity Study in Rats

Dose *	1.9 µg/kg/day	9.0 µg/kg/day	58 µg/kg/day
$C_{0.25\,h}$ (nmol/L)	M, F	M, F	M, F
Week 1 (d 7)	0.30, 0.41	1.3, 1.4	6.4, 10.4
Week 49 (d 345)	0.40, 0.86	0.93, 1.6	7.4, 7.0
Week 76 (d 530)	0.53, 0.52	2.3, 3.2	4.5, 5.5
AUC (nmol.h/L)			
Week 1 (d 7)	-	1.94, 2.27[a]	11.6, 16.1[b]
Week 49 (d 345)	-	2.23, 2.86[a]	17.9, 18.6[b]
Week 76 (d 530)	-	3.74, 4.14[a]	14.9, 14.0[b]
Mean			15.5

a = 0 - 4.5 h, b = 0 - 8.5 hour.

Conclusion: Treatment of Sprague-Dawley rats with inhalation formoterol for 2 years induced a non-statistically significant increase in the incidence of leiomyomas in the uterus and ovary at 58 µg/kg/day. No such findings were observed at lower doses (2 and 9 µg/kg/day). This leiomyoma is considered as a treatment related effect based on scientific understanding of the carcinogenesis of beta agonists. Detailed information can be found in the summary and evaluation section of this review.

2. 2-year oral (gavage) carcinogenicity study in mice (submission of 1/16/96, vol. 10.1 - 10.9, p45)

Testing lab:	[redacted]
Study number:	85133
Study dates:	April 30, 1992 - May 20, 1994, Report date - Sept. 8, 1995
GLP:	Yes
Dose:	0 (C), 0 (C), 0.1 (L), 0.5 (M), 2.5 (H) mg/kg/day
Batch No.	DSD:5, 12, 20, 42; DTA: 6,13, 21, 44; and DTK: 7, 22, 45

Methods:

Three groups of Swiss mice [redacted], *Mus musculus,* 60/sex/dose) were given by gavage 0.1, 0.5, and 2.5 mg/kg/day of formoterol fumarate dihydrate 2-years to study its carcinogenic potential. At the start of the dosing, these animals were 6 weeks of age. The vehicle consisted of 0.77 gram citric acid (monohydrate), 2.25 g sodium hydrogen phosphate (dihydrate), 8.5 gram of sodium chloride, and proper amount of formoterol per litter of distilled water. The animals were sacrificed at week 104 to 106 of dosing for necropsy and histological examinations.

Clinical signs:	Daily to weekly
Body weight:	Weekly
Food consumption:	Weekly
Ophthalmology:	pretest and week 104
Clinical pathology:	hematology $_{\text{pretest, terminal}}$, blood chemistry $_{\text{pretest, terminal}}$
Plasma drug level:	Day 14 and month, 6, 12 and 22 at hours 0.17, 0.5, 1, 2 and 4
Pathology:	

Organ weights:	Adrenals, brain, heart, kidneys, liver, lungs, ovaries, testes, prostate, spleen, testes, thymus, uterus
Necropsy:	Preterminal and terminal sacrifice
Histopathology:	Sepcimens from all rats and relevant gross changes of the following organs: Adrenals, aorta, bone and marrow, brain, bronchi, cecum, colon, duodenum, epididymides, esophagus, eyes, ileum, jejunum, heart, kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes, mammary glands, nasal cavity and sinuses, optic nerves, ovaries, pancreas, pharynx, pituitary, prostate, rectum, salivary glands, skeletal muscle, spinal cord, stomach, spleen, testes, treacha (interim analysis), thymus, thyroid and parathyroids, urinary bladder, uterus, vagina

Results:

Mortality: Figure 4 and Table 11 show survival and mortality rate as a function of time. There was no apparent differences in mortalities between treated and control groups (Table 1 and Fig. 4). Survival rates ranged from 42 - 55 % in the males and 33 - 55% in the females.

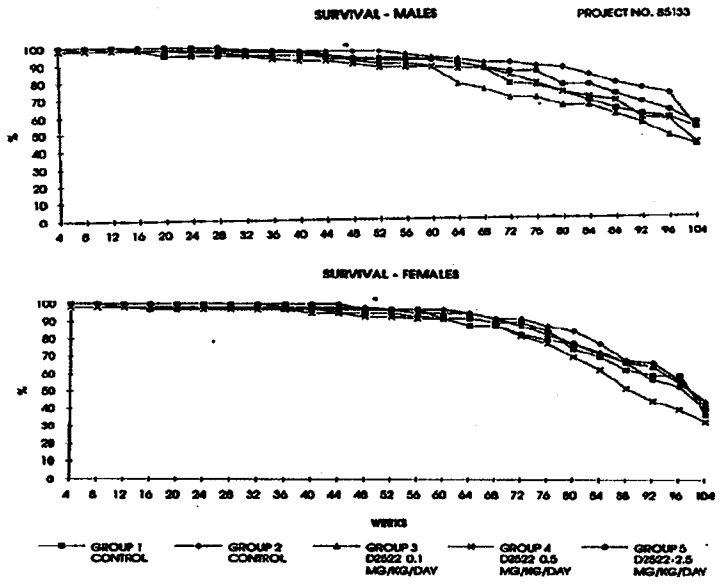


Fig 4. Survival as a function of time in the 2-year carcinogenicity study in mice.

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Table 11. Mortality and Survival Rate During the Carcinogenicity Study in Mice

Group	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5
Mortality (incidence)					
Male	29	29	35	34	27
Female	38	35	33	40	36
Survival rate (%)					
Male	52	52	42	43	55
Female	37	42	45	33	40

Clinical signs: No treatment-related effects were observed.
Palpable mass: No treatment-related effect were seen (Table 12).

Table 12. Incidences of palpable mass during the carcinogenicity study in mice

Group	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5
Male	1	6	1	1	5
Female	4	1	6	4	4

Body weight: Statistically significant increases in mean body weight (up to 7%) were seen in the first 1 - 1 1/2 years of the treatment (Fig . 5). This difference became less evident as the treatment continued. Numerical expressions can be found in Table 13. This change in body weight was generally dose-related.

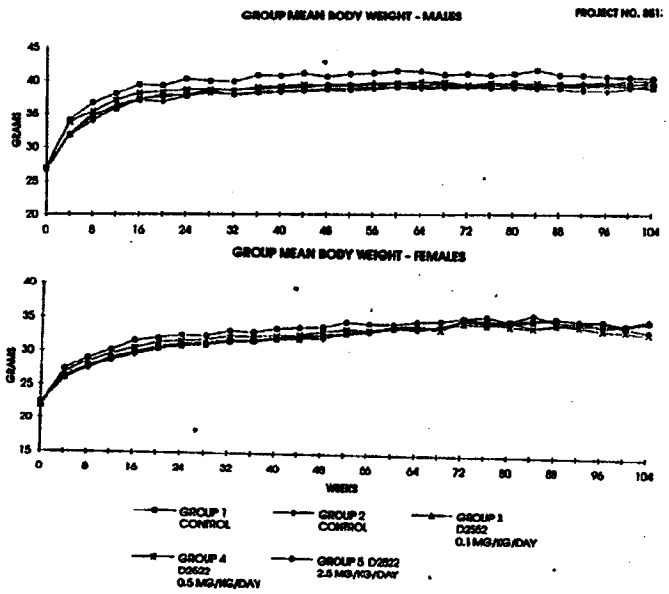

Fig 5. Mean body weight as a function of time in the 2-year carcinogenicity study in mice.

Table 13. Mean Body Weight in the 2-year Oral Carcinogenicity Study in Mice

	Male					Female				
Dose (μg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Absolute (g)										
Week 0	26.9	26.5	26.8	26.8	26.9	22.3	22.0	21.9	22.0	21.9
Week 26	38.2	37.8	38.5	38.4	40.5**	31.0	31.1	30.8	31.6	32.8**
Week 52	39.0	38.6	39.5	39.4	41.1**	33.2	33.3	33.0	33.7	34.8**
Week 78	39.5	39.3	39.2	40.1	41.3*	35.0	35.5	35.0	35.0	35.1
Week 104	39.1	39.6	39.9	40.1	40.6	35.0	35.4	35.7	35.6	34.1
Relative (%@)										
Week 0	-	-	-	-	-	-	-	-	-	-
Week 26	-	-	↑1.3	↑1.1	↑6.6	-	-	-	↑1.8	↑5.6
Week 52	-	-	↑1.8	↑1.5	↑5.9	-	-	-	↑1.4	↑4.7
Week 78	-	-	-	↑1.8	↑4.8	-	-	-	-	-
Week 104	-	-	↑1.4	↑1.9	↑3.2	-	-	-	-	↓3.1

* P<0.05, ** P < 0.01.

@ Percentage of mean of the controls.

Food consumption (weekly): The high dose males showed an increase in food consumption for most of the study while the high dose females showed an temporary increase in food consumption at early phase of the dosing.

Ophthalmology: No treatment-related effects were observed.

Clinical chemistry: No toxicologically significant effects were observed.

Organ weights: No significant findings were observed. A trend of increases in lung and heart weight was evident (Table 14), but none of this changes reached statistically significant level.

Table 14. Organ weights in the 2-year Oral carcinogenicity study of formoterol in juvenile Mice (terminal)

	Male				Female			
Groups	I, II	III	IV	V	I, II	III	IV	V
Dose(mg/kg/day)	0	0.1	0.5	2.5	0	0.1	0.5	2.5
Body weight (g)	32.4	32.2	32.8	33.3	29.3	29.2	27.5	27.7
Heart (g)	0.238	0.244	0.238	0.265	0.194	0.197	0.200	0.202
Lung (g)	0.301	0.317	0.272	0.335	0.267	0.310	0.279	0.336
relative (%)	0.933	0.991	0.824	1.01	0.923	1.07	1.02	1.23
Testes/ovary (g)	0.193	0.200	0.193	0.189	0.227	0.375	0.339	0.179
Uterus (g)	-	-	-	-	1.57	1.01	0.90	0.71

Pathology:

Necropsy: The most significant findings in gross pathology was a dose-related increase in the incidences of enlarged ovary and increased incidences of uterus mass in the females (Table 15). Incidences of masses in several organs of interests are also listed in the table for convenience. The interest in heart derives from it being the target organ of formoterol toxicity in general toxicity studies. Lung is the port of entry for clinical medications. Leiomyomas of the uterus in rodents is known to be associated with beta agonist treatment.

Table 15. Gross pathology in a 2-year Oral carcinogenicity Study of Formoterol in Mice

	Male					Female				
Dose (mg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Preterminal sacrifice (n)	29	29	38	34	28	38	35	33	41	38
Heart, mass	0	1	4	1	2	1	1	0	2	2
Ovary, enlargement						1	2	1	7	11
Uterus, mass						8	8	10	13	13
Vagina, mass						0	0	0	0	1
Terminal sacrifice (n)	31	31	22	26	32	22	25	27	19	22
Heart, mass	0	0	1	0	1					
area raised	1	1	1	0	5	0	0	2	0	2
Lung, area depressed	1	1	1	0	5	1	0	1	1	3
area raised	1	0	0	3	4	0	1	1	0	1
Eye, opacity						7	5	8	9	11
Uterus, mass						3	9	10	6	9
Total (n)	60	60	60	60	60	60	60	60	60	60
Heart, mass	0	1	5	1	3	1	1	2	0	2
Lung, mass	8	5	8	5	7	8	7	5	4	4
Ovary, mass						6	5	6	9	6
enlargement						4	3	4	7	13
Uterus, mass						11	17	20	19	22
Eye, opacity						17	13	10	14	14

Histopathology:

Non-neoplastic findings: Table 16 presents noticeable microscopic non-neoplastic findings. Increased incidences of pneumonia and incidences of amyloidosis in the testis and liver were observed in the high dose males. Incidences of cardiac lesions were similar in all groups. The severity of myocardial lesions were dose-related in the males but the differences were minimal. The incidences and severity of lung injuries were similar in all groups.

Table 16. Non-neoplastic Findings in a 2-year Oral carcinogenicity Study of Formoterol in Mice (N = 60)

	Male					Female				
Dose (mg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Heart, atrial thrombosis	3	3	9	4	8	4	5	1	4	4
myocardiofibrosis/deg.	20	25	25	28	28	22	16	15	13	23
average grade	2.5	2.4	2.0	2.6	3.1	2.0	2.0	3.0	2.3	2.3
endo/epicarditis	9	8	8	6	9	4	5	8	6	9
Liver, amyloidosis	5	9	8	8	13					
Lung, pneumonia	5	4	7	1	9	8	8	7	2	4
Stomach, erosion, granular mucosa	4	6	3	3	9	5	3	0	4	6
Testis, amyloidosis	7	5	6	8	12	-	-	-	-	-
Uterus, endomet. hyperplasia	-	-	-	-	-	22	23	26	19	22

Neoplastic findings: A dose-dependent and statistically significant increase ($P < 0.0002$, trend test) in the incidence of leiomyomas of the uterus was seen in all treated groups (Table 17). The trend was evident in both the pre-terminally and terminally sacrificed animals. The overall statistical analysis of the tumor incidences verse control groups is presented in Table 18. Other observations (Table 19) appeared to be spontaneous in nature and causal responses were lacking.

Table 17. Neoplastic lesions (Pre- and terminal) in a 2-year Oral carcinogenicity Study of Formoterol in Mice

	Male					Female				
Dose (mg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Preterminal sacrifice										
N	29	29	38	34	28	38	35	33	41	38
Liver/ hemangioma	0	1	0	1	0	0	0	0	0	3
carcinoma/hepatocyte	1	0	2	0	3					
Spleen/ hemangiosarcoma						1	2	0	2	3
Ovary/ carcinoma						0	0	0	0	1
leiomyoma						0	0	0	0	1
Uterus/ leiomyoma*						**0**	**3**	**2**	**6**	**7**
Terminal sacrifice (n)						22	25	27	19	22
Liver/ hemangioma	1	2	0	1	2				1	
carcinoma/hepatocyte	2	1	1		1	1			1	
Uterus/ leiomyoma						**0**	**1**	**5**	**5**	**6**
Total						**0/60**	**4/60**	**7/60**	**11/60**	**13/60**

* Bold indicates significant findings.

Table 18. Incidences and Statistical Analysis of Uteral Leiomyomas in Female Mice

Group	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5
Leiomyoma:					
incidence	0/60	4/60	7/60	11/60	13/60
rate (%)	0	6.7	11.6	18.3	21.7
Fisher's exact test value					
Treated vs C1	-		0.0065	0.0003	0.0001
Treated vs C2		-	0.264	0.0476	0.0169
Treat vs mean of C1 & C2			0.036	0.0009	0.0001

Table 19. Detailed Neoplastic lesions (Total) in a 2-year Oral carcinogenicity Study of Formoterol in Mice*

	Male					Female				
Group	I	II	III	IV	V	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Adrenal/ neuroblastoma		1								
cortical adenoma			2	1		1		1		
pheochromacytoma	1				1		1			
Bone marrow/mast cell tumor					1					
hemangiosarcoma	2			2						
myeloid leukemia			1					1		
Brain/ schwannoma	1									
Harderian gland/ adenoma	1/1	-	1/2	0/1	0/1	0/2	0/2	1/1	1/2	0/1
Jejunum/ adenosarcoma		1			1	1	2	0	2	
Kidney, carcinoma						1				1
Liver/ adenoma/hepatocyte	8	7	10	13	11	4	1	4	2	2
carcinoma/hepatocyte	3	1	3	0	4	1			1	
hemangiosarcoma	2	4	0	2	2	3	2	1	2	1
hemangioma	1	3	0	2	2	0	0	0	1	3
Lung/ alveo/broncho adenoma	14	11	9	13	12	12	17	18	10	15
carcinoma	5	3	4	1	5	4	2	4	3	2
carcinoma, undifferent'd					1				1	
sarcoma, undifferent'd									1	
Lymph node/ lymphosarcoma	3	6	5	4	3					
histiocytic sarcoma	1	4	3	3	3					
hemangioma									1	
Pancreas/ adenoma / islet c.	1								1	
Prostate/ adenocarcinoma		1								
Seminal vesicle		1/59								
Spleen/ hemangiosarcoma	4	1	3	1		1	3	0	2	3
Stomach/ all tumors		1						1	1	
Testes/ adenocarcinoma aden.		1		1						
Pituitary G/ adenoma		1	1		1		2	2	2	1
Thyroid G/ foll. adenoma				1	1					
carcinoma/C-cell										
Parathyriod G/ adenoma						1	0	1	0	1
Mammary G/ adenocarcinoma										
Ovary/ tumor/granulose cell						1				
cysadenoma								3	1	1
Carcinoma, undiff.										1
leiomyoma										1
Ureter/ trans. cell papilloma	1/7	0/3	0/12	0/10	0/4					
U. bladder/leiomyosarcoma					1	1				
Uterus/ leiomyoma						**0**	**4**	**7**	**11**	**13**
other tumors						12	10	14	11	10
Skin/ miscellaneous, total	0/4	3/12	0/14	0/13	0/7	0/15	0/12	1/8	2/7	0/9

* N = 60/group unless specified.
Few other incidental tumors are not listed.

Plasma drug levels: Plasma formoterol concentrations were analyzed by Astra Draco using coupled column liquid chromatography. Results were reported in Report No. 843-RD-0349. The limits of quantitation were 0.50, and 0.15 nmol/l when 0.3 ml and 1.0 ml plasma was

Table 19. Detailed Neoplastic lesions (Total) in a 2-year Oral carcinogenicity Study of Formoterol in Mice*

	Male					Female				
Group	I	II	III	IV	V	I	II	III	IV	V
Dose (mg/kg/day)	0	0	0.1	0.5	2.5	0	0	0.1	0.5	2.5
Adrenal/ neuroblastoma		1								
cortical adenoma			2	1		1		1		
pheochromacytoma	1				1		1			
Bone marrow/mast cell tumor					1					
hemangiosarcoma	2			2						
myeloid leukemia			1					1		
Brain/ schwannoma	1									
Harderian gland/ adenoma	1/1	-	1/2	0/1	0/1	0/2	0/2	1/1	1/2	0/1
Jejunum/ adenosarcoma		1			1	1	2	0	2	
Kidney, carcinoma						1				1
Liver/ adenoma/hepatocyte	8	7	10	13	11	4	1	4	2	2
carcinoma/hepatocyte	3	1	3	0	4	1			1	
hemangiosarcoma	2	4	0	2	2	3	2	1	2	1
hemangioma	1	3	0	2	2	0	0	0	1	3
Lung/ alveo/broncho adenoma	14	11	9	13	12	12	17	18	10	15
carcinoma	5	3	4	1	5	4	2	4	3	2
carcinoma, undifferent'd					1				1	
sarcoma, undifferent'd									1	
Lymph node/ lymphosarcoma	3	6	5	4	3					
histiocytic sarcoma	1	4	3	3	3					
hemangioma									1	
Pancreas/ adenoma / islet c.	1								1	
Prostate/ adenocarcinoma		1								
Seminal vesicle		1/59								
Spleen/ hemangiosarcoma	4	1	3	1		1	3	0	2	3
Stomach/ all tumors		1						1	1	
Testes/ adenocarcinoma aden.		1		1						
Pituitary G/ adenoma		1	1		1		2	2	2	1
Thyroid G/ foll. adenoma				1	1					
carcinoma/C-cell										
Parathyriod G/ adenoma						1	0	1	0	1
Mammary G/ adenocarcinoma										
Ovary/ tumor/granulose cell						1				
cysadenoma								3	1	1
Carcinoma, undiff.										1
leiomyoma										1
Ureter/ trans. cell papilloma	1/7	0/3	0/12	0/10	0/4					
U. bladder/leiomyosarcoma					1	1				
Uterus/ leiomyoma						**0**	**4**	**7**	**11**	**13**
other tumors						12	10	14	11	10
Skin/ miscellaneous, total	0/4	3/12	0/14	0/13	0/7	0/15	0/12	1/8	2/7	0/9

* N = 60/group unless specified.
Few other incidental tumors are not listed.

Plasma drug levels: Plasma formoterol concentrations were analyzed by Astra Draco using coupled column liquid chromatography. Results were reported in Report No. 843-RD-0349. The limits of quantitation were 0.50, and 0.15 nmol/l when 0.3 ml and 1.0 ml plasma was

used, respectively. Blood samples were collected at hours 0.17, 0.5, 1.0, 2.0 and 4.0 post-dosing on months 0.5, 6, 12 and 22 of the treatment. Sample sizes per group ranged from 3/sex (LD) to 10/sex (HD at early phase of the treatment). Plasma levels were below the LOQ and could not be determined for most time in the low dose animals. Mean AUC and Cmax (if applicable) are listed in Table 20. The data were too limited and scattered to allow general conclusions about difference in sexes and treatment duration.

Table 20. Plasma Formoterol levels* $C_{0.25\,hr}$ (nmol/L) in the 2-year Oral carcinogenicity Study in Mice

	Male			Female		
Dose (mg/kg/day)	0.1	0.5	2.5	0.1	0.5	2.5
AUC $_{0\text{-}4\,h}$ (nmol.h/L)						
Week 2	-	1.1	7.1	-	1.1	5.1
Month 6	-	-	7.1	-	-	5.8
Month 12	-	-	6.5	-	-	3.1
Month 22	-	-	24	-	-	17
Cmax (nM)						
Week 2	0.2	1.5	5.2	0.2	0.6	3.6
Month 6	0.3	0.9	3.2	0.5	0.4	7.5
Month 12	0.7	0.8	6.7	0.2	1.4	2.3
Month 22	2.0	-	13	2.0	-	8.3
T max (h)						
Week 2	0.5	0.5	0.5	0.5	0.5	0.5
Month 6	0.2	1.0	1.0	0.2	1.0	0.5
Month 12	0.2	0.5	2.0	0.2	0.5	0.2
Month 22	0.2	-	0.2	0.2	-	0.5

Conclusion: Treatment of Swiss mice with oral (gavage) formoterol at 0.1, 0.5 and 2.5 mg/kg/day for 2 years induced a statistically significant and dose-dependent increase in the incidence of leiomyomas in the uterus.

OVERALL SUMMARY AND EVALUATION
of the Carcinogenicity Studies of formoterol

Carcinogenicity potential of formoterol was tested in two 2-year bioassays in rats (inhalation) and mice (oral gavage). This section follows such an order: an introduction to the drug, summaries and evaluations of each individual study, and the overall evaluation of the carcinogenicity potential of the drug. Statistician Reviewer for this application was Moh-Jee Ng (See reviews dated 10-NOV-97 and 18-NOV-1997).

A. Background:

Formoterol is a highly selective long-acting β_2-agonist that possesses potent bronchospasmolytic activity. Its proposed indication is asthma in adult patients. The drug has been approved in Japan (1986) and several other countries since then.

Formoterol is readily absorbed after oral and inhalation administration. The peak concentrations are reached 0.5 - 1 hour after oral and intratracheal administration. Distribution of the drug after inhalation exposure is in the following order: trachea > lung > kidney > liver plasma > heart > brain. Half-lives of the drug after inhalation range 2 to 4 hours. Glucoronide conjugation and O-demethylation are the major pathways of metabolism (Fig. 6). Formoterol is eliminated through both urine and the bile and an enterohepatic circulation may exists. Bioavailability after inhalation in animals is unknown. Approximately a half of the drug in the plasma is protein-bond. Formoterol readily crosses the placenta to reach the fetus and excreted into the milk.

Repeated exposures of formoterol (up to 1 year) in rats and dogs indicate that the heart is the target organ of toxicity. Cardiac changes are apparent in almost all toxicity studies, regardless the route of administration. Changes in the heart are both functional and morphological: tachycardia, changes in EKG, cardiac hypertrophy, and cardiac necrosis/fibrosis. Tachycardia occurs in dogs at doses as low as 0.4 μg/kg/day (IH) and 2 mg/kg/day (PO). Tachycardia can also be associated with epitopic arrhythmia. Severe cardiac effects from large doses may even result in deaths.

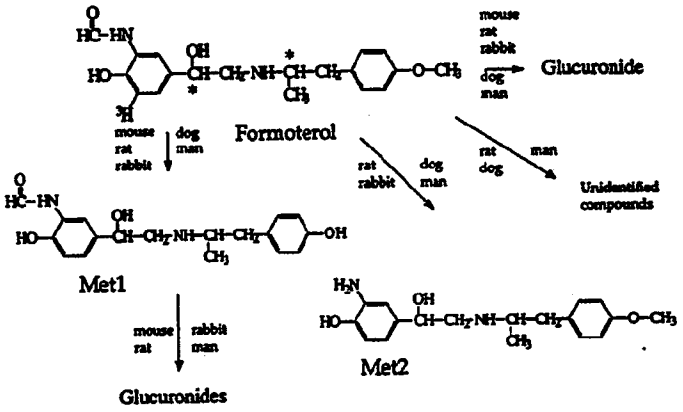

Fig. 6. Metabolic pathway (tentative) of formoterol fumarate

Formoterol was not genotoxic in these assays: *Salmonella typhimurium* TA 1535, TA1537, TA98, TA100; L5178Y mouse lymphoma cell thymidine kinase forward mutation assay; the *in vivo* rat micronucleus assay and the *in vitro* human lymphocytes chromosome aberration assay. However, a weak mutagenic effect was seen in *Salmonella* strain T1538 in the presence of S9 liver fraction at high at high concentrations (4300 μg/plate).

B. Rat studies:

Summary:

Sprague-Dawley rats (50/sex/dose) were given through nose-only inhalation formoterol at 1.9, 9.0 and 58 µg/kg/day (total body deposition) in lactose for 2 years. Two additional groups received lactose only to serve as controls. Mean $AUC_{0-8.5\ h}$ of the high dose groups was 15.5 nmol.h/L. The mean AUC for the low and mid dose groups were not available because of low plasma drug levels. About 59% of mortality occurred during the treatment. There was no difference in mortality rates, nor in clinical signs and appearance of palpable masses between treatments and sexes. Mean body weights were generally decreased in the treated groups, but a clear dose-response relationship was lacking for most the study. Only the males showed a trend of decrease in the mean body weight in a dose-dependent fashion at week 75. Compared to the controls, the mean body weight of the treated animals was decreased by 9, 10 and 12% for the low, mid and high dose groups, respectively. The females, on other hand, showed the most remarkable decreases in body weight in the mid dose group (up to 12.5% at terminal sacrifice). At the final termination, the respective mean body weight decreases for the LD, MD and HD groups were 8.2%, 8.8% and 7.1% in the males and 4.5%, 12.5% and 2.6%, compared to the mean of the control groups. Changes in food consumption generally followed the trend in body weight changes in both sexes.

Electrocardiography (EKG) analysis indicated a dose-related increase in the heart rate. This increase in heart rate lasted at least 30 minutes pose dosing. Tolerance in the increase in the heart rate tended to develop in the second year of the treatment. Increased episodes of epitopic premature contraction of heart was also noticed during the first year of exposure. The most noticeable change in clinical pathology laboratory analysis was a dose-related decrease in serum glucose levels (18 - 28%). Changes in other clinical laboratory parameters were either incidental or minimal and were of little toxicological significance.

Gross pathology revealed statistically significant increases ($P < 0.05$ and/or 0.01) in the both absolute and relative organ weights of the heart (males and females) and lung (males) in the mid and high dose groups. A trend of a dose-related increase of lung weight was apparent in the females, although none of the increases reached a statistically significant level.

The only noticeable neoplastic finding was the increased incidence of ovary and uteral leiomyomas in high dose females (one each). No such a tumor was found in the control, low and mid dose groups. No remarkable non-neoplastic findings were revealed. The incidences of myofibrosis and/or necrosis in the treated groups was similar to the controls. A slight increase in the incidence of the Leidig cell hyperplasia in the testis was observed in the high dose males (1/50-C vs 4/50-HD).

Evaluation:

As usual, validity of the rat carcinogenicity study is evaluated by the achievement of the maximum tolerated dose (MTD), the number of animals surviving the study, statistically analysis of the tumor incidence and the time of tumor onset, pharmacokinetics, and mechanism of action. This study probably achieved, if not exceeded, the MTD based on the decrease in the mean body weights. The actual and the most decreases in mean body weight were about 12% for the high dose group males at week 76 and for the mid dose females at terminal sacrifice. This dose selection seems close enough to the MTD.

The dose selection for the carcinogenicity study was based a 6-month inhalation study. In that study, rats were exposed to formoterol at 26, 128, and 853 µg/kg/day (inhaled dose). Dose-related increase in mean body weight was observed (2 - 10%). The mid dose group showed a mean body weight increase of 6.5%. Cardiac fibrosis was detected in the mid and high dose groups (incidences: 0/40-C, 0/40-LD, 2/40-MD, and 6/40-HD). With exception of slight increase in the heart rate (15%), the low dose group did not show any abnormalities. The sponsor elected to use the mid dose in the 6-month study (130 µg/kg/day in inhaled dose corresponding to 58 µg/kg/day for the total body burden) as the high dose in the carcinogenicity study. This dose selection seemed reasonable given the 6-month study results.

Pharmacokinetic data also suggested the acceptance of the dose selection. Sufficient systemic exposure of formoterol was achieved in rats. Mean $AUC_{0-8.5\ h}$ of the high dose rats (15.5 nmol.h/L) was 50 times the human $AUC_{0-24\ h}$ (0.31 nmol.h/L) in clinical use. These pharmacokinetic data seemed to be good quality although plasma levels was measured in only one animal per time. The data was rather consistent over time and between sexes (Table 10). Overall, the dose selection seems acceptable.

With regard to the animal numbers, fewer rats/dose for the terminal examination is a shortcoming of the study. Most treated groups (5/6) in this study had only 15 - 19 rats survived the study, with an overall survival rate of 32 - 40% (Table 1). However, their survival rates were not different from the controls. The *Toxicological Principles for the Safety of Assessment of Direct Food Actives and Color Additives Used in Food "Red Book II", 1993 (draft)* indicates that a appropriately designed study should have at least 25 animals/sex/dose surviving through the experiment. Recent CDER policies deem 20 animals/sex/dose to be acceptable. Too few animals for the terminal sacrifice may be a deficiency of the study.

Leiomyomas (one in ovary and the other in uterus) were observed in two high dose females and might be a treatment-related effect, although a statistical significance was lacking (P = 0.035 at terminal sacrifice). Mesovarian leiomyoma is a rare tumor in Sprague-Dawley rats. Data Base showed a mesovarian leiomyoma incidence of 0.05% (4/7748) in the reference controls. The formoterol study had an higher tumor rate of 4.5% (1/22). Also, this observation was consistent with other beta-agonist such as albuterol which are known to cause mesovarian tumors in rats (Kelly *et al., L Amer Col Toxicol,* 1993;12:13-21). The rarity of mesovarian leiomyoma and scientific understanding of this drug class indicate that this tumor is a treatment-related effect. The lack of statistical significance should not prevent one from

classifying the leiomyoma as a treatment-related effect. The uteral leiomyoma should also be considered as treatment-related effect for the same reasons. Furthermore, a dose-related and significant increase in uteral leiomyomas was observed in the female mice in a 2-year carcinogenicity study (Study No. 85133). Finally, the close location of leiomyomas as well as the same tumor type suggest that a analysis of the combined incidences of leiomyomas in the rats be appropriate. The combined leiomyoma rate of 4.0% (2/50, the overall incidence rate) far exceeds the historical reference (0.05%). Therefore, it is proper to conclude that formoterol treatment results in an increase in the incidence of leiomyomas of the mesovarium and uterus in rats.

Other noticeable neoplastic and non-neoplastic findings that are interesting but are of little scientific significance included changes in the testes, brain, and the heart. Findings in the testis included slight increases in the incidences of tumor and hyperplasia of the Leidig cells in the treated male. The respective incidences were 2/100, 4/50, 0/50 and 2/50 for the tumors; and 2/100, 1/50, 0/50 and 4/50 for the hyperplasia for the control, low, mid and high dose groups. The lack of a dose-response relationship in tumor incidences and the closeness to the background levels suggest little toxicological concern. The lack of the increase in tumor incidence also undermines the significance of the hyperplasia.

Findings in the brain included a dose-related increase in the incidences of pituitary adenoma remnant in the brain in the females (incidences: 2/100-C, 2/50-LD, 4/50-MD and 5/50-HD). Pituitary adenoma remnant is a non-neoplastic microscopic finding and a possible indication of tumor regression. This findings are not considered toxicologically meaningful, based on the prevalence of pituitary tumors in the females: 87/100-C, 45/50-LD, 42/50-MD and 33/50-HD.

The absence of pathological finding in the heart is somewhat unexpected. A 6-month inhalation study (T2860) in the same strain of rats showed only 23% increase in the heart rate and a significant increase in the incidences of myocardial fibrosis in the males (5/20 for 72 µg/kg/day vs 1/20 for the control). Rats (males) in the 2-year carcinogenicity study showed an increases in heart rate by up 34%. If the increase in the heart rate is a good indicator of cardiac toxicity, cardiac lesions in the 2-year carcinogenicity study should be at least as severe and prevalent as the 6-month toxicity study. Myocardial fibrosis and/or necrosis is known to be associated with beta-agonist use.

Conclusion:

Treatment of Sprague-Dawley rats with inhalation formoterol for 2 years induced a non-statistically significant increase in the incidence of leiomyomas in the uterus and ovary in the females at 58 µg/kg/day (pulmonary deposition), but not at lower doses (2 and 9 µg/kg/day). A few deficiencies (fewer animals for terminal sacrifice, the limited pharmacokinetic data, and the appropriateness of MTD) were evident, but do not change the study conclusions.

C. Mouse gavage study.

Summary

Swiss mice [redacted], 60/sex/dose) were given by oral gavage formoterol at 0.1, 0.5, and 2.5 mg/kg/day for 2 years to study carcinogenicity potential of the drug. Two additional groups received vehicle only and served as controls. The vehicle consisted of citric acid, sodium phosphate, sodium chloride, and water. Mean AUC $_{0-4\,h}$ of the high dose groups was 20.5 nmol.h/L. Mean AUC for the mid and low dose animals could not be accurately determined because of the low plasma drug levels. Overall, about 44% of the animals survived the study. There were no apparent differences in mortality rates, nor in clinical signs and appearance of palpable masses between treatments and sexes. Number of animals per group at the end of the study ranged from 19 to 32; but the high dose and the control groups had at least 22 rats/sex for terminal sacrifice. Mean body weights were generally increased in the treated groups, especially in the first one and a half years. This increase was clearly dose-related and was up to 5 - 7% in the high dose groups. Changes in food consumption generally followed the body weight changes in both sexes. No significant effects were observed in the clinical pathological examinations.

Gross pathology revealed dose-related increases in the incidences of ovary enlargement and masses in the ovary in the females. A slight increase in the incidence of masses in the uterus was also evident. A slight and non-statistical increase in the both organ weights of the heart and lung was seen in the mid and high dose groups. Uterus weight seemed to be lower in all treated groups, but none of the decreases was statistically significant.

No remarkable findings were seen in the non-neoplastic changes upon microscopic examinations. The average degree of myocardiofibrosis/necrosis was slightly increased in the high dose males only (2.5-C vs 3.1-HD). Other changes included increases in the incidences of pneumonia (9/120-C vs 9/60-HD), amyloidosis of the liver (13/120-C vs 13/60-HD) and testis (12/120-C vs 12/60) in the high dose males, and endo/epicarditis (9/120-C vs 9/60-HD) in the high females.

The only significant neoplastic findings was the dose-related and statistically significant increases in the incidences of leiomyoma in the uterus in the females (Incidences: 4/120-C, 7/60-LD, 11/60-MD, and 13/60-HD). The same trend was also apparent in the preterminally sacrificed animals (incidences: 3/73-C, 2/33-LD, 6/41-MD, and 7/38-HD). The incidences of other tumor findings were similar among the treated and control groups and were regarded as spontaneous. A statistical analysis of combined tumors incidences did not reveal any additional findings. The pre-terminally-sacrificed high dose females showed few incidences (3) of hepatohemangioma and was statistically significant (P = 0.008). However, there was no apparent difference in the combined incidences of liver hemangioma/sarcoma (3/60-C1, 2/60-C2, 1/60-LD, 3/60-MD, and 4/60-HD).

Evaluation

Dose selection of the mouse study was acceptable based on plasma drug levels. Mean $AUC_{0-4\,h}$ of the high dose groups (20.5 nmol.h/L) was 65 times the AUC ($AUC_{0-24\,h}$ = 0.31 nmol.h/L) of the maximum clinical dose in humans. The MTD, that is based on the decrease in body weight, probably was not achieved if the increase in mean body weight is not considered as an index of toxicity. The high dose animals showed an increase in mean body weight of 5 - 7%. This observation is consistent with other chronic studies in mice and rats. Overall, the achievement of sufficient systemic exposure suggests that this study be a valid study. Sufficient animals survived the study and at least 22 rats/sex were available for terminal sacrifice.

Dose selection is also reasonable based on the dose ranging study in this species. A 3-month dose ranging study showed significant adverse effects at the 10 mg/kg/day level: decreased motor activity, cyanosis, and remarkable increase (up to 20 fold) serum liver functional enzymes. None of these effects was apparent in the mid dose group (1.0 mg/kg/day). The sponsor elected to use 2.5 mg/kg/day, a dose higher than the non-effective dose but lower than the toxic dose, in their carcinogenicity study. Such a dose selection seemed reasonable although a high dose (probably 5.0 mg/kg/day) could have been better.

A major and significant finding in this study is the dose-related increase in the incidence of leiomyomas in the uterus in the females. This leiomyoma is considered as a formoterol-induced effect based on its dose-response relationship, and pharmacological and toxicological understanding of formoterol and other beta-agonists.

The increase in the incidences of hepatohemangioma in the pre-terminally sacrificed high dose females is probably not a treatment-related effect. The actual tumor incidences were 0/72, 0/33, 0/41 and 3/38 ($P < 0.008$) for the control, low, mid and high dose groups, respectively. Hemangioma is a common benign tumor. The Fisher's test value for the high dose group is higher than the current standard of a critical p-value for a common tumor (0.005). Also, the terminally sacrificed females did not show any market differences in tumor incidences between the treated and control animals. In addition, the males failed to show a treatment-related effect in the incidences of hemangioma at either pre-terminal or terminal sacrifices. Furthermore, hemangiosarcoma, a malignant tumor and more of safety concern, was lower in the treated groups. Finally, the combined incidences of liver hemangioma/sarcoma were similar in all female groups: 3/60-C1, 2/60-C2, 3/60-LD, 4/60-MD, and 4/60-HD. The lacking of difference in the incidences of terminally-sacrificed animals, and in the combined incidences of hemangioma and hemangiosarcoma, and the actual lower incidences of hemangiosarcoma in the treated groups suggest the observation of the observation of hemangioma in the preterminal females may not be formoterol treatment-related.

Conclusion

Treatment of Swiss mice rats with oral formoterol at 0.1, 0.5 and 2.5 mg/kg/say for 2 years induced a dose-related and statistically significant increase in the incidence of leiomyomas in the uterus in the female mice. The observed tumor incidences were 4/120, 7/60, 11/60, and 13/60 for the control, low dose, mid dose, and high dose groups, respectively.

C. Overall Evaluation and conclusion

Carcinogenicity potential of formoterol, a selective $beta_2$-agonist, was evaluated in two 2-year rodent bioassays in rats and mice. Sprague-Dawley rats were exposed to the drug by nose-only inhalation at total body burden doses of 1.9, 9.0 and 58 μg/kg/day. Swiss mice were exposed to the drug by oral gavage at 0.1, 0.5, and 2.5 mg/kg/day. Both studies are considered to be valid based upon a thorough evaluation of the study design, dose selection and animal survival rates.

The rats study showed a low incidence (2) of leiomyomas of the mesovarium and uterus in the high dose females. Leiomyoma, a rare tumor in rats, is known to be associated with beta agonists in rats (Kelly *et al., L Amer Col Toxicol,* 1993;12:13-21). The incidence of leiomyomas (4%) in the high dose group was much higher than the historic background (0.05%).

The female mice clearly showed a dose-related and statistically significant increase in the incidence of leiomyomas. Also, all treated groups possessed higher tumor incidences than the highest background level. In addition, leiomyoma is known to be associated with other beta agonists in CD-1 mice (*Gibson et al., Toxicol Pathol,* 1987;15:457-467). Thus, it is appropriate to conclude that formoterol is carcinogenic in mice.

Both the 2-year rat and mouse bioassays showed formoterol may induce leiomyomas in the uterus and/or ovary in the female rodents. In addition, beta agonists have been known to cause leiomyomas in these animals. Therefore, formoterol should be classified as a rodent carcinogen. The relevance of these findings to humans, however, is not known at present.

Hilary Sheevers, Ph.D.
Pharmacologist/Toxicologist Team Leader

Luqi Pei, V.M.D., Ph.D.
Pharmacologist/Toxicologist Reviewer

Ori: IND HFD-570/Division File
HFD-570/Drs. Pei/ Dr. Sheevers

Appendix 7: Minutes of Executive CAC Meeting dated April 14, 1998

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Executive CAC
4/14/98

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Conrad Chen, Ph.D. Alternate Member
Hilary Sheevers, Ph.D. Team Leader
Luqi Pei, D.V.M., Ph.D., Presenting Reviewer

Author of Draft: Luqi Pei, D.V.M., Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND #: —
Drug Name: **Formoterol Fumarate Dihydrate**
Sponsor: **Astra**

Formoterol fumarate is selective beta 2 agonist and non-genotoxic. It is present in the free base form under biological conditions. Metabolic pathways of formoterol are qualitatively similar between animals and humans: glucoronidation and sulfation; however, they may differ quantitatively. Recent reports suggest that formoterol may also be metabolized to ——— that possesses weak pharmacological activity. Protein binding of the drug was consistent across species: 54.1 - 57.7% in rats, rabbits, dogs and humans.

Mouse Carcinogenicity Study

Swiss mice ———, 60/sex/dose) were given by oral gavage formoterol at 0.1, 0.5, and 2.5 mg/kg/day for 2 years. Two additional groups received vehicle only and served as controls. Plasma formoterol AUC in the high dose group was 65 times the human AUC. A dose-related and statistically significant increases ($P < 0.0001$) in the incidences of leiomyoma in the uterus was found in the females (Incidences: 4/120-C, 7/60-LD, 11/60-MD, and 13/60-HD). The same trend was also apparent in the preterminally sacrificed animals (incidences: 3/73-C, 2/33-LD, 6/41-MD, and 7/38-HD). Leiomyomas are known to be associated with beta agonist treeament in this species.

Concerns about mouse PK data were raised. This was prompted by the findings that no AUC data were obtained from the mid and low dose groups in mice while very low AUC was obtained in humans. The mid dose mice, if not the low dose mice, seemed to possess higher drug levels based on single time point estimates than humans. The sponsor explained that the inability to calculate AUC data for mouse is was due to the difference in their use of different analytical methods for rodents and humans. The assay for mouse samples, performed in 1992, had lower sensitivity and lower selectivity than the improved assay for human samples (1995) which had a 30-fold lower detection limit.

Rat Carcinogenicity Study

Sprague-Dawley rats (50/sex/dose) were given formoterol through nose-only inhalation at doses of 1.9, 9.0 and 58 µg/kg/day (total body deposition) in lactose for 2 years. Two additional groups received lactose only to serve as controls. Plasma formoterol AUC in the high dose group was 50 times the human AUC. The only noticeable neoplastic finding was a non-statistically significant increase in the incidence of ovary and uterine leiomyomas in high dose females (one each). No such a tumor was found in the control, low and mid dose groups. Mesovarian leiomyomas is a rare tumor in rats but is also known to be associated with beta agonist treatment in this species. The laboratory background rate of this tumor was 4/7748.

Executive CAC Recommendations and Conclusions:

1). Both studies were acceptable based on the plasma exposure between animals and humans, and no evidence of genotoxicity for the drug; the respective plasma AUCs in mice and rats were 65 and 50 times human AUC at the highest doses tested.

2). The observed leiomyomas in mice were considered a drug-related effect.

3). The leiomyomas in female reproductive organs in rats are a typical beta 2 agonist effect and should be considered a drug-related effect considering the exceptionally low spontaneous rate, even though the increase in tumor incidence is not statistically significant.

10/23/98

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD 570
/Team leader, HFD-
/Reviewer, HFD-Pei
/ HFD-024

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Appendix 8: ——— - Miscellaneous telephone conversations between the sponsor and FDA reviewer.

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Division of Pulmonary Drug Products
Food and Drug Administration

Telephone Conversation Note

IND No. ——

Attendants: Ms. Roberta Tucker
Dr. Luqi Pei, FDA

Date: September 13, 1996 (9:35 AM)
Initiated by: Dr. Pei

Subject: Inquiry on formoterol's effect on male fertility: Sperm and behavior evaluations

Notes:

I asked the sponsor to provide information about any sperm and behavior evaluations that had been conducted with formoterol or any literature evidence associated with other beta$_2$-agonists. She agreed to provide a response soon.

Background:

Several studies (T2579, T3136, T3015, T3160 and T3137) submitted by the sponsor showed that formoterol affected the male reproductive system: the decreased weight and/or size of the testis, and scrotal hyperemia in both rats and dogs. Histologic changes were usually absent. However, the only fertility study (T3015) showed that formoterol at oral dose of 15 mg/kg/day in rats decreased male fertility (decreased pregnancy rate and failed to mate) accompanied by smaller testis and epididymides. According to Zenick *et al* (*Assessment of Male Reproductive System, in: The Principle and Methods of Toxicology, 1994, p 937-988*), histological examinations may not detect most subtle lesions induced by chemicals. Sperm and behavior evaluations may help delineate concerns about the effect of formoterol on the male reproductive system.

cc:
N:\INL —— \pharm\96-09-13.tel
HFD-570/Dr. Sheevers

Division of Pulmonary Drug Products
Food and Drug Administration

Telephone Conversation Note

IND No. ~~~~

Attendants: Dr. Luqi Pei, FDA
Mr. Paul Damiani, Astra USA

Date: October 28, 1996 (1:00 PM)
Initiated by: Dr. Luqi Pei of FDA

Subject: Request for historical controls of cardiac lesions in dogs.

Background:

An one-month inhalation toxicity study formoterol in dogs (T3120) showed dsoe-related increases in the incidences of the cardiac fibrosis (males: 0/6-C, 1/6-LD, 1/6-MD, 5/6-HD). Dose levels were 0.1, 0.4 and 2.1 mcg/kg/day for the low, mid and high dose groups respectively. The sponsor argued that these lesions in the low and mid dose groups were spontaneous. However, none of the control animals in the experiment showed the cardiac lesions. Furthermore, only one of the 28 control dogs in the entire toxicology program showed this lesion.

Note:

I asked the sponsor to submit historical control of this lesion in dogs to support their claim. Mr. Damisani agreed to provide the data later.

Division of Pulmonary Drug Products
Food and Drug Administration

Telephone Conversation Note

IND No. ———

Attendants: Mr. Paul Damiani, Astra UAS (508-366-1100 ext. 4772)
Dr. Luqi Pei, FDA

Date: April 16, 1998 (8:58 AM)
Initiated by: Luqi Pei

Subject: Inquiry on formoterol plasma levels in the mouse carcinogenicity study:

Notes:

I asked the sponsor to provide a clarification about their apparent difficulty in obtaining reliable AUC data in the low and mid dose groups in the mouse carcinogenicity study which may have higher drug levels than humans at clinical dose. Astra seemed to have good data in humans even though they may have lower plasma drug concentrations. Mr. Damiani agreed to provide a quick response.

Background:

In the discussion of the formoterol carcinogenicity studies on April 14, 1998, the Executive Carcinogenicity Assessment Committee looked into the plasma drug levels for risk assessment. The committee noticed that the sponsor seemed to have good plasma AUC data (mean of 20 nmol.h/L on month 22) in the high dose groups (2.5 mg/kg/day), but seemed to have difficulty to pick up the drug at the mid (0.5 mg/kg/day) and low dose groups (0.1 mg/kg/day). The dose level in the mid dose group was 1/5 of the high dose. On the other hand, the sponsor seemed to have good human AUC data although their plasma drug concentration may be much lower (0.31 nmol.h/L at 24 μg, bid).

cc:
N:\IND —— \pharm\98-04-16.tel
HFD-570/Dr. Sheevers/ Mr. Jani

Appendix 9: Addendum to Review of Review of [redacted] Amendment #170 dated May 29, 2002

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW
Addendum to Review of Amendment #170 dated May 29, 2002

IND number: —
Review number: #04
Sequence number/date/type of submission: #170/September 20, 2001/Amendment
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: AstraZeneca LP
1800 Concord Pike
P.O. Box 8355
Wilmington, DE 19803-8355
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: March 1, 2006

Drug:

Trade name:
Generic name (list alphabetically): Formoterol fumarate dihydrate
Chemical name: Formoterol fumarate dihydrate, (R*,R*)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]phenyl]formamide, (E)-2-butendioate (2:1), dihydrate
Molecular formula/molecular weight:
Formoterol fumarate dihydrate, $C_{42}H_{56}N_4O_{14}$ / 840.9 g/mole

Relevant INDs/NDAs/DMFs:
IND 63,394 (Symbicort, AstraZeneca)
NDA 21-929 (Symbicort, AstraZeneca)
DMF 10378 (1,1,1,2,3,3,3-heptafluoropropane (HFA-227), IPACT-II)

Drug class: β_2-adrenergic agonist

Indication: Asthma

Route of administration: Inhalation

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

Studies reviewed within this submission:
Formoterol: 3-month inhalation (HFA pMDI) toxicity study in the rat.

Reviewer: Timothy W. Robison, Ph.D., D.A.B.T. IND No. —

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

In this addendum to the review of the 3-month inhalation toxicology study with formoterol HFA pMDI in rats (— Amendment #170; Review #03 dated May 29, 2002), the conclusions regarding histopathological findings in the lung consisting of alveolar histiocytosis, pneumonitis, and congestion induced by the excipients in the vehicle formulation (i.e., PVP K-25 and PEG-600) have been revised.

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group. Target doses of formoterol fumarate dihydrate for the low, mid, and high dose groups were 0.090, 0.280, and 0.890 mg/kg/day, respectively. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively.

Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively. These doses of excipients were significantly higher than those used in 3-month inhalation bridging toxicology studies with Symbicort HFA pMDI in rats and dogs.

There were no changes in conclusions that the target organ of toxicity for formoterol was the heart and that histopathological findings were evident in the adrenal cortex from the vehicle-control group as compared to the air-control group.

Changes were evident in the lung for the vehicle-control group. Alveolar histiocytosis was observed in 8 of 20 (40%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control group. The incidence of alveolar histiocytosis in the vehicle-control group exceeded the published background occurrence in young rats of 16-20% (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). Pneumonitis was observed in 9 of 20 (45%) rats in the vehicle-control group as compared to 4 of 20 (20%) rats in the air-control groups. Acute or chronic inflammation consisting of small aggregates of lymphoid cells around bronchioles and small vessels is a relatively common finding in rats observed with a background occurrence of 56%, while more extensive inflammation (e.g., alveolitis, bronchiolitis, pneumonitis) occurs at a lower background incidence of 18-20% (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). Congestion was observed in 4 of 20 (20%) rats in the vehicle-control group as compared to 2 of 20 (10%) rats in the air-control group. It is possible that congestion may have been related to the procedure used to sacrifice animals (Handbook of Toxicology, 2nd Edition, CRC Press, Pages 702-703). In the initial review of this study provided in Amendment #170 under IND — (Review dated May 29, 2002), given that findings were observed in all groups including the air-control and scientific references reporting high incidences of spontaneous inflammation in the lungs

Reviewer: Timothy W. Robison, Ph.D., D.A.B.T. IND No. ~

of control rats, these findings were not identified as adverse. However, based upon experiences with alveolar histiocytosis in recent years and local toxicity observed with PVP K-25 from another application, these findings are now re-interpreted to be the result of local toxicity induced by excipients in the vehicle formulation (PVP K-25 and PEG-1000).

In the initial submission of IND 63,994, the sponsor provided 3-month inhalation bridging toxicology studies with Symbicort HFA pMDI in rats and dogs that included PVP K-25, PEG-1000, and HFA-227. In addition, the sponsor provided several nonclinical studies, conducted with another inhalation drug product ~~~~~ that contained relatively similar excipients, PVP K-30, PEG-600, and HFA-227. These studies consisted of a 6-month inhalation toxicity with rats, 6- and 12-month inhalation toxicity studies with dogs, and a 24-month inhalation carcinogenicity study with rats. In our review of the initial submission for IND 63,394, we agreed that PVP K-30 and PEG-600 appeared to be closely related to PVP K-25 and PEG-1000, respectively. Further, PVP K-30 and PEG-600 could be used for the safety assessment of PVP K-25 and PEG-1000, respectively. In all of these studies, there was no evidence of local toxicity induced by the vehicle.

The 3-month toxicity study with Symbicort in dogs (Table 2) as well as the 6-month toxicity with rats, 6- and 12-month toxicity studies with dogs, and a 24-month carcinogenicity study with rats that used closely related excipients, PVP K-30 and PEG-600 (Table 4), provided sufficient dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product. The 3-month toxicity study with Symbicort in rats used very low doses of the vehicle (i.e., 2-fold of human doses, see Table 1).

Table 1: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in rats.

Compound	Rats		Clinical doses in Symbicort µg/g LW/day	Rat to human dose ratio
	Deposited dose µg/kg/day	Deposited dose µg/g LW/day		
PVP K-25	0.03	0.006	0.0028	2.1
PEG-1000	8.6	1.72	0.8492	2.0

Notes: The deposition factor for rats was approximately 8.6 to 9.4%. Lung weight values for rats and humans used in calculations were 1.5 g and 1000 g, respectively.

Table 2: Dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation bridging toxicology study with Symbicort in dogs.

Compound	Rats		Clinical doses in Symbicort µg/g LW/day	Dog to human dose ratio
	Deposited dose µg/kg/day	Deposited dose µg/g LW/day		
PVP K-25	0.42	0.038	0.0028	13.6
PEG-1000	126	11.455	0.8492	13.5

Notes: The deposition factor for dogs was 100%. Lung weight values for dogs and humans used in calculations were 110 g and 1000 g, respectively.

Table 3: Inhaled dose (µg/kg/day) and deposited doses (µg/g lung weight/day) of excipients, PVP K-30 and PEG-600, in the vehicle-control group from inhalation toxicology studies

Excipient	6-month rat study		24-month rat study		6-month dog study		12-month dog study	
	Inhaled dose µg/kg	Deposited dose µg/g LW	Inhaled dose µg/kg	Deposited dose µg/g LW	Inhaled dose µg/kg	Deposited dose µg/g LW	Inhaled dose µg/kg	Deposited dose µg/g LW
PVP K-25	18.4	0.36	110	1.12	2.9	0.26	1.9	0.17
PEG-1000	2209.6	44.2	13000	131.9	344.2	31.3	222.2	20.2

a. The deposition factor for rats was 10%.
b. A close-fitting face mask and aerosol delivery mouth tube system was used to deliver aerosols directly to the buccal cavity of the dog. Deposition factors for dogs in the original reviews have been revised to 100%.

Table 4: Rat to human and dog to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of similar excipients, povidone K-30 and PEG-600, in vehicle-control groups of studies

Clinical Doses of Excipients, PVP K-25 and PEG-1000		Dose ratios based upon nonclinical studies with PVP K-30 and PEG 600			
		6-month Rat to Human ratio	24-month Rat to Human ratio	6-month Dog to Human ratio	12-month Dog to Human ratio
Excipients	Clinical doses in Symbicort, µg/g LW/day				
Povidone K25	0.0028	128.6	400	94.2	61.7
PEG 1000	0.8492	52	155.4	36.8	23.8

Doses of PVP K-25 and PEG-1000 in the 3-month study with Formoterol HFA pMDI (Table 5) were generally lower than those used with PVP K-30 and PEG-600 (Tables 3 and 4), respectively; however, no toxicity was evident in studies with PVP K-30 and PEG-600 following 1 or 2 years of treatment.

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Reviewer: Timothy W. Robison, Ph.D., D.A.B.T. IND No. —

Table 5: Rat to human dose ratios for clinical doses of excipients, PVP K-25 and PEG-1000, in the Symbicort HFA pMDI drug product based upon comparison to doses of the same excipients in the 3-month inhalation toxicology study with Formoterol HFA pMDI in rats.

Excipients	3-month rat study		Clinical doses in Symbicort, µg/g LW/day	Rat to Human Ratios
	Deposited dose, µg/kg/day	Deposited dose, µg/g LW/day		
PVP K-25	1.14	0.228	0.0028	81.4
PEG-1000	211.3	42.26	0.8492	49.8

Notes: Rat body and lung weights were estimated to be 300 g and 1.5 g, respectively. Human lung weight was estimated to be 1000 g.

Based upon re-evaluation of histopathological findings in the lung from the 13-week inhalation toxicology study with rats that received formoterol HFA pMDI, it appears that PVP K-25 and PEG-1000 were more toxic than PVP K-30 and PEG-600. Therefore, studies with PVP K-30 and PEG-600 cannot be used to bridge PVP K-25 and PEG-1000, respectively. The sponsor needs to conduct a 6-month inhalation toxicology study with PVP K-25 and PEG-1000 in rats to determine if acceptable safety margins can be established.

Recommendation: The sponsor should be requested to conduct a 6-month inhalation toxicology study with PVP K-25 and PEG-1000 in rats to determine if acceptable safety margins can be established. This request will be conveyed under NDA 21-929.

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

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

cc: list:
IND — HFD-570
JacksonC, 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
3/1/2006 05:05:14 PM
PHARMACOLOGIST

Joseph Sun
3/3/2006 10:22:43 AM
PHARMACOLOGIST
I concur.

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Appendix 10: Review of IND — Amendment #170 dated May 29, 2002

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PHARMACOLOGY/TOXICOLOGY COVER SHEET

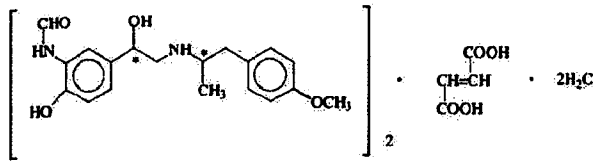
IND number: ——
Review number: #03
Sequence number/date/type of submission: #170/September 20, 2001/Amendment
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: AstraZeneca LP

Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: May 29, 2002

Drug:

Trade name:
Generic name (list alphabetically): Formoterol fumarate dihydrate
Code name:
Chemical name:
Formoterol fumarate dihydrate, (R*,R*)-(±)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]phenyl]formamide, (E)-2-butendioate (2:1), dihydrate (asterisks denote asymmetric carbon atoms)
CAS registry number:
Mole file number:
Molecular formula/molecular weight:
Formoterol fumarate dihydrate, $C_{42}H_{56}N_4O_{14}$ / 840.9 g/mole
Structure:



Relevant INDs/NDAs/DMFs:
IND 63,394 (Symbicort, AstraZeneca)
NDA 20-831 (Foradil®, Novartis)
NDA 21-279 (Foradil®, Novartis)
DMF 10378 (1,1,1,2,3,3,3-heptafluoropropane (HFA-227), IPACT-II)

Drug class: β_2-adrenergic agonist

Indication: Asthma

i

Clinical formulation: A dry powder inhaler (Turbuhaler®) will be used in the Phase 3 program.

Route of administration: Inhalation

Proposed clinical protocol: This material was taken from the Annual Report in IND —— Amendment #143 dated March 30, 2001. The Phase 3 program consists of three studies in adults and adolescents: one long-term (12-month) safety and two, 12-week pivotal safety and efficacy studies in patients with asthma. All studies will be conducted using the "M3" Turbuhaler device. The proposed clinical dose is 9 µg BID (i.e., total of 18 µg/day). A pediatric program will be conducted concurrently. This program consists of one study: a 12-week pivotal safety and efficacy trial in children with asthma.

256 - Twelve-week safety and efficacy trial.

The primary objective is to assess the effectiveness and safety of Oxis-formoterol 9 µg BID by Turbuhaler® compared to placebo in adults and adolescents (≥12 years of age) with asthma. Patients will receive study medication for 12 weeks. The primary efficacy variable will be the 12-hr average FEV_1 time curve at 12 weeks. Approximately 200 patients will be randomized into the trial with a 1 to 1 ratio of active treatment to placebo.

259 - Twelve-week safety and efficacy trial.

The primary objective is to assess the effectiveness and safety of Oxis-formoterol 9 µg BID by Turbuhaler® compared to placebo in adults and adolescents (≥12 years of age) with asthma. Patients will receive study medication for 12 weeks. The primary efficacy variable will be the 12-hr average FEV_1 time curve at 12 weeks. Approximately 200 patients will be randomized into the trial with a 1 to 1 ratio of active treatment to placebo.

264 - Twelve-week safety and efficacy trial (Pediatric Study).

The primary objective is to assess the effectiveness and safety of Oxis-formoterol 9 µg BID by Turbuhaler® compared to placebo in children (4 to 11 years of age) with asthma. Patient will receive study medication for 12 weeks. The primary efficacy variable will be the 12-hr average FEV_1 time curve at 12 weeks. Approximately 200 patients will be randomized into the trial with a 1 to 1 ratio of active treatment to placebo.

268 - Twelve-month safety trial in Adults and Adolescents.

The primary objective of this study is to assess the safety of Oxis-formoterol 9 µg BID by Turbuhaler® (in addition to standard treatment) compared to standard treatment. The primary variable will be incidence of reported adverse events. Approximately 675 patients will be randomized in a 2:1 ratio to receive Oxis-Formoterol or to continue standard treatment.

Previous clinical experience: This material was taken from the Investigator's Brochure provided in IND — ' Amendment #143 dated March 30, 2001.

The tolerability of formoterol Turbuhaler® in healthy subjects has been assessed in 4 single dose studies (4.5 to 72 µg), 2 multiple-dose (1-week; 9 and 18 µg BID) studies and 1 cumulative-dose study (13.5+13.5+27 = 54 µg) including a total of 134 subjects.

The tolerability of formoterol has been assessed in adult asthmatics as follows: single doses of 4.5, 18 and 54 µg to 29 subjects, a cumulative dose study with 90 µg formoterol administered over 3 hr to 48 subjects, a three-day study with formoterol doses of 54 and 90 µg in 28 subjects, and a 4-week study with 9 µg BID or cumulative doses (13.5 + 13.5 µg + 27 µg) given on 5 days, one week apart involving 13 patients.

Formoterol turbuhaler dose-response studies comprised five placebo-controlled, crossover, single-dose studies (2.25 to 36 µg; 170 patients) and 1 placebo-controlled, parallel-group one-week study (4.5, 9, and 18 µg/day, 165 patients).

Six clinical studies have been performed in adults asthmatics, evaluating the clinical efficacy of twice-daily treatment with formoterol Turbuhaler® compared with placebo or regular treatment with a short-acting inhaled bronchodilator, terbutaline sulfate (Bricanyl® Turbuhaler®) q.i.d, regarding lung function, asthma symptoms and use of rescue inhaled bronchodilators. Formoterol doses, treatment durations, and numbers of patients were as follows: a 4-week study with formoterol doses of 4.5, 9, and 18 µg BID in 221 patients, a 12-week study with a formoterol dose of 9 µg BID in 343 patients, a 12-week study with a formoterol dose of 4.5 µg BID in 397 patients, a 6-month study with a formoterol dose of 18 µg BID in 239 patients, a one-year study with 100 or 400 µg budesonide BID ± 9 µg formoterol BID in 852 patients, and a one-year study with 100 µg budesonide BID + 9 µg formoterol BID or 400 µg budesonide + placebo in 60 patients.

Three efficacy studies with active control in adult asthmatics have been conducted. Formoterol at 9 µg BID was compared with Foradil® at 12 µg BID using 66 patients in a 2 x 2-week crossover study with a 2-week washout period. Formoterol at 18 µg BID was compared with Foradil® at 24 µg BID using 66 patients in a 2 x 2-week crossover study with a 2-week washout period. Formoterol at 9 µg BID was compared to Formoterol 9 µg BID + Budesonide Turbuhaler at 200 µg BID or Budesonide Turbuhaler at 200 µg BID using 21 patients in a 3 x 4-week crossover study.

Two long-term clinical studies involving a total of 201 patients were performed with formoterol Turbuhaler®, primarily to evaluate safety. In a 12-month study, formoterol was administered at 9 or 18 µg BID to 188 patients. In another 12-month study, formoterol was administered at 9 µg BID to 13 patients.

Two efficacy studies have been performed in children: one single-dose study with 68 children with doses of 4.5 to 36 µg, and one study where 248 children were randomized to 3-month BID treatment with formoterol (4.5 or 9 µg BID).

The effect of formoterol Turbuhaler in patients with chronic obstructive pulmonary disease has been evaluated in five studies as follows: a single dose study with 12 patients (4.5 and 18 µg), a one-week crossover study with doses of 4.5, 9, and 18 µg BID in 35 patients, a twelve week study with doses of 4.5, 9, and 18 µg BID in 687 patients, a 12-week study with a dose of 18 µg BID in 187 patients, and a single dose + 3 weeks (i.e., 18 µg + 9 µg BID) study in 21 patients.

Other studies have included patients who used 4.5 µg formoterol as their "as-needed" medication and adults and children who used formoterol Turbuhaler as protection against exercise-induced bronchoconstriction. Miscellaneous studies included a 14-day study with doses of 4.5 µg BID, 18 µg BID, or 9 µg QD in 72 patients to evaluate the protective effect against methacholine challenge and a single dose (9 µg) study with 24 patients to evaluate endurance performance.

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

Introduction and drug history:

Formoterol fumarate dihydrate is a β_2-adrenergic agonist under development for the treatment of asthma. The preclinical assessment of this proposed drug product is essentially complete. In the present amendment, the sponsor provided a 13-week nose-only inhalation toxicology study with a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 that was administered to rats. This bridging study is intended to support the clinical development of a formoterol HFA pMDI formulation.

Studies reviewed within this submission:

1. Acute toxicity (MTD) in dogs of KWD 2183 given orally in single doses: plasma concentration study.
2. General toxicity study of formoterol fumarate dihydrate (D2522) in lactose given to rats by the inhalation route for 6 months. Plasma concentration study.
3. Plasma levels of formoterol fumarate dihydrate (D2522) lactose in rats. Satellite study to study number 91055: Oncogenicity study in rats given D2522 lactose by the inhalation route for 104 weeks.
4. General toxicity study of formoterol fumarate dihydrate (D2522) in lactose given to dogs by the inhalation route for one month. Plasma concentration study.
5. Effect on the respiratory tract and male reproductive organs in young rats given formoterol fumarate (D2522) in lactose by the inhalation route for 3 months. Plasma concentration study.
6. Biotransformation of R,R- and S,S-formoterol by O-demethylation. Liver enzyme identification and effect of formoterol on selected CYP substrates.
7. Metabolite pattern of formoterol in the rat after intratracheal administration.
8. Formoterol: 3-month inhalation (HFA pMDI) toxicity study in the rat.

Studies <u>not</u> reviewed within this submission: None.

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

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PHARMACOLOGY/TOXICOLOGY REVIEW

III. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Rats

Title: General Toxicity Study of Formoterol Fumarate Dihydrate (D2522) in Lactose Given to Rats by the Inhalation Route for 6 Months. Plasma Concentration Study.

Report number: 843-RD-0335
Volume #, and page #: Volume 1, Pages 1-33
Conducting laboratory and location: Safety Assessment
AB Astra
Sodertalje, Sweden

Pharmacokinetics Laboratory
AB Draco
Box 34
S-221 00 Lund
Sweden
Date of study initiation: November 29, 1992
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Formoterol fumarate dihydrate (D2522), batch 100/92 was used for preparation of a micronized powder mix of — D2522 in lactose (1493-1, batch 300/92).
Formulation/vehicle: Micronized lactose monohydrate (1448-1, batch 203/92) was prepared for use as a test formulation for control animals.

Dosing:
Species/strain: Sprague-Dawley rats ——————
#/sex/group or time point (main study): Toxicokinetic groups that received treatment with formoterol fumarate dihydrate (D2522) consisted of 8 rats/sex/group. An additional 4 rats/sex/group were included as reserves. The control group consisted of 2 rats/sex/group.
Age: Not provided.
Weight: Not provided.
Doses in administered units: Target inhaled doses of D2522 were 25, 140, and 800 µg/kg/day. Actual inhaled doses on days of blood sampling were 26.5-34.0, 100-148, and 927-1112 µg/kg/day, respectively.
Route, form, volume, and infusion rate: Animals were exposed by nose-only inhalation. The powder mix was used to generate a powder aerosol by a —— dust feed mechanism at the site of animal exposure.

Methods: Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 60 min per day as a dry powder aerosol to rats for periods up to 6 months. Plasma concentrations of D2522 were measured in rats following one week or 6 months of inhalation exposure. Target inhaled doses of D2522 were 25, 140, and 800 µg/kg/day. Actual inhaled doses on days of blood sampling were 26.5-34.0, 100-148, and 927-1112 µg/kg/day, respectively. This study was part of a 6-month general toxicology study (Study number 92110). Toxicokinetic data for all treatment groups was provided in the current report. Blood samples for measurement of plasma drug levels were collected on days 8 and 176 at 0.25, 0.5, 0.75, 1, 2, 4, 8, and 24 hr after completion of dosing. One rat/sex/group was used for each time point. In the control group, blood samples were collected at 0.5 and 2 hr. Toxicokinetic animals were discarded without examination after completion of blood sampling on day 8 or 176. Plasma concentrations of D2522 were measured by coupled column liquid chromatography with electrochemical detection. The limit of quantification was 0.20 nmol/L with 2.0 mL plasma. In this report, the sponsor did not appear to distinguish between the salt and base forms of drug.

Results: Deposited doses for low, mid, and high dose groups were 2.4-3.2, 9.0-12.6, and 80.6-94.5 µg/kg/day, respectively. For the low dose group, plasma concentrations of D2522 could be followed only from the start of dosing to 4 hr after completion of dosing (i.e., $AUC_{0\text{-}5hr}$). For mid and high doses, plasma concentrations of D2522 could be followed from the start of dosing to 8 hr after completion of dosing (i.e., $AUC_{0\text{-}9hr}$). AUC values increased with elevating doses on days 8 and 176, although, increases were less than dose proportional. On day 176, exposure was generally higher in male rats as compared to female rats. For female rats at the high dose, exposure was less on day 176 as compared to day 8. Drug half-life was approximately 2 hr for male and female rats.

Target inhaled, actual inhaled, and deposited doses for male and female rats exposed to formoterol fumarate dihydrate (D2522) by nose-only inhalation.

Target Inhaled Dose µg/kg/day	Actual Inhaled Dose, µg/kg/day				Deposited Dose, µg/kg/day			
	Day 8		Day 176		Day 8		Day 176	
	Males	Females	Males	Females	Males	Females	Males	Females
0	0	0	0	0	-	-	-	-
25	31.2	34.0	26.5	31.4	2.9	3.2	2.4	2.8
140	100	109	138	148	9.0	9.8	11.8	12.6
800	927	1013	975	1112	80.6	88.2	82.9	94.5

Toxicokinetic parameters for D2522 in male and female rats exposed to formoterol fumarate dihydrate (D2522) by nose-only inhalation.

Target Inhaled Dose µg/kg/day	$AUC_{0\text{-}5hr}$, nmole·hr/L				$AUC_{0\text{-}9hr}$, nmole·hr/L				T½, hr			
	Day 8		Day 176		Day 8		Day 176		Day 8		Day 176	
	M	F	M	F	M	F	M	F	M	F	M	F
25	3.51	2.79	4.11	3.46	-	-	-	-	-	-	-	-
140	12.2	12.2	16.2	12.4	14.4	14.3	19.1	15.4	1.4	2.0	1.9	2.3
800	51.9	65.0	51.9	38.1	60.3	73.6	60.2	42.6	2.0	1.7	1.9	1.8

Title: Plasma Levels of Formoterol Fumarate Dihydrate (D2522) Lactose in Rats. Satellite Study to Study No. 91055: Oncogenicity Study in Rats given Formoterol Fumarate Dihydrate (D2522) Lactose by the Inhalation Route for 104 Weeks.

Report number: 843-RD-0336
Volume #, and page #: Volume 1, 1-47 to 1-79
Conducting laboratory and location: Safety Assessment
AB Astra
Sodertalje, Sweden

Pharmacokinetics Laboratory
AB Draco
Box 34
S-221 00 Lund
Sweden

Date of study initiation: Not provided in this report.
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Formoterol fumarate dihydrate (batch 100/91) was used for preparation of a micronized powder mix of [redacted] formoterol fumarate dihydrate in lactose (1493-1, batch 105/91 and 1493-1, batch 113/91)
Formulation/vehicle: Not provided in this report.

Dosing:
Species/strain: Sprague-Dawley rats [redacted]WI,BR) [redacted]

#/sex/group or time point (main study): Toxicokinetic groups consisted of 8 rats/sex/group. There were a total of nine groups.
Age: Not provided.
Weight: Not provided.
Doses in administered units: Target inhaled doses were 5, 25, and 125 µg/kg/day
Route, form, volume, and infusion rate: Exposure was by nose-only inhalation. The powder mix was used to generate a powder aerosol by a [redacted] dust feed mechanism at the site of animal exposure.

Methods: Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 30 min per day as a dry powder aerosol to rats for periods up through 76 weeks. Plasma concentrations of D2522 were measured in rats after 1, 49, and 76 weeks of exposure. Target inhaled doses of D2522 were 5, 25, and 125 µg/kg/day. Actual inhaled doses on days of blood sampling were 3.1-7.3, 17-24, and 82-147 µg/kg/day, respectively. This study was part of an oncogenicity study in rats given D2522 lactose by the inhalation route for 104 weeks (Study number 91055). Blood samples for measurement of plasma drug levels were collected on days 7, 345 (346), and 532 (533) at 0.25, 0.5, 0.75, 1, 2, 4, 8, and 24 hr after completion of dosing. One rat/sex/group was used for each time point. Toxicokinetic animals were discarded without examination after completion of blood sampling on day 8 or 176. Plasma concentrations of D2522 were measured by coupled column liquid chromatography with

electrochemical detection. The limit of quantification was 0.20 nmol/L with 2.0 mL plasma. In this report, the sponsor did not appear to distinguish between the salt and base forms of drug.

Results: Deposited doses for the low, mid, and high dose groups on days of blood sampling were approximately 0.26-0.66, 1.5-2.2, and 7.1-14 µg/kg/day, respectively. Given that 7 animals were found dead and 4 animals were sacrificed in a moribund condition (dose groups not specified) during the course of treatment, the sponsor decided to discontinue the toxicokinetic study after 76 weeks of treatment. Plasma drug concentrations could not be followed as long for low and mid dose groups as compared to the high dose group. AUC values could not be calculated for the low dose group. AUC values for the mid dose group were calculated from the start of dosing to 4 hr after completion of dosing. AUC values for the high dose group were calculated from the start of dosing to 4 and 8 hr after completion of dosing. Peak plasma drug concentrations were observed at 15 min, the first time point after completion of dosing. C_{max} and AUC values on days 7, 345-346, and 532-533 increased with elevating dose. On days 7 and 345-346, increases in C_{max} and AUC values with elevating dose were approximately dose proportional. However, during week 76, C_{max} and AUC values for the high dose group were generally lower than those observed during weeks 1 and 49. Actual and deposited doses as well as C_{max} and AUC values in female rats were generally slightly higher than those observed in male rats. These effects may be related to lower body weights of female rats. These comparisons should be interpreted with some caution given that only one rat/sex/group was used for each time point.

Actual and deposited doses for rats exposed by nose-only inhalation to formoterol fumarate dihydrate at target inhaled doses of 5, 25, and 125 µg/kg/day.

Target Inhaled Dose µg/kg/day	Actual Inhaled Dose, µg/kg/day						Deposited Dose, µg/kg/day					
	Day 7		Day 345-346		Day 532-533		Day 7		Day 345-346		Day 532-533	
	M	F	M	F	M	F	M	F	M	F	M	F
5	5.3	6.2	6.1	7.3	3.1	3.7	0.48	0.56	0.55	0.66	0.26	0.31
25	20	23	20	24	17	22	1.7	2.0	1.9	2.2	1.5	2.0
125	124	147	82	99	108	125	12	14	7.1	8.5	9.8	11

C_{max} values (i.e., plasma drug levels at 15 min after completion of dosing) in rats exposed by nose-only inhalation to formoterol fumarate dihydrate at target inhaled doses of 25 and 125 µg/kg/day.

Target Inhaled Doses µg/kg/day	Plasma drug levels at 15 min after completion of dosing, nmole/L					
	Week 1		Week 49		Week 76	
	Male	Female	Male	Female	Male	Female
5	0.30	0.41	0.40	0.86	0.53	0.52
25	1.3	1.4	0.93	1.6	2.3	3.2
125	6.4	10.4	7.4	7.0	4.5	5.5

Systemic exposure (AUC) in rats exposed by nose-only inhalation to formoterol fumarate dihydrate at target inhaled doses of 25 and 125 µg/kg/day.

Target Inhaled Dose µg/kg/day	$AUC_{0\text{-}4.5hr}$						$AUC_{0\text{-}8.5hr}$					
	Day 7		Day 345-346		Day 532-533		Day 7		Day 345-346		Day 532-533	
	M	F	M	F	M	F	M	F	M	F	M	F
25	1.94	2.27	2.23	2.86	3.74	4.14	-	-	-	-	-	-
125	9.81	15.3	15.1	14.9	11.2	11.1	11.6	16.9	17.9	18.6	14.9	14.0

Title: Effects on the Respiratory Tract and Male Reproductive Organs in Young Rats Given Formoterol Fumarate (D2522) in Lactose by the Inhalation Route for 3 Months. Plasma Concentration Study.

Report number: 843-RD-0367
Volume #, and page #: Volume 1, Pages 1-115 to 1-149
Conducting laboratory and location: Astra Safety Assessment
Astra AB
Sodertalje, Sweden

Bioanalytical Chemistry
Astra Draco AB
Lund, Sweden
Date of study initiation: January 29, 1995
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Formoterol fumarate dihydrate (D2522; batch 311/92, purity —) was used for preparation of a micronized powder mix of — D2522 in lactose (batch 25-803-48, 701/95).
Formulation/vehicle: Micronized lactose monohydrate (batch 1448-1, 105/94) was used as the test formulation for control animals.

Dosing:
Species/strain: Wistar Hanlbm: WIST (SPF) rats —. After mating, dams were allowed to give birth at Astra Safety Assessment. The sponsor stated that dams were not seen as part of the study, but were only a means of support for the pups.
#/sex/group or time point (main study): Not provided.
Age: Pups were 16-18 days old at the start of dosing.
Weight: Not provided.
Doses in administered units: Target inhaled doses were 25, 140, and 800 µg/kg/day.
Route, form, volume, and infusion rate: Nose-only inhalation exposure

The powder mix was used to generate a dry particle aerosol by a — dust feed mechanism at the site of animal exposure. The animals were exposed to aerosolized D2522 in a nose-only inhalation chamber daily for 30 min.

Methods: Formoterol fumarate dihydrate (D2522) was administered daily as a dry powder aerosol for 3 months to young rats (16-18 days old at the start of dosing). Target inhaled doses were 25, 140, and 800 µg/kg/day. Plasma concentrations of D2522 were measured on days 6, 25, and 88. This toxicokinetic study was associated with Study number 94143 entitled "Effects on the respiratory tract and male reproductive organs in young rats given formoterol fumarate (D2522) in lactose by the inhalation route for 3 months." Animals used for blood sampling on day 6 were subjected to a restricted pathology examination. Remaining satellite animals were killed after completion of blood sampling without autopsy. The plasma concentration of D2522 was determined by coupled column liquid chromatography with electrochemical

detection. The limits of quantitation were 0.5 and 0.2 nmole/L with 0.5 and 2 mL of plasma, respectively.

Results: Deposited doses for low, mid, and high dose groups were 2.54, 13.2, and 67.6 µg/kg/day, respectively. Peak plasma levels were obtained within 15 min after completion of dosing, indicating rapid absorption of drug from the lungs. C_{max} and AUC values increased with elevating dose on days 6, 25, and 88, although, increases were generally less than dose proportional. C_{max} and AUC values were generally higher in male rats as compared to female rats over the entire treatment period. C_{max} and AUC values were relatively constant over the 3-month treatment period as young pups developed into adult animals. Thus, toxicokinetic parameters of D2522 did not appear to change during continuous dosing of young rats up to adult age.

Target inhaled, actual inhaled, and deposited doses of formoterol fumarate dihydrate administered to young rats for 3 months.

Target Inhaled Doses, µg/kg/day	Actual Inhaled Doses, µg/kg/day	Deposited Dose, µg/kg/day
25	26.6	2.54
140	144	13.2
800	794	67.6

Peak plasma concentration (C_{max}) of D2522 after administration of D2522 by nose-only inhalation exposure to young rats for periods up to 3 months.

Target Inhaled Dose, µg/kg/day	Sex	C_{max} (nmole/L)		
		Day 6	Day 25	Day 88
25	M	2.9	1.8	1.9
	F	1.9	1.8	1.1
140	M	9.3	6.7	14.2
	F	9.1	6.8	14.8
800	M	48.7	46.6	47.2
	F	38.6	32.7	25.6

Systemic exposure to D2522 (i.e., AUC, nmole·hr/L) after administration of D2522 by nose-only inhalation exposure to young rats for periods up to 3 months.

Target Inhaled Dose, µg/kg/day	Sex	$AUC_{0\text{-}4.5hr}$		
		Day 6	Day 25	Day 88
25	M	3.38	3.42	3.43
	F	2.32	2.65	2.50
140	M	13.50	10.14	25.65
	F	11.13	7.53	28.01
800	M	64.07 69.13[a]	58.73 67.11[a]	74.04 86.24[a]
	F	61.85 Not Determined[a]	40.38 44.39[a]	43.87 49.97[a]

a. $AUC_{0\text{-}8.5hr}$

Dogs

Title: Acute Toxicity (MTD) in Dogs of KWD 2183 (Bambuterol) Given Orally in Single Doses: Plasma Concentration Study.

Report number: 843-RD-0242
Volume #, and page #: Volume 1, Pages 1-13
Conducting laboratory and location: Safety Assessment
AB Astra
Sodertalje, Sweden

Pharmacokinetics Laboratory
AB Draco
Box 34
S-221 00 Lund
Sweden

Date of study initiation: February 18, 1987
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Bambuterol hydrochloride (KWD 2183, batch 303/84, content: —)
Formulation/vehicle: The drug powder was packed in gelatin capsules.

Dosing:
Species/strain: Beagle dogs
#/sex/group or time point (main study): 2 male and 2 female dogs
Age: Not provided.
Weight: Not provided.
Doses in administered units: 250 and 500 µmole/kg.
Route, form, volume, and infusion rate: The drug was administered by the oral route as a powder packed in gelatin capsules.

Methods: Plasma concentrations of KWD 2183 (bambuterol) and its hydrolysis product, terbutaline, were measured after oral administration of single doses of bambuterol at 250 and 500 µmole/kg to dogs. This study was conducted in connection

with an acute toxicity study in which KWD 2183 was administered to dogs as single oral doses (Study number 86089). The dose of 250 µmole/kg was administered to 2 dogs/sex. The dose of 500 µmole/kg was administered to the same dogs, six days later. Blood samples for measurement of plasma drug concentrations were collected before dosing and at 1, 2, 4, 6, and 24 hr after dosing. An esterase inhibitor, D2456, was added to blood samples to prevent the hydrolysis of bambuterol. Bambuterol and terbutaline concentrations were measured by liquid chromatography with mass spectrometry. The limits of quantitation for bambuterol and terbutaline were 6.7 µmole/L and 157 nmole/L, respectively.

Results: Bambuterol was rapidly absorbed following an oral dose of 250 µmole/kg with a T_{max} of ~1 hr. Absorption was delayed with a larger dose of 500 µmole/kg, as the T_{max} was ~4 hr. C_{max} values for bambuterol were dose proportional. Addition of D2456 to blood samples to prevent the breakdown of bambuterol by plasma esterases was apparently ineffective. The sponsor reported that the terbutaline plasma concentrations increased during repeated thawing of samples. The plasma terbutaline concentrations were probably overestimated by a factor of 2 or more. T_{max} values for terbutaline ranged from 2 to 6 hr.

Dose	Bambuterol		Terbutaline	
µmole/kg	T_{max}, hr	C_{max}, µmol/L	T_{max}, hr	C_{max}[a], µmol/L
250	~1 hr	65-92 µmol/L	2-6 hr	5.8 µmole/L
500	4 hr	135-172 µmol/L	6 hr	5.3 µmole/L

a. The sponsor reported that plasma terbutaline concentrations increased during repeated thawing of samples. The plasma samples were thawed on one occasion for determination of bambuterol and on a second occasion for determination of terbutaline. Terbutaline plasma concentrations were probably overestimated by a factor of 2 or more.

Title: General Toxicity Study of Formoterol Fumarate Dihydrate (D2522) in Lactose Given to Dogs by the Inhalation Route for One Month: Plasma Concentration Study.

Report number: 843-RD-0343
Volume #, and page #: Volume 1, Pages 1-80 to 1-114
Conducting laboratory and location: Astra Safety Assessment
Astra AB
Sodertalje, Sweden

Bioanalytical Chemistry
Astra Draco AB
Lund, Sweden

Date of study initiation: August 31, 1993
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: Formoterol fumarate dihydrate (D2522), batch 100/92 was used to manufacture a micronized powder mix of —— formoterol fumarate dihydrate in lactose (batch 1493-1 103/92).

Formulation/vehicle: Micronized lactose monohydrate (batch 1448-1 203/92) was administered to control animals.

Dosing:
Species/strain: Beagle dogs
#/sex/group or time point (main study): 3 dogs/sex/group
Age: Not provided.
Weight: Not provided.
Doses in administered units: Formoterol fumarate dihydrate was administered at doses of 0.5, 2.7, and 15 µg/kg/day. Control animals received lactose monohydrate only.
Route, form, volume, and infusion rate: Inhalation exposure.

During inhalation exposures, dogs were kept in slings and received the aerosol through a mask. The dry particle aerosol was delivered by a dust feeder. The exposure was conducted using a nose-only system. The inhaled dose was calculated for each animal using a microprocessor controlled signal system. During exposures, the system simultaneously measured the concentration of aerosol particles and the inhalation flow of the animal. The exposure ended when the preset dose was delivered. The duration of each inhalation exposure was variable, although, the target was 3 min.

Methods: Systemic exposure to formoterol was assessed following inhalation exposure of beagle dogs to a micronized powder mix of formoterol fumarate dihydrate in lactose. Target doses of formoterol fumarate dihydrate were 0.5, 2.7, and 15 µg/kg/day. Control animals received lactose monohydrate only. The treatment duration was 1 month. Blood for measurement of plasma drug concentrations was collected on days 0, 6, and 21 immediately after completion of dosing and at 0.083, 0.167, 0.5, 1, 2, 4, 8, and 24 hr after dosing. Quantities of formoterol were measured by coupled column liquid chromatography with electrochemical detection. The limit of quantification was 0.15 nmol/L when 1.0 mL plasma was used.

Results: On days of blood sampling (i.e., days 0, 6, and 21), mean deposited doses for low, mid, and high dose groups were 0.07, 0.4, and 2 µg/kg/day, respectively. For the low dose group, only a few blood samples contained drug concentrations above the limit of quantitation (i.e., 15 nmole/L). No calculations of AUC could be performed for the low dose group. For the mid dose, AUC values were calculated from the start of dosing up to 4 hr after completion of dosing. For the high dose, AUC values were calculated from the start of dosing up to 24 hr after completion of dosing. Peak plasma drug levels were observed within 10 min after completion of dosing, indicating rapid drug absorption. C_{max} values on days 0, 6, and 21 increased with elevating dose, and increases were approximately dose proportional. The increase in AUC from the mid to high dose was approximately dose proportional.

Target inhaled, actual inhaled, and deposited doses for male and female dogs exposed to formoterol fumarate dihydrate in lactose by nose-only inhalation (e.g., mean of days 0, 6, and 21).

Target Inhaled Dose, µg/kg/day	Actual Inhaled Dose, µg/kg/day	Deposited Dose, µg/kg/day

0.5	0.510	0.070
2.7	2.794	0.395
15	15.28	1.963

C_{max} values for male and female dogs exposed to formoterol fumarate dihydrate in lactose by nose-only inhalation.

Target Inhaled Dose, µg/kg/day	C_{max}, nmole/L		
	Day 0	Day 6	Day 21
0.5	~0.18	~0.18	~0.20
2.7	0.66	0.59	0.70
15	5.8	5.1	5.2

Systemic exposure for male and female dogs exposed to formoterol fumarate dihydrate in lactose by nose-only inhalation.

Target Inhaled Dose, µg/kg/day	AUC_{0-4hr}, nmole·hr/L			AUC_{0-24hr}, nmole·hr/L		
	Day 0	Day 6	Day 21	Day 0	Day 6	Day 21
2.7	1.11	1.09	1.09	-	-	-
15	7.19	7.30	7.83	16.7	16.2	18.7

Metabolism:

In Vitro

Title: Biotransformation of R,R- and S,S-Formoterol by O-Demethylation. Liver Enzyme Identification and Effect of Formoterol on Selected CYP Substrates.

Report number: 843-RD-0395
Volume #, and page #: Volume 1, 1-150 to 1-183
Conducting laboratory and location: Astra Draco AB
Lund, Sweden
Date of study initiation: Not specified.
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: Not specified.

Methods: Formoterol is a racemic mixture of the enantiomer pair with the absolute configurations RR/SS where the pharmacological effect appears to reside principally in the RR-enantiomers. In humans, the major elimination route of formoterol is glucuronidation of the phenolic hydroxy group, but O-demethylated formoterol (metabolite Met1) as well as two glucuronide conjugates of Met1 have also been detected in urine. The biotransformation of R,R- and S,S-formoterol by O-demethylation to the metabolites Met1-RR and Met1-SS, respectively, was studied in human liver microsomes in order to identify the cytochrome P450 (CYP) enzymes(s) involved. Possible effects of the enantiomers on biotransformation of selected CYP substrates were also evaluated.

For inhibition and interaction studies and for determination kinetic constants (i.e., V_{max}, K_m), pooled liver microsomes from six human subjects (two females and four males) were used. Microsomes from a human lymphoblastoid cell line expressing

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human CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, CYP2F1, and CYP3A4 and an insect cell line expressing CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP4A11 were obtained from ——. Plasma membranes from E. coli with expressed CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4 were prepared. The O-demethylation reaction kinetics were assessed with R,R-, S,S-, and racemic formoterol incubated with human liver microsomes (1 mg/mL protein concentration) at concentrations ranging from 1 to 500 µM. For correlation studies, microsomes (1 mg/mL microsomal protein) from ten different human livers (six females and four males; ——) were incubated with enantiomers (0.2 and 200 µM) or the racemate (0.2 and 200 µM) for 2 hr.

Mechanism-based inhibitors, furafyllin, disulfiram, and troleandomycin, were preincubated with microsomes for 20 min prior to addition of formoterol (1 µM). Other inhibitors included quinidine and ketaconazole. For inhibition studies with cloned isozymes, the substrate concentration was 100 µM for the enantiomers and 200 µM for the racemate. Antisera against CYP2D6 and CYP2C (CYP2C8/9/19) were also used in inhibition studies. When interactions of one enantiomer on the other was assessed, 100 µM of labeled enantiomer was added together with 100 µM of the unlabeled opposite enantiomer. For isozymes expressed in a lymphoblastoid cell line, 1 mg/mL microsomal protein was used, and the incubation time was 3 hr. For isozymes expressed in insect cells or bacteria, a isozyme concentration of 0.1 nmol/mL was used, and the incubation time was 2 hr.

Interaction with different isozyme substrates was evaluated by mixing three concentrations (1, 10, or 100 µM) of R,R- and S,S-formoterol with human liver microsomes prior to addition of 10 µM of the selected substrate and 1 mM NADPH. The following cytochrome 450 isozyme substrate reactions were tested: ethoxyresorufin O-deethylation (CYP1A2), coumarin 7-hydroxylation (CYP2A6), bufuralol 1'-hydroxylation (CYP2D6), chlorzoxazone 6-hydroxylation (CYP2E1), budesonide-6β-hydroxylation (CYP3A), and testosterone 6β-hydroxylation (CYP3A).

Results:

Enzyme Kinetics: The formation rate (i.e., V_{max}) of Met1-SS was always higher than for Met1-RR, regardless of plotting method. However, affinities (i.e., K_m) were relatively similar. Eadie-Hofstee plots were biphasic indicating the involvement of several enzymes (i.e., V_{max1} and V_{max2}, and K_{m1} and K_{m2}).

Enzyme kinetics observed with substrate concentrations ranging from 1 to 500 µM.

Plotting Method	V_{max}, pmol/min/mg microsomal protein			K_m, µM		
	Met1-RR	Met1-SS	Racemate	Met1-RR	Met1-SS	Racemate
Lineweaver-Burk	43	87	40	69	65	54
Eadie-Hofstee	21 / 103	75 / 160	23 / 87	32 / 252	56 / 203	30 / 196

Correlation Studies: Regression analysis of the formation rate of Met1-RR and Met1-SS and various cytochrome P450 isozyme reactions was evaluated. The highest correlation for Met1-RR was found versus dextromethorphan O-demethylation

(CYP2D6) with r^2 = 0.44 and 0.83 for substrate concentrations of 0.1 and 100 µM, respectively. These results suggest that CYP2D6 may be more important at high concentrations of R,R-formoterol. Met1-SS showed highest correlation versus benzphetamine N-demethylation (unknown isozyme) with r^2 = 0.55 and 0.41 for substrate concentrations of 0.1 and 100 µM, respectively. No correlation was found between Met1-SS formation and CYP3A4. The racemate showed highest correlation versus benzphetamine N-demethylation with r^2 = 0.70 at a substrate concentration of 0.2 µM and versus caffeine N3-demethylation (CYP1A) with r^2 = 0.42 for a substrate concentration of 0.2 µM. There was no correlation between the formation rate of Met1-RR and Met1-SS at either 0.1 or 100 µM substrate concentration, respectively.

Inhibition Studies: For the formation of Met1-RR, the highest inhibition was observed with quinidine (CYP2D6 inhibitor) where a concentration of 1 µM decreased formation by 50%. Ketoconazole at 1 µM (general CYP3A inhibitor) decreased the formation of Met1-SS by approximately 40%. This ketoconazole concentration was relatively high and other isozymes may have been effected. Inhibition studies with antisera against CYP2D6 and CYP2C decreased Met1-RR formation by 15-20% and 10%, respectively. For O-demethylation of the racemate, highest inhibition was observed with 100 µM budesonide (CYP3A substrate) and 100 µM quinidine, which decreased formation by 60% and 40%, respectively. These concentrations required with the racemate were significantly higher than those required with enantiomers.

Metabolism by cDNA-expressed human cytochrome P450 isozymes: Met1-RR and Met1-SS were only formed from lymphoblastoid cells expressing CYP2D6. Insect cells expressing CYP2D6 and CYP2C19 were able to produce high concentrations of Met1 forms, although, CYP2D6 was significantly more active. Low or insignificant levels were found with cells expressing CYP2A6, CYP2C8, CYP2E1, and CYP4A11. Cell membrane preparations from E. coli expressing CYP2D6 were able to produce both Met1 forms. Effects on O-demethylation with one enantiomer in the presence of the other were greatest for CYP2D6 and CYP2C9.

Effects on CYP substrate reactions: Greatest effects were observed with bufuralol 1'-hydroxylation, catalyzed by CYP2D6, which was decreased by approximately 40% in the presence of 100 µM RR- or SS-formoterol.

These studies suggest that O-demethylation of R,R- and S,S-formoterol was catalyzed by more than one cytochrome P450 isozyme (i.e., CYP2D6 and CYP2C). There was a high correlation between Met1-RR and CYP2D6; however, there appeared to be no correlation between Met1-SS and CYP2D6. There was uncertainty regarding CYP isozymes catalyzing O-demethylation of S,S-formoterol. The relative contributions of CYP3A, CYP2C, CYP1A2, CYP2E1, CYP2A6, CYP2D6, and CYP2B6 in the human liver are 28, 18, 13, 7, 4, 2, and 0.2%, respectively. The total capacity of CYP2C is greater than CYP2D6 with regard to substrates that bind to both isozymes. However, with R,R-formoterol, there appears to be a preference for CYP2D6, given the higher correlation coefficient for this isozyme, possibly due to higher affinity. Although, with S,S-formoterol, this is not known.

Reviewer: Timothy W. Robison, Ph.D. IND No.

Rats

Title: Metabolite Pattern of Formoterol in the Rat After Intratracheal Administration.

Report number: 843-RD-0410-01
Volume #, and page #: Volume 1, 1-184 to 1-213
Conducting laboratory and location: AstraZeneca R&D
Lund, Sweden
Date of study initiation: Not provided.
GLP compliance: No
QA report: yes ( ) no (X)
Drug, lot #, radiolabel, and % purity: ^{3}H-formoterol (Batch number 360/2101) in phosphate/citric acid buffer, pH 6.3, batch number DZG 10
Formulation/vehicle: Not applicable.

Dosing:
Species/strain: Male rats (Strain not specified).
#/sex/group or time point (main study): Approximately 3 rats per group
Age: Not specified.
Weight: Not specified.
Doses in administered units: 50 µg/kg (intratracheal or intravenous)
Route, form, volume, and infusion rate: Intratracheal or intravenous administration

Methods: The metabolic profile of formoterol was assessed at different time points in plasma, lungs, trachea, adrenals, stomach, and kidneys after intratracheal administration and in urine after intratracheal and intravenous administration of ^{3}H-formoterol in the rat. The biological samples used for metabolic profiling were obtained from the study entitled "The disposition of [^{3}H]-formoterol in the rat following intratracheal and intravenous administration ——— project number 163209). Urine samples analyzed in the present study were collected at 0-12, 12-24, and 24-48 hr from 3 male rats/group after intratracheal and intravenous administration of ^{3}H-formoterol. Plasma, lungs, trachea, adrenal glands, kidneys, and stomach were obtained at 0.083, 0.25, 1, and 4 hr after intratracheal administration of ^{3}H-formoterol to rats. Samples were processed for identification and measurement of radiolabeled metabolites. The metabolite pattern was analyzed in all samples by liquid chromatography-radiochromatography. The identification of metabolites in urine was performed by LC-mass spectrometry.

Results:
Qualitatively, the same metabolite pattern in urine was obtained following either intratracheal or intravenous administration of formoterol at 50 µg/kg. Radiolabeled drug products identified in urine were intact formoterol, the phenol glucuronide of formoterol (FG1), the O-demethylation product (Met1), and two glucuronides of this primary metabolite (Met1G1 and Met1G2). The main metabolite observed during the 48-hr sampling period was the formoterol glucuronide followed by Met1 and its two glucuronides.

Radioactivity levels in plasma, lungs, trachea, adrenal glands, kidneys, and stomach were assessed after intratracheal administration of ^{3}H-formoterol to rats. For radiolabeled drug products in plasma, unchanged formoterol ranged from 72.0% at 5 min to 25.6% at 4 hr, and formoterol glucuronide, the main metabolite, ranged from 7.4% at 5 min to 57.9% at 4 hr. The O-demethylated formoterol (Met1) ranged from 1.5 to 5.2% and its two glucuronides (Met1G1 and Met1G2) each ranged from 1 to 2.6%. In the lungs, unchanged formoterol was the main component at all time points. Met1 ranged from 0.8% at 5 min to 3.3% at 4 hr. In the trachea, adrenal glands, and stomach, unchanged formoterol was the main component at all time points (>90%). In the kidneys, unchanged formoterol ranged from 80.6% at 5 min to 52.9% at 4 hr. FG1 ranged from 2.8% at 5 min to 21.4% at 4 hr. Met 1 ranged from 5.4% at 5 min, to a peak of 15% at 15 min, and 3.3% at 4 hr. Met1G1 and Met1G2 were both ≤1%.

PK/TK summary:

Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 60 min per day as a dry powder aerosol to rats for periods up to 6 months. Plasma concentrations of D2522 were measured in rats following one week or 6 months of inhalation exposure. Target inhaled doses of D2522 were 25, 140, and 800 µg/kg/day. Deposited doses were 2.4-3.2, 9.0-12.6, and 80.6-94.5 µg/kg/day, respectively. AUC values increased with elevating doses on days 8 and 176, although, increases were less than dose proportional. On day 176, exposure was generally higher in male rats as compared to female rats. For female rats at the high dose, exposure was less on day 176 as compared to day 8. Drug half-life was approximately 2 hr for male and female rats.

Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 30 min per day as a dry powder aerosol to rats for periods up through 76 weeks. Plasma concentrations of D2522 were measured in rats after 1, 50, and 76 weeks of exposure. Target inhaled doses of D2522 were 5, 25, and 125 µg/kg/day. Deposited doses were approximately 0.26-0.66, 1.5-2.2, and 7.1-14 µg/kg/day, respectively. Peak plasma drug concentrations were observed at 15 min, the first time point after completion of dosing. C_{max} and AUC values on days 7, 345-346, and 532-533 increased with elevating dose. On days 7 and 345-346, increases in C_{max} and AUC values with elevating dose were approximately dose proportional. However, during week 76, C_{max} and AUC values for the high dose group were generally lower than those observed during weeks 1 and 49. Actual and deposited doses as well as C_{max} and AUC values in female rats were generally slightly higher than those observed in male rats.

Formoterol fumarate dihydrate (D2522) was administered daily as a dry powder aerosol for 3 months to young rats (16-18 days old at the start of dosing). Target inhaled doses were 25, 140, and 800 µg/kg/day. Deposited doses for low, mid, and high dose groups were 2.54, 13.2, and 67.6 µg/kg/day, respectively. Peak plasma levels were obtained within 15 min after completion of dosing, indicating rapid absorption of drug from the lungs. C_{max} and AUC values increased with elevating dose on days 6, 25, and 88, although, increases were generally less than dose proportional. C_{max} and AUC values were generally higher in male rats as compared to female rats over the entire

treatment period. C_{max} and AUC values were relatively constant over the 3-month treatment period as young pups developed into adult animals. Thus, toxicokinetic parameters of D2522 did not appear to change during continuous dosing of young rats up to adult age.

Plasma concentrations of KWD 2183 (bambuterol) and its hydrolysis product, terbutaline, were measured after oral administration of single doses of bambuterol at 250 and 500 µmole/kg to dogs. Bambuterol was rapidly absorbed following a dose of 250 µmole/kg with a T_{max} of ~1 hr. Absorption was delayed with a larger dose of 500 µmole/kg, as the T_{max} was ~4 hr. C_{max} values for bambuterol were dose proportional.

Systemic exposure to formoterol was assessed following inhalation exposure of beagle dogs to a micronized powder mix of --- formoterol fumarate dihydrate in lactose. Target doses of formoterol fumarate dihydrate were 0.5, 2.7, and 15 µg/kg/day. On days of blood sampling (i.e., days 0, 6, and 21), mean deposited doses for low, mid, and high dose groups were 0.07, 0.4, and 2 µg/kg/day, respectively. No calculations of AUC could be performed for the low dose group. Peak plasma drug levels were observed within 10 min after completion of dosing, indicating rapid drug absorption. C_{max} values on days 0, 6, and 21 increased with elevating dose, and increases were approximately dose proportional. The increase in AUC from the mid to high dose was approximately dose proportional.

The biotransformation of R,R- and S,S-formoterol by O-demethylation to the metabolites Met1-RR and Met1-SS, respectively, was studied in human liver microsomes in order to identify the cytochrome P450 (CYP) enzymes(s) involved. Results indicated that O-demethylation of R,R- and S,S-formoterol was catalyzed by more than one cytochrome P450 isozyme (i.e., CYP2D6 and CYP2C). There was a high correlation between Met1-RR and CYP2D6; however, there appeared to be no correlation between Met1-SS and CYP2D6. There was uncertainty regarding identities of CYP isozymes catalyzing O-demethylation of S,S-formoterol.

The metabolic profile of formoterol in urine was assessed after intratracheal and intravenous administration of ^{3}H-formoterol to male rats at 50 µg/kg. The metabolic profile of formoterol was also assessed at different time points in plasma, lungs, trachea, adrenals, stomach, and kidneys after intratracheal administration of ^{3}H-formoterol to rats at 50 µg/kg. Qualitatively, the same metabolite pattern in urine was obtained following either intratracheal or intravenous administration of formoterol at 50 µg/kg. Radiolabeled drug products identified in urine were intact formoterol, the phenol glucuronide of formoterol (FG1), the O-demethylation product (Met1), and two glucuronides of this primary metabolite (Met1G1 and Met1G2). The main metabolite observed during the 48-hr sampling period was the formoterol glucuronide followed by Met1 and its two glucuronides. For radiolabeled drug products in plasma, unchanged formoterol ranged from 72.0% at 5 min to 25.6% at 4 hr, and formoterol glucuronide, the main metabolite, ranged from 7.4% at 5 min to 57.9% at 4 hr. The O-demethylated formoterol (Met1) ranged from 1.5 to 5.2% and its two glucuronides (Met1G1 and Met1G2) each ranged from 1 to 2.6%. In the trachea, lungs, adrenal glands, and stomach, unchanged formoterol was the main component at all time points (>90%). In the kidneys, unchanged formoterol ranged from 80.6% at 5 min to 52.9% at 4 hr. FG1

ranged from 2.8% at 5 min to 21.4% at 4 hr. Met 1 ranged from 5.4% at 5 min, to a peak of 15% at 15 min, and 3.3% at 4 hr. Met1G1 and Met1G2 were both ≤1%.

PK/TK conclusions:

Formoterol fumarate dihydrate (D2522) was administered daily as a dry powder aerosol for 3 months to young rats (16-18 days old at the start of dosing). C_{max} and AUC values were relatively constant over the 3-month treatment period as young pups developed into adult animals. Thus, toxicokinetic parameters of D2522 did not appear to change during continuous dosing of young rats up to adult age.

The O-demethylation of R,R- and S,S-formoterol was catalyzed by more than one cytochrome P450 isozyme (i.e., CYP2D6 and CYP2C). There was a high correlation between Met1-RR and CYP2D6; however, there was uncertainty regarding the identities of CYP isozymes catalyzing O-demethylation of SS-formoterol.

The metabolic profile of formoterol in rats was assessed after intratracheal and intravenous administration of ^{3}H-formoterol at a dose of 50 µg/kg. Radiolabeled drug products identified in urine and tissues were intact formoterol, the phenol glucuronide of formoterol (FG1), the O-demethylation product (Met1), and two glucuronides of this primary metabolite (Met1G1 and Met1G2).

IV. GENERAL TOXICOLOGY:

Subchronic Toxicity

Rats

Study title: Formoterol: Three Month Inhalation (HFA pMDI) Toxicity Study in Rats.

Key study findings:

♦ In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group. Target doses of formoterol fumarate dihydrate for the low, mid, and high dose groups were 0.090, 0.280, and 0.890 mg/kg/day, respectively. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control).

♦ The mid dose was identified as the NOAEL due to the death of one animal and histopathological findings in the heart at the high dose.

♦ The heart was the target organ of toxicity. A number of changes were observed in other organs, although, treatment relationships were unclear.

♦ In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the high dose group. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat was characteristic of cardiac lesions produced by β-adrenergic agonists.

♦ Changes were evident in the lungs and adrenal cortex for vehicle-control and formoterol treatment groups as compared to the air-control group. The incidence and severity of alveolar histiocytosis in the lungs were increased for male and female rats in vehicle-control and formoterol treatment groups as compared to the air-control group. Diffuse fatty changes in the adrenal cortices were evident for male and female rats in vehicle-control and formoterol treatment groups as compared to no similar findings in the air-control group. Both of these histopathological changes have been characterized as common findings in laboratory rats.

♦ Deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively.

Study no: 96195-1
Volume #, and page #: Volume 2, Pages 1 to 397
Conducting laboratory and location: Astra Safety Assessment
Astra Charnwood
Bakewell Road
Loughborough
Leics
LE11 5RH
England
Date of study initiation: June 10, 1997
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, radiolabel, and % purity: The test formulation, Formoterol HFA pMDI, consisted of pressurized metered dose inhalers (pMDIs) containing micronized formoterol fumarate dihydrate (batch number: 4056H, purity ——), polyvinylpyrrolidone (PVP) K-25, polyethylene glycol (PEG) 1000, and HFA-227 (propellant) delivering a nominal 4.7 or 47 µg formoterol fumarate dihydrate per actuation from a 25 µL valve. The pMDIs batch numbers P5170 (4.7 µg/actuation) and P5171 (47 µg/actuation) were supplied by Astra Charnwood.

Test Formulations

Used in Groups	Material	% w/w	Output (µg/actuation)
3, 7	Formoterol fumarate dihydrate PVP K-25 PEG 1000 HFA-227		
4-5, 8-9	Formoterol fumarate dihydrate PVP K-25 PEG 1000		

	HFA-227			

Formulation/vehicle: The vehicle control formulation consisted of pressurized metered dose inhalers (pMDIs) that delivered polyvinylpyrrolidone (PVP) K-25, polyethylene glycol (PEG) 1000, and HFA-227 (propellant) from a 25 µL valve. The pMDIs (batch number P5256) were supplied by Astra Charnwood.

Vehicle-control formulation

Groups	Material	%, w/w	Output (µg/actuation)
2, 6	PVP K-25 PEG 1000 HFA-227		

Methods (unique aspects): A formoterol pMDI formulation containing formoterol fumarate dihydrate, PVP K-25, PEG-1000, and HFA-227 was administered by nose-only inhalation to 10 rats/sex/group at target formoterol fumarate dihydrate doses of 0.090, 0.280, and 0.890 mg/kg/day (actual doses of 0.098, 0.307, and 0.936 mg/kg/day, respectively) for 3 months. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control).

Dosing:

Species/strain: Sprague-Dawley (CD)BR) rats

#/sex/group or time point (main study): 10 rats/sex/group

Satellite groups used for toxicokinetics or recovery: Satellite groups were included for toxicokinetic blood sampling. The control group consisted of 2 rats/sex/group and the three treatment groups, each consisted of 16 rats/sex/group.

Group #	Main/TK	Treatment	Number of animals
1	Main	Air-control	10 rats/sex/group
2	Main	Vehicle-control	10 rats/sex/group
3	Main	Low dose	10 rats/sex/group
4	Main	Mid dose	10 rats/sex/group
5	Main	High dose	10 rats/sex/group
6	TK	Vehicle-control	2 rats/sex/group
7	TK	Low dose	16 rats/sex/group
8	TK	Mid dose	16 rats/sex/group
9	TK	High dose	16 rats/sex/group

Age: Animals were approximately 6-8 weeks at the start of dosing.

Weight: Body weight ranges were 246-330 g for male rats and 162-229 g for female rats at the start of treatment.

Doses in administered units: The total inhaled dose for each group was calculated from aerosol concentrations and group mean body weights.

$$\text{Dose}^{a} = \frac{\text{(Aerosol concentration, µg/L) x Time (min) x Min Volume (L/min)}}{\text{Body weight (g)}}$$

a. Please note that the sponsor has expressed doses in units of mg/kg/day in this study.

Appears This Way
On Original

Minute volume =

Mean doses of formoterol fumarate dihydrate (mg/kg/day) from weeks 1-13.

Group	Chamber Conc. µg/L	Body weight, g		Minute Volume, L/min		Time, min	Total inhaled dose, mg/kg/day		Deposited Dose, mg/kg/day	
		M	F	M	F		M	F	M	F
3	2.73	288.2-470.2	201.6-281.5	0.176-0.243	0.139-0.173	60	0.090	0.105	0.008	0.009
4	8.65	290.9-480.2	201.7-279.9	0.177-0.247	0.139-0.173	60	0.283	0.331	0.023	0.027
5	26.4	292.6-465.5	199.9-283.6	0.178-0.242	0.138-0.174	60	0.863	1.008	0.067	0.078

An aerosol sample was collected from each exposure chamber (formoterol treated groups only) for the duration of each exposure period. Samples were collected onto pressed glass fiber filters held in an open faced filter holder inserted into a port in the side of the exposure chamber at the mid point. All filters were retained for determination of the amount of formoterol using a HPLC method.

Aerosol concentrations of excipients were not measured during the study; however, doses of excipients were estimated. Doses of PVP K-25 and PEG-1000 were estimated from the formoterol fumarate dihydrate doses using the ratios of the three materials in the pMDI formulation on the assumption that aerosol generation efficiencies were similar. The HFA-227 dose was estimated using an aerosol generation efficiency of 100% given the assumption that as a gas, deposition losses within the equipment were minimal.

The excipient doses for the vehicle control were assumed to be identical to those for the high dose group, since the vehicle-control formulation and aerosol generation conditions matched those of the high dose.

Total inhaled and lung burden doses (mg/kg/day) of formoterol fumarate dihydrate and estimated total inhaled doses (mg/kg/day) for excipients, PVP K-25 and PEG 1000, and the propellant, HFA-227.

Group	Total or deposited dose	Formoterol fumarate dihydrate			PVP K-25	PEG-1000	HFA-227
		Male	Female	Combined	M and F	M and F	M and F
1	Total Inhaled	0	0	0	0	0	0
	Deposited	0	0	0			
2 and 6	Total Inhaled	0	0	0	0.0114	2.113	1703
	Deposited	0	0	0			
3 and 7	Total Inhaled	0.090	0.105	0.098	0.00730	0.738	1431
	Deposited	0.008	0.009	0.009			
4 and 8	Total Inhaled	0.283	0.331	0.307	0.00372	0.693	568
	Deposited	0.023	0.027	0.025			
5 and 9	Total Inhaled	0.863	1.008	0.936	0.0114	2.113	1703
	Deposited	0.067	0.078	0.073			

The deposition fractions for the low, mid, and high dose groups were estimated to be 8.9, 8.3, and 7.8%, respectively, based upon measured particle size ranges. The particle size distribution of the aerosol in each exposure chamber (formoterol treatment

groups only) was determined at approximate one-month intervals during the study. The samples were collected with cascade impactors and the quantity of formoterol deposited on each stage was determined using a HPLC method.

Particle size distribution (MMAD ± GSD, µm).

Group	Day 7	Day 23	Day 51	Day 79
3				
4				
5				

Route, form, volume, and infusion rate: Daily treatment consisted of a single nose-only inhalation exposure of 60 min.

Exposure to test and control formulations was performed using a nose-only exposure system. The rats were restrained in clear molded plastic tubes, tapered at one end. Each animal's nose protruded through a hole in the tapered end. Each restraint tube was connected to the exposure chamber by way of a push-fit through rubber "O" ring in the chamber wall. The exposure chambers were operated with a dynamic airflow, designed to facilitate an even distribution of the test atmosphere.

Aerosols were generated from the pMDIs by an automated pMDI actuator and shaker device, which actuated the pMDIs directly into the top of the chamber. The actuation and shaking frequency were controlled by a timer unit, which also recorded the cumulative number of actuations. The pMDIs were shaken prior to each actuation. Aerosol concentrations were controlled by adjusting pMDI actuation rates and exhaust air as required.

Observations and times:
Clinical signs: Animals were monitored for mortality and/or moribundity twice per day. Animals were observed for clinical signs of toxicity before, during, and after each exposure. Physical examinations were conducted once per week.
Body weights: Body weights were measured weekly.
Food consumption: Food and water consumption by each cage of animals was measured daily. Weekly individual and group mean food and water consumption was calculated. Food consumption was not measured for animals in toxicokinetic groups.
Ophthalmoscopy: Not performed.
EKG: Not performed.
Hematology: Blood samples for measurements of hematology parameters were collected during weeks 5 and 13.
Clinical chemistry: Blood samples for measurements of clinical chemistry parameters were collected during weeks 5 and 13.
Urinalysis: Urine samples for measurements of urinalysis parameters were measured during weeks 5 and 13.
Gross pathology: After a minimum of 13 consecutive weeks of treatment, animals were sacrificed using a predetermined sequence in which males preceded females. Organs and tissues designated for microscopic examinations were fixed either whole or as sections in 10% buffered formalin. The lungs were inflated with formalin vapor before being placed in the fixative. The eyes were fixed in Davidson's fluid and testes in

Bouin's fixative. An additional sample of liver was fixed in 80% ethanol for possible demonstration of glycogen content, but was not examined. Bone marrow smears were collected at necropsy from a femur of each animal, but were not examined.
Organs weighed: Absolute and relative (to brain weight) organ weights were determined for the adrenal glands, brain, kidneys, heart, liver, lungs, ovaries, prostate, spleen, testes, thymus, thyroid gland (left), and uterus.
Histopathology: Samples from all tissues from all animals in the air-control, vehicle-control, and high dose groups were processed into paraffin blocks. Sections were cut at approximately 4 µm and stained with hematoxylin and eosin. Tissue sections from Groups 1, 2, and 5 were examined by light microscopy. Sections of heart, lungs, and adrenal glands were examined from remaining groups.
Toxicokinetics: Blood samples for measurements of plasma drug levels were collected on days 9 and 85 at 0.25, 0.5, 0.75, 1, 2, 4, 8, and 24 hr after the completion of the exposure period. Animals in toxicokinetic groups were sacrificed and discarded without examination.

Results:

Mortality: One female rat (#92F) in the high dose group collapsed shortly after blood collection for clinical pathology on day 87. Other clinical signs included cold-to-touch and eye(s) half shut. This animal was subsequently sacrificed in a moribund condition. Histopathological findings for this animal were as follows: trachea, glandular ectasia; lungs, alveolar histiocytosis; heart, inflammation, focal, chronic; urinary bladder, transitional cell hyperplasia, simple, diffuse; thymus, atrophy and lymphocytolysis; mesenteric lymph node, lymphocytolysis; cervical lymph nodes, plasma cell hyperplasia; adrenal cortices, congestion, unilateral; and ovaries, congestion, bilateral. The cause of death was unclear, although, the sponsor reported that this animal might have become overheated during blood collection. Further, treatment with the test article, formoterol, might have exacerbated effects produced by overheating.

Clinical signs: One female rat (#94F) in the high dose group displayed several clinical signs following blood collection for clinical pathology on day 87 that included unsteady gait, hunched posture, irregular and shallow respiration, and sensitivity to external stimuli. Other nonspecific clinical signs included stained anogenital area, loose feces, hair loss on the head and one limb. This animal subsequently recovered. The cause of these clinical signs was unclear, although, the sponsor reported that this animal might have become overheated during blood collection. Further, treatment with the test article, formoterol, might have exacerbated effects produced by overheating.

Body weights: Body weights were elevated for female treatment groups, which could be attributed to the pharmacological effects of β-adrenergic agonists.

Body weights for male air-controls at weeks –1 and 14 were 249.2 and 469.6 g, respectively. Body weight gains for male rats in the vehicle-control, low dose, mid dose, and high dose groups from weeks –1 to 14 were 98.0, 106.8, 110.8, and 104.5% of the air-control, respectively. Body weights for female air-controls at weeks –1 and 14 were 185.3 and 259.0 g, respectively. Body weight gains for female rats in the vehicle-

control, low dose, mid dose, and high dose groups from weeks –1 to 14 were 93, 121.8, 128.3, and 131.9% of the air-control, respectively.

Food and Water consumption: Overall food consumption during the study period was increased for male and female treatment in general accordance with body weight gains. Overall water consumption was elevated for the male and female vehicle-control groups, although, no changes were noted in male or female treatment groups.

Food consumption for male rats in the low, mid, and high dose groups was increased to 105.4, 109.4, and 107% of the air-control (29.8 g/rat/day), respectively. Food consumption for female rats in the low, mid, and high dose groups was increased to 105.7, 109.9, and 109.9% of the air-control (21.2 g/rat/day), respectively.

Water consumption for the male and female vehicle control groups were elevated to 112.3 and 111.5% of air-controls (34.4 and 29.4 mL/animal/day), respectively, although, water spillage may have contributed to these increases.

Hematology: Increased white cell counts (i.e., neutrophils, lymphocytes, eosinophils and basophils) were evident for male and female rats in vehicle-control, low dose, mid dose, and high dose groups at weeks 5 and/or 13 as compared to the air-control. These changes suggest some differences between the vehicle-control and air-control groups; however, many of these differences might also be attributed to normal variations of control levels.

Week 5: White blood cell counts for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 140.3, 138.6, 182, and 162.1% of the air-control (6.225 x 10^9/L), respectively. Neutrophil counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 119.4, 129.9, 120.5, and 151.7% of the air-control (1.296 x 10^9/L), respectively. Neutrophil counts for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 124.25, 128.6, 142.5, and 128.1% of the air-control (0.928 x 10^9/L), respectively. Lymphocyte counts for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 144, 144.7, 193.9, and 172.5% of the air-control (4.833 x 10^9/L), respectively. Basophil counts for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 183.3, 166.7, 258.3, and 200% of the air-control (0.012 x 10^9/L), respectively.

Week 13: White blood cell counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 126.4, 129.8, 129.2, and 117.9% of the air-control (7.760 x 10^9/L), respectively. White blood cell counts for female rats in low, mid, and high dose groups were increased to 144.7, 182.2, and 161.3% of the air-control (5.420 x 10^9/L), respectively. Neutrophil counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 204.55, 147.9, 180.5, and 125.4% of the air-control (1.187 x 10^9/L), respectively. Neutrophil counts for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 125.9, 116.7, 170, and 128% of the air-control (0.962 x 10^9/L), respectively. Lymphocyte counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 112, 128.9, 122.9, and 120.6% of the air-control (5.874 x 10^9/L),

respectively. Lymphocyte counts for female rats in low, mid, and high dose groups were increased to 158.6, 193.45, and 173.8% of the air-control (3.957 x 10^9/L), respectively. Eosinophil counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 112, 128.5, 136.1, and 115.8% of the air-control (0.158 x 10^9/L), respectively. Basophil counts for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 200, 180, 200, and 210% of the air-control (0.010 x 10^9/L), respectively. Basophil counts for female rats in low, mid, and high dose groups were increased to 140, 200, and 220% of the air-control (0.010 x 10^9/L), respectively.

Clinical chemistry: For male and female treatment groups, glucose and triglyceride levels were decreased, while cholesterol levels were increased. These changes might be attributed to the effects of a β-adrenergic agonist. Other observed changes were generally small and their toxicological significance was unclear.

Week 5: Glucose levels for male rats in low, mid, and high dose groups were decreased to 91.4, 88.2, and 84.9% of the air-control (8.56 mmol/L), respectively. Glucose levels for female rats in the low, mid, and high dose groups were decreased to 88.5, 82.3, and 82.7% of the air-control (8.76 mmol/L), respectively. Creatinine levels for male rats in mid and high dose groups were increased to 105.8 and 104.6% of the air-control (54.9 µmol/L), respectively. Creatinine levels for female rats in the high dose group were increased to 112.5% of the control (69.6 µmol/L), respectively. Cholesterol levels for male rats in low, mid, and high dose groups were increased to 110.9, 112.4, and 112.65% of the air-control (1.455 mmol/L), respectively. Cholesterol levels for female rats in the low, mid, and high dose groups were increased to 116.8, 120.7, and 119.7% of the control (1.573 mmol/L), respectively. Triglyceride levels for male rats in low, mid, and high dose groups were decreased to 84.8, 85.2, 73.9% of the air-control (1.014 mmol/L), respectively. Aspartate aminotransferase activities for male rats in the low, mid, and high dose groups were increased to 121.4, 121.7, and 118.7% of the control (80.8 IU/L), respectively. Alanine aminotransferase activities for male rats in the low, mid, and high dose groups were increased to 111, 119.2, and 116.6% of the air-control (73.9 IU/L), respectively. Albumin/globulin ratios for female rats in low, mid, and high dose groups were decreased to 92.5, 89.0, and 93.8% of the air-control (1.46%), respectively.

Week 13: Glucose levels for male rats in low, mid, and high dose groups were decreased to 92.9, 84.8, and 88.4% of the air-control (8.07 mmol/L), respectively. Glucose levels for female rats in mid and high dose groups were decreased to 82 and 86.6% of the control (8.37 mmol/L), respectively. Urea levels for male rats in low, mid, and high dose groups were increased to 114.7, 117.7, and 129.5% of the air-control (4.75 mmol/L), respectively. Creatinine levels for male rats in mid and high dose groups were increased to 106.4 and 109.6% of the air-control (59.4 µmol/L), respectively. Cholesterol levels for male rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 106.4, 110.9, 113.6, and 116.5% of the air-control (1.528 mmol/L), respectively. Triglyceride levels for male rats in vehicle-control, low dose, mid dose, and high dose groups were decreased to 91.4, 79.8, 86.2, and 83.4% of the air-control (1.020 mmol/L), respectively. Sodium levels for male rats in the low, mid, and high dose groups were increased to 102.4, 103, and 103.6% of the control (138.44

mmol/L), respectively. Sodium levels for female rats in the vehicle-control, low dose, mid dose, and high dose groups were increased to 97.1, 96.8, 96.8, and 93.5% of the air-control (148.80 mmol/L), respectively. Potassium levels for female rats in the low, mid, and high dose groups were increased to 106.4, 105.7, and 106.6% of the air-control (4.533 mmol/L), respectively. Aspartate aminotransferase activities for male rats in the low, mid, and high dose groups were increased to 117.7, 111.8, and 114.5% of the control (79.1 IU/L), respectively. Alanine aminotransferase (ALT) activities for male rats in the low, mid, and high dose groups were increased to 115, 117.3, and 122.5% of the control (61.3 IU/L), respectively. ALT activity for female rats in the high dose group was increased to 122.3% of the control (104.8 IU/L), respectively. Albumin to globulin ratios for female rats in mid and high dose groups were decreased to 88.75 and 88.1% of the air-control (1.60%), respectively.

Urinalysis: There were no toxicologically significant changes of urinalysis parameters.

Week 5: Urinary osmolalities for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 107.3, 112, 110.9, and 118.5% of the air-control (1290.6 mOsmol/kg), respectively.

Week 13: Urinary osmolalities for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 107.1, 114.7, 128.2, and 140.2% of the air-control (1290.6 mOsmol/kg), respectively. Urinary volumes for male rats in vehicle-control and treatment groups were increased to 108.4-149.4% of the air-control (8.30 mL), respectively.

Organ weights: Statistically significant increases in absolute and relative heart weights were observed for male and female treatment groups that appeared to correlate with histopathological findings. Increases of absolute and relative weights for the lungs, thymus, and spleen were observed for female treatment groups, although, the relationship to treatment was unclear.

Heart: Absolute heart weights for male rats in low, mid, and high dose groups were increased to 113.1, 111.7, and 117.2% of the air-control (1.45 g), respectively. Relative heart weights for male rats in low, mid, and high dose groups were increased to 116, 116, and 120.8% of the air-control (57.65% of brain weight), respectively. Absolute heart weights for female rats in low, mid, and high dose groups were increased to 114.7, 116.9, and 123.5% of the air-control (0.90714 g), respectively. Relative heart weights for female rats in low, mid, and high dose groups were increased to 116.4, 116.9, and 124% of the air-control (41.41% of brain weight), respectively.

Lungs: Absolute lung weights for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 110.8, 123.7, 114.7, and 117.7% of the air-control (2.32 g), respectively. Relative lung weights for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 111.55, 125.3, 115.4, and 117.6 % of the air-control (105.6% of brain weight), respectively. These changes may possibly correlate with findings of alveolar histiocytosis; however, similar changes of absolute and relative lung weights for male rats were not observed.

Thymus: Absolute thymus weights for female rats in vehicle-control, mid dose, and high dose groups were increased to 114.75, 109.3, and 117% of the air-control (0.23947 g), respectively. Relative thymus weights for female rats in vehicle-control, mid dose, and high dose groups were increased to 115.8, 110.3, and 117.8% of the air-control (10.86% of brain weight), respectively.

Spleen: Absolute spleen weights for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 109, 105, 114.9, and 116.8% of the air-control (0.53880 g), respectively. Relative spleen weights for female rats in vehicle-control, low dose, mid dose, and high dose groups were increased to 109.9, 106.6, 115.5, and 117.4% of the air-control (24.54% of brain weight), respectively.

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

Histopathology: The heart was the target organ of toxicity. A number of changes were observed in other organs, although, treatment relationships were unclear.

In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the high dose groups. The incidence of inflammation was slightly increased for male and female rats in the high dose group. Epicarditis was observed for one female rat in the high dose group. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat (94F) was characteristic of cardiac lesions produced by β-adrenergic agonists.

Changes were evident in the lungs and adrenal cortex for vehicle-control and formoterol treatment groups as compared to the air-control group. The incidence and severity of alveolar histiocytosis in the lungs were increased for male and female rats in vehicle-control and formoterol treatment groups as compared to the air-control group. These changes in the lungs were characterized as common findings for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices were evident for male and female rats in vehicle-control and formoterol treatment groups as compared to no findings for the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704), although, these changes were not evident in the concurrent control.

A number of findings were isolated to male and female rats in the high dose group. In general, the incidences of these findings were low and their relationship to treatment was unclear. Inflammation of the aorta was observed for three female rats in the high dose group. Thymic atrophy and lymphocytolysis were observed for female rats in the high dose group. Centrilobular hypertrophy was observed in the liver for 1 female rat in the high dose group. Histiocytosis was observed in the cervical lymph nodes for two male rats in the high dose group. Inflammation of the tracheal carina was observed for one female rat in the high dose group. Arteritis/periarteritis was evident in the stomach for two female rats (91F and 94F) in the high dose group. Arteritis/periarteritis was also evident in pancreas, cecum, and mesenteric lymph node

for one of these female rats (94F) in the high dose group. The incidences of cortical cyst, tubular degeneration/regeneration, and lymphoid cell infiltration in the kidneys were increased for male and/or female rats in the high dose group.

The incidence and severity of hemosiderin deposits in the spleen for female rats in the vehicle-control group were increased as compared to the air-control group.

Organ/Tissue	Air-Control		Vehicle-Control		Formoterol Low dose		Formoterol Mid dose		Formoterol High dose	
	M	F	M	F	M	F	M	F	M	F
Heart										
-number examined	10	10	10	10	10	10	10	10	10	10
-inflammation (total)	3	2	3	1	3	0	1	4	5	4
Grade 1	2	2	3	1	3	0	1	4	5	3
Grade 2	1	0	0	0	0	0	0	0	0	1
-Fibrosis (total)	0	0	2	0	1	0	0	1	0	0
Grade 1	0	0	2	0	1	0	0	1	0	0
-Myocyte degeneration (total)	1	0	0	0	1	0	0	0	2	1
Grade 1	1	0	0	0	1	0	0	0	2	0
Grade 2	0	0	0	0	0	0	0	0	0	1
-Epicarditis (total)	0	0	0	0	0	0	0	0	0	1
Grade 1	0	0	0	0	0	0	0	0	0	1
Lungs										
-number examined	10	10	10	10	10	10	10	10	10	10
-vascular mineralization (total)	7	1	9	1	5	2	3	9	6	6
Grade 1	5	1	7	1	5	2	3	9	6	6
Grade 2	2	0	2	0	0	0	0	0	0	0
-congestion	1	1	4	0	3	1	6	2	2	2
Grade 1	1	0	4	0	1	1	6	2	1	2
Grade 2	0	1	0	0	2	0	0	0	1	0
-alveolar histiocytosis	1	3	5	3	6	3	3	7	3	7
Grade 1	0	3	3	3	3	3	2	7	2	6
Grade 2	1	0	1	0	2	0	1	0	1	1
Grade 3	0	0	1	0	1	0	0	0	0	0
-pneumonitis	3	1	6	3	7	5	4	5	1	4
Grade 1	3	1	3	3	6	5	3	5	1	4
Grade 2	0	0	3	0	1	0	1	0	0	0
Adrenal Cortices										
-number examined	10	10	10	10	10	10	10	10	10	10
-diffuse fatty change	0	0	3	1	2	0	4	0	3	1
Grade 1	0	0	2	1	2	0	3	0	3	0
Grade 2	0	0	1	0	0	0	1	0	0	0
Grade 3	0	0	0	0	0	0	0	0	0	1

Extraorbital lacrimal gland										
-number examined	10	10	10	10	-	-	-	-	10	10
-lymphoid cell infiltration (total)	2	2	3	0					5	0
Grade 1	2	1	3	0					5	0
Grade 2	0	1	0	0					0	0
-atrophy (total)	0	0	0	0					1	0
Grade 1	0	0	0	0					1	0
Aorta										
-number examined	10	10	10	10	-	-	-	-	10	10
-inflammation (total)	0	0	0	0					0	3
Grade 1	0	0	0	0					0	2
Grade 2	0	0	0	0					0	1
Thymus										
-number examined	10	10	10	10	-	-	-	-	10	10
-atrophy (total)	0	0	0	0					0	2
Grade 1	0	0	0	0					0	1
Grade 3	0	0	0	0					0	1
-lymphocytolysis (total)	0	0	0	0					0	2
Grade 1	0	0	0	0					0	1
Grade 2	0	0	0	0					0	1
Spleen										
-number examined	10	10	10	10	-	-	-	-	10	10
-increased hematopoiesis (total)	2	4	4	2					2	6
Grade 1	1	3	4	1					1	4
Grade 2	1	1	0	1					1	2
-hemosiderin deposits (total)	0	0	0	5					1	1
Grade 1	0	0	0	2					0	1
Grade 2	0	0	0	2					1	0
Grade 3	0	0	0	1					0	0
Liver										
-number examined	10	10	10	10	-	-	-	-	10	10
-centrilobular hypertrophy	0	0	0	0					0	1
Grade 1	0	0	0	0					0	1
Cervical lymph node										
-number examined	10	10	10	10	-	-	-	-	10	10
-histiocytosis	0	0	0	0					2	0
Grade 1	0	0	0	0					2	0
Tracheal Carina										
-number examined	10	10	10	10	-	-	-	-	10	10
-inflammation	0	0	0	0					0	1
Grade 2	0	0	0	0					0	1

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Kidneys										
-number examined	10	10	10	10	-	-	-	-	10	10
-cortical cyst (total)	0	0	0	0					0	2
-tubular degeneration/ regeneration (total)	0	0	0	0					0	1
Grade 3	0	0	0	0					0	1
-lymphoid cell infiltration (total)	2	2	1	1					4	2
Grade 1	2	2	1	1					4	2
Stomach										
-number examined	10	10	10	10	-	-	-	-	10	10
-arteritis/periarteritis (total)	0	0	0	0					0	2
Grade 1	0	0	0	0					0	1
Grade 2	0	0	0	0					0	1
-medial hemorrhage (total)	0	0	0	0					0	1
Grade 1	0	0	0	0					0	1
Pancreas										
-number examined	10	10	10	10	-	-	-	-	10	10
-arteritis/periarteritis (total)	0	0	0	0					0	1
Grade 1	0	0	0	0					0	1
Mesenteric lymph node										
-number examined	10	10	10	10	-	-	-	-	10	10
-lymphocytolysis (total)	0	0	0	0					0	1
Grade 2	0	0	0	0					0	1
-arteritis/periarteritis (total)	0	0	0	0					0	1
Grade 1	0	0	0	0					0	1
Cecum										
-number examined	10	10	10	10	-	-	-	-	10	10
-arteritis/periarteritis	0	0	0	0					0	1
Grade 1	0	0	0	0					0	1

Toxicokinetics: Systemic exposure to formoterol increased with elevating dose in both male and female rats. For male rats, AUC and C_{max} values tended to be higher on day 85 as compared to day 9. Dose proportionality was evident for female rats on day 9 and male rats on day 85. Although pharmacokinetic data are not available for the clinical dose of 9 µg BID, 24 µg BID produced an $AUC_{0\text{-}24hr}$ of 0.31 nmol·hr/L. The AUC at the mid dose (i.e., NOAEL; 25.3 nmol·hr/L) was approximately 82 times the AUC produced with a clinical dose of 24 µg BID.

Toxicokinetic parameters for formoterol in rats after inhalation of target doses at 0.090, 0.280, and 0.890 mg/kg/day for 9 or 85 days.

Target Dose mg/kg/day	Sex	AUC[a] (nmol·hr/L)		C_{max} (nmol/L)		T½ (hr)	
		Day 9	Day 85	Day 9	Day 85	Day 9	Day 85
0.090	Males	6.72	7.39	2.4	3.9	3.15	3.11
	Females	5.50	5.87	2.6	3.2	2.49	4.60
0.280	Males	26.74	28.22	11.4	13.8	3.09	1.90
	Females	28.61	22.43	10.0	8.2	3.59	3.61
0.890	Males	40.96	105.8	17.2	39.6	2.64	2.83
	Females	88.47	53.16	55.2	22.4	2.29	2.24

a. AUC0-9hr for 0.090 mg/kg/day and AUC0-24hr for 0.280 and 0.890 mg/kg/day

Summary of individual study findings:

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group at target formoterol fumarate dihydrate doses of 0.090, 0.280, and 0.890 mg/kg/day (actual doses of 0.098, 0.307, and 0.936 mg/kg/day, respectively). Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). The mid dose was identified as the NOAEL due to the death of one animal and histopathological findings in the heart at the high dose.

The heart was the target organ of toxicity. A number of changes were observed in other organs, although, treatment relationships were unclear.

Increased absolute and relative heart weights were observed for male and female rats in all formoterol groups. In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the high dose groups. The incidence of inflammation was slightly increased for male and female rats in the high dose group. Epicarditis was observed for one female rat in the high dose group. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat (94F) was characteristic of cardiac lesions produced by β-adrenergic agonists.

Changes were evident in the lungs and adrenal cortex for vehicle-control and formoterol treatment groups as compared to the air-control group. The incidence and severity of alveolar histiocytosis in the lungs were increased for male and female rats in vehicle-control and formoterol treatment groups as compared to the air-control group. This change in the lungs was characterized as a common finding for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices were evident for male and female rats in vehicle-control and formoterol treatment groups as compared to no findings for the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704).

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A number of findings were isolated to male and female rats in the high dose group. In general, the incidences of these findings were low and their relationship to treatment was unclear. Inflammation of the aorta was observed for three female rats in the high dose group. Thymic atrophy and lymphocytolysis were observed for female rats in the high dose group. Centrilobular hypertrophy was observed in the liver for 1 female rat in the high dose group. Histiocytosis was observed in the cervical lymph nodes for two male rats in the high dose group. Inflammation of the tracheal carina was observed for one female rat in the high dose group. Arteritis/periarteritis was evident in the stomach for two female rats (91F and 94F) in the high dose group. Arteritis/periarteritis was also evident in pancreas, cecum, and mesenteric lymph node for one of these female rats (94F) in the high dose group. The incidences of cortical cyst, tubular degeneration/regeneration, and lymphoid cell infiltration in the kidneys were increased for male and/or female rats in the high dose group.

As stated above, there were some differences in histopathological findings between vehicle- and air-control groups. The incidence and severity of alveolar histiocytosis in the lungs were increased for the vehicle-control group as compared to the air-control group. Diffuse fatty changes in the adrenal cortices were evident for vehicle-control groups as compared to no findings in the air-control group. Alveolar histiocytosis was characterized as a common finding for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704). Thus, deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively.

Although pharmacokinetic data are not available for the clinical dose of 9 µg BID, 24 µg BID produced an $AUC_{0\text{-}24hr}$ of 0.31 nmol·hr/L. The AUC at the mid dose (i.e., NOAEL; 25.3 nmol·hr/L) was approximately 82 times the AUC produced with a clinical dose of 24 µg BID.

Toxicology summary:

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group. Target doses of formoterol fumarate dihydrate for the low, mid, and high dose groups were 0.090, 0.280, and 0.890 mg/kg/day, respectively. Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). The mid dose was identified as the NOAEL due to the death of one animal and histopathological findings in the heart at the high dose. The heart was the target organ of toxicity. A number of changes were observed in other organs, although, treatment relationships were unclear. In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the

high dose groups. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat was characteristic of cardiac lesions produced by β-adrenergic agonists. Changes were evident in the lungs and adrenal cortex for vehicle-control and formoterol treatment groups as compared to the air-control group. The incidence and severity of alveolar histiocytosis in the lungs were increased for male and female rats in vehicle-control and formoterol treatment groups as compared to the air-control group. Diffuse fatty changes in the adrenal cortices were evident for male and female rats in vehicle-control and formoterol treatment groups as compared to no similar findings in the air-control group. Both of these histopathological changes have been characterized as common findings in laboratory rats. Deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively. The AUC at the mid dose (i.e., NOAEL; 25.3 nmol·hr/L) was approximately 82 times the AUC (i.e., $AUC_{0\text{-}24hr}$ = 0.31 nmol·hr/L) produced with a clinical dose of 24 µg BID.

Toxicology conclusions:

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to rats. Deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively.

Histopathology Inventory for IND # ___

Study	3-month inhalation study (96195-1)
Species	Dogs
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	
Gross lesions	?
Harderian gland	X
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X (Extraorbital)
Larynx	X
Liver	X*
Lungs	X*
Lymph nodes, cervical	X
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Lymph nodes, bronchial	X
Mammary Gland	X
Nasal cavity	X
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	X
Pituitary	X
Prostate	X*

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

X, histopathology performed
*, organ weight obtained

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

Formoterol fumarate dihydrate is a β_2-adrenergic agonist under development for the treatment of asthma. To support the continued clinical development of formoterol, the sponsor has submitted several toxicokinetic studies in rats and dogs, two metabolism studies, and a 13-week inhalation toxicology study in rats with a formoterol HFA pMDI formulation.

Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 60 min per day as a dry powder aerosol to rats for periods up to 6 months. Plasma concentrations of D2522 were measured in rats following one week or 6 months of inhalation exposure. Target inhaled doses of D2522 were 25, 140, and 800 µg/kg/day. Deposited doses were 2.4-3.2, 9.0-12.6, and 80.6-94.5 µg/kg/day, respectively. AUC values increased with elevating doses on days 8 and 176, although, increases were less than dose proportional. On day 176, exposure was generally higher in male rats as compared to female rats. For female rats at the high dose, exposure was less on day 176 as compared to day 8. Drug half-life was approximately 2 hr for male and female rats.

Formoterol fumarate dihydrate (D2522) in lactose was administered by inhalation exposure for 30 min per day as a dry powder aerosol to rats for periods up through 76 weeks. Plasma concentrations of D2522 were measured in rats after 1, 50, and 76 weeks of exposure. Target inhaled doses of D2522 were 5, 25, and 125 µg/kg/day. Deposited doses were approximately 0.26-0.66, 1.5-2.2, and 7.1-14 µg/kg/day, respectively. Peak plasma drug concentrations were observed at 15 min, the first time point after completion of dosing. C_{max} and AUC values on days 7, 345-346, and 532-533 increased with elevating dose. On days 7 and 345-346, increases in C_{max} and AUC values with elevating dose were approximately dose proportional. However, during week 76, C_{max} and AUC values for the high dose group were generally lower than those observed during weeks 1 and 49. Actual and deposited doses as well as C_{max} and AUC values in female rats were generally slightly higher than those observed in male rats.

Formoterol fumarate dihydrate (D2522) was administered daily as a dry powder aerosol for 3 months to young rats (16-18 days old at the start of dosing). Target inhaled doses were 25, 140, and 800 µg/kg/day. Deposited doses for low, mid, and high dose groups were 2.54, 13.2, and 67.6 µg/kg/day, respectively. Peak plasma levels were obtained within 15 min after completion of dosing, indicating rapid absorption of drug from the lungs. C_{max} and AUC values increased with elevating dose on days 6, 25, and 88, although, increases were generally less than dose proportional. C_{max} and AUC values were generally higher in male rats as compared to female rats over the entire treatment period. C_{max} and AUC values were relatively constant over the 3-month treatment period as young pups developed into adult animals. Thus, toxicokinetic parameters of D2522 did not appear to change during continuous dosing of young rats up to adult age.

Plasma concentrations of KWD 2183 (bambuterol) and its hydrolysis product, terbutaline, were measured after oral administration of single doses of bambuterol at 250 and 500 µmole/kg to dogs. Bambuterol was rapidly absorbed following a dose of 250 µmole/kg with a T_{max} of ~1 hr. Absorption was delayed with a larger dose of 500 µmole/kg, as the T_{max} was ~4 hr. C_{max} values for bambuterol were dose proportional.

Systemic exposure to formoterol was assessed following inhalation exposure of beagle dogs to a micronized powder mix of [redacted] formoterol fumarate dihydrate in lactose. Target doses of formoterol fumarate dihydrate were 0.5, 2.7, and 15 µg/kg/day. On days of blood sampling (i.e., days 0, 6, and 21), mean deposited doses for low, mid, and high dose groups were 0.07, 0.4, and 2 µg/kg/day, respectively. No calculations of AUC could be performed for the low dose group. Peak plasma drug levels were observed within 10 min after completion of dosing, indicating rapid drug absorption. C_{max} values on days 0, 6, and 21 increased with elevating dose, and increases were approximately dose proportional. The increase in AUC from the mid to high dose was approximately dose proportional.

The biotransformation of R,R- and S,S-formoterol by O-demethylation to the metabolites Met1-RR and Met1-SS, respectively, was studied in human liver microsomes in order to identify the cytochrome P450 (CYP) enzymes(s) involved. Results indicated that O-demethylation of R,R- and S,S-formoterol was catalyzed by more than one cytochrome P450 isozyme (i.e., CYP2D6 and CYP2C). There was a

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high correlation between Met1-RR and CYP2D6; however, there appeared to be no correlation between Met1-SS and CYP2D6. There was uncertainty regarding identities of CYP isozymes catalyzing O-demethylation of S,S-formoterol.

The metabolic profile of formoterol in urine was assessed after intratracheal and intravenous administration of ^{3}H-formoterol to male rats at 50 µg/kg. The metabolic profile of formoterol was also assessed at different time points in plasma, lungs, trachea, adrenals, stomach, and kidneys after intratracheal administration of ^{3}H-formoterol to rats at 50 µg/kg. Radiolabeled drug products identified in urine and tissues were intact formoterol, the phenol glucuronide of formoterol (FG1), the O-demethylation product (Met1), and two glucuronides of this primary metabolite (Met1G1 and Met1G2).

In a 13-week nose-only inhalation toxicology study, a formoterol pMDI formulation containing formoterol fumarate dihydrate, polyvinylpyrrolidone (PVP) K-25, polyethylene glycol 1000 (PEG-1000), and HFA-227 was administered to 10 rats/sex/group at target formoterol fumarate dihydrate doses of 0.090, 0.280, and 0.890 mg/kg/day (actual doses of 0.098, 0.307, and 0.936 mg/kg/day, respectively). Deposited doses were 0.009, 0.025, and 0.073 mg/kg/day, respectively. Two similarly sized control groups were exposed daily to either an excipients-only pMDI aerosol formulation (vehicle-control) or to air only (air-control). The mid dose was identified as the NOAEL due to the death of one animal and histopathological findings in the heart at the high dose.

The heart was the target organ of toxicity. A number of changes were observed in other organs, although, treatment relationships were unclear.

Increased absolute and relative heart weights were observed for male and female rats in all formoterol groups. In the heart, the incidence and severity of myocyte degeneration were increased for male and female rats in the high dose groups. The incidence of inflammation was slightly increased for male and female rats in the high dose group. Epicarditis was observed for one female rat in the high dose group. The Grade 2 myocyte degeneration in the region of the papillary muscle for one female rat (94F) was characteristic of cardiac lesions produced by β-adrenergic agonists.

Changes were evident in the lungs and adrenal cortex for vehicle-control and formoterol treatment groups as compared to the air-control group. The incidence and severity of alveolar histiocytosis in the lungs were increased for male and female rats in vehicle-control and formoterol treatment groups as compared to the air-control group. This change in the lungs was characterized as a common finding for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices were evident for male and female rats in vehicle-control and formoterol treatment groups as compared to no findings for the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704).

A number of findings were isolated to male and female rats in the high dose group. In general, the incidences of these findings were low and their relationship to treatment was unclear. Inflammation of the aorta was observed for three female rats in the high dose group. Thymic atrophy and lymphocytolysis were observed for female rats in the high dose group. Centrilobular hypertrophy was observed in the liver for 1 female rat in the high dose group. Histiocytosis was observed in the cervical lymph nodes for two male rats in the high dose group. Inflammation of the tracheal carina was observed for one female rat in the high dose group. Arteritis/periarteritis was evident in the stomach for two female rats (91F and 94F) in the high dose group. Arteritis/periarteritis was also evident in pancreas, cecum, and mesenteric lymph node for one of these female rats (94F) in the high dose group. The incidences of cortical cyst, tubular degeneration/regeneration, and lymphoid cell infiltration in the kidneys were increased for male and/or female rats in the high dose group.

As stated above, there were some differences in histopathological findings between vehicle- and air-control groups. The incidence and severity of alveolar histiocytosis in the lungs were increased for the vehicle-control group as compared to the air-control group. Diffuse fatty changes in the adrenal cortices were evident for vehicle-control groups as compared to no findings in the air-control group. Alveolar histiocytosis was characterized as a common finding for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704). Thus, deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively.

Although pharmacokinetic data are not available for the clinical dose of 9 µg BID, 24 µg BID produced an $AUC_{0\text{-}24hr}$ of 0.31 nmol·hr/L. The AUC at the mid dose (i.e., NOAEL; 25.3 nmol·hr/L) was approximately 82 times the AUC produced with a clinical dose of 24 µg BID.

General Toxicology Issues:

Under IND 63,394, the sponsor is developing a Symbicort (Budesonide/Formoterol) HFA pMDI formulation for the treatment of asthma. The Symbicort formulation contains excipients, PVP K-25 and PEG-1000, and the propellant, HFA-227. These excipients and the propellant are identical to those used in the 3-month inhalation study with the formoterol HFA pMDI formulation in rats.

In this 3-month inhalation toxicology study with the formoterol HFA pMDI formulation in rats, there were some differences in histopathological findings between vehicle- and air-control groups. The incidence and severity of alveolar histiocytosis in the lungs were increased for the vehicle-control group as compared to the air-control group. Diffuse fatty changes in the adrenal cortices were evident for vehicle-control groups as compared to no findings in the air-control group. Alveolar histiocytosis was characterized as a common finding for rats dosed by the inhalation route. Further, these changes in the lungs were not specific to excipients or the test article as they were also observed in the air-control group. Diffuse fatty changes in the adrenal cortices, observed as cytoplasmic vacuolation of adrenal cortical cells, have been characterized as common findings in laboratory rats (CRC Handbook of Toxicology, Page 704). Thus, deposited doses of excipients and the propellant in the vehicle-control group could be characterized as qualified NOAELs. Deposited doses of PVP K-25, PEG-1000, and HFA-227 in the vehicle-control group were 0.001, 0.2, and 1703 mg/kg/day, respectively.

Rat to human exposure ratios for excipients in clinical doses of the Symbicort HFA pMDI formulation in adults with a body weight of 50 kg (based upon excipient levels in vehicle-controls of the 3-month inhalation toxicology study with the Formoterol HFA pMDI formulation in rats).

Clinical Doses				Rat to Human exposure ratio
	µg/actuation	4 actuations/day	µg/kg/day	
Povidone K25	0.7	2.8	0.056	17.9
PEG 1000	212.3	849.2	16.984	11.8
HFA 227	70750	283000	5660	301

Rat to human exposure ratio for excipients in clinical doses of the Symbicort HFA pMDI formulation in children (6-11 years) with a body weight of 20 kg (based upon excipient levels in vehicle-controls of the 3-month inhalation toxicology study with the Formoterol HFA pMDI formulation in rats).

Clinical Doses				Rat to Human exposure ratio
	µg/actuation	4 actuations/day	µg/kg/day	
Povidone K25	0.7	2.8	0.14	7.1
PEG 1000	212.3	849.2	42.46	4.7
HFA 227	70750	283000	14150	120

This 3-month inhalation toxicology study with the formoterol HFA pMDI formulation in rats provides additional evidence that adequate safety margins appear to exist for excipients, povidone K-25 and PEG-1000, in the clinical Symbicort HFA pMDI formulations.

Recommendations: None.

Reviewer: Timothy W. Robison, Ph.D. IND No.

Labeling with basis for findings: None.

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

Supervisor signature: Concurrence - _____
Robin Huff, Ph.D.
Non-Concurrence - _____
(see memo attached)

cc: list:
OstroffC
HuffR
RobisonT

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/s/

Timothy Robison
5/29/02 05:20:20 PM
PHARMACOLOGIST

Robin Huff
5/30/02 01:46:06 PM
PHARMACOLOGIST
I concur.

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10 Trade Secret / Confidential

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Timothy Robison
5/22/2006 02:08:37 PM
PHARMACOLOGIST

Joseph Sun
5/22/2006 02:33:08 PM
PHARMACOLOGIST
I concur.

3/14/06

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: March 14, 2006

FROM: **Alan C. Schroeder, Ph.D., ONDQA**

THROUGH: **Blair Fraser, Ph.D., Branch Chief, ONDQA**

SUBJECT: **Consult Request for N21-929 (Symbicort Inhalation Aerosol)**

TO: **Ching-Long J. Sun, Ph.D., Supervisory Pharmacologist, HFD-570**

Please evaluate the safety of the proposed drug substance limits for formoterol fumarate dihydrate for the following: [redacted]. Please evaluate the safety of the proposed drug substance limits for formoterol fumarate dihydrate *micronized, conditioned* for the following: [redacted] impurities, and foreign particles. See the two sets of acceptance criteria proposed in the original NDA, Section 3.2.S.4.1 for unmicronized and micronized drug substance (formoterol fumarate). Note that the justification of these specifications, found in section 3.2.S.4.5 of the original NDA, includes a discussion of the basis for safety of the proposed specification for foreign particles in the formoterol drug substance. These foreign particles are not otherwise identified. Note that the proposed acceptance criterion for the [redacted] is less for the unmicronized drug substance [redacted] than for the micronized, conditioned drug substance [redacted].

Please evaluate the drug product for safety of foreign particles (see specifications, original NDA, sections P5.1, for proposed limits on foreign particles in the drug product). A sample of foreign particles was characterized and particles were observed that may (for example) be derived from [redacted]. See data in Section P.2 (Attachment 4) of the original NDA. Note that not all particles listed are likely to have been derived from the drug product: some may have originated in the sample preparation materials or equipment used for analysis. See Section P5.6 (pages 43 and 44) of the original NDA for a safety evaluation of the foreign particles.

Please evaluate the drug product for safety of budesonide impurities and degradation products, and for safety of formoterol impurities and degradation products. (See proposed specifications in NDA, section P5.1).

For the record, following is information that I previously provided informally, which pertains to a safety assessment of acceptance criteria for drug substance (formoterol fumarate) impurities/degradants for this drug product (in an e-mail on December 21, 2006).

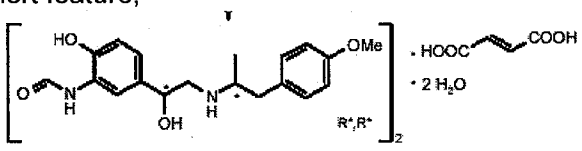
This pertains to the formoterol fumarate drug substance (see page 403 of the Pharmacology Written Summary, Section 2.6.2 of this NDA 21-929). Drug product

batch number P5993 contains drug substance batch number 1743/98. The drug substance shelf life limits are shown along with the batch data for relevant impurities, for batch 1743/98 used in drug product batch P5993. According to ICH Q3A, is the qualification limit (or whichever is lower, and that would be here). Therefore, impurities with limits of up to do not need to be further qualified (unless there is structural alert concern, see below). Proposed limits for impurities respectively, yet the data in this batch are respectively, which are not sufficient to qualify these proposed limits.

There are two batches used in some other toxicology studies which have data for the impurities that exceed or come close to the proposed drug substance limits for these impurities. See page 446 of the Pharmacology Written Summary, Section 2.6.2 of this NDA for this information (drug substance batch numbers 115/90 and 131/90).

Additional information: might be considered to have a structural alert because it contains a :

Even the drug substance itself, as well as some of its impurities, contains a structural alert feature,



Formoterol fumarate dihydrate
$C_{42}H_{56}N_4O_{14}$
[840.9]

Additional comments:
It may be noted that the proposed specifications for the budesonide drug substance are identical to those proposed in this applicant's NDA 21-949 for Budesonide Turbuhaler. These specifications have been found to be adequate, and therefore a duplicate safety assessment is not needed here for budesonide drug substance.

A request for safety evaluation of leachables data is not being submitted at this time, pending a response from the applicant for additional information and clarifications.

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/s/

Prasad Peri
3/14/2006 03:51:59 PM

11/10/05

NDA Pharmacology Fileability Check List

NDA No: 21-929

Date of submission: September 23, 2005

Date of Fileability meeting: November 9, 2005

Information to Sponsor Yes ( ) No (X)

Date of check list: November 9, 2005

(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	(X)	( )	( )
others	(X)	( )	( )

(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 ( ) No (X) NA ( )

If no, state why not?
The same formulation was used in 3-month bridging toxicology studies with rats and dogs as compared to the to be marketed product.

If yes, has the applicant made an appropriate effort to repeat the studies using the to be marketed product, to bridge the studies or to explain why such repetition or bridging should not be required? Yes (X) No ( ) NA ( )

The sponsor conducted single dose and 3-month bridging studies in rats and dogs using the to be marketed formulation produced from a pilot scale batch.

(5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose multiples expressed in either mg/m^2 or comparative systemic exposure levels) and in accordance with 201.57? Yes (X) No ( ).

(6) Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion? Yes (X) No ( ) NA ( )

(7) On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? Yes (X) No ( ) NA ( )

If not, has the applicant submitted a rationale to justify the alternative route?
Yes ( ) No ( ) NA

(8) Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes ( ) No ( ) NA (X)

(9) Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? Yes (X) No ( ) NA ( )

(10) Are there any outstanding preclinical issues? Yes ( ) No (X)
If yes, identify those below.

(11) From a preclinical perspective, is this NDA fileable? Yes (X) No ( )

If no, state below why it is not.

(12) Should any additional information/data be requested? Yes (X) No ( )

In consultation with the Chemistry reviewer, additional information may need to be requested for qualifications of impurities, extractables, and leachables.

NDA Planning Timeline

NDA No.: 21-949

Date of planning timeline: November 9, 2005

PDUFA Due Date: July 23, 2005

Projected review completion date: May 23, 2006

	Milestone Dates
Pharmacology and ADME Toxicology	2-23-06
General toxicity studies	Completed
Carcinogenicity studies and mutagenicity studies	Completed
a. Statistical consult request for CA studies	Completed
b. Submission of CA studies for CAC concurrence	Completed
Reproductive studies	Completed
Special studies and Others	4-23-06
Labeling	5-23-06

Signatures (optional):

Reviewer Signature _____
Timothy W. Robison, Ph.D.

Supervisor Signature _____
C. Joseph Sun, Ph.D.
Concurrence Yes ___ **No** ___

cc:
NDA 21-929, HFD-570 Division Files
JacksonC, HFD-570
GunkelJ, HFD-570
SchroederA
SunC, HFD-570
RobisonT, HFD-570

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/s/

Timothy Robison
11/16/2005 10:15:09 AM
PHARMACOLOGIST

Joseph Sun
11/18/2005 11:31:34 AM
PHARMACOLOGIST
I concur.