a. Human exposure ($AUC_{0\text{-}24hr}$ = 109 pg·hr/mL) from 091-026 preliminary data (after 2 weeks of daily inhalation treatment of arformoterol in COPD subjects with a dose of 50 µg/day.
b. $AUC_{0.5\text{-}2hr}$ (No sampling after 2 hr time point).

Rats

Study title: **A 24-Month Inhalation Oncogenicity Study of (R,R)-Formoterol in Rats.**

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks. The sponsor did not have ECAC concurrence for dose selection. The sponsor did not contact the Division prior to early termination of groups as described below.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups.

The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks.

Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was also achieved at this dose.

Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and it appears that the treatment period was insufficient. There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively; however, histopathological examinations of organs and tissues were incomplete for these animals. The sponsor should be asked to complete the histopathological evaluations of organs and tissues for males and females in the 200 µg/kg/day group and lower doses if appropriate to adequately assess the carcinogenic potential of (R,R)-formoterol in rats.

The incidences of cyst(s) in the ovaries and oviducts were significantly increased for female treatment groups.

Evaluation of tumor findings:
For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 200 µg/kg/day group when the high dose group was excluded

For the thyroid gland, the combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day slightly exceeded the upper range of the historical control background. It should be noted that histopathological examination of the thyroid gland was incomplete for females in the 200 µg/kg/day group as only 37 of 60 animals were examined. Histopathological examinations were also incomplete for the female 40 and 100 µg/kg/day groups, although the period of treatment was identical to the 400 µg/kg/day group.

Study no.: — 312051
Volume #, and page #: Volumes 1-20, Pages 1-7392
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: January 19 and 27, 2000 (Initiation of dose administration for males and females, respectively; Designated as study week 0)
GLP compliance: Yes.
QA report: yes (X) no ( )
Drug, lot #, and % purity: (R,R)-formoterol tartrate, Lot number 021 5012 Log No.: 4517A] (Purity, 100.7% from Certificate of Analysis)
CAC concurrence: No.

The (R,R)-formoterol doses in the current study were based upon a 1-month inhalation toxicology with rats. The ECAC did not concur (see attached minutes in Appendix 1). In this subchronic study, test article-related effects were reported by the sponsor at 400 µg/kg/day. According to the sponsor, systemic exposure levels at 100 and 400 µg/kg/day after 1 month of inhalation treatment were at least 90- and 450-fold, respectively, higher than clinical exposure with a therapeutic dose of (R,R)-formoterol.

Methods
Doses: 0, 40, 100, 200, and 400 µg/kg/day

Group	Treatment	Target Inhaled Dose[a], µg/kg/day	Number of Animals	
			Males	Females
1	Vehicle-Control 1	0	60	60
2	Vehicle-Control 2	0	60	60
3	(R,R)-Formoterol	40	60	60
4	(R,R)-Formoterol	100	60	60

5	(R,R)-Formoterol	200	60	60
6	(R,R)-Formoterol	400[b]	60	60

a. Daily dose levels were controlled by the combination of the exposure duration and exposure concentration, based upon the delivered drug on the filter as determined by chemical analysis.
b. The exposure concentration for the high dose male group was targeted to provide a dosage level of 200 µg/kg/day for the first and second days of exposure and then targeted to provide a dose level of 400 µg/kg/day starting on the third day of exposure.

Target concentrations were calculated as follows:

$$\text{Target concentration (µg/L)} = \frac{\text{Target dose (µg/kg) x Body weight (kg)}}{\text{Minute volume (L/min) x Exposure duration (min)}}$$

Target concentrations were calculated at study initiation and periodically during the study using the most recent body weight data.

Mean exposure concentrations

Dose, µg/kg/day	Free base concentration (µg/L)	
	Males	Females
40	2.2	2.0
100	5.4	4.9
200	11.1	9.8
400	20.8	19.0

Animal exposures were conducted using the ⊂ ⊐ directed-flow nose-only exposure systems assembled with six 12-port modules that operated under dynamic conditions. Two systems were dedicated for (R,R)-formoterol exposures and a third system for control (saline aerosol) exposures. Control groups 1 and 2 were exposed using System 1, the 40 and 100 µg/kg/day groups were exposed using System 2, and the 200 and 400 µg/kg/day groups were exposed using System 3. Male and female rats from each of the six groups were exposed separately, using separate generation and exposure periods. Animals were placed in nose-only exposure restraint tubes during exposure periods. b(4)

For the test article exposures of each group, liquid droplet aerosol atmospheres were generated using nebulizer-based systems. The modified ⊂ ⊐ Collision nebulizer was selected for use based on its stable and efficient aerosolization and production of aerosols with particle sizes (MMAD) <2 µm. b(4)

Actual exposure concentrations of (R,R)-formoterol were determined by chemical analyses of aerosol samples collected on filters. The protocol required collection of two filter samples during each animal exposure period. For a small fraction of the total exposures, only one filter was collected due to technical errors or problems. From the start of the study on January 19, 2000 through April 6, 2001, the filters collected from all test article exposures (7 days/week) were analyzed. From April 7, 2001 to the termination of the study on January 16, 2002, samples from only two days per week (2

days/week) were analyzed. For the saline aerosol (control) exposures, two filter samples were collected during one exposure period approximately each week. Each sample was collected from an unused animal exposure port using 25-mm glass-fiber filters held in an in-lined filter holder. The sample volume was calculated by multiplying the sampling airflow rate by the sample collection time.

The mass of formoterol free base on each filter was determined using an HPLC method. The actual exposure concentrations (as free base) were calculated by dividing the analytically determined mass of free base by the sample volume. The estimated dose (µg/kg) for each test article treatment group was calculated from the exposure concentration using the following equation:

Estimated inhaled dose (µg/kg/day) = Exposure concentration (µg/L) x minute volume/mean body weight (L/min/kg) x Duration (min)

Mean concentrations (Sponsor's data)

Dose µg/kg/day	Sex	Exposure Conc. µg/L	Target Conc. µg/L	Minute Volume/BW L/min/kg	Exposure Duration min	Estimated Dose µg/kg/day	% of Target Dose
40	M	2.1	2.1	0.64	30	40.3	101.6
	F	2.0	1.9	0.70	30	42.3	106.3
100	M	5.4	5.3	0.64	30	102.8	101.5
	F	4.9	4.8	0.70	30	103.8	103.3
200	M	11.1	10.8	0.64	30	209.9	102.6
	F	9.9	9.5	0.70	30	207.0	103.6
400	M	20.7	20.8	0.65	30	401.0	99.6
	F	18.9	18.9	0.70	30	399.7	100.3

Estimated inhaled doses

Target dose, µg/kg/day	Actual dose[a], µg/kg/day		Deposited dose[b], µg/kg/day	
	Males	Females	Males	Females
40	40.5	42.0	4.0	4.2
100	101.6	102	10.2	10.2
200	209.1	206.1	20.9	20.6
400	398.1	397.2	39.8	39.7

a. Actual exposure concentrations for each group were used with the exposure duration, group mean body weight, and minute volume to calculate the inhaled dose (µg/kg).
b. A 10% deposition factor was used to calculate the deposited dose.

Aerosol particle size determinations were performed for the low and high concentrations of (R,R)-formoterol. The frequency of measurements did not meet the original requirements of the study protocol, but was considered sufficient to characterize the aerosol characteristics in terms of particle size distribution. Particle size determinations were performed using a 7-stage cascade impactor. Glass-fiber filters were used as collection substrates. Formoterol free base collected on the substrates was chemically analyzed using a HPLC method. Particle size was calculated based on impactor stage cutoffs. Aerosol particle was expressed as MMAD ± GSD.

Mean particle size, MMAD ± GSD

Dose, µg/kg/day	Mean particle size ± GSD
40	0.6 ± 1.36 µM
400	0.8 ± 1.77 µM

Operating parameters of exposure systems

Parameter	0-1	0-2	40	100	200	400
Temperature, °C	20	20	21	21	21	21
Relative Humidity, %	50	51	59	59	54	53

Basis of dose selection (MTD, MFD, AUC etc.): MTD for males based upon decreased survival in the 400 µg/kg/day group; however, these animals were sacrificed during week 92. MTD for males and females

Species/strain: Male and female Sprague-Dawley — :CD®(SD)IGS BR] rats were obtained from [redacted] b(4)

Number/sex/group (main study): 60 rats/sex/group

Route, formulation, volume: The vehicle or (R,R)-formoterol tartrate was administered as an aerosolized saline solution by nose-only exposure. Two concurrent control groups were exposed to an aerosol of the vehicle, 0.9% sodium chloride for injection USP, at a level matching the saline concentration in the test atmosphere for the high dose of 400 µg/kg/day.

Frequency of dosing: Animals were exposed for approximately 30 min/day, 7 days/week for periods up to 104 weeks.

Satellite groups used for toxicokinetics or special groups: Satellite groups for toxicokinetic assessments consisted of 15 rats/sex/group and received (R,R)-formoterol at inhaled doses of 40, 100, 200, and 400 µg/kg/day.

Age: At the start of treatment, male and female rats were 8 and 9 weeks old, respectively. Body weight ranges were 238 to 310 g for males and 182 to 256 g for females.

Animal housing: All animals were housed individually in clean, wire mesh cages suspended above cage-board. Animals were housed in rooms that adjoined the exposure room by internal doors; therefore, animals were not transported through facility corridors.

Restriction paradigm for dietary restriction studies: No.

Drug stability/homogeneity: Solutions of (R,R)-formoterol in 0.9% saline were prepared. The solutions were mixed with a magnetic stirrer until uniform and throughout use; solutions were filtered through a 0.22-µm [redacted] filter. The dosing solutions were prepared approximately once or twice weekly and were stored refrigerated. Stability of the test article in the vehicle for 8 days under refrigerated conditions was determined in a previous study. b(4)

Dual controls employed: Yes.

Interim sacrifices: See Deviations for original study protocol.

Deviations from original study protocol: The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92 (referred to as study week 92). All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. These early sacrifices for females were

collectively referred to as week 91. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101 (collectively referred to as week 101).
Other: A pre-study health screen was conducted on 10 rats/sex. Blood was collected from each animal and gross necropsy examinations were conducted. Blood was used for used for determinations of white cell counts and assessment of serum antibody profiles. ⊏ ⊐ reported that none of serum antibody tests (CARB, MPul, PVM, RCV/SDA, Reo, Sendai, LCM, Parvo, H-1, KRV) were positive. Carcasses were discarded after completion of necropsy examinations. b(4)

Fifteen rats/sex were assigned to sentinel groups. These animals were housed in the same rooms as the animals assigned to the main study and were used to provide biological samples for diagnosis of possible disease conditions. Sentinel animals were observed for mortality/moribundity twice daily. Physical examinations were conducted weekly. Body weights were measured as described for main study animals. For animals that survived to the end of the study or were sacrificed in a moribund condition, blood was collected and gross necropsy examinations were conducted. Tissues were collected and preserved for possible microscopic examination. Bone marrow smears were not collected. The sponsor reported that there were no indications that required follow-up using the sentinel animals.

Observation times
Mortality: Animals were observed twice daily for moribundity/mortality.
Clinical signs: Clinical examinations of animals were conducted daily and when removed from exposure tubes. Detailed physical examinations were conducted on all animals weekly. All male and female rats were examined weekly starting at study weeks 33 and 27, respectively, until completion of the study for the presence of palpable masses. The time of onset, location, size, appearance, and progression of each mass were recorded throughout the study period.
Body weights: Body weights were measured weekly from one week prior to the start of treatment through week 12. Thereafter, body weights were collected monthly throughout the study.
Food consumption: Food consumption was measured weekly, beginning one week prior to the start of treatment through week 12. Thereafter, food consumption was measured monthly through week 100, and week 103 for the surviving male groups.
Clinical Pathology: Blood and urine samples for clinical pathology determinations (hematology, serum chemistry, and urinalysis) were collected from 10 rats/sex/group from control group 1 and the 40, 100, 200, and 400 µg/kg/day groups at week 51. In addition, blood smears were prepared for all animals at the scheduled necropsy and animals sacrificed in a moribund condition; however, blood smears (differential leukocyte counts) were not evaluated.
Organ weights: Absolute and relative organ weights were determined for the adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus, and uterus.
Histopathology: Animals in a moribund condition or at scheduled necropsies were sacrificed with the use of isoflurane, and necropsies were conducted on all animals. Protocol-specified tissues were trimmed according to standard operating procedures

and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 µm, mounted on glass microscope slides and stained with hematoxylin and eosin. Microscopic examinations were performed on all tissues from all animals euthanized in extremis or that died spontaneously and for all animals in control group 1, control group 2, and the 400 µg/kg/day group. The heart, lung, liver, kidneys, nasal tissues, pituitary gland, and tissue masses were also examined from all animals in the 40, 100 and 200 µg/kg/day groups. In addition, the ovaries and spleen were examined from all females in the 40, 100, and 200 µg/kg/day groups. All gross lesions from all animals were examined. Remaining tissues from the 40, 100, and 200 µg/kg/day groups were processed to stained microscope slides and retained for possible future examination. A histopathology peer review was performed by an outside Pathologist as described for the mouse carcinogenicity study. As stated above, not all tissues were examined for lower dose groups. Complete examinations of tissues were limited to control and high dose groups.
Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected from toxicokinetic animals during weeks 51 and 78 at 0.5, 1, 2, 6, and 24 hr following exposure. Three rats/sex/group were used for each time point. Plasma samples were analyzed for drug exposure concentration using a LC/MS/MS method.

Results

Mortality: There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group (dose mortality trend Cox p-value = 0.0006 and Kruskal-Wallis p-value = 0.0005). Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups (Cox p-value = 0.2095 and Kruskal-Wallis p-value = 0.1402). The sponsor reported that the survival rate of female rats in the 100 µg/kg/day group was significantly lower than that of the pooled control as well as each individual control group; however, there was no effect on survival for female rats in the 200 µg/kg/day group. Although, not statistically significant, survival was slightly decreased for females in the 400 µg/kg/day.

The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day group during weeks 91 and 92 (referred to as study week 92). All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. These early sacrifices for females were collectively referred to as week 91. The control group 1 rats were sacrificed "to provide a comparative group to aid in defining and describing organ weight and morphologic changes in the early terminated groups." The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101 (collectively referred to as week 101). The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks. Early sacrifices of males in control group 1 and the 400 µg/kg/day group and females in control group 1 and the 100 and 400 µg/kg/day groups were inappropriate as defined by the guidance

These sacrifices appear to deviate from the Draft Guidance for Industry, Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001). Further, there was no consultation with the Division prior to any of these early sacrifices. The guidance states that if survival of the high dose group falls below 50% or 20-30 surviving animals after week 80, the study should be continued, either stopping dosing of animals in the high dose group or terminating only the high dose group, because the comparison of at least the control and low/middle doses would still be informative (the high dose comparison would depend on the situation). A study could be terminated early when the survival of the control or low dose group is reduced to 20-25% of the original number of animals.

Text Table 1: Survival at Study Weeks 25, 52, 77, 80, 90 and 104 for Males and at Study Weeks 25, 52, 77, 80, 89 and 101 for Females - Number and Percentage of Animals Surviving

	MALES						FEMALES						
GROUP	1	2	3	4	5	6	GROUP	1[a]	2	3	4	5	6[a]
DOSE	0	0	40	100	200	400	DOSE	0	0	40	100	200	400
STUDY WEEK							STUDY WEEK						
25	60 100%	59 98%	60 100%	59 98%	58 97%	59 98%	25	59 100%	60 100%	59 98%	58 97%	60 100%	59 100%
52	56 93%	58 97%	57 95%	55 92%	55 92%	52 87%	52	56 95%	59 98%	56 93%	54 90%	58 97%	56 95%
77	43 72%	50 83%	50 83%	48 80%	39 65%	37 62%	77	47 80%	44 73%	43 72%	38 63%	47 78%	36 61%
80	42 70%	47 78%	46 77%	43 72%	37 62%	35 58%	80	45 76%	43 72%	41 68%	34 57%	42 70%	36 61%
90	34 57%	37 62%	34 57%	34 57%	32 53%	22 37%	89	32 54%	33 55%	34 57%	22 37%	34 57%	27 46%
104	NA	24 40%	24 40%	23 38%	21 35%	NA	101	NA	24 40%	17 28%	NA	24 40%	NA

[a] = There were 60 animals/sex/group at study initiation of dosing. Mortality data corrected for accidental deaths (*i.e.*, procedural errors).

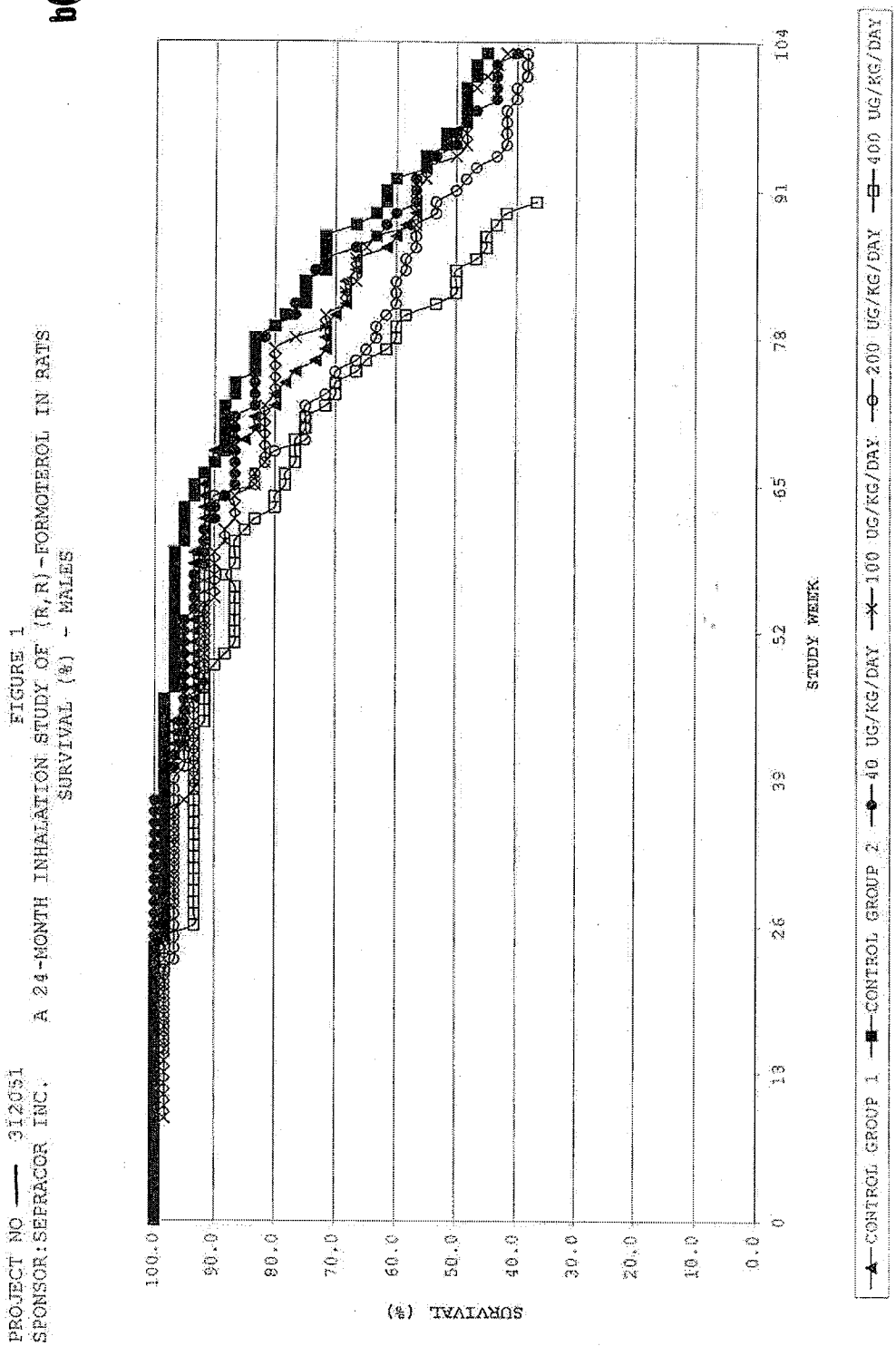
NA = Not Applicable

Appears This Way On Original

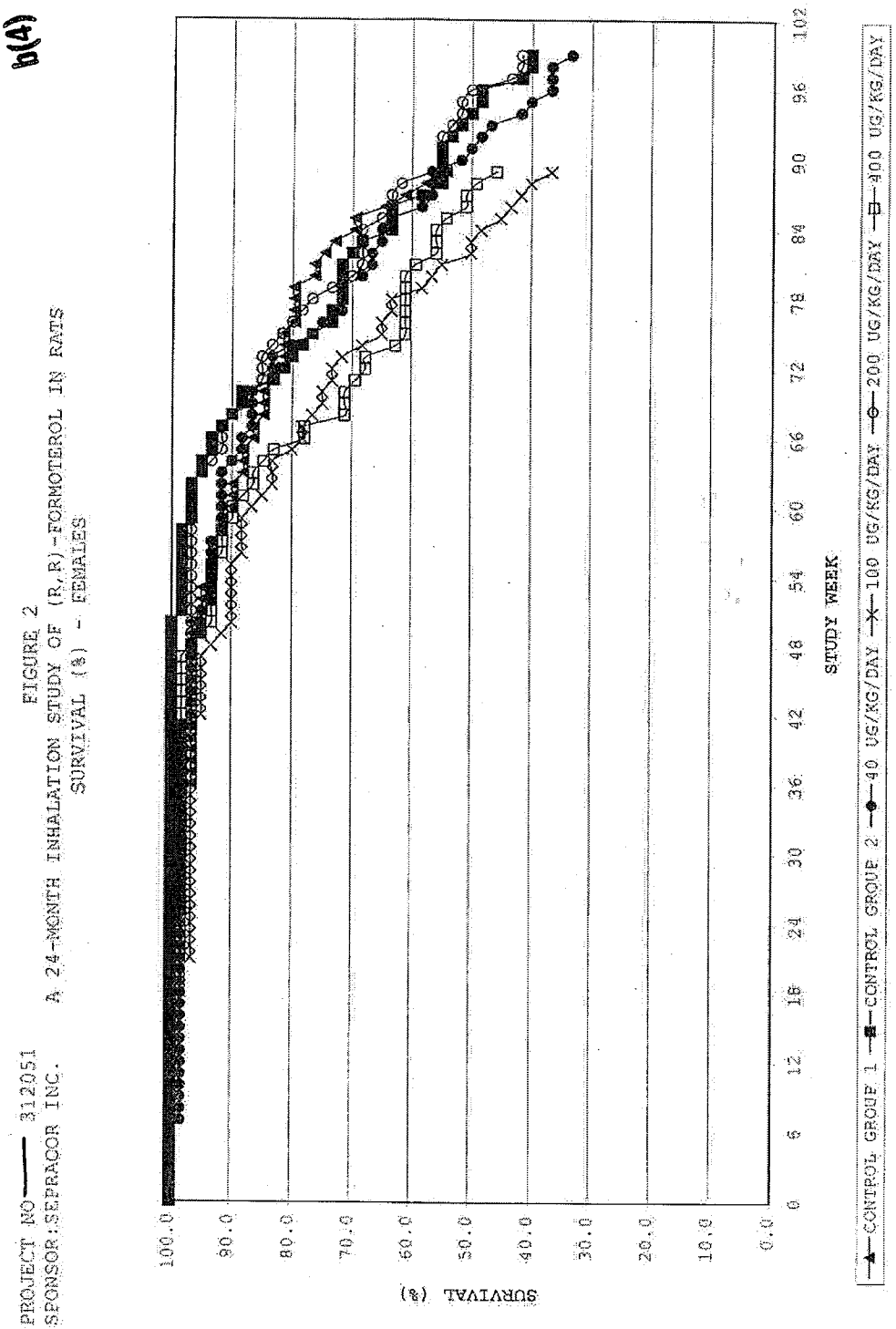
Animal Disposition

Disposition	Males						Females					
	0-1	0-2	40	100	200	400	0-1	0-2	40	100	200	400
Found dead	14	16	18	14	25	22	8	8	14	12	16	7
Euthanized in extremis	1	1	1	2	1	0	2	0	2	2	1	0
Euthanized in extremis, physical condition	9	12	13	12	8	16	2	10	15	9	12	12
Euthanized in extremis, size/condition masses	2	4	1	4	2	0	15	15	10	14	5	10
Found dead after exposure	1	3	3	5	3	3	0	2	2	2	2	3
Found dead in exposure tube, prior to exposure	0	0	0	0	0	0	0	1	0	1	0	0
Total	27	36	36	37	39	41	27	36	43	40	36	32
Interim necropsy Week 92 or 91	33	0	0	0	0	19	32	0	0	20	0	27
Primary necropsy Week 104 or 102	0	24	24	23	21	0	0	24	17	0	24	0
Accidental Death	0	0	0	0	0	0	1	0	0	0	0	1

b(4)


PROJECT NO —— 312051
SPONSOR:SEPRACOR INC.
FIGURE 1
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
SURVIVAL (%) - MALES
SURVIVAL (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
13
26
39
52
65
78
91
104
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

b(4)


PROJECT NO—— 312051
SPONSOR:SEPRACOR INC.
FIGURE 2
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
SURVIVAL (%) - FEMALES
SURVIVAL (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

Clinical signs: A number of nonspecific clinical and physical signs were observed for control and treatment groups. In some cases, clinical and/or physical signs were elevated in treatment groups, although incidences were relatively low in relation to the total number of animals per group and these signs were not test article-specific. Thus, the relationships of these clinical and/or physical signs to treatment with (R,R)-formoterol were unclear.

Body weights: Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was achieved at this dose.

Body weight and body weight gain

Parameter	Male rats						Female rats					
	0-1	0-2	40	100	200	400	0-1	0-2	40	100	200	400
Wk 0	275	275	275	275	275	275	212	213	213	213	213	213
Wk 25	566	565	572	560	549	527	319	319	337	341	328	334
BW, % of Control	-	-	101%	99%	97%	93%	-	-	106%	107%	103%	105%
BW gain Wk_{0-25}, % of control	-	-	103%	99%	95%	87%	-	-	116%	119%	108%	113%
Wk 53	635	657	603	604	576	548	363	362	368	379	350	359
BW, % of Control	-	-	96%	96%	91%	87%	-	-	102%	105%	97%	99%
BW gain Wk_{0-53}, % of control	-	-	92%	92%	85%	77%	-	-	103%	110%	91%	97%
Wk 77	623	618	602	590	567	518	398	399	396	419	376	384
BW, % of Control	-	-	97%	95%	91%	84%	-	-	99%	105%	95%	96%
BW gain Wk_{0-77}, % of control	-	-	95%	91%	85%	70%	-	-	98%	110%	87%	92%
Wk 89	597	603	590	578	546	503	405	399	395	399	383	380
BW, % of Control	-	-	115%	110%	98%	83%	-	-	98%	99%	95%	95%
BW gain Wk_{0-89}, % of control	-	-	97%	93%	83%	70%	-	-	96%	98%	90%	88%
Wk 104	NA	581	556	557	521	NA	NA	416	406	NA	372	NA
BW, % of Control		-	96%	96%	90%			-	98%		89%	
BW gain		-	92%	92%	80%			-	95%		78%	

$Wk_{0\text{-}104/101}$, % of control												

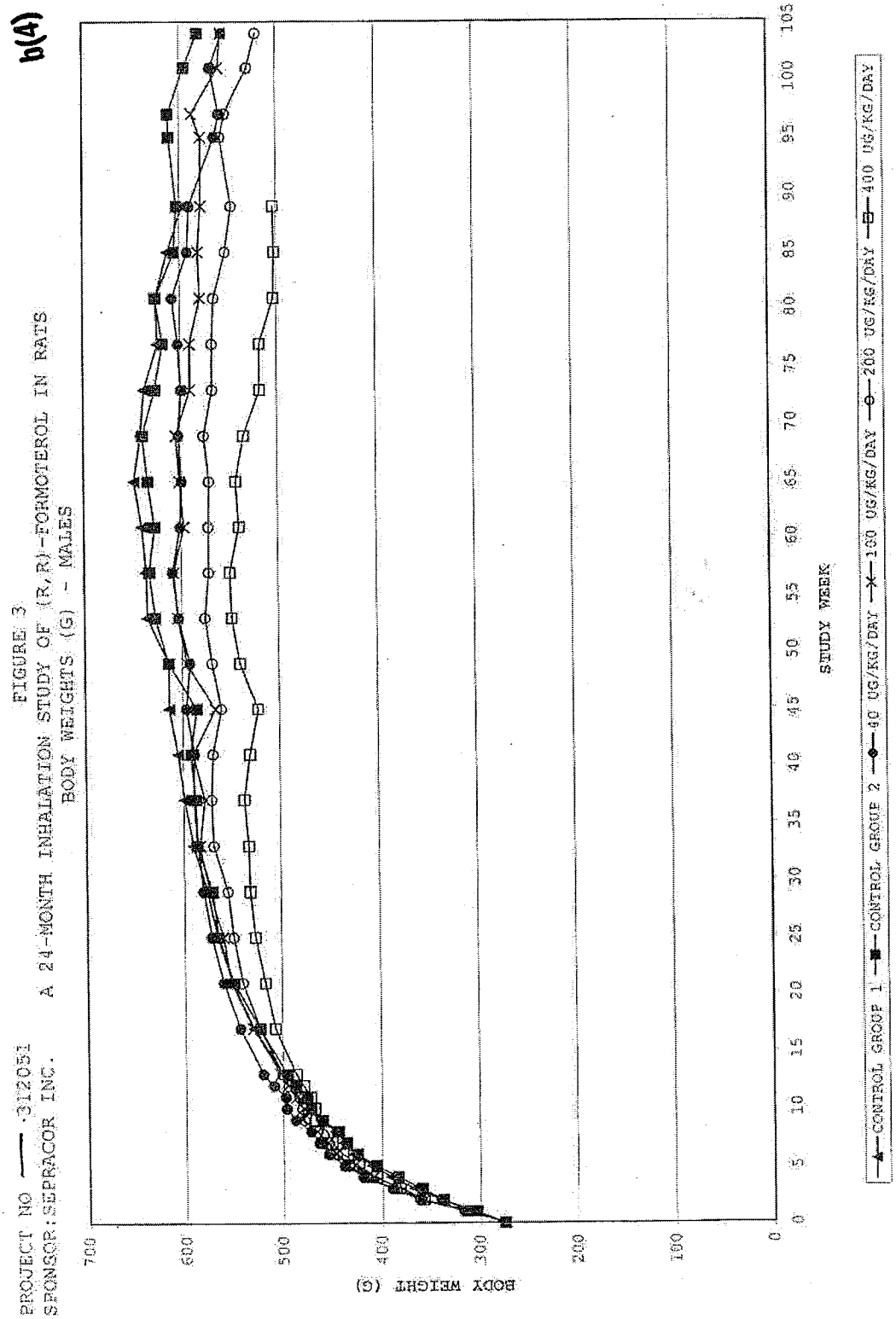

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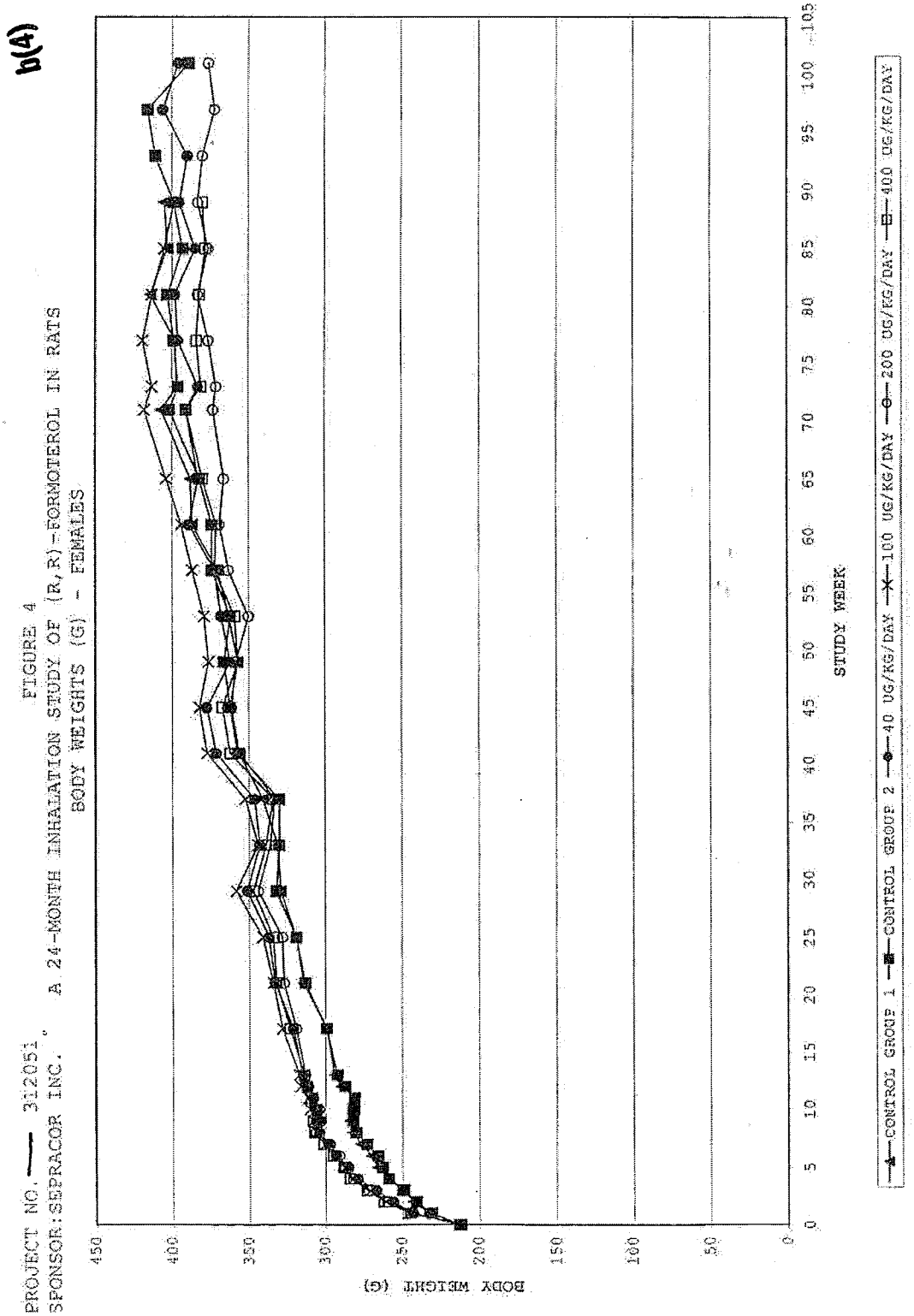
FIGURE 3
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
BODY WEIGHTS (G) - MALES
PROJECT NO —— .312051
SPONSOR:SEPRACOR INC.
BODY WEIGHT (G)
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY


b(4)
PROJECT NO. — 312051
SPONSOR:SEPRACOR INC.
FIGURE 4
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
BODY WEIGHTS (G) - FEMALES
BODY WEIGHT (G)
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

Food consumption: There were no treatment-related effects on food consumption.

Hematology (Week 51): Decreased white blood cell counts, attributed to decreases of neutrophils, lymphocytes, and monocytes, were observed for female treatment groups. Decreased platelet counts were observed for females at 100, 200, and 400 µg/kg/day. There were no significant changes of hematology parameters for male treatment groups.

Week 51 hematology parameters for female control and treatment groups

Parameter	Females				
	0-1	40	100	200	400
White blood cells $10^3/\mu L$	9.6	7.1 (74%)	6.9* (72%)	6.6* (69%)	5.6* (58%)
Neutrophils $10^3/\mu L$	3.1	1.9 (61%)	1.8 (58%)	1.9 (61%)	1.3* (42%)
Neutrophils %	32	26 (81.3%)	27 (84%)	28 (87%)	23 (72%)
Lymphocytes $10^3/\mu L$	5.4	4.5 (83%)	4.3 (80%)	4.0 (74%)	3.7 (69%)
Lymphocytes %	57	65 (114%)	62 (109%)	62 (109%)	66 (116%)
Monocytes $10^3/\mu L$	0.9	0.6 (67%)	0.7 (78%)	0.6 (67%)	0.5 (56%)
Platelets $10^3/\mu L$	1071	1054	970 (91%)	903* (84%)	923 (86%)

Clinical Chemistry (Week 51): Glucose levels and amylase activities were decreased for male and female treatment groups. These changes could be attributed to the pharmacological activity of the β_2-adrenergic agonist, arformoterol. Blood urea nitrogen (BUN) levels were slightly increased for male treatment groups; however, there was no evidence of treatment-related histopathological changes in the kidneys. Potassium levels and the A/G ratio were slightly increased for female treatment groups. Phosphorus levels were increased for females in the 400 µg/kg/day group.

Week 51 clinical chemistry parameters for male and female control and treatment groups

Parameter	Males					Females				
	0-1	40	100	200	400	0-1	40	100	200	400
Glucose mg/dL	125	104* (83%)	91* (73%)	87* (70%)	79* (63%)	119	79* (66%)	87* (73%)	74* (62%)	87* (73%)
Amylase U/L	2063	1601* (78%)	1477* (72%)	1450* (70%)	1319* (64%)	1413	1218 (86%)	1152* (81%)	1099* (78%)	1135* (80%)
BUN mg/dL	15.3	17.4 (114%)	18.6* (122%)	17.6 (115%)	19.0* (124%)					
Potassium mEq/L						4.56	5.35* (117%)	5.29* (116%)	5.54* (122%)	5.41* (119%)
Phosphorus mg/dL						5.0	5.1	5.5	5.2	6.2* (124%)
A/G ratio						1.42	1.57 (111%)	1.60 (113%)	1.57 (111%)	1.67 (118%)

Urinalysis (Week 51): Urine volumes for female treatment groups were increased to 140-173% of the control (5.5 mL). There were no changes of urinalysis parameters for male treatment groups.

Organ Weights: Absolute and relative ovaries weights were increased for all female treatment groups sacrificed at weeks 91 and 101, which corresponded with histopathological findings of ovarian cysts. Absolute and/or relative spleen weights were decreased for males in the 400 µg/kg/day group sacrificed at week 92 and females in the 200 µg/kg/day sacrificed at week 101. It is unclear if these differences corresponded with histopathological findings of hemosiderin pigment. Relative heart weights were slightly increased for male treatment groups at weeks 92 and 104 and females in the 100 and 400 µg/kg/day groups at week 91; however, there was no dose-response relationship or corresponding histopathological findings. Increased heart weights might be attributed to the pharmacological properties of the β_2-adrenergic agonist, formoterol. Differences were also noted for absolute and/or relative liver, thymus, lung, kidney, and brain weights as shown in the table below; however, these differences were generally small and there were no corresponding histopathological findings.

Absolute and relative organ weights for male control and treatment groups at necropsies during weeks 92 and 104

Organ Weight	0-1	0-2	40	100	200	400
Week 92 Necropsy						
Liver, g	17.68					15.10* (85%)
Liver, g/100 g BrW	810.310					671.083* (83%)
Thymus, g	0.0792					0.0649 (82%)
Thymus, g/100 g BrW	3.696					2.8488 (77%)
Heart, g/100 g BW	0.321					0.370* (115%)
Spleen, g/100 g BrW	47.277					40.621* (86%)
Lungs, g/100 g BW	0.417					0.470 (113%)
Kidneys, g/100 g BrW	188.955					170.692* (90%)
Brain g/100 g BW	0.378					0.440* (116%)
Week 104 Necropsy						
Heart, g/100 g BW		0.327	0.381 (117%)	0.412 (126%)	0.399 (122%)	
Heart, g/100 g BrW		87.224	97.610 (112%)	105.245 (121%)	97.211 (111%)	
Lung, g/100 g BW		0.408	0.447 (110%)	0.469 (115%)	0.482 (118%)	

Absolute and relative organ weights for female control and treatment groups at necropsies during weeks 91 and 101

Organ Weight	0-1	0-2	40	100	200	400
Week 91 Necropsy						
Ovaries, g	0.1309			0.2483 (190%)		0.3448* (263%)
Ovaries, g/100 g BW	0.032			0.063 (197%)		0.092* (288%)
Ovaries, g/100 g BrW	6.528			12.474 (191%)		17.479* (268%)
Thymus, g	0.0957			0.0842 (88%)		0.1111 (116%)
Thymus, g/100 g BW	0.023			0.022		0.030 (130%)
Thymus, g/100 g BrW	4.780			4.277		5.596 (117%)
Heart, g/100g BW	0.365			0.418* (115%)		0.410* (112%)
Lungs g/100g BW	0.437			0.487 (111%)		0.521* (119%)
Week 101 Necropsy						
Ovaries, g		0.1551	0.1905 (123%)		0.3196 (206%)	
Ovaries, g/100 g BW		0.040	0.048 (120%)		0.088 (220%)	
Ovaries, g/100 g BrW		7.806	9.777 (125%)		16.357 (210%)	
Spleen, g		1.04	1.25 (120%)		0.76 (73%)	
Spleen, g/100 g BW		0.269	0.313 (116%)		0.205 (76%)	
Spleen, g/100 g BrW		52.644	64.938 (123%)		38.746 (73.60%)	

Gross pathology: The incidences of cyst(s) in the oviducts were increased for females at 100, 200, and 400 µg/kg/day. The incidences of cysts in the ovaries were increased for all female treatment groups. These findings correlate with histopathological findings of cysts in the oviducts and ovaries.

There were other gross pathological findings that were increased for male and female treatment groups, although, incidences were generally low and these findings were not test article-specific. Thus, relationships to treatment were unclear.

Gross pathological findings for female rats

Organ/Tissue	Time point	0-1	0-2	40	100	200	400
Oviducts -cyst(s)	Unscheduled deaths	2	1	2	4	6	7
	Interim Necropsy, week 91	1	NA	NA	4	NA	4
	Primary necropsy, week 101	NA	2	0	NA	7	NA
	Total	3	3	2	8	13	11

Ovaries -cyst(s)	Unscheduled deaths	5	5	17	8	12	16
	Interim Necropsy, week 91	11	NA	NA	10	NA	22
	Primary necropsy, week 101	NA	7	8	NA	11	NA
	Total	16	12	25	18	23	38

Histopathology:

Non-neoplastic:

The incidences of cyst(s) in the ovaries and oviducts were significantly increased for female treatment groups.

The incidence of hemosiderin in the spleen was slightly increased for male and female treatment groups.

Incidence of alveolar macrophages and edema in the lungs were slightly increased for male and female treatment groups. The incidence of granulomatous inflammation in the lungs was slightly increased for female treatment groups.

There were other histopathological findings that were increased for male and female treatment groups, although, incidences were generally low and these findings were not test article-specific. Thus, relationships to treatment were unclear.

Histopathological findings for all females (i.e., unscheduled deaths, interim necropsy at week 91, and primary necropsy at week 101). Data is displayed as number of animals with finding over total number of animals examined.

Organ/Tissue	0-1	0-2	40	100	200	400
Ovaries -cyst(s)	18/59	15/59	30/60	28/60	38/60	45/59
Oviducts -cyst(s)	2/59	0/59	3/43	2/44	4/42	8/60
Spleen -pigment, hemosiderin, minimal-moderate	38/60	36/60	40/60	41/60	47/60	49/60
Lung -alveolar macrophages, minimal-moderate	24/60	21/60	34/59	34/60	34/60	30/60
-edema, minimal-severe	1/60	1/60	1/60	2/60	8/60	4/60
-inflammation, granulomatous, minimal-moderate	6/60	3/60	7/59	15/60	11/60	12/60

Histopathological findings for all males (i.e., unscheduled deaths, interim necropsy at week 91, and primary necropsy at week 101). Data is displayed as number of animals with finding over total number of animals examined.

Organ/Tissue	0-1	0-2	40	100	200	400
Lungs						
-alveolar macrophages, minimal-severe	31/59	29/59	38/59	44/60	36/58	39/60
-edema, minimal-severe	1/59	2/59	3/60	3/60	6/58	5/60
Spleen						
-pigment, hemosiderin, minimal-moderate	4/60	4/60	6/38	6/41	10/40	7/60
-depletion, lymphoid, mild-severe	1/60	2/60	4/38	4/41	6/40	6/60
Nasal level 3						
-degeneration, olfactory epithelium, minimal-mild	0/60	0/60	0/60	060	1/60	5/60
Nasal level 5						
Metaplasia, mild-severe	0/60	0/59	0/60	0/60	0/60	3/60

Neoplastic:

For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 200 µg/kg/day group when the high dose group was excluded (p-value of pairwise comparison with mid-high dose group 0.0176<0.05). There were no findings in the 400 µg/kg/day group, which was terminated early. The trend test was positive without the high dose group (0.0123<0.025), however, it was negative with the high dose group (0.2336>0.025).

For the thyroid gland, the combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. It should be noted that surviving females in control group 1 and the 100 and 400 µg/kg/day group were sacrificed in week 91 whereas surviving females in other groups were sacrificed in week 101. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day slightly exceeded the upper range of the historical control background. Further, histopathological examination of the thyroid gland was incomplete for females in the 200 µg/kg/day group as only 37 of 60 animals were examined. Examination of females in the 40 and 100 µg/kg/day groups was also incomplete, although the period of treatment was identical to the 400 µg/kg/day group. Based upon historical control incidences, thyroid c-cell adenoma and carcinoma are relatively common tumors for Sprague-Dawley rats. Based upon available data the combined incidence of c-cell adenoma and carcinoma, trend tests with and without the high dose group were negative and the p-value of pairwise comparison with the mid high dose group was not statistically significant.

For the uterus, the incidence of benign endometrial stromal polyps was increased for females in the 400 µg/kg/day group, although this was within the upper range of the historical control background. This finding was not statistically significant.

For the soft tissue of the thorax, the incidences of malignant liposarcoma were increased for females in the 100 and 400 µg/kg/day groups, although a dose response was not evident. From the testing laboratory, 4 liposarcoma from a total of 7 control tissues have been examined, which yields an incidence of 57.14%; however, the sample size is small and it is unclear if it could explain the observed tumor findings. These findings were not statistically significant.

Neoplastic findings for all males (i.e., unscheduled deaths, interim necropsy at week 91, and primary necropsy at week 101). Data is displayed as number of animals with finding and the total number of animals examined (S = metastatic, B= benign, and M = malignant).

Tissue/Organ	0-1[a]	0-2	40	100	200	400[a]	Historical control[b]
Soft Tissue, Thorax							
Number examined	1	1	0	2	2	0	
M liposarcoma	0	0	-	1	2	-	
Brain							
Number examined	60	60	36	37	39	60	
S carcinoma, pars distalis, pituitary	0	0	0	0	1	0	
Lungs							
Number examined	59	59	60	60	58	60	
S carcinoma, adrenal cortex	0	0	0	1	0	1	
M carcinoma, hepatocellular, unknown	0	0	0	0	1	0	
S liposarcoma, soft-tissue, thorax	0	0	0	0	1	0	
Pituitary							
Number examined	59	59	59	60	60	58	
M carcinoma, pars distalis	0	0	0	0	1	0	
Prostate							
Number examined	60	60	40	45	46	60	
M carcinoma, transitional cell	0	0	0	0	0	1	
B Schwannoma, benign	0	0	0	0	1	0	
Testes							
Number examined	59	58	43	44	43	59	
B adenoma, interstitial cell	1	1	1	1	2	2	2.35% (1.43-7.14%)
Thymus							
Number examined	56	56	36	37	38	56	
M liposarcoma	0	0	0	0	0	1	
Tail							
Number examined	32	37	40	38	38	36	
M neurofibrosarcoma	0	0	0	0	1	0	
Adrenal cortex							
Number examined	60	60	49	49	53	60	
M carcinoma	0	0	1	1	0	1	0.65% (0.77-2.00%)
Adrenal medulla							
Number examined	60	60	48	47	53	59	
M pheochromocytoma, malignant	0	0	0	0	1	0	1.63% (1.43-5.00%)
Systemic tumors							

Number examined	1	5	1	4	0	1	
B mesothelioma, benign	0	0	0	0	-	1	

a. Surviving animals in control group 1 and the 400 µg/kg/day group were sacrificed during week 92.
b. Historical control data from [redacted] (March 2001).
c. Historical control data from the testing laboratory are shown in brackets [ ]. b(4)

Neoplastic findings for all females (i.e., unscheduled deaths, interim necropsy at week 91, and primary necropsy at week 101). Data is displayed as number of animals with finding and the total number of animals examined (S = metastatic, B= benign, and M = malignant).

Tissue/Organ	0-1[a]	0-2	40	100[a]	200	400[a]	Historical control[b]
Thyroid gland							
Number examined	60	60	43	41	37	60	
B adenoma, C-cell	2	3	1	6	3	4	7.17%[4.45%][c]
B adenoma, C-cell, multiple	0	1	1	0	2	0	(2.86-16.67%)
M carcinoma, C-cell	0	0	0	0	1	0	0.46%[0.68%]
B adenoma and M carcinoma	0	0	0	0	1	0	(0.77-4.00%)
Total (adenoma + carcinoma, C-cell)	2 (3.3%)	4 (6.7%)	2 (4.7%)	6(14.6%)	7(18.9%)	4 (6.7%)	7.63%
Uterus							
Number examined	60	60	50	45	42	60	
B polyp, endometrial stromal	2 (3.3%)	2	1	3	2	5 (8.3%)	2.37%[4.08%] (0.91-11.67%)
Soft Tissue, Thorax							
Number examined	0	2	2	8	1	5	
M liposarcoma	-	2	2	5	0	4	
M liposarcoma, multiple, fatal	-	0	0	1	0	1	
Total	-	2	2	6	0	5	[4/7; 57.14%]
S liposarcoma, abdominal soft tissue	-	0	0	0	1	0	
Adrenal Medulla							
Number examined	58	59	54	54	56	60	
B pheochromocytoma, complex, benign	0	0	0	0	1	0	2.14%[3.11%] (1.43-10.00%)
M pheochromocytoma, malignant	0	0	0	0	1	0	0.75%[0.35%] (1.43-8.33%)
Total (pheochromocytoma, benign + malignant)	0	0	0	0	2	0	2.89%
Kidney							
Number examined	60	60	60	60	60	60	
lipoma	0	0	0	0	0	1	0.17% (0.50-1.67%)
Liver							
Number examined	60	60	60	60	60	60	
S leiomomyosarcoma, stomach, glandular	0	0	0	0	0	1	
Lungs							
Number examined	60	60	59	60	60	60	
M liposarcoma	0	0	0	0	1	0	

S liposarcoma, soft tissue, thorax	0	1	1	2	0	3	
S fibrosarcoma, skeletal muscle	0	0	0	0	1	0	
M carcinoma, unknown	0	0	0	0	0	1	
S liposarcoma, soft tissue, abdomen	0	0	0	0	1	0	
Mammary gland							
Number examined	60	58	53	52	49	59	
M adenocarcinoma	10	7	15	16	14	15	23.71% (8.57-58.33%)
Nerve, Sciatic							
Number examined	60	60	43	40	36	59	
M sarcoma undifferentiated	0	0	0	0	0	1	
Lymph node, Bronchial							
Number examined	44	47	26	27	27	38	
S adenocarcinoma, mammary gland	0	0	0	0	1	0	
Ovaries							
Number examined	59	59	60	60	60	59	
B granulosa cell tumor, benign	0	0	0	0	1	0	0.06% (1.67%)
B-adenoma, sex cord stromal	0	0	0	0	0	1	
Paws							
Number examined	29	23	24	11	19	13	
M myxosarcoma	0	0	0	0	0	1	
Skeletal muscle							
Number examined	60	60	41	40	36	60	
M fibrosarcoma	0	0	0	0	1	0	
Skin							
Number examined	60	60	49	47	43	60	
M fibrosarcoma	0	0	1	0	0	1	0.23% (1.43-2.00%)
B fibroma	0	1	0	1	1	2	0.58% (0.91-3.33%)
B pilomatricoma	0	0	0	0	0	1	0.06% (0.50%)
Spleen							
Number examined	60	60	60	60	60	60	
S adenocarcinoma, mammary gland	0	0	0	0	0	1	
Thymus gland							
Number examined	59	55	44	37	38	60	
S adenocarcinoma, mammary gland	0	0	0	0	1	0	
Cervix							
Number examined	60	59	43	42	37	60	
B polyp, endometrialstromal	0	0	0	0	0	1	
B granular cell tumor, benign	0	0	0	0	0	1	0.12% (1.43%)
Soft Tissue, Abdomen							
Number examined	0	0	1	0	1	2	
M liposarcoma	-	-	0	-	1	0	
S liposarcoma, stomach glandular	-	-	0	-	0	1	

Stomach, Glandular							
Number examined	60	60	43	40	36	60	
M leiomyosarcoma	0	0	0	0	0	1	
Systemic tumors							
Number examined	3	2	1	1	3	2	
B hemangioma	0	0	0	0	1	0	

a. Surviving animals in control group 1 and the 100 and 400 µg/kg/day groups were sacrificed during week 91.
b. Historical control data from ⊂ ⊐ (March 2001).
c. Historical control data from the testing laboratory are shown in brackets [ ]. b(4)

Toxicokinetics: For males and females at 12 and 18 months, C_{max} and AUC values for (R,R)-formoterol increased with elevating dose. For males at 12 months, C_{max} and AUC values increased in an approximate dose proportional manner. For males at 18 months, C_{max} and AUC values with doses from 40 to 200 µg/kg/day generally increased in a less than dose proportional manner; however, increases of these values from 200 to 400 µg/kg/day were significantly greater than dose proportional (possibly suggestive of saturation). For females at 12 and 18 months, C_{max} values increased in a dose proportional manner; however, AUC values generally increased in a slightly less than dose proportional manner. No sex-related differences of C_{max} and AUC values were observed with doses from 40 to 200 µg/kg/day; however, at 400 µg/kg/day, C_{max} and AUC values were significantly greater for males. C_{max} and AUC values with doses from 40 to 200 µg/kg/day were generally comparable at 12 and 18 months; however, for the dose of 400 µg/kg/day, these values at 18 months were significantly greater than values at 12 months. Systemic exposures for male and female rats at doses of 40, 100, 200, and 400 µg/kg/day ranged from 22.8 to 621.1-fold greater than clinical exposure at the proposed therapeutic dose.

Toxicokinetic parameters for (R,R)-formoterol in male rats

Dose µg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
40	12	0.5	897	4330	39.7
	18	1.0	359	2480	22.8
100	12	0.5	1430	6710	61.6
	18	0.5	2500	7660	70.3
200	12	0.5	2950	12200	111.9
	18	0.5	2660	11600	106.4
400	12	0.5	6820	24900	228.4
	18	0.5	11200	67700	621.1

a. Human exposure ($AUC_{0\text{-}24hr}$ = 109 pg·hr/mL) from 091-026 preliminary data (after 2 weeks of daily inhalation treatment of arformoterol in COPD subjects with a dose of 50 µg/day; Human dosage was based on a body weight of 50 kg.

Toxicokinetic parameters for (R,R)-formoterol in female rats

Dose µg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
40	12	0.5	591	4130	37.9
	18	0.5	501	3830	35.1
100	12	0.5	1650	6680	61.3

	18	0.5	1330	5870	53.9
200	12	0.5	2410	9790	89.8
	18	0.5	2140	8740	80.2
400	12	0.5	4450	15800	145
	18	0.5	4920	22900	210

a. Human exposure ($AUC_{0\text{-}24hr}$ = 109 pg·hr/mL) from 091-026 preliminary data (after 2 weeks of daily inhalation treatment of arformoterol in COPD subjects with a dose of 50 µg/day; Human dosage was based on a body weight of 50 kg.

Histopathology inventory (optional)

Study	2-year carcinogenicity study	2-year carcinogenicity study
Species	Mice	Rats
Adrenals	X	X*
Aorta	X	X
Bone Marrow smear	X (not evaluated)	X (Not evaluated)
Bone (femur)		
Brain	X*	X*
Cecum	X	X
Cervix	X (w/uterus and vagina)	X (w/uterus and vagina)
Clitoral gland	X	X
Colon	X	X
Duodenum	X	X
Epididymis	X* (w/testes)	X
Esophagus	X	X
Eye	X (w/optic nerves)	X (w/optic nerve)
Fallopian tube		
Gall bladder	X	
Gross lesions	X (including masses)	X (including masses)
Harderian gland	X	X
Heart	X*	X*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X*	X
Lachrymal gland	X (extraorbital)	X
Larynx		X
Liver	X*	X*
Lungs	X (w/bronchi)	X*
Lymph nodes, bronchial		X
Lymph nodes, cervical		
Lymph nodes mandibular	X	X
Lymph nodes, mediastinal		X
Lymph nodes, mesenteric	X	X

Mammary Gland	X	X
Nasal cavity		X (6 cross sections)
Optic nerves	X (w/eyes)	
Ovaries	X (w/oviducts)	X* (w/oviducts)
Pancreas	X	X
Parathyroid	X* (w/thyroid)	X (w/thyroid)
Peripheral nerve		
Pharynx		X
Pituitary	X*	X
Preputial gland	X	X
Prostate	X	X
Rectum	X	X
Salivary gland	X (submandibular)	X (submandibular)
Sciatic nerve	X	X
Seminal vesicles	X	X
Skeletal muscle	X	X
Skin	X	X (inguinal)
Spinal cord	X	X
Spleen	X*	X*
Sternum	X (bone w/marrow)	X (w/marrow)
Stomach	X	X
Testes	X* (w/epididymides)	X*
Thymus	X*	X*
Thyroid	X* (w/parathyroids)	X (w/parathyroid)
Tongue	X	X
Trachea	X	X
Urinary bladder	X	X
Uterus	X* (w/cervix and vagina)	X* (w/cervix and vagina)
Vagina	X (w/uterus and cervix)	X (w/uterus and cervix)
Zymbal gland	X	X

X, histopathology performed
*, organ weight obtained

2.6.6.9 Discussion and Conclusions

Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up to 104 weeks.

Survival was significantly decreased for males and females in the 25 mg/kg/day group indicating the MTD was exceeded. Surviving males in the 25 mg/kg/day group were sacrificed up to 6 months early. Sacrifice of these animals was inappropriate and it appears that the treatment period was insufficient. Histopathological examination of tissues was complete for males at 5 and 25 mg/kg/day and females at 25 mg/kg/day. The treatment periods appeared to be sufficient for males at 5 mg/kg/day (sacrificed at week 95) and females at 25 mg/kg/day (sacrificed at week 92). Survival was reduced for males at 5 mg/kg/day; however, it did not reach a level of statistical significance.

The incidences of uterine endometrial stromal polyps, combined incidences of uterine endometrial stromal polyps and stromal cell sarcoma, and combined incidences of

uterine and cervical endometrial stromal polyps and stromal cell sarcoma were significantly increased for female treatment groups; however dose-response relationships were not present given that tumor incidences for the high dose group were lower than the low and mid dose groups due to decreased survival and early termination of surviving animals at the high dose.

The carcinogenic assessment of (R,R)-formoterol in male mice appears acceptable given that (1) there was evidence that the MTD was exceeded for males at 25 m/kg/day, although the treatment period was insufficient (2) the treatment period was adequate for males at 5 mg/kg/day and survival was reduced, although it did not reach a level of statistical significance, and (3) the incidence and severity of cardiomyopathy were increased for male treatment groups, although the correlation between cardiomyopathy and decreased survival was weak. Systemic exposure to (R,R)-formoterol in males at 5 mg/kg/day was >25-fold of exposure at the clinical dose, although metabolism of (R,R)-formoterol in mice and humans have not been examined.

Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks. The sponsor did not contact the Division prior to early termination of groups.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups. Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was achieved at this dose.

For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 200 µg/kg/day group when the high dose group was excluded.

For the thyroid gland, the combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. It should be noted that surviving females in control group 1 and the 100 and 400 µg/kg/day group were sacrificed in week 91 whereas surviving females in other groups were sacrificed in week 101. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day slightly exceeded the upper range of the historical control background. Further, histopathological examination of the thyroid gland was incomplete for females in the 200 µg/kg/day group as only 37 of 60 animals were examined. Histopathological examinations were also incomplete for females in the 40 and 100 µg/kg/day groups, although the treatment period was identical to the 400 µg/kg/day group. The sponsor should be asked to complete the histopathological evaluations of

organs and tissues for males and females in the 200 µg/kg/day group and lower doses if appropriate.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

(R,R)-formoterol was negative in the in vitro bacterial reverse mutation assay, in vitro Chinese hamster ovary cell chromosomal aberration assay, and in vivo mouse micronucleus assay.

The sponsor evaluated the carcinogenic potential of (R,R)-formoterol with 2-year mouse and rat carcinogenicity studies. There was no concurrence from the ECAC for either study. The sponsor did not consult with the Division prior to early termination of treatment groups in either study.

Mouse Carcinogenicity Study: Mice received (R,R)-formoterol at oral doses of 0, 1, 5, and 25 mg/kg/day for periods up to 2 years.

For mice, survival was significantly decreased for males and females at 25 mg/kg/day. Thus, the MTD was exceeded at 25 mg/kg/day. The male 25 mg/kg/day group was terminated at week 77 and the duration of treatment was considered inadequate. The female 25 mg/kg/day group and male 5 mg/kg/day group were terminated at weeks 92 and 95, respectively. Durations of treatment for these two groups were considered adequate. Survival was reduced for males at 5 mg/kg/day, although it did not reach a level of statistical significance. These early sacrifices appear to deviate from the Draft Guidance for Industry, Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals (May 2001). Histopathological evaluations of all tissues were conducted for control groups 1 and 2, the male 5 mg/kg/day group, and the male and female 25 mg/kg/day groups.

The incidences of uterine endometrial stromal polyps, combined incidences of uterine endometrial stromal polyps and stromal cell sarcoma, and combined incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma were significantly increased for female treatment groups; however dose-response relationships were not present given that tumor incidences for the high dose group were lower than the low and mid dose groups due to decreased survival and early termination of surviving animals at the high dose.. Based upon tumor findings in the uterus and cervix combined, (R,R)-formoterol is tumorigenic in female mice.

The carcinogenic assessment of (R,R)-formoterol in male mice appears acceptable given that (1) there was evidence that the MTD was exceeded for males at 25 m/kg/day, although the treatment period was insufficient (2) the treatment period was adequate for males at 5 mg/kg/day and survival was reduced, although it did not reach a level of statistical significance, and (3) the incidence and severity of cardiomyopathy were increased for male treatment groups, although the correlation between cardiomyopathy

and decreased survival was weak. Systemic exposure to (R,R)-formoterol in males at 5 mg/kg/day was >25-fold of exposure at the clinical dose, although metabolism of (R,R)-formoterol in mice and humans have not been examined.

There were differences in tumor results from the present study with (R,R)-formoterol as compared to studies with racemic formoterol conducted under NDA 20-831. For racemic formoterol, the incidence of hepatocarcinomas was increased in the dietary study at doses of 20 and 50 mg/kg in females and 50 mg/kg in males, but not at doses up to 5 mg/kg in either males or females. Also in the dietary study, the incidence of uterine leiomyomas and leiomyosarcomas was increased at doses ≥2 mg/kg. Increases in leiomyomas of the rodent female genital tract have been similarly demonstrated with other β agonist drugs. In the present study with (R,R)-formoterol, slight increases in the combined incidences of hepatocellular adenoma and carcinoma were observed for female treatment groups, which may correlate with findings observed in studies with racemic formoterol. In contrast, the incidences of uterine leiomyoma and leiomyosarcoma displayed no relationship to treatment as compared to studies with racemic formoterol; however, the combined incidences of uterine and cervical endometrial stromal polyps and stromal cell sarcoma were increased for female treatment groups.

Rat carcinogenicity study: Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 2 years.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. Trend analysis indicated no effects on survival for female treatment groups. The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and it appears that the duration of treatment was insufficient.

Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was also achieved at this dose.

For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 200 µg/kg/day group when the high dose group was excluded.

For the thyroid gland, the combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. It should be noted that surviving females in control group 1 and the 100 and 400

µg/kg/day group were sacrificed in week 91 whereas surviving females in other groups were sacrificed in week 101. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day slightly exceeded the upper range of the historical control background. Further, histopathological examination of the thyroid gland was incomplete for females in the 200 µg/kg/day group as only 37 of 60 animals were examined.

There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively; however, histopathological examinations of organs and tissues were incomplete for these animals. The sponsor should be asked to complete the histopathological evaluations of organs and tissues for males and females in the 200 µg/kg/day group and lower doses if appropriate to adequately assess the carcinogenic potential of (R,R)-formoterol in rats.

These results of the present study with (R,R)-formoterol differ from study results with racemic formoterol, most likely due to differences in systemic drug exposure given the significantly lower doses used in this inhalation study. The carcinogenic potential of racemic formoterol fumarate was evaluated in 2-year drinking water and dietary studies with rats. The incidence of ovarian leiomyomas was increased at doses of ≥15 mg/kg in the drinking water study and at 20 mg/kg in the dietary study, but not at dietary doses up to 5 mg/kg. In the dietary study, the incidence of benign ovarian theca cell tumors was increased at doses ≥0.5 mg/kg. Increases in leiomyomas of the rodent female genital tract have been similarly demonstrated with other beta-agonist drugs. These findings were not evident in present study with (R,R)-formoterol administered by the inhalation route to rats.

Systemic exposures in mice and rats as compared to the clinical dose
Systemic exposures to (R,R)-formoterol in mice and rats at doses used in 2-year carcinogenicity studies exceeded the clinical exposure with a dose of 50 µg/day by significantly greater than 25-fold. It is noted that there has been significant variations in measurements of clinical exposure to (R,R)-formoterol (i.e., AUC of 33.8 pg·hr/mL at the human clinical dose of 48 µg/day, Study number 091-004 versus $AUC_{0\text{-}24hr}$ of 109 pg·hr/mL at the clinical dose of 50 µg/day in COPD patients, Study number 091-026 preliminary data). Accurate measurements of systemic exposures to formoterol in nonclinical and clinical studies were a significant problem with NDA 20-831 (Novaritis, Foradil Aerolizer). Plasma protein binding of ^{3}H-(R,R)-formoterol was somewhat lower in mice and rats as compared to humans (i.e., mouse, 1 to 100 ng/mL, 28.2-33.7%; rat, 1 to 100 ng/mL, 37.2-48.9%; human, 0.25 to 1 ng/mL, 52.1-64.8%), which suggests greater levels of free drug in mice and rats Metabolism of (R,R)-formoterol has not been examined in mice and humans. The sponsor has provided metabolism data for rats and dogs (i.e., preclinical species used in toxicology studies).

Recommendations:
Based upon tumor findings in the uterus and cervix from the 2-year carcinogenicity study, (R,R)-formoterol was tumorigenic in female mice.

The carcinogenic assessment of (R,R)-formoterol in male mice appears adequate. If the ECAC considers it inadequate, there is the option of using the 25-fold AUC approach. Systemic exposure to (R,R)-formoterol in males at 5 mg/kg/day was >25-fold of exposure at the clinical dose; however, metabolism of (R,R)-formoterol in mice and humans have not been examined.

The rat carcinogenicity study appears to be inadequate. Potential treatment-related neoplastic findings were evident in the soft tissue of the thorax and possibly thyroid gland.

There was evidence that a maximum tolerated dose was obtained for males and females at 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively, based upon decreased absolute body weights; however, histopathological examinations of organs and tissues were incomplete for these animals. The sponsor should be asked to complete the histopathological evaluations of organs and tissues for males and females in the 200 µg/kg/day group to adequately assess the carcinogenic potential of (R,R)-formoterol in rats. The sponsor should consider conducting histopathological examination in all dose groups (i.e., 40, 100, and 200 µg/kg/day groups). The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the males and females in the 200 µg/kg/day group, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group.

The above recommendations are pending on concurrence from the ECAC.

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

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

Non-Concurrence - _____
(see memo attached)

cc: list:
IND 55,302 Division File, HFD-570

GreenA, HFD-570
GunkelJ, HFD-570
ZhouF, HFD-570
SunC, HFD-570
RobisonT, HFD-570

APPENDIX/ATTACHMENTS (Appendices 2 thru 5 will be attached in version sent to ECAC).
Appendix 1: Minutes of Executive CAC Meeting dated September 7, 1999
Appendix 2: Neoplastic findings for the mouse carcinogenicity study.
Appendix 3: Non-neoplastic findings for the mouse carcinogenicity study.
Appendix 4: Neoplastic findings for the rat carcinogenicity study.
Appendix 5: Non-neoplastic findings for the rat carcinogenicity study.

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
4/29/05 04:49:31 PM
PHARMACOLOGIST

Joseph Sun
5/2/05 11:25:33 AM
PHARMACOLOGIST
I concur.

Appears This Way
On Original

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

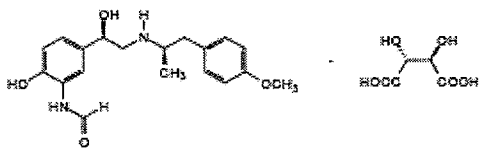
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-912
Review number: #01
Sequence number/date/type of submission:
#000/December 12, 2005/Initial Submission
March 29, 2006/BP
April 27, 2006/BP
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Sepracor, Inc.
84 Waterford Drive
Marlborough, MA 01752
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: June 6, 2006

Drug:

Trade name:
Generic name: Arformoterol Tartrate Inhalation Solution
Code name: Arformoterol, (R,R)-Formoterol-L-tartrate
Chemical name: (R,R)-(-)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide-(R,R)-2,3-dihydroxybutanedioate (1:1 salt)
CAS registry number:
Molecular formula/molecular weight: $C_{23}H_{30}N_2O_{10}$ / MW 494.5
Structure:



Relevant INDs/NDAs/DMFs:
IND 55,302 (R,R-Formoterol, Sepracor, Inc.)
NDA 20-831 (Foradil, Novartis)

Drug class: β_2-Adrenergic Bronchodilator

Intended clinical population: Arformoterol Tartrate Inhalation Solution is indicated for twice daily (morning and evening) long-term maintenance treatment of

bronchoconstriction in patients with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema.

Clinical formulation: Arformoterol Tartrate Inhalation Solution is supplied in 2-mL unit-dose, low-density polyethylene (LDPE) vials. Each 2-mL unit-dose vial contains 15 mcg of arformoterol (22 mcg of the tartrate salt) in a sterile, isotonic saline solution, pH-adjusted to 5.0 with citric acid and sodium citrate.

• The recommended dosage of Arformoterol Tartrate Inhalation Solution for COPD patients is 15 mcg administered twice a day (morning and evening) by nebulization. A total daily dose greater than 30 mcg (15 mcg twice daily) is not recommended. Arformoterol Tartrate Inhalation Solution should only be administered by nebulizer by the inhalation route.

Route of administration: Oral Inhalation (Nebulization)

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

Studies reviewed within this submission:
A 24-Month Inhalation Oncogenicity Study of (R,R)-Formoterol in Rats.

Studies not reviewed within this submission: None.

TABLE OF CONTENTS

2.6.6 TOXICOLOGY

2.6.6.5 Carcinogenicity

Background:
Study results of the rat carcinogenicity study with R,R-formoterol were discussed by the Executive Carcinogenicity Assessment Committee on May 10, 2005. The conclusions and recommendations of the committee were as follows:

1. Surviving males and females in the 400 µg/kg/day group were sacrificed up to 3 months early. Sacrifice of these animals was inappropriate and the treatment period was judged to be inadequate for that group.

2. There was evidence that a maximum tolerated dose was obtained for males and females in the 200 µg/kg/day group that received treatment for periods up to 104 and 101 weeks, respectively, based upon decreased absolute body weights; however, histopathological examinations of organs and tissues were incomplete for these animals. The findings of the study were judged to be inconclusive because of incomplete histopathological examinations of organs and tissues for males and females in the 200 µg/kg/day group and lower dose groups.

a. The sponsor should complete the histopathological evaluations of organs and tissues for males and females in the 200 µg/kg/day group and lower dose groups if appropriate. The sponsor is reminded that if they plan to conduct the histopathological evaluation of tissues from only the males and females in the 200 µg/kg/day group, they will also need to conduct histopathological examination of other dose groups under any of the following circumstances: a) any macroscopic findings in the low or mid dose groups for that particular tissue or organ, b) an increase in the incidence of tumors (rare or common) observed in the high dose animals for a particular tissue or organ even if the increase is not statistically significant, c) any increase in tumors that should be analyzed across tissue sites as well as by tissue site (e.g. hemangiosarcoma, lymphoma; see McConnell et al., JNCI 76:283, 1986) will necessitate that all relevant tissues from that dose level and the next lower dose level(s) be examined, or d) an excessive decrease in body weight or survival in the examined dose group.

b. The Committee also noted that the controls need to be reexamined by the pathologist along with drug treatment groups in order to have a valid comparison.

c. A study report of histopathological examinations of organs and tissues needs to be reviewed by the Division and evaluated by the Executive CAC before the FDA could find the study to be adequate.

Based upon conclusions and recommendations from the ECAC meeting dated May 10, 2005, the sponsor requested that study pathologists examine all remaining (unexamined) tissues for the animals from the 40, 100, and 200 µg/kg/day groups (i.e.,

animals from these groups that survived the terminal primary necropsy and females in the 100 µg/kg/day group that were sacrificed early). To achieve valid comparisons with control tissues that were examined previously, it was necessary to re-establish diagnostic criteria, including grading criteria and diagnostic thresholds or baselines. Microscopic slides of all relevant tissues from control groups 1 and 2 that corresponded to the additional tissues to be examined in the 40, 100, and 200 µg/kg/day groups were reviewed contemporaneously, together with the originally recorded data for those tissues. This data was provided in the present submission.

Rats

Study title: **A 24-Month Inhalation Oncogenicity Study of (R,R)-Formoterol in Rats (Amended Report, September 30, 2005).**

All sections except Histopathology were previously reviewed by the ECAC on May 10, 2005.

Key study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model:
Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks. The sponsor did not have ECAC concurrence for dose selection.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups. The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks. The sponsor did not contact the Division prior to early termination of groups during weeks 90-92.

Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was also achieved at this dose.

Non-neoplastic findings were observed in the ovaries, oviducts, spleen, lung, heart, mesenteric lymph nodes, parathyroid gland, nasal cavities, and exorbital lacrimal gland. The most notable findings were increased incidences of cyst(s) in the ovaries and oviducts for female treatment groups.

Evaluation of tumor findings:
For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day exceeded mean incidences from the historical control background data of the testing laboratory. Increases at 100 and 200 µg/kg/day were significant using trend analysis. However, only the increase at 200 µg/kg/day was statistically significant by pairwise comparison.

For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 100 and 200 µg/kg/day group when the high dose group was excluded (Trend analysis). However, there were no statistically significant increases using pairwise comparison. There were no findings in the 400 µg/kg/day group, which was terminated early.

For the skin, the combined incidence of fibroma and fibrosarcoma were significantly increased for females in the 400 µg/kg/day group by trend analysis; however, this was not statistically significant by pairwise comparison. The incidence of fibroma + fibrosarcoma at 400 µg/kg/day exceeded mean incidences of the historical control data, but may have been within upper ranges.

For the uterus and cervix combined, the incidence of benign endometrial stromal polyps was increased for females in the 400 µg/kg/day group, although this was not statistically significant. The incidence at 400 µg/kg/day exceeded the mean incidence of the historical control data; however it was within the upper range. In the 2-year mouse carcinogenicity study with R,R-formoterol, there were statistically significant increased incidences of endometrial stromal polyps in the uterus and cervix.

Based upon increased incidences of thyroid C-cell adenoma and carcinoma in female treatment groups, R,R-formoterol is tumorigenic in rats.

Systemic exposure at 18 month in rats that received 40 µg/kg/day R,R-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times systemic exposure with a clinical dose of 15 µg BID.

Study no.: —-312051
Volume #, and page #: Volumes 1-20, Pages 1-7392
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: January 19 and 27, 2000 (Initiation of dose administration for males and females, respectively; Designated as study week 0)
GLP compliance: Yes.
QA report: yes (X) no ( )

Drug, lot #, and % purity: (R,R)-formoterol tartrate, Lot number 021 5012 — Log No.: 4517A] (Purity, 100.7% from Certificate of Analysis) b(4)
CAC concurrence: No.

The (R,R)-formoterol doses in the current study were based upon a 1-month inhalation toxicology with rats. The ECAC did not concur (see attached minutes in Appendix 1). In this subchronic study, test article-related effects were reported by the sponsor at 400 µg/kg/day. According to the sponsor, systemic exposure levels at 100 and 400 µg/kg/day after 1 month of inhalation treatment were at least 90- and 450-fold, respectively, higher than clinical exposure with a therapeutic dose of (R,R)-formoterol.

Methods
Doses: 0, 40, 100, 200, and 400 µg/kg/day

Group	Treatment	Target Inhaled Dose[a], µg/kg/day	Number of Animals	
			Males	Females
1	Vehicle-Control 1	0	60	60
2	Vehicle-Control 2	0	60	60
3	(R,R)-Formoterol	40	60	60
4	(R,R)-Formoterol	100	60	60
5	(R,R)-Formoterol	200	60	60
6	(R,R)-Formoterol	400[b]	60	60

a. Daily dose levels were controlled by the combination of the exposure duration and exposure concentration, based upon the delivered drug on the filter as determined by chemical analysis.
b. The exposure concentration for the high dose male group was targeted to provide a dose of 200 µg/kg/day for the first two days of treatment and then targeted to provide a dose of 400 µg/kg/day starting on the third day and thereafter.

Target concentrations were calculated as follows:

$$\text{Target concentration (µg/L)} = \frac{\text{Target dose (µg/kg) x Body weight (kg)}}{\text{Minute volume (L/min) x Exposure duration (min)}}$$

Target concentrations were calculated at study initiation and periodically during the study using the most recent body weight data.

Mean exposure concentrations

Dose, µg/kg/day	Free base concentration (µg/L)	
	Males	Females
40	2.2	2.0
100	5.4	4.9
200	11.1	9.8
400	20.8	19.0

b(4)

Animal exposures were conducted using the [redacted] directed-flow nose-only exposure systems assembled with six 12-port modules that operated under dynamic conditions. Two systems were dedicated for (R,R)-formoterol exposures and a third

system for control (saline aerosol) exposures. Control groups 1 and 2 were exposed using System 1, the 40 and 100 µg/kg/day groups were exposed using System 2, and the 200 and 400 µg/kg/day groups were exposed using System 3. Male and female rats from each of the six groups were exposed separately, using separate generation and exposure periods. Animals were placed in nose-only exposure restraint tubes during exposure periods.

For the test article exposures of each group, liquid droplet aerosol atmospheres were generated using nebulizer-based systems. The modified C J Collision nebulizer was selected for use based on its stable and efficient aerosolization and production of aerosols with particle sizes (MMAD) <2 µm. b(4)

Actual exposure concentrations of (R,R)-formoterol were determined by chemical analyses of aerosol samples collected on filters. The protocol required collection of two filter samples during each animal exposure period. For a small fraction of the total exposures, only one filter was collected due to technical errors or problems. From the start of the study on January 19, 2000 through April 6, 2001, the filters collected from all test article exposures (7 days/week) were analyzed. From April 7, 2001 to the termination of the study on January 16, 2002, samples from only two days per week (2 days/week) were analyzed. For the saline aerosol (control) exposures, two filter samples were collected during one exposure period approximately each week. Each sample was collected from an unused animal exposure port using 25-mm glass-fiber filters held in an in-lined filter holder. The sample volume was calculated by multiplying the sampling airflow rate by the sample collection time.

The mass of formoterol free base on each filter was determined using an HPLC method. The actual exposure concentrations (as free base) were calculated by dividing the analytically determined mass of free base by the sample volume. The estimated dose (µg/kg) for each test article treatment group was calculated from the exposure concentration using the following equation:

Estimated inhaled dose (µg/kg/day) = Exposure concentration (µg/L) x minute volume/mean body weight (L/min/kg) x Duration (min)

Mean concentrations (Sponsor's data)

Dose µg/kg/day	Sex	Exposure Conc. µg/L	Target Conc. µg/L	Minute Volume/BW L/min/kg	Exposure Duration min	Estimated Dose µg/kg/day	% of Target Dose
40	M	2.1	2.1	0.64	30	40.3	101.6
	F	2.0	1.9	0.70	30	42.3	106.3
100	M	5.4	5.3	0.64	30	102.8	101.5
	F	4.9	4.8	0.70	30	103.8	103.3
200	M	11.1	10.8	0.64	30	209.9	102.6
	F	9.9	9.5	0.70	30	207.0	103.6
400	M	20.7	20.8	0.65	30	401.0	99.6
	F	18.9	18.9	0.70	30	399.7	100.3

Estimated inhaled doses

Target dose, µg/kg/day	Actual dose[a], µg/kg/day		Deposited dose[b], µg/kg/day	
	Males	Females	Males	Females
40	40.5	42.0	4.0	4.2
100	101.6	102	10.2	10.2
200	209.1	206.1	20.9	20.6
400	398.1	397.2	39.8	39.7

a. Actual exposure concentrations for each group were used with the exposure duration, group mean body weight, and minute volume to calculate the inhaled dose (µg/kg).
b. A 10% deposition factor was used to calculate the deposited dose.

Aerosol particle size determinations were performed for the low and high concentrations of (R,R)-formoterol. The frequency of measurements did not meet the original requirements of the study protocol, but was considered sufficient to characterize the aerosol characteristics in terms of particle size distribution. Particle size determinations were performed using a 7-stage cascade impactor. Glass-fiber filters were used as collection substrates. Formoterol free base collected on the substrates was chemically analyzed using a HPLC method. Particle size was calculated based on impactor stage cutoffs. Aerosol particle was expressed as MMAD ± GSD.

Mean particle size, MMAD ± GSD

Dose, µg/kg/day	Mean particle size ± GSD
40	0.6 ± 1.36 µM
400	0.8 ± 1.77 µM

Operating parameters of exposure systems

Parameter	0-1	0-2	40	100	200	400
Temperature, °C	20	20	21	21	21	21
Relative Humidity, %	50	51	59	59	54	53

Basis of dose selection (MTD, MFD, AUC etc.): No concurrence from the ECAC was obtained for dose selection. Survival was significantly decreased for males in the 400 mg/kg/day group; however, these animals were sacrificed during week 92 without Division consultation. A MTD was evident for males and females at 200 µg/kg/day based upon an approximate 10% decrease of absolute body weight at the end of treatment,

Species/strain: Male and female Sprague-Dawley — CD®(SD)IGS BR] rats were obtained from ⊏ ⊐ b(4)

Number/sex/group (main study): 60 rats/sex/group

Route, formulation, volume: The vehicle or (R,R)-formoterol tartrate was administered as an aerosolized saline solution by nose-only exposure. Two concurrent control groups were exposed to an aerosol of the vehicle, 0.9% sodium chloride for injection USP, at a level matching the saline concentration in the test atmosphere for the high dose of 400 µg/kg/day.

Frequency of dosing: Animals were exposed for approximately 30 min/day, 7 days/week for periods up to 104 weeks.

Satellite groups used for toxicokinetics or special groups: Satellite groups for toxicokinetic assessments consisted of 15 rats/sex/group and received (R,R)-formoterol at inhaled doses of 40, 100, 200, and 400 µg/kg/day.
Age: At the start of treatment, male and female rats were 8 and 9 weeks old, respectively. Body weight ranges were 238 to 310 g for males and 182 to 256 g for females.
Animal housing: All animals were housed individually in clean, wire mesh cages suspended above cage-board. Animals were housed in rooms that adjoined the exposure room by internal doors; therefore, animals were not transported through facility corridors.
Restriction paradigm for dietary restriction studies: No.
Drug stability/homogeneity: Solutions of (R,R)-formoterol in 0.9% saline were prepared. The solutions were mixed with a magnetic stirrer until uniform and throughout use; solutions were filtered through a 0.22-µm ⊏ ⊐ filter. The dosing solutions were prepared approximately once or twice weekly and were stored refrigerated. Stability of the test article in the vehicle for 8 days under refrigerated conditions was determined in a previous study. b(4)
Dual controls employed: Yes.
Interim sacrifices: See Deviations for original study protocol.
Deviations from original study protocol: The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92 (referred to as study week 92). All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. These early sacrifices for females were collectively referred to as week 91. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101 (collectively referred to as week 101). There was no consultation with the Division prior to these early sacrifices.
Other: A pre-study health screen was conducted on 10 rats/sex. Blood was collected from each animal and gross necropsy examinations were conducted. Blood was used for determinations of white cell counts and assessment of serum antibody profiles. ⊏ ⊐ reported that none of serum antibody tests (CARB, MPul, PVM, RCV/SDA, Reo, Sendai, LCM, Parvo, H-1, KRV) were positive. Carcasses were discarded after completion of necropsy examinations. b(4)

Fifteen rats/sex were assigned to sentinel groups. These animals were housed in the same rooms as the animals assigned to the main study and were used to provide biological samples for diagnosis of possible disease conditions. Sentinel animals were observed for mortality/moribundity twice daily. Physical examinations were conducted weekly. Body weights were measured as described for main study animals. For animals that survived to the end of the study or were sacrificed in a moribund condition, blood was collected and gross necropsy examinations were conducted. Tissues were collected and preserved for possible microscopic examination. Bone marrow smears were not collected. The sponsor reported that there were no indications that required follow-up using the sentinel animals.

Observation times

Mortality: Animals were observed twice daily for moribundity/mortality.

Clinical signs: Clinical examinations of animals were conducted daily and when removed from exposure tubes. Detailed physical examinations were conducted on all animals weekly. All male and female rats were examined weekly starting at study weeks 33 and 27, respectively, until completion of the study for the presence of palpable masses. The time of onset, location, size, appearance, and progression of each mass were recorded throughout the study period.

Body weights: Body weights were measured weekly from one week prior to the start of treatment through week 12. Thereafter, body weights were collected monthly throughout the study.

Food consumption: Food consumption was measured weekly, beginning one week prior to the start of treatment through week 12. Thereafter, food consumption was measured monthly through week 100, and week 103 for the surviving male groups.

Clinical Pathology: Blood and urine samples for clinical pathology determinations (hematology, serum chemistry, and urinalysis) were collected from 10 rats/sex/group from control group 1 and the 40, 100, 200, and 400 µg/kg/day groups at week 51. In addition, blood smears were prepared for all animals at the scheduled necropsy and animals sacrificed in a moribund condition; however, blood smears (differential leukocyte counts) were not evaluated.

Organ weights: Absolute and relative organ weights were determined for the adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes, thymus, and uterus.

Histopathology:

Animals in a moribund condition or at scheduled necropsies were sacrificed with the use of isoflurane, and necropsies were conducted on all animals. Protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 µm, mounted on glass microscope slides and stained with hematoxylin and eosin. The initial histopathologic examination consisted of all tissues from animals in control group 1, control group 2, and the 400 µg/kg/day group. For the 40, 100 and 200 µg/kg/day groups, histopathologic examination included all tissues from animals that died or were euthanized in extremis during the study. Further, selected potential target tissues (heart, lungs, liver, kidneys, nasal cavities, pituitary gland, tissue masses and gross from all animals and ovaries and spleen from all females) were included in the histopathologic evaluation for rats in the 40, 100, and 200 µg/kg/day groups that survived to study termination.

Based upon conclusions and recommendations from the ECAC meeting dated May 10, 2005, the sponsor requested that study pathologists examine all remaining (unexamined) tissues for the animals from the 40, 100, and 200 µg/kg/day groups (i.e., animals from these groups that survived the terminal primary necropsy as well as females in the 100 µg/kg/day group that were sacrificed early). To achieve valid comparisons with control tissues that were examined previously, it was necessary to re-establish diagnostic criteria, including grading criteria and diagnostic thresholds or baselines. Microscopic slides of all relevant tissues from control groups 1 and 2 that corresponded to the additional tissues to be examined in the 40, 100, and 200

µg/kg/day groups were reviewed contemporaneously, together with the originally recorded data for those tissues. A pathology peer review was conducted. Following the completion of the histopathologic examination of the terminal sacrifice animals in the 40, 100, and 200 µg/kg/day groups, the peer-review process was performed for the tissues from 10% of animals in control group 2 and the 200 µg/kg/day group and newly diagnosed neoplasms for the 40, 100, and 200 µg/kg/day groups. The thyroid gland was considered a new potential target tissue and thus was examined as part of the review for all groups.

Toxicokinetics: Blood samples for measurement of plasma drug concentrations were collected from toxicokinetic animals during weeks 51 and 78 at 0.5, 1, 2, 6, and 24 hr following exposure. Three rats/sex/group were used for each time point. Plasma samples were analyzed for drug exposure concentration using a LC/MS/MS method.

Statistical analysis: As a result of the above-mentioned additional microscopic examination, the following statistical analysis statement was now applicable to all protocol-defined tissues. The incidence of each tumor type that occurred in any organ was analyzed with a one-sided trend test using the dose coefficients, as well as with pairwise comparisons of active treatment groups with the controls (control group 1, control group 2 and pooled control). At the request of the sponsor the following criteria for statistical analysis were applied: • A Tumor Data Set (TDS) was created from [ ] data that included neoplasms of all animals in all groups. • All tumor statistics were performed with inclusion of the data from control groups 1 and 2 and the 40, 100, and 200 µg/kg/day (400 µg/kg/day excluded) and also with the inclusion of all groups. • For proliferative lesions of thyroid C-cells, statistical analyses were performed for adenoma alone, carcinoma alone, combined adenoma and carcinoma, and combined hyperplasia, adenoma and carcinoma. Although the hyperplasia is not a neoplasm, the statistical methods used for analyses of combined hyperplasia, adenoma and carcinoma were the same as those used for combined tumors. When findings were combined and there existed multiple findings in the same tissue, an animal was only counted once. For example, if an animal had both an adenoma and a carcinoma of thyroid C-cells, this animal was counted only once for combined adenoma and carcinoma.

b(4)

Results

Mortality: There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group (dose mortality trend Cox p-value = 0.0006 and Kruskal-Wallis p-value = 0.0005). Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups (Cox p-value = 0.2095 and Kruskal-Wallis p-value = 0.1402). The sponsor reported that the survival rate of female rats in the 100 µg/kg/day group was significantly lower than that of the pooled control as well as each individual control group; however, there was no effect on survival for female rats in the 200 µg/kg/day group. Although, not statistically significant, survival was slightly decreased for females in the 400 µg/kg/day.

The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day group during weeks 91 and 92 (referred to as study week 92). All surviving females in

control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. These early sacrifices for females were collectively referred to as week 91. The control group 1 rats were sacrificed "to provide a comparative group to aid in defining and describing organ weight and morphologic changes in the early terminated groups." The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101 (collectively referred to as week 101). The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks. Early sacrifices of males in control group 1 and the 400 µg/kg/day group and females in control group 1 and the 100 and 400 µg/kg/day groups were inappropriate. There was no consultation with the Division prior to any of these early sacrifices.

Text Table 1: Survival at Study Weeks 25, 52, 77, 80, 90 and 104 for Males and at Study Weeks 25, 52, 77, 80, 89 and 101 for Females - Number and Percentage of Animals Surviving

	MALES						FEMALES						
GROUP	1	2	3	4	5	6	GROUP	1[a]	2	3	4	5	6[a]
DOSE	0	0	40	100	200	400	DOSE	0	0	40	100	200	400
STUDY WEEK							STUDY WEEK						
25	60 100%	59 98%	60 100%	59 98%	58 97%	59 98%	25	59 100%	60 100%	59 98%	58 97%	60 100%	59 100%
52	56 93%	58 97%	57 95%	55 92%	55 92%	52 87%	52	56 95%	59 98%	56 93%	54 90%	58 97%	56 95%
77	43 72%	50 83%	50 83%	48 80%	39 65%	37 62%	77	47 80%	44 73%	43 72%	38 63%	47 78%	36 61%
80	42 70%	47 78%	46 77%	43 72%	37 62%	35 58%	80	45 76%	43 72%	41 68%	34 57%	42 70%	36 61%
90	34 57%	37 62%	34 57%	34 57%	32 53%	22 37%	89	32 54%	33 55%	34 57%	22 37%	34 57%	27 46%
104	NA	24 40%	24 40%	23 38%	21 35%	NA	101	NA	24 40%	17 28%	NA	24 40%	NA

[a] = There were 60 animals/sex/group at study initiation of dosing. Mortality data corrected for accidental deaths (*i.e.*, procedural errors).

NA = Not Applicable

Appears This Way On Original

Animal Disposition

Disposition	Males						Females					
	0-1	0-2	40	100	200	400	0-1	0-2	40	100	200	400
Found dead	14	16	18	14	25	22	8	8	14	12	16	7
Euthanized in extremis	1	1	1	2	1	0	2	0	2	2	1	0
Euthanized in extremis, physical condition	9	12	13	12	8	16	2	10	15	9	12	12
Euthanized in extremis, size/condition masses	2	4	1	4	2	0	15	15	10	14	5	10
Found dead after exposure	1	3	3	5	3	3	0	2	2	2	2	3
Found dead in exposure tube, prior to exposure	0	0	0	0	0	0	0	1	0	1	0	0
Total	27	36	36	37	39	41	27	36	43	40	36	32
Interim necropsy Week 92 or 91	33	0	0	0	0	19	32	0	0	20	0	27
Primary necropsy Week 104 or 102	0	24	24	23	21	0	0	24	17	0	24	0
Accidental Death	0	0	0	0	0	0	1	0	0	0	0	1

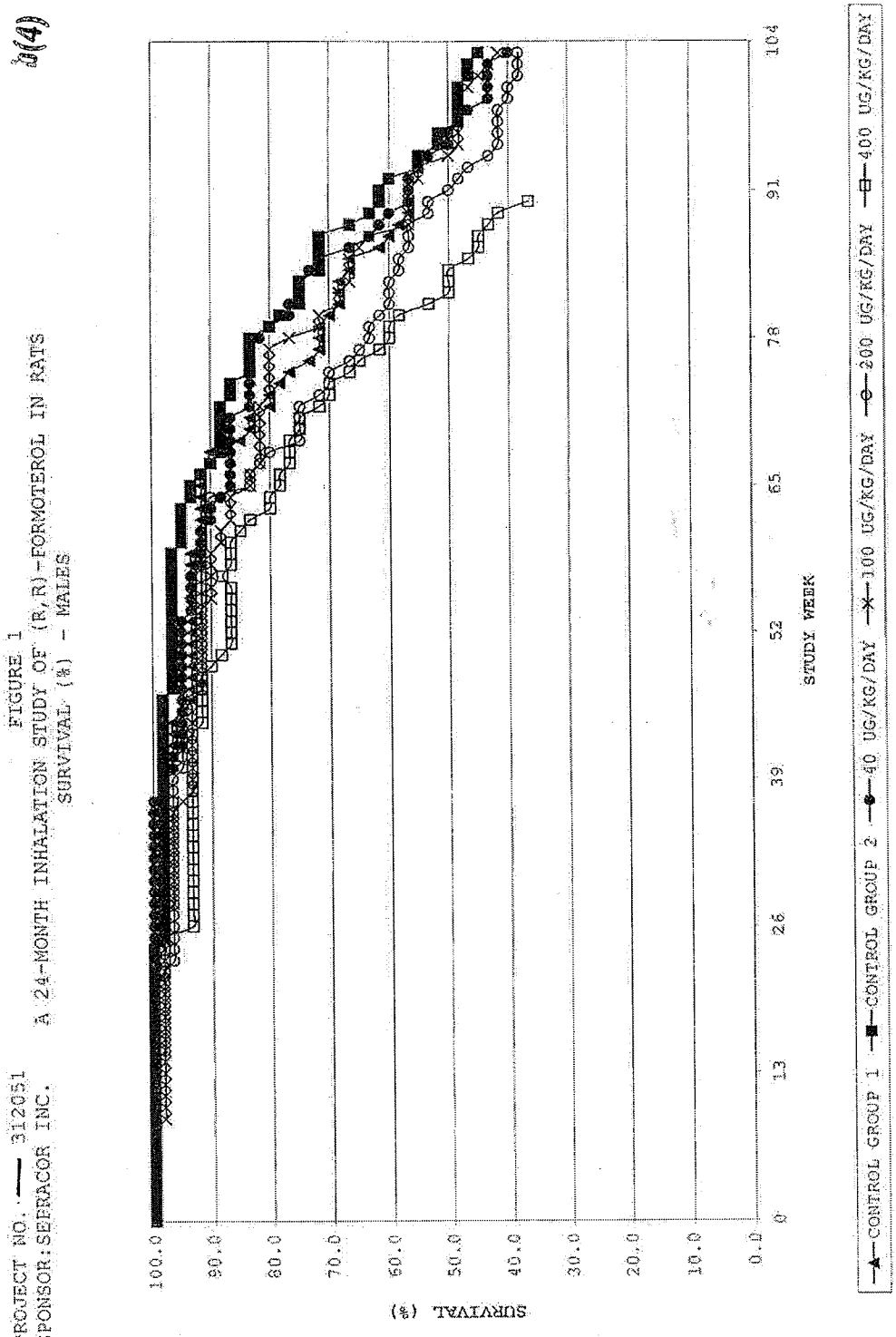

PROJECT NO. — 312051
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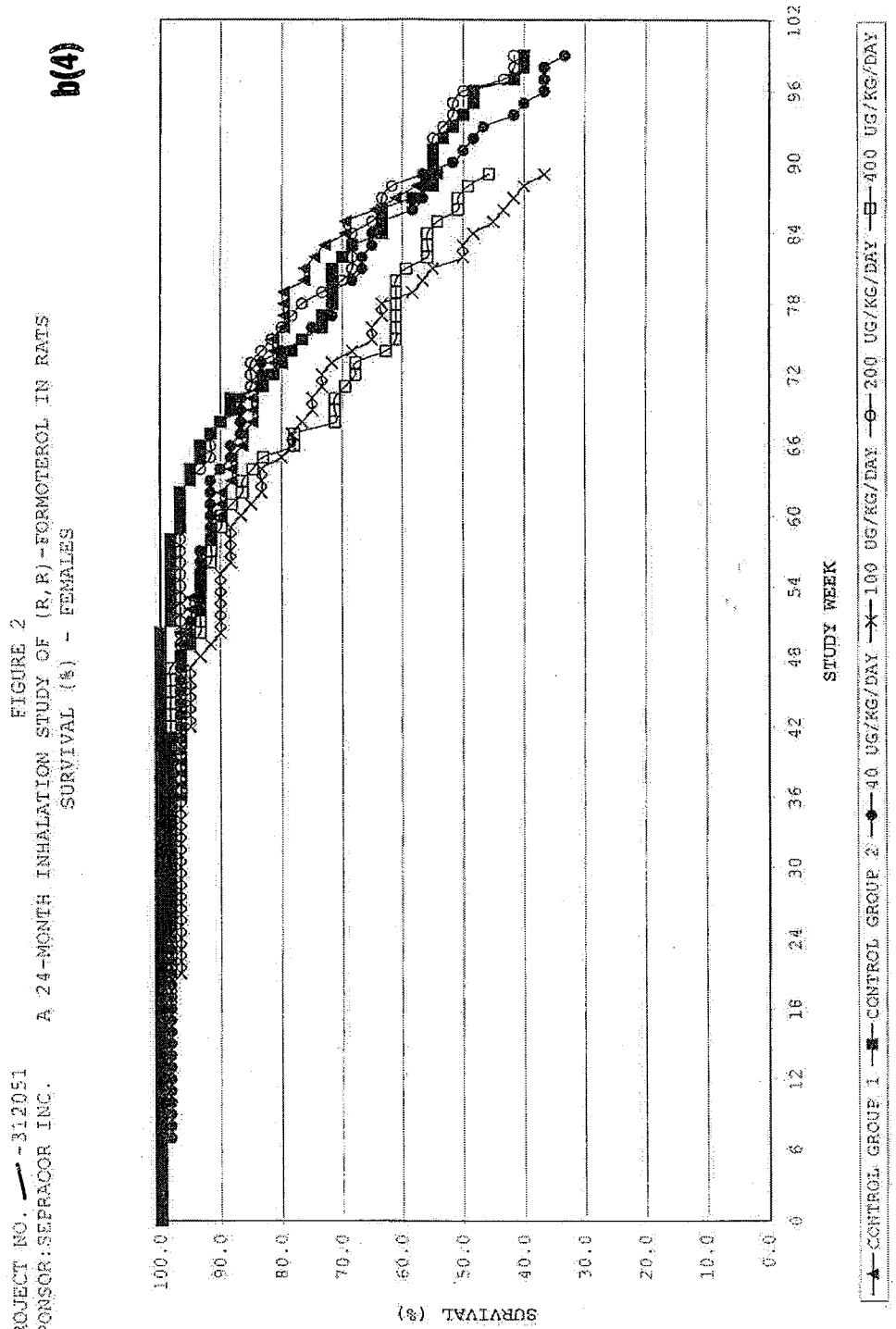
FIGURE 1
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
SURVIVAL (%) - MALES
SURVIVAL (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
13
26
39
52
65
78
91
104
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY


PROJECT NO. —-312051
SPONSOR:SEPRACOR INC.
FIGURE 2
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
SURVIVAL (%) - FEMALES
b(4)
SURVIVAL (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

Clinical signs: A number of nonspecific clinical and physical signs were observed for control and treatment groups. In some cases, clinical and/or physical signs were elevated in treatment groups, although incidences were relatively low in relation to the total number of animals per group and these signs were not test article-specific. Thus, the relationships of these clinical and/or physical signs to treatment with (R,R)-formoterol were unclear.

Body weights: Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was achieved at this dose.

Body weight and body weight gain

Parameter	Male rats						Female rats					
	0-1	0-2	40	100	200	400	0-1	0-2	40	100	200	400
Wk 0	275	275	275	275	275	275	212	213	213	213	213	213
Wk 25	566	565	572	560	549	527	319	319	337	341	328	334
BW, % of Control	-	-	101%	99%	97%	93%	-	-	106%	107%	103%	105%
BW gain Wk_{0-25}, % of control	-	-	103%	99%	95%	87%	-	-	116%	119%	108%	113%
Wk 53	635	657	603	604	576	548	363	362	368	379	350	359
BW, % of Control	-	-	96%	96%	91%	87%	-	-	102%	105%	97%	99%
BW gain Wk_{0-53}, % of control	-	-	92%	92%	85%	77%	-	-	103%	110%	91%	97%
Wk 77	623	618	602	590	567	518	398	399	396	419	376	384
BW, % of Control	-	-	97%	95%	91%	84%	-	-	99%	105%	95%	96%
BW gain Wk_{0-77}, % of control	-	-	95%	91%	85%	70%	-	-	98%	110%	87%	92%
Wk 89	597	603	590	578	546	503	405	399	395	399	383	380
BW, % of Control	-	-	115%	110%	98%	83%	-	-	98%	99%	95%	95%
BW gain Wk_{0-89}, % of control	-	-	97%	93%	83%	70%	-	-	96%	98%	90%	88%
Wk 104/101	NA	581	556	557	521	NA	NA	416	406	NA	372	NA
BW, % of Control		-	96%	96%	90%			-	98%		89%	
BW gain $Wk_{0-104/101}$, % of control		-	92%	92%	80%			-	95%		78%	

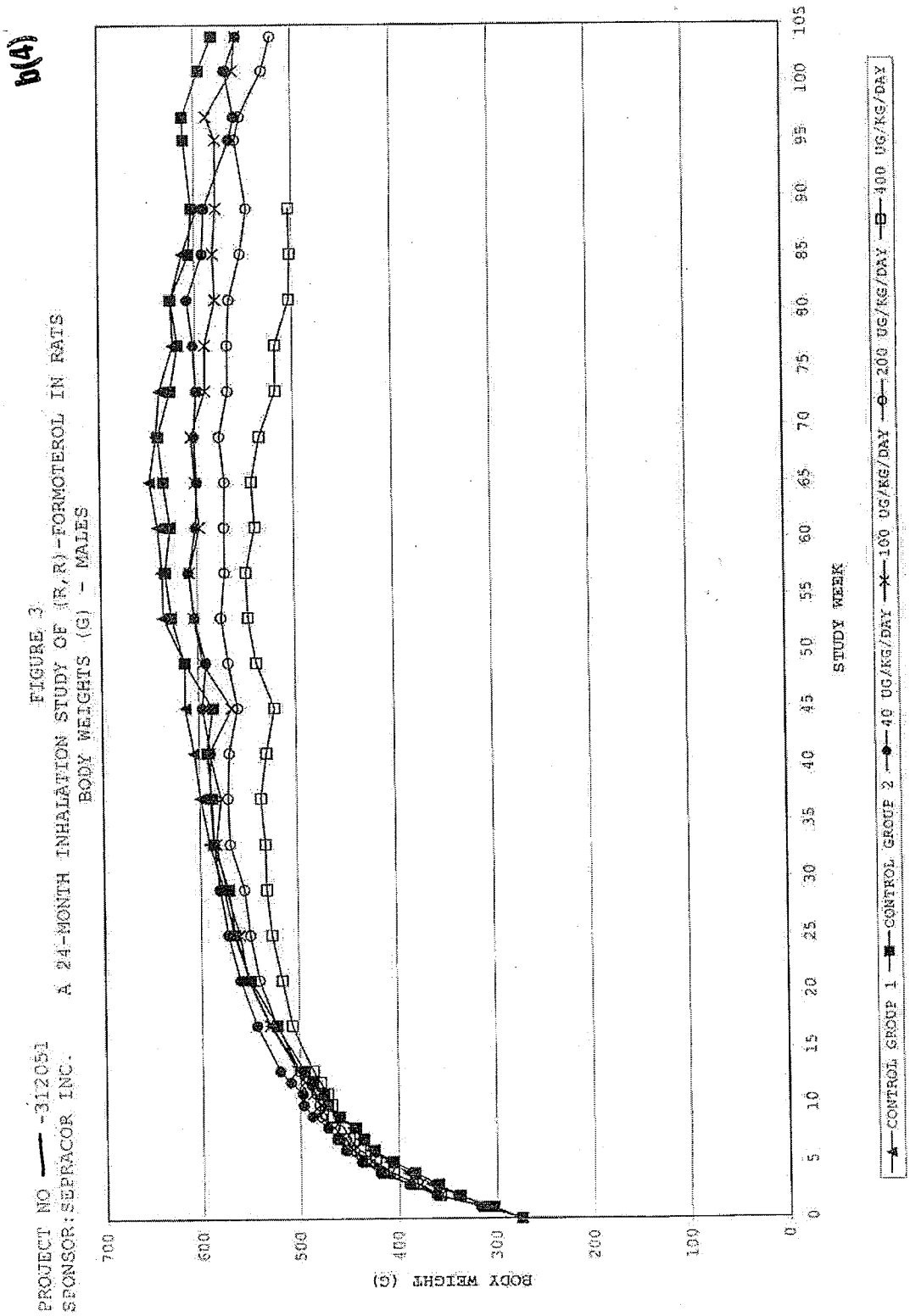

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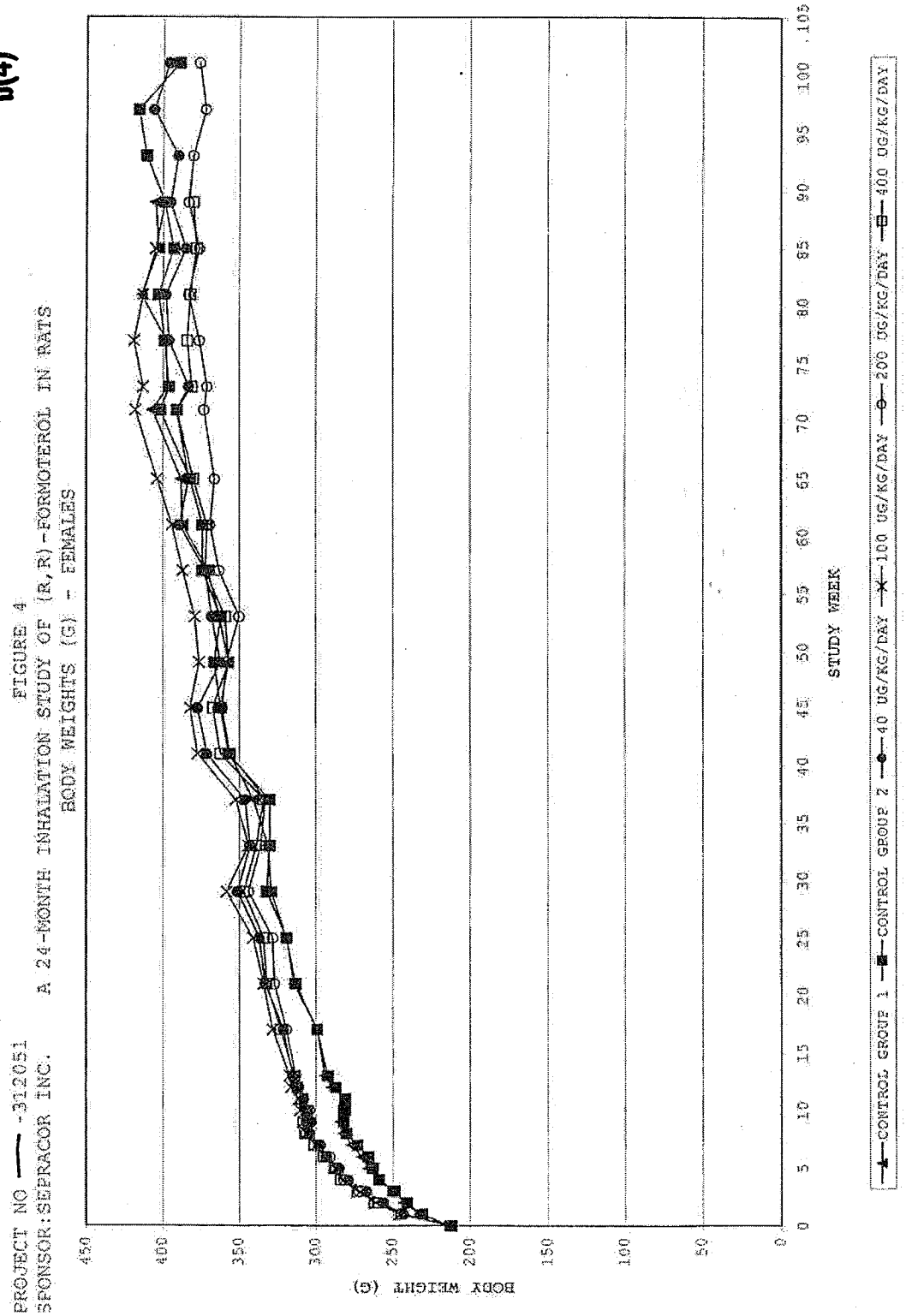
FIGURE 3
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
BODY WEIGHTS (G) - MALES
PROJECT NO. —-312051
SPONSOR:SEPRACOR INC.
BODY WEIGHT (G)
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

b(4)


PROJECT NO — -312051
SPONSOR:SEPRACOR INC.
FIGURE 4
A 24-MONTH INHALATION STUDY OF (R,R)-FORMOTEROL IN RATS
BODY WEIGHTS (G) - FEMALES
BODY WEIGHT (G)
STUDY WEEK
CONTROL GROUP 1
CONTROL GROUP 2
40 UG/KG/DAY
100 UG/KG/DAY
200 UG/KG/DAY
400 UG/KG/DAY

Food consumption: There were no treatment-related effects on food consumption.

Hematology (Week 51): Decreased white blood cell counts, attributed to decreases of neutrophils, lymphocytes, and monocytes, were observed for female treatment groups. Decreased platelet counts were observed for females at 100, 200, and 400 µg/kg/day. There were no significant changes of hematology parameters for male treatment groups.

Week 51 hematology parameters for female control and treatment groups

Parameter	Females				
	0-1	40	100	200	400
White blood cells $10^3/\mu L$	9.6	7.1 (74%)	6.9* (72%)	6.6* (69%)	5.6* (58%)
Neutrophils $10^3/\mu L$	3.1	1.9 (61%)	1.8 (58%)	1.9 (61%)	1.3* (42%)
Neutrophils %	32	26 (81.3%)	27 (84%)	28 (87%)	23 (72%)
Lymphocytes $10^3/\mu L$	5.4	4.5 (83%)	4.3 (80%)	4.0 (74%)	3.7 (69%)
Lymphocytes %	57	65 (114%)	62 (109%)	62 (109%)	66 (116%)
Monocytes $10^3/\mu L$	0.9	0.6 (67%)	0.7 (78%)	0.6 (67%)	0.5 (56%)
Platelets $10^3/\mu L$	1071	1054	970 (91%)	903* (84%)	923 (86%)

Clinical Chemistry (Week 51): Glucose levels and amylase activities were decreased for male and female treatment groups. These changes could be attributed to the pharmacological activity of the β_2-adrenergic agonist, arformoterol. Blood urea nitrogen (BUN) levels were slightly increased for male treatment groups; however, there was no evidence of treatment-related histopathological changes in the kidneys. Potassium levels and the A/G ratio were slightly increased for female treatment groups. Phosphorus levels were increased for females in the 400 µg/kg/day group.

Week 51 clinical chemistry parameters for male and female control and treatment groups

Parameter	Males					Females				
	0-1	40	100	200	400	0-1	40	100	200	400
Glucose mg/dL	125	104* (83%)	91* (73%)	87* (70%)	79* (63%)	119	79* (66%)	87* (73%)	74* (62%)	87* (73%)
Amylase U/L	2063	1601* (78%)	1477* (72%)	1450* (70%)	1319* (64%)	1413	1218 (86%)	1152* (81%)	1099* (78%)	1135* (80%)
BUN mg/dL	15.3	17.4 (114%)	18.6* (122%)	17.6 (115%)	19.0* (124%)					
Potassium mEq/L						4.56	5.35* (117%)	5.29* (116%)	5.54* (122%)	5.41* (119%)
Phosphorus mg/dL						5.0	5.1	5.5	5.2	6.2* (124%)
A/G ratio						1.42	1.57 (111%)	1.60 (113%)	1.57 (111%)	1.67 (118%)

Urinalysis (Week 51): Urine volumes for female treatment groups were increased to 140-173% of the control (5.5 mL). There were no changes of urinalysis parameters for male treatment groups.

Organ Weights: Absolute and relative ovaries weights were increased for all female treatment groups sacrificed at weeks 91 and 101, which corresponded with histopathological findings of ovarian cysts. Absolute and/or relative spleen weights were decreased for males in the 400 µg/kg/day group sacrificed at week 92 and females in the 200 µg/kg/day sacrificed at week 101. It is unclear if these differences corresponded with histopathological findings of hemosiderin pigment. Relative heart weights were slightly increased for male treatment groups at weeks 92 and 104 and females in the 100 and 400 µg/kg/day groups at week 91; however, there was no dose-response relationship or corresponding histopathological findings. Increased heart weights might be attributed to the pharmacological properties of the β_2-adrenergic agonist, formoterol. Differences were also noted for absolute and/or relative liver, thymus, lung, kidney, and brain weights as shown in the table below; however, these differences were generally small and there were no corresponding histopathological findings.

Absolute and relative organ weights for male control and treatment groups at necropsies during weeks 92 and 104

Organ Weight	0-1	0-2	40	100	200	400
Week 92 Necropsy						
Liver, g	17.68					15.10* (85%)
Liver, g/100 g BrW	810.310					671.083* (83%)
Thymus, g	0.0792					0.0649 (82%)
Thymus, g/100 g BrW	3.696					2.8488 (77%)
Heart, g/100 g BW	0.321					0.370* (115%)
Spleen, g/100 g BrW	47.277					40.621* (86%)
Lungs, g/100 g BW	0.417					0.470 (113%)
Kidneys, g/100 g BrW	188.955					170.692* (90%)
Brain g/100 g BW	0.378					0.440* (116%)
Week 104 Necropsy						
Heart, g/100 g BW		0.327	0.381 (117%)	0.412 (126%)	0.399 (122%)	
Heart, g/100 g BrW		87.224	97.610 (112%)	105.245 (121%)	97.211 (111%)	
Lung, g/100 g BW		0.408	0.447 (110%)	0.469 (115%)	0.482 (118%)	

Absolute and relative organ weights for female control and treatment groups at necropsies during weeks 91 and 101

Organ Weight	0-1	0-2	40	100	200	400
Week 91 Necropsy						
Ovaries, g	0.1309			0.2483 (190%)		0.3448* (263%)
Ovaries, g/100 g BW	0.032			0.063 (197%)		0.092* (288%)
Ovaries, g/100 g BrW	6.528			12.474 (191%)		17.479* (268%)
Thymus, g	0.0957			0.0842 (88%)		0.1111 (116%)
Thymus, g/100 g BW	0.023			0.022		0.030 (130%)
Thymus, g/100 g BrW	4.780			4.277		5.596 (117%)
Heart, g/100g BW	0.365			0.418* (115%)		0.410* (112%)
Lungs g/100g BW	0.437			0.487 (111%)		0.521* (119%)
Week 101 Necropsy						
Ovaries, g		0.1551	0.1905 (123%)		0.3196 (206%)	
Ovaries, g/100 g BW		0.040	0.048 (120%)		0.088 (220%)	
Ovaries, g/100 g BrW		7.806	9.777 (125%)		16.357 (210%)	
Spleen, g		1.04	1.25 (120%)		0.76 (73%)	
Spleen, g/100 g BW		0.269	0.313 (116%)		0.205 (76%)	
Spleen, g/100 g BrW		52.644	64.938 (123%)		38.746 (73.60%)	

Gross pathology: The incidences of cyst(s) in the oviducts were increased for females at 100, 200, and 400 µg/kg/day. The incidences of cysts in the ovaries were increased for all female treatment groups. These findings correlate with histopathological findings of cysts in the oviducts and ovaries.

There were other gross pathological findings that were increased for male and female treatment groups, although, incidences were generally low and these findings were not test article-specific. Thus, relationships to treatment were unclear.

Gross pathological findings for female rats

Organ/Tissue	Time point	0-1	0-2	40	100	200	400
Oviducts -cyst(s)	Unscheduled deaths	2	1	2	4	6	7
	Interim Necropsy, week 91	1	NA	NA	4	NA	4
	Primary necropsy, week 101	NA	2	0	NA	7	NA
	Total	3	3	2	8	13	11

Ovaries -cyst(s)	Unscheduled deaths	5	5	17	8	12	16
	Interim Necropsy, week 91	11	NA	NA	10	NA	22
	Primary necropsy, week 101	NA	7	8	NA	11	NA
	Total	16	12	25	18	23	38

Histopathology:

Non-neoplastic:

Non-neoplastic findings were observed in the ovaries, oviducts, spleen, lung, heart, mesenteric lymph nodes, parathyroid gland, nasal cavities, and exorbital lacrimal gland.

For the ovaries and oviducts, incidences of cyst(s) were significantly increased for female treatment groups.

For the spleen, incidences of hemosiderin were slightly increased for male and female treatment groups.

For the lungs, incidences of alveolar macrophages, congestion, and edema in the lungs were slightly increased for male and female treatment groups. The incidence of granulomatous inflammation in the lungs was slightly increased for female treatment groups.

Incidences of cardiomyopathy were increased slightly for females in the 40, 100, and 200 mg/kg/day groups.

For the mesenteric lymph nodes, incidences of hemorrhage were increased for females in the 100, 200, and 400 µg/kg/day groups and all male treatment groups.

For the parathyroid gland, incidences of hyperplasia were increased for females in the 40, 100, and 200 µg/kg/day groups.

For nasal cavity levels 1 to 5, there were slight increases in incidences of subacute inflammation, exudate, squamous metaplasia, metaplasia, and/or foreign body for female treatment groups. For nasal cavity levels 3 and 5, increased incidences of degeneration of the olfactory epithelium and metaplasia, respectively, were observed for males in the high dose group.

For the exorbital lacrimal gland, there were increased alterations of the Harderian gland for males in the 200 mg/kg/day group.

There were other histopathological findings that were increased for male and female treatment groups, although, incidences were generally low and these findings were not test article-specific. Thus, relationships to treatment were unclear.

Non-neoplastic findings for all female rats from the control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups.

Organ/Tissue	0-1[a]	0-2[c]	40[c]	100[b]	200[c]	400[a]
Ovaries						
-cyst	18/59	15/59	30/60	28/60	38/60	45/59
-atrophy, minimal-moderate	48/59	43/59	33/60	35/60	32/60	30/59
Oviducts						
-cyst	2/59	0/59	3/60	2/60	4/58	8/60
Uterus						
-cyst	6/60	10/60	7/60	9/60	15/60	11/60
Cervix						
-cyst	0/60	2/59	1/60	0/60	1/60	1/60
Spleen						
-pigment, hemosiderin	38/60	36/60	40/60	41/60	47/60	49/60
Lung						
-congestion	4/60	4/60	11/59	7/60	12/60	6/60
-macrophages, alveolar, minimal-moderate	24/60	21/60	34/59	34/60	34/60	30/60
-edema	1/60	1/60	1/59	2/60	8/60	4/60
-inflammation, granulomatous	6/60	3/60	7/59	15/60	11/60	12/60
Heart						
-cardiomyopathy, minimal-severe	17/60	23/60	27/60	26/60	29/60	17/60
Mesenteric LN						
-histiocytosis, sinus, minimal-mild	1/60	0/60	0/60	1/60	2/60	5/59
-hemorrhage	4/60	4/60	3/60	6/60	7/60	9/59
Parathyroid						
-hyperplasia, minimal-mild	2/49	2/54	4/54	7/52	4/51	1/49
Nasal Level 1						
-inflammation, subacute, minimal-severe	20/60	20/60	15/60	20/60	28/60	24/60
-exudate, minimal-severe	9/60	6/60	13/60	17/60	17/60	12/60
-metaplasia, squamous, minimal-mild	0/60	0/60	0/60	3/60	3/60	2/60
-foreign body, minimal-mild	0/60	1/60	2/60	0/60	1/60	3/60
Nasal Level 2						
-foreign body, minimal-mild	0/60	1/60	4/60	1/60	3/60	4/60
-metaplasia, squamous, minimal-mild	0/60	0/60	0/60	2/60	1/60	1/60
Nasal Level 3						
-exudate, minimal-severe	5/60	0/60	6/60	4/60	8/60	8/60
-foreign body, minimal-mild	0/60	1/60	3/60	1/60	5/60	5/60
Nasal Level 4						
-inflammation, subacute, minimal-moderate	2/59	1/60	0/60	2/60	5/60	2/60
-metaplasia, minimal	0/59	0/60	0/60	0/60	1/60	0/60
-foreign body, minimal-mild	1/59	1/60	4/60	2/60	3/60	3/60
Nasal Level 5						
-foreign body, minimal-mild	1/59	1/60	3/60	0/60	4/60	1/60
-inflammation, subacute, minimal-	0/59	0/60	0/60	1/60	1/60	1/60

mild						

a. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91.
b. All remaining females in the 100 µg/kg/day group were sacrificed during week 92.
c. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101.

Non-neoplastic findings for all male rats from the control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups.

Organ/Tissue	0-1[a]	0-2[b]	40[b]	100[b]	200[b]	400[a]
Spleen						
-pigment, hemosiderin, minimal-moderate	4/60	4/60	7/60	6/60	13/60	7/60
-depletion, lymphoid, mild-severe	1/60	2/60	4/60	5/60	6/60	6/60
-fibrosis, capsular, minimal-mild	0/60	1/60	0/60	0/60	1/60	3/60
Lungs						
-congestion, minimal-severe	2/59	5/59	5/60	4/60	5/58	8/60
-macrophages, alveolar, minimal-severe	31/60	2959	38/60	44/60	36/58	39/60
-edema, minimal-severe	1/60	2/59	3/60	3/60	6/58	5/60
Seminal vesicles						
-increased secretion, minimal-severe	0/60	0/60	2/60	3/60	4/60	3/60
Mesenteric LN						
-hemorrhage, minimal-mild	1/60	1/60	6/60	4/60	5/60	4/60
Medullary LN						
-pigment, brown, minimal-moderate	4/57	0/56	4/56	2/58	7/51	0/58
-inflammation, granulomatous, minimal-moderate	0/57	0/56	1/56	1/58	2/51	1/58
Lacrimal gland, exorbital						
-alteration, Harderian gland, minimal-severe	25/60	31/59	23/60	32/60	37/59	23/60
Thymus						
-hemorrhage, minimal-moderate	1/56	1/56	0/59	0/56	1/55	3/56
Nasal Level 3						
-degeneration, olfactory epithelium, minimal-mild	0/60	0/60	0/60	0/60	1/60	5/60
Nasal Level 5						
-metaplasia, mild-severe	0/60	0/59	0/60	0/60	0/60	3/60

a. The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92.
b. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks.

Neoplastic: There were potential treatment-related tumor findings in the thyroid gland, thoracic soft tissue, skin, and uterus + cervix as described below.

Thyroid gland C-cell adenoma and carcinoma: For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. It should be noted that surviving females in control-1 and 400 µg/kg/day groups were sacrificed in week 91, surviving females in the 100 µg/kg/day group were sacrificed in week 92, and surviving females in other groups were sacrificed in week 101. The combined incidence of c-cell adenoma and carcinoma for females at 100 and 200 µg/kg/day exceeded mean incidences from the historical control background data of the testing laboratory. Statistical significance was achieved when comparing control-1, control-2, 40 µg/kg/day, 100 µg/kg/day, and 200 µg/kg/day groups (P-value = 0.0010, Exact method) by trend analysis. Significant increases were observed at 200 µg/kg/day using pairwise comparison. However, statistical significance was not achieved when comparing control-2, 40 µg/kg/day, and 200 µg/kg/day groups by trend analysis (P-value = 0.0097, Exact method). Historical control data indicates that thyroid c-cell adenoma and carcinoma are relatively common tumors for Sprague-Dawley rats.

Incidences of C-cell adenoma, C-cell carcinoma, and C-cell hyperplasia in the thyroid gland for all female rats from control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups.

Findings in the thyroid gland	Females						
	0-1[a]	0-2[c]	40[c]	100[b]	200[c]	400[a]	Trend P-value (Exact method)
N =	60	60	60	60	60	60	
C cell adenoma	2	3	2	9	9	5	
C cell adenoma, multiple	0	1	1	0	1	0	
C cell carcinoma	0	0	0	0	2	0	
C cell adenoma + carcinoma	2 (3.3%)	4 (6.7%)	3 (5%)	9 (15%)	11 (18.3%)	5 (8.3%)	0.0010[d]
Pairwise comparison, P-value (Exact method)[e]			0.6041	0.0147	0.0054		
C cell hyperplasia	17	10	10	14	11	13	
C cell adenoma, carcinoma, and hyperplasia	19	13	12	21	21	17	

a. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91.
b. All remaining females in the 100 µg/kg/day group were sacrificed during week 92.
c. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101.
d. P-value = 0.0010 (Exact method) was obtained by comparing control-1, control-2, 40 µg/kg/day, 100 µg/kg/day, and 200 µg/kg/day groups. A p-value cutoff of 0.005 was used.
e. For pairwise comparison, statistical significance was achieved at p values <0.01 for common tumors.

Historical control incidence of thyroid gland C-cell adenoma and C-cell carcinoma for female Sprague-Dawley rats

Thyroid gland	Test laboratory	March 2004
C-cell adenoma	5.13%	7.21% (2.86-16.67%)
C-cell carcinoma	1.28%	0.85% (0.56-11.43%)
C-cell adenoma + carcinoma	6.41%	8.06%

b(4)

It is suggested that there is a progression of proliferation changes of thyroid C-cells (parafollicular cells) from hyperplasia (non-neoplastic) to adenoma (benign) and potentially to carcinoma. C-cell hyperplasia is characterized by a diffuse or focal increase in the number of morphologically normal C-cells. C-cells are located within the basal lamina of thyroid follicles and compression or distortion of follicular architecture by the proliferating cells is observed for larger focal (nodular) lesions. C-cell adenoma is a benign neoplasm that is defined as a usually well demarcated proliferation of C-cells greater in size than the diameter of 5 contiguous thyroid follicles. Since the size and shape of thyroid follicles can vary considerably, the diagnostic criterion used to differentiate C-cell adenoma from hyperplasia is arbitrary. C-cell carcinoma is a malignant tumor consisting of solid to irregular groups of neoplastic cells that may closely resemble those of the adenoma. The tumor may involve the entire lobe of the thyroid and/or extend to the margin of the lobe.

Thyroid C-cells produce and secrete calcitonin, which acts in concert with parathyroid hormone to maintain the concentration of calcium in extracellular fluids within narrow limits. In the presence of excess calcitonin, blood calcium along with phosphorus are reduced. Calcitonin has been proposed as a biomarker for thyroid C-cell proliferative lesions in humans and rats. In rats, the C-cell population of the thyroid progressively increases with age. Incidence rates for both C-cell neoplasms and C-cell hyperplasia have positive correlation with age in the rat. Life-long food restriction diminishes age-related increases of calcitonin levels. The β-adrenergic agonist activity of (R,R)-formoterol as well as dietary effects of long-term treatment could be involved in the development of C-cell adenomas in rats. β-adrenergic agonists produce hypercalcemia in thyroid-parathyroidectomized rats and calcitonin treatment counteracts this increased. β-adrenergic agonists have been shown to affect calcium homeostasis with increases in serum calcitonin, while β-adrenergic antagonists have the opposite effect.

Thoracic soft tissue liposarcoma: For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 100 and 200 µg/kg/day group as assessed by trend analysis. No statistical differences were found using pairwise comparison. There were no findings in the 400 µg/kg/day group, which was terminated early and therefore excluded from the statistical analysis.

Incidence of thoracic soft tissue liposarcoma for all male rats from the control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups.

Organ/Tissue	0-1[a]	0-2[b]	40[b]	100[b]	200[b]	400[a]	Trend P-value (Asymptotic method)
Soft tissue – Thorax -Number examined -M liposarcoma	1 (60) 0	1 (60) 0	0 (60) 0	2 (60) 1 (1.7%)	2 (60) 2 (3.3%)	0 (60) 0	0.0237[c] *0.0085*[d]
Pairwise comparison, P-value (Exact method)				0.4815 *0.3238*	0.2179 *0.0959*		

a. The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92.
b. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks.
c. The dose-tumor linear trend in male rats was statistically significant for malignant liposarcoma in thoracic soft tissue with a p-value of 0.0237 (Control-2, and 40, 100, and 200 mg/kg/day). See associated pairwise comparison p-values in regular text.
d. The dose-tumor linear trend in male rats was statistically significant for malignant liposarcoma in thoracic soft tissue with a p-value of 0.0085 (Control-1, Control-2, and 40, 100, and 200 mg/kg/day). See associated pairwise comparison p-values in *italic text*.

Skin fibroma and fibrosarcoma: For the skin, the combined incidence of fibroma and fibrosarcoma were significantly increased for females in the 400 µg/kg/day group by trend analysis. However, statistical significance was not achieved with pairwise comparison. The incidence of fibroma + fibrosarcoma at 400 µg/kg/day exceeded mean incidences of the historical control data, but may have been within upper ranges. Incidences of skin fibroma and fibrosarcoma from [redacted] (March 2004) were 0.60% (0.91-4.29%) and 0.34% (1.43-2.00%), respectively b(4)

Incidence of skin fibroma, fibrosarcoma, and fibroma + fibrosarcoma for all female rats from the control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups (B = benign, M = malignant, and S = metastatic).

Organ/Tissue	0-1[a]	0-2[c]	40[c]	100[b]	200[c]	400[a]	Trend P-value (Asymptotic method)
Skin							
-B fibroma	0/60	1/60	0/60	1/59	1/60	2/60	
-M fibrosarcoma	0/60	0/60	1/60	0/59	0/60	1/60	
-fibroma + fibrosarcoma	0/60	1/60 (1.7%)	1/60 (1.7%)	1/59 (1.7%)	1/60 (1.7%)	3/60 (5%)	0.0219[d]
Pairwise comparison, P-value (exact method)[e]			0.5210	0.5321	0.5578	0.0730	

a. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91.
b. All remaining females in the 100 µg/kg/day group were sacrificed during week 92.
c. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101.
d. P value = 0.0219 (Asymptotic method) was obtained by comparing Control-1, Control-2, 40 µg/kg/day, 100 µg/kg/day, 200 µg/kg/day, and 400 µg/kg/day groups. A p-value cutoff of 0.025 was used.
e. For pairwise comparison, statistical significance for common and rare tumors was achieved at 0.05 and 0.01 for, respectively.

Endometrial stromal polyps in the uterus, cervix, and uterus + cervix: For the uterus and cervix combined, the incidence of benign endometrial stromal polyps was increased for females in the 400 µg/kg/day group, although this was not statistically significant. The incidence at 400 µg/kg/day exceeded the mean incidence of the historical control data; however it was within the upper range. The historical control incidence of endometrial stromal polyps in the uterus from ⊏ ⊐ (March 2004) is 2.99% (0.91-11.67%). b(4)

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Incidences of endometrial stromal polyps in the uterus, cervix, and uterus + cervix for all female rats from the control groups 1 and 2 and the 40, 100, 200, and 400 µg/kg/day groups (B = benign, M = malignant, and S = metastatic).

Organ/Tissue	Females						
	0-1[a]	0-2[c]	40[c]	100[b]	200[c]	400[a]	P-value (Exact method)
Uterus -B polyp, endometrial stromal	2/60	3/60	1/60	4/60	3/60	5/60	
Cervix -B polyp, endometrial stromal	0/60	0/59	0/60	0/60	0/60	1/60	
Uterus + Cervix -B polyp, endometrial stromal	2/60 (3.3%)	3/60 (5%)	1/60 (1.7%)	4/60 (6.7%)	3/60 (5%)	6/60 (10%)	0.0274[d]

a. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91.
b. All remaining females in the 100 µg/kg/day group were sacrificed during week 92.
c. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101.
d. P-value = 0.0274 (Exact method) was obtained by comparing Control-1, Control-2, 40 µg/kg/day, 100 µg/kg/day, 200 µg/kg/day, and 400 µg/kg/day groups. A p-value cutoff of 0.005 was used.

In the 2-year mouse carcinogenicity study with R,R-formoterol, there were statistically significant increased incidences of endometrial stromal polyps in the uterus and cervix.

Toxicokinetics: For males and females at 12 and 18 months, C_{max} and AUC values for (R,R)-formoterol increased with elevating dose. For males at 12 months, C_{max} and AUC values increased in an approximate dose proportional manner. For males at 18 months, C_{max} and AUC values with doses from 40 to 200 µg/kg/day generally increased in a less than dose proportional manner; however, increases of these values from 200 to 400 µg/kg/day were significantly greater than dose proportional (possibly suggestive of saturation). For females at 12 and 18 months, C_{max} values increased in a dose proportional manner; however, AUC values generally increased in a slightly less than dose proportional manner. No sex-related differences of C_{max} and AUC values were observed with doses from 40 to 200 µg/kg/day; however, at 400 µg/kg/day, C_{max} and AUC values were significantly greater for males. C_{max} and AUC values with doses from 40 to 200 µg/kg/day were generally comparable at 12 and 18 months; however, for the dose of 400 µg/kg/day, these values at 18 months were significantly greater than values at 12 months. Systemic exposures for male and female rats at doses of 40, 100, 200, and 400 µg/kg/day ranged from 35.9 to 981.2-fold greater than clinical exposure at the proposed therapeutic dose.

Toxicokinetic parameters for (R,R)-formoterol in male rats

Dose µg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
40	12	0.5	897	4330	**62.8**
	18	1.0	359	2480	**35.9**
100	12	0.5	1430	6710	**97.3**
	18	0.5	2500	7660	**111.0**
200	12	0.5	2950	12200	**176.8**
	18	0.5	2660	11600	**168.1**
400	12	0.5	6820	24900	**360.9**
	18	0.5	11200	67700	**981.2**

a. From Clinical Study 091-026, human exposure (AUC) was 69 pg·hr/mL after 2 weeks of inhalation treatment of 15 µg BID arformoterol in COPD patients.

Toxicokinetic parameters for (R,R)-formoterol in female rats

Dose µg/kg/day	Time point months	T_{max} hr	C_{max} pg/mL	$AUC_{0.5\text{-}24hr}$ pg·hr/mL	Exposure margin[a]
40	12	0.5	591	4130	**59.9**
	18	0.5	501	3830	**55.5**
100	12	0.5	1650	6680	**96.8**
	18	0.5	1330	5870	**85.1**
200	12	0.5	2410	9790	**141.9**
	18	0.5	2140	8740	**126.7**
400	12	0.5	4450	15800	**229**
	18	0.5	4920	22900	**331.9**

a. From Clinical Study 091-026, human exposure (AUC) was 69 pg·hr/mL after 2 weeks of inhalation treatment of 15 µg BID arformoterol in COPD patients.

Histopathology inventory (optional)

Study	2-year carcinogenicity study
Species	Rats
Adrenals	X*
Aorta	X
Bone Marrow smear	X (Not evaluated)
Bone (femur)	
Brain	X*
Cecum	X
Cervix	X (w/uterus and vagina)
Clitoral gland	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X (w/optic nerve)
Fallopian tube	
Gall bladder	
Gross lesions	X (including masses)
Harderian gland	X

Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X
Lachrymal gland	X
Larynx	X
Liver	X*
Lungs	X*
Lymph nodes, bronchial	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mediastinal	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	X (6 cross sections)
Optic nerves	
Ovaries	X* (w/oviducts)
Pancreas	X
Parathyroid	X (w/thyroid)
Peripheral nerve	
Pharynx	X
Pituitary	X
Preputial gland	X
Prostate	X
Rectum	X
Salivary gland	X (submandibular)
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X (inguinal)
Spinal cord	X
Spleen	X*
Sternum	X (w/marrow)
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X (w/parathyroid)
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X* (w/cervix and vagina)
Vagina	X (w/uterus and cervix)
Zymbal gland	X

X, histopathology performed
*, organ weight obtained

2.6.6.9 Discussion and Conclusions

Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was also achieved at this dose.

incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day exceeded mean incidences from the historical control background data of the testing laboratory. Increases at 100 and 200 µg/kg/day were significant using trend analysis. However, only the increase at 200 µg/kg/day was statistically significant by pairwise comparison. It

Systemic exposure at 18 month in rats that received 40 µg/kg/day R,R-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times systemic exposure with a clinical dose of 15 µg BID.

2.6.6.10 Tables and Figures

Two-year carcinogenicity study with rats that received R,R-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day. It should be noted that the Control-1 and 400 µg/kg/day groups for males and females as well as the female 100 µg/kg/day group were terminated early.

Tumor Finding	Evaluation
Thyroid gland: increased incidence of C cell adenoma and carcinoma in females at 100 and 200 µg/kg/day	Statistically significant by trend test and pairwise comparison and exceeded spontaneous incidences from the testing laboratory
Thoracic soft tissue: increased incidence of liposarcoma in males at 100 and 200 µg/kg/day	Statistically significant by trend test, but not by pairwise comparison
Skin: increased incidences of fibroma and fibrosarcoma in females at 400 µg/kg/day	Statistically significant by trend test, but not by pairwise comparison
Uterus and Cervix: increased incidence of endometrial stromal polyps for females in the 400 µg/kg/day group	Not statistically significant and within the upper range of the historical control. This tumor type was statistically significant in the mouse carcinogenicity study.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

Based upon conclusions and recommendations from the ECAC meeting dated May 10, 2005, the sponsor requested that study pathologists examine all remaining (unexamined) tissues for the animals from the 40, 100, and 200 µg/kg/day groups (i.e., animals from these groups that survived the terminal primary necropsy). To achieve valid comparisons with control tissues that were examined previously, it was necessary to re-establish diagnostic criteria, including grading criteria and diagnostic thresholds or

baselines. Microscopic slides of all relevant tissues from control groups 1 and 2 that corresponded to the additional tissues to be examined in the 40, 100, and 200 µg/kg/day groups were reviewed contemporaneously, together with the originally recorded data for those tissues. This data was provided in the present submission.

Rats received (R,R)-formoterol at inhaled doses of 0, 40, 100, 200, and 400 µg/kg/day for periods up to 104 weeks. The sponsor did not have ECAC concurrence for dose selection.

There was a statistically significant decrease in the survival rate for male rats in the 400 µg/kg/day group. Trend analysis indicated no treatment-related effects on survival for female (R,R)-formoterol groups. The sponsor sacrificed all surviving males in control group 1 and the 400 µg/kg/day during weeks 91 and 92. All surviving females in control group 1 and the 400 µg/kg/day group were sacrificed during weeks 90 and 91, and all remaining females in the 100 µg/kg/day group were sacrificed during week 92. The remaining females in control group 2 and the 40 and 200 µg/kg/day groups were sacrificed during weeks 100 and 101. The males in control group 2 and the 40, 100, and 200 µg/kg/day groups were exposed for 104 weeks. The sponsor did not contact the Division prior to early termination of groups during weeks 90-92.

Absolute body weight was decreased for male rats in the 400 µg/kg/day. Absolute body weight for female rats in the 400 µg/kg/day was unaffected through week 89. Decreases (~10%) of absolute body weight were observed for male and female rats in the 200 µg/kg/day group toward the end of the treatment period. The approximate 10% decrease of absolute body weight for males and females in the 200 µg/kg/day group suggests a maximum tolerated dose was also achieved at this dose.

Non-neoplastic findings were observed in the ovaries, oviducts, spleen, lung, heart, mesenteric lymph nodes, parathyroid gland, nasal cavities, and exorbital lacrimal gland. The most notable findings were increased incidences of cyst(s) in the ovaries and oviducts for female treatment groups.

For the thyroid gland, combined incidences of c-cell adenoma and carcinoma were increased for females in the 100 and 200 µg/kg/day groups as compared to controls. The combined incidence of c-cell adenoma and carcinoma for females at 200 µg/kg/day exceeded mean incidences from the historical control background data of the testing laboratory. Increases at 100 and 200 µg/kg/day were significant using trend analysis. However, only the increase at 200 µg/kg/day was statistically significant by pairwise comparison.

For the soft tissue of the thorax, the incidences of malignant liposarcoma were significantly increased for males in the 100 and 200 µg/kg/day group when the high dose group was excluded (Trend analysis). However, there were no statistically significant increases using pairwise comparison. There were no findings in the 400 µg/kg/day group, which was terminated early.

For the skin, the combined incidence of fibroma and fibrosarcoma were significantly increased for females in the 400 µg/kg/day group by trend analysis; however, this was not statistically significant by pairwise comparison. The incidence of fibroma + fibrosarcoma at 400 µg/kg/day exceeded mean incidences of the historical control data, but may have been within upper ranges.

For the uterus and cervix combined, the incidence of benign endometrial stromal polyps was increased for females in the 400 µg/kg/day group, although this was not statistically significant. The incidence at 400 µg/kg/day exceeded the mean incidence of the historical control data; however it was within the upper range. In the 2-year mouse carcinogenicity study with R,R-formoterol, there were statistically significant increased incidences of endometrial stromal polyps in the uterus and cervix.

Based upon increased incidences of thyroid C-cell adenoma and carcinoma in female treatment groups, R,R-formoterol is tumorigenic in rats.

Systemic exposure at 18 month in rats that received 40 µg/kg/day R,R-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times systemic exposure with a clinical dose of 15 µg BID.

Recommendations: Based upon increased incidences of thyroid C-cell adenoma and carcinoma in female treatment groups, R,R-formoterol is tumorigenic in rats. Systemic exposure at 18 month in rats that received 40 µg/kg/day R,R-formoterol, where there were no treatment-related tumor findings, was approximately 35.9 to 55.5 times systemic exposure with a clinical dose of 15 µg BID.

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

Supervisor signature: Concurrence - _____
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8/3/2006 11:46:20 AM
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Joseph Sun
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I concur.

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Timothy Robison
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Joseph Sun
6/7/2006 10:32:12 AM
PHARMACOLOGIST
I concur.

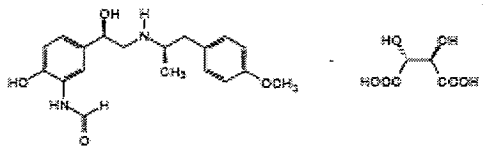
PHARMACOLOGY/TOXICOLOGY REVIEW
Chemistry Consult # 1

NDA number: NDA 21-912
Date/type of submission: December 12, 2005/#000
Request date: June 14, 2006
Sponsor and/or agent: Sepracor, Inc.
84 Waterford Drive
Marlborough, MA 01752
Manufacturer for drug substance: Same

Reviewer name: Timothy W. Robison, Ph.D., D.A.B.T.
Division name: Pulmonary and Allergy Products
HFD #: 570
Review completion date: August 3, 2006

Drug:

Trade name:
Generic name: Arformoterol Tartrate Inhalation Solution
Code name: Arformoterol, (R,R)-Formoterol-L-tartrate
Chemical name: (R,R)-(-)-N-[2-hydroxy-5-[1-hydroxy-2-[[2-(4-methoxyphenyl)-1-methylethyl]amino]ethyl]phenyl]formamide-(R,R)-2,3-dihydroxybutanedioate (1:1 salt)
CAS registry number:
Molecular formula/molecular weight: $C_{23}H_{30}N_2O_{10}$ / MW 494.5
Structure:



Response to Chemistry Consult Requested by Chien Hua Niu, Ph.D. and Prasad Peri, Ph.D.

Description of the Consult: This consult request is for a safety assessment of drug substance impurity, desformoterol (proposed level of NMT — % w/w in the drug substance), drug product impurity limits (all impurities), and levels of extractables ⊏ ⊐ leachables in the drug product. The leachables were identified as to be ⊏ ⊐ ⊏ ⊐ as indicated in the 4/18/06 amendment. ⊏ ⊐ impurities and degradants of (R,R)-formoterol were noted to contain a structural alert similar or identical to that found in ⊏ ⊐ See the Request for Chemistry Consult attached in the Appendix for further details.

b(4)

9 Page(s) Withheld

✓ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-2

Appendix: Chemistry Consult dated June 14, 2006

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE FOOD AND DRUG ADMINISTRATION			REQUEST FOR CONSULTATION	
TO: *(Division/Office)* Dr. Tim Robison Pharmacology			FROM: Chien Hua Niu/ Prasad Peri--HFD-570	
DATE: 06/14/2006	IND NO.:	NDA NO.: NDA21-912	TYPE OF DOCUMENT: Original NDA	DATE OF DOCUMENT: 12/27/04
NAME OF DRUG Brovana Inhalation Solution		PRIORITY CONSIDERATION: 3	CLASSIFICATION OF DRUG: S	DESIRED COMPLETION DATE: 7/13/06
NAME OF APPLICANT: Sepracor Inc.				

REASON FOR REQUEST

I. GENERAL

☐ NEW PROTOCOL	☐ PRE-NDA MEETING	☐ REPONSE TO DEFICIENCY LETTER
☐ PROGRESS REPORT	☐ END OF PHASE II MEETING	☐ FINAL PRINTED LABELING
☐ NEW CORRESPONDENCE	☐ RESUBMISSION	☐ LABELING REVISION
☐ DRUG ADVERTISING	☐ SAFETY/EFFICACY	☐ ORIGINAL NEW CORRESPONDENCE
☐ ADVERSE REACTION REPORT	☐ PAPER NDA	☐ FORMULATIVE REVIEW
☐ MANUFACTURING CHANGE/ADDITION	☐ CONTROL SUPPLEMENT	☑ OTHER *(Specify below)*
☐ MEETING PLANNED BY _____		

II. BIOMETRICS

STATISTICAL EVALUATION BRANCH	STATISTICAL APPLICATION BRANCH
☐ TYPE A OR B NDA REVIEW	☐ CHEMISTRY
☐ END OF PHASE II MEETING	☐ PHARMACOLOGY
☐ CONTROLLED STUDIES	☐ BIOPHARMACEUTICS
☐ PROTOCOL REVIEW	☐ OTHER
☐ OTHER	

III. BIOPHARMACEUTICS

☐ DISSOLUTION	☐ DEFICIENCY LETTER RESPONSE
☐ BIOAVAILABILITY STUDIES	☐ PROTOCOL-BIOPHARMACEUTICS
☐ PHASE IV STUDIES	☐ *IN-VIVO* WAIVER REQUEST

IV. DRUG EXPERIENCE

☐ PHASE IV SURVEILLANCE/EPIDEMIOLOGY PROTOCOL	☐ SUMMARY OF ADVERSE EXPERIENCE
☐ DRUG USE e.g. POPULATION EXPOSURE, ASSOCIATED DIAGNOSES	☐ POISON RISK ANALYSIS
☐ COMPARATIVE RISK ASSESSMENT ON GENERIC DRUG GROUP	☐ CASE REPORTS OF SPECIFIC REACTIONS *(List below)*
☐ REVIEW OF MARKETING EXPERIENCE, DRUG USE AND SAFETY	

V. SCIENTIFIC INVESTIGATIONS

☐ CLINICAL	☐ PRECLINICAL

COMMENTS/SPECIAL INSTRUCTIONS *(Attach additional sheets if necessary)*: Please evaluate impurity levels permitted by the following specifications for excipients in the drug product:

This pharm/tox consult is for evaluating the safety and risk assessment of Drug Substance impurity (Desformoterol: proposed level of NMT [redacted] in DS), Drug Product impurity limits (all impurities), levels of Extractables [redacted] and Leachables in the Drug Product. See NDA dated 03-JAN-2006 for data.

The leachables are identified as to be [redacted] as indicated in the 4/18/06 amendment. See tables listing extractables and leachables Drug substance impurities limits and Drug Product impurities. Reference is made to some structural alert compounds.

b(4)

SIGNATURE OF REQUESTER	METHOD OF DELIVERY *Check one)* ☐ MAIL ☑ HAND
SIGNATURE OF RECEIVER	SIGNATURE OF DELIVERER

cc: Orig. NDA21-912 HFD-570/Div. File/PPeri/JafariL

4 Page(s) Withheld

X Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Pharm/Tox-3

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/s/

Blair Fraser
6/14/2006 11:52:35 AM

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/s/

Timothy Robison
8/3/2006 02:44:04 PM
PHARMACOLOGIST .

Joseph Sun
8/3/2006 02:48:57 PM
PHARMACOLOGIST
I concur.

NDA Pharmacology Fileability Check List

NDA No: 21-912

Date of submission: December 12, 2005

Date of Fileability meeting: February 3, 2006

Information to Sponsor Yes ( ) No (X)

Date of check list: February 3, 2006

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

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

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

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

(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 ( ) NA (X)

If no, state why not?

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 ( ) No ( ) NA (X)

(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 (X) No ( ) NA ( )

(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 ( ) No (X)

NDA Planning Timeline

NDA No.: 21-912

Date of planning timeline:

PDUFA Due Date: October 12, 2006

Projected review completion date: August 12, 2006

	Milestone Dates
Pharmacology and ADME	July 12, 2006
Toxicology	
General toxicity studies	Completed
Carcinogenicity studies and mutagenicity studies	July 12, 2006
a. Statistical consult request for CA studies	May 12, 2006
b. Submission of CA studies for CAC concurrence	June 12, 2006
Reproductive studies	July 12, 2006
Special studies and Others	July 12, 2006
Labeling	August 12, 2006

Signatures (optional):

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

Supervisor Signature _____
C. Joseph Sun, Ph.D.

Concurrence Yes ___ **No** ___

cc:
NDA 21-912, HFD-570 Division Files
JafariL, HFD-570
DurmwiczA, HFD-570
SunC, HFD-570
RobisonT, HFD-570

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/s/

Timothy Robison
2/3/2006 04:40:39 PM
PHARMACOLOGIST

Joseph Sun
2/6/2006 12:51:44 PM
PHARMACOLOGIST
I concur.