Table 3. Inhibition of Inflammatory Cell Functions by Budesonide

Model	Trigger	Result	Reference
PBM expression of HLA-DR	allergen	inhibition at 10^{-8} M	20
AMØ expression of HLA-DR	---	inhibition at 10^{-8} M	30
neutral endopeptidase expression in transformed human epithelial cells	---	up regulation at 10^{-7} M	31
neutral endopeptidase expression in epithelial cell line	---	no change	32
T-cell proliferation of PBMs	Con A, allergen, anti-CD3-Ab	inhibition at 10^{-9}-10^{-7} M	19, 20, 33
T-cell proliferation of PBLs	PHA, anti-CD3-Ab, anti-CD2 + CD28-Ab	inhibition at 10^{-11}-10^{-7} M	24, 34
NK cell cytotoxicity	---	inhibition	35
Eosinophil survival	conditioned medium of nasal polyps	inhibition at 10^{-8} M	36
Differentiation of eosinophil progenitor basophils	GM-CSF	inhibition at 10^{-9} M	37
Eosinophil respiratory burst	Ab to integrins	inhibition at 10^{-8} M	38
Eosinophil locomotion	IL-3, IL-5	inhibition at 10^{-9} M	21
ICAM-1 expression in bronchial epithelial cells	TNFα	inhibition at 10^{-6} M	39
ICAM-1 expression in bronchial epithelial cell line	IFNγ	inhibition at 10^{-8} M	40
E-selectin, ICAM-1, VCAM-1 expression on umbilical vein endothelial cells	IL-1β, TNFα, IL-4, FMLP, PAF	inhibition at 10^{-7} M	41
Secretion of RANTES and MCP-4 by bronchial cell line	TNFα	inhibition at 10^{-9} M	42, 43
Release of eosinophil chemotactic factors by PBMs	allergen	inhibition at 10^{-8} M	21
PBM cytotoxicity on fibroblasts	PMA	inhibition at 10^{-9} M	44
PBN cytotoxicity on fibroblasts	PMA	inhibition at 10^{-8} M	44
Induction of myofibroblast phenotype in fibroblast cell line	TGF-β1	inhibition at 10^{-8} M	45
Induction of mast cell growth factor in human lung fibroblasts	---	inhibition at 10^{-7} M	46

Abbreviations: PBM = peripheral blood monocytes; PBN = peripheral blood neutrophils; AMØ = alveolar macrophages; PBL = peripheral blood lymphocytes; LPS = lipopolysaccharide; Ab = antibody; PHA = phytohemagglutinin, PMA = phorbol myristate acetate. Reference indicates submission numbers listed on pp 3-8 of this review.

In Vivo Studies

Effects of budesonide in animal inflammation models are summarized in table 4, below.

Table 4. Effects of Inhaled or Intratracheal Budesonide in *In Vivo* Efficacy Models

Model	Trigger	Result	Ref.*
Sensitized guinea pig tracheal plasma exudation	allergen or toluene diisocyanate	inhibits late phase exudation	60
Rat tracheal plasma exudation	bradykinin, PAF	inhibits exudation	49,62
Rat nasal plasma exudation	capsaicin, substance P	inhibits exudation	61
TNFα release from rat airways or AMØ ex vivo	LPS	inhibits TNFα release	2,66
Bronchopulmonary inflammation in rat	Sephadex beads	inhibits pulmonary edema and endothelin accumulation	63-65
Neutrophil-rich pulmonary inflammation in rat or guinea pig	Endotoxin	inhibits neutrophil influx, lung injury and blood leukopenia	67,68
Lung function and inflammatory cell influx in dogs	ozone	inhibits ↑ airway resistance and inflammatory cell influx but not AHR	54,55
Allergic rat	allergen	inhibits LAR, inflammatory cell influx and LTE_4 excretion	59
Allergic guinea pig	allergen	inhibits AHR	50
Allergic rabbit	allergen	inhibits AHR and inflammatory cell influx	51
Allergic pig	allergen	inhibits LAR and LTE4 excretion	56
Allergic dog	allergen	inhibits AHR, eosinophil influx, and eosinophil precursors in bone marrow	52,53
Allergic sheep	allergen	inhibits LAR and AHR	57
Regrowth of airway epithelium	Mechanical damage	no inhibition	69

Abbreviations: PAF = platelet activating factor; LAR = late airway response; AHR = airway hyperreactivity; LPS = lipopolysaccharide; AMØ = alveolar macrophage. * Reference indicates submission numbers listed on pp 3-8 of this review.

Budesonide inhibited tracheal and nasal plasma exudation in response to challenge with antigen or other triggers such as bradykinin, capsaicin, substance-P, or PAF. Budesonide inhibited LPS-stimulated TNFα release from rat airways or macrophages. Endotoxin-mediated neutrophil influx, lung injury and leukopenia were inhibited by budesonide in rats or guinea pigs. Increased airway resistance and inflammatory cell influx after ozone challenge in dogs was inhibited by budesonide. Budesonide was studied in allergen challenge models in sensitized rats, guinea pigs, rabbits, dogs, sheep and pigs. In these models budesonide inhibited late airway responses, airway hyperreactivity, inflammatory cell influx and leukotriene (LTE_4) excretion. The sponsor calculated the effective dose of budesonide in the *in vivo* models as lung deposited fraction in µg/kg. Effective dose ranged from 2-40 µg/kg with a median of 10 µg/kg. Despite the potential for corticosteroids to delay healing, budesonide did not inhibit regrowth of airway epithelium denuded by mechanical injury.

Summary of Pharmacology

Budesonide is a potent glucocorticoid consisting of two stereoisomers. The R-isomer is more potent than the S-isomer. The racemic budesonide mixture is more potent than dexamethasone and less potent than fluticasone propionate. For lipophilic glucocorticoids like budesonide, the topical pharmacological potency (e.g. skin blanching in humans and inhibition of ear edema in rats) is proportional to glucocorticoid binding affinity. Consistent with its glucocorticoid activity, budesonide inhibits a variety of inflammatory processes *in vitro*. Budesonide inhibited production of cytokines, growth factors, or chemotactic factors by monocytes, macrophages, lymphocytes, or respiratory epithelium. Budesonide also inhibited activation, proliferation, cytotoxicity, survival or locomotion of monocytes, macrophages, lymphocytes, or eosinophils. These effects occurred at physiologically relevant concentrations between 10^{-9} M and 10^{-7} M. Any of these might contribute to anti-inflammatory efficacy *in vivo*, but the relative importance of the possible mechanisms is unknown. Inhaled or intratracheal budesonide was also effective in animal models of airway inflammation mediated by allergen challenge or by other triggers *in vivo*. Inflammatory activities inhibited by budesonide include: plasma exudation, mediator release (TNFα, endothelin, leukotrienes), lung injury, cell influx, late airway responses, and airway hyperreactivity. These *in vivo* effects occurred at estimated lung deposited doses of 2-40 µg/kg. Similar to known clinical effects of inhaled corticosteroids, budesonide was more effective at inhibiting the delayed manifestations of allergen challenge (e.g. eosinophil influx, late airway responses, airway hyperreactivity) than preventing the immediate anaphylactic response.

PHARMACOKINETICS/TOXICOKINETICS

New pharmacokinetic studies and literature references are reviewed here. The pharmacokinetic profile established in previous submissions is summarized in the Overall Summary and Evaluation.

PK PARAMETERS:

Pharmacokinetic Study of Budesonide. Budesonide Concentration in Plasma.
Astra Draco Report # 850-RD-0390, volume 16, page 42

Methods:
Plasma budesonide was measured in male Sprague-Dawley rats, ~7 weeks of age weighing 227 - 299 g. ^{14}C-Budesonide (batch CFQ8905), 97% purity by HPLC, was synthesized at [redacted] Specific radioactivity was 1.96 Gbq/mmol; the fraction of unlabelled budesonide was 14.9% by [redacted] Budesonide chemical assay was by [redacted] with a lower limit of quantitation of [redacted] when 1 mL of plasma was analyzed. ^{14}C-Budesonide dissolved at 100 µg mL in ethanol/saline (1/9) was used for intravenous and subcutaneous administration. ^{14}C-Budesonide dissolved at 250 µg/mL in ethanol/saline (1/9) plus 0.9% Tween 80 was used for intratracheal (i.t.) instillation. A dose of 100 µg/kg was

administered to different groups by the i.v. (n = 3 per time point), s.c. (n = 3 per time point) or i.t. (n = 5 per time point) routes. Sampling times were 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hr after dosing. For intratracheal administration only, animals were anesthetized with pentobarbital. Reported values are for authentic budesonide, not metabolites.

Results:

Table 5. PK Parameters of Budesonide in Rats

Intravenous	Subcutaneous	Intratracheal
$AUC_{0-\infty}$ = 75.6 nmol·hr/L	$AUC_{0-\infty}$ = 83.6 nmol·hr/L	$AUC_{0-\infty}$ = 112.0 nmol·hr/L
t½ = 2.1 hr	t½ = 1.8 hr	t½ = 4.2 hr
clearance = 3.1 L/hr/kg	Cmax = 77.0 nM	Cmax = 80.7 nM
Vss = 3.7 L/kg	Tmax = 0.25 hr	Tmax = 0.08 hr
bioavailability = 100%	bioavailability = 111%	bioavailability = 148%
AUC B/R* = 38%	AUC B/R* = 42%	AUC B/R* = 43%

AUC B/R = plasma AUC for budesonide/plasma AUC for total radioactivity

Absorption was relatively rapid by either the s.c. or i.t. routes. Bioavailability by the s.c. route was slightly greater, and bioavailability by the intratracheal route was 50% greater than by the i.v. route. Plasma half life was similar by the i.v. and s.c. routes but ~2 times longer by the i.t. route. This is probably an artifact of the pentobarbital anesthesia which could compete for budesonide P450 metabolism and could decrease hepatic blood flow; these factors could also account for the greater bioavailability by the i.t. route. Although the rate of metabolism by the i.t. route was slower, the extent of metabolism was similar by all three routes as determined by the ratio of budesonide/total radioactivity.

Bioavailability of Inhaled Nebulised Suspension or Dry Powder Formulation of Budesonide in Intubated Beagle Dogs.

Astra Draco Report # 850-RD-0402, volume 16, page 121

Methods: Female beagle dogs approximately 1.5 years old weighing 11.8 - 13.3 kg were used in this study. Plasma levels of budesonide were compared after inhalation of dry powder, inhalation of nebulized aqueous suspension, or intravenous infusion on different days in the same 5 dogs. The dry powder was generated by a [redacted] particle size was [redacted] MMAD; the nebulizer suspension contained 0.5 mg budesonide and 0.2 mg polysorbate 80/mL, particle size was [redacted] MMAD; the intravenous solution contained 25 µg/mL budesonide in normal saline with 10% ethanol. Presented dose was calculated by a [redacted] system that simultaneously measured aerosol concentration ([redacted] method) and inhalation flow. Animals were anesthetized (Pentothal) and intubated during dosing. Doses were administered over 5-10 minutes. Blood samples were drawn before and at 0.25, 0.5, 1, 2, 4, 6, and 8 hours after dosing. Budesonide was assayed by a [redacted] method with a lower limit of detection of [redacted]

Results:

Table 6. PK Parameters of Budesonide in Dogs

Intravenous	Dry Powder	Nebulizer Suspension
Dose = 19 µg/kg	Dose = 40 µg/kg	Dose = 3 µg/kg
$AUC_{0-\infty}$ = 28.9 nmol·hr/L	$AUC_{0-\infty}$ = 33.7 nmol·hr/L	$AUC_{0-\infty}$ = 2.7 nmol·hr/L
AUC/dose = 1.52	AUC/dose = 0.84	AUC/dose = 0.90
t½ = 2.7 hr	t½ not reported	t½ not reported
clearance = 1.6 L/hr/kg	Cmax = 22.5 nM	Cmax = 2.8 nM
Vss = 2.1 L/kg	Tmax = 17 min	Tmax = 11 min
bioavailability = 100%	bioavailability = 55.5%	bioavailability = 59.6%

Systemic absorption was rapid after inhalation with peak plasma levels occurring between 9-33 minutes after dosing. Bioavailability was similar (~60%) for the two inhalation formulations. Budesonide was rapidly eliminated with a plasma t½ less than three hours after i.v. administration. Although the t½ was not reported after inhalation, the plasma concentration vs time curves for inhalation were parallel to that for i.v. administration.

TOXICOKINETIC SUMMARIES

Plasma levels from new toxicology studies are summarized in table 7, page 16. Plasma concentration time curves in young rats and dogs are shown in figure 2, page 16. Methods are described with the main studies in the Toxicology section. Plasma levels from the dog inhalation PK study above and from human PK studies are also shown for comparison. Peak plasma concentrations in adult rats and dogs were similar for comparable doses (e.g. $C_{max} \approx 20$ nM at ~ 40 µg/kg). AUC values were higher in adult dogs than in adult rats at comparable doses. In adult rats there were no obvious differences between males and females and no significant accumulation of budesonide after repeated administration. In immature rats C_{max} and AUC values were consistently lower in females than in males, particularly at day 22. C_{max} did not change consistently from day 1 to 22 in immature rats; in young males C_{max} was slightly higher at day 22, in young females C_{max} was lower on day 22. In both young male and female rats, AUC was substantially lower on day 22. The steeper plasma concentration vs time curve (figure 2) indicates that the decreased AUC on day 22 was due to more rapid elimination. In young dogs C_{max} and AUC increased from day 30 to 86 of treatment. Possible explanations include drug accumulation or increased deposition or increased absorption over time with no change in elimination. Deposition may have been more efficient in the older dogs as they become acclimatized to the face mask and dosing procedure, or normal growth may have resulted in an increase of pulmonary vs oropharyngeal deposition. C_{max} and AUC in children 3.5 to 13 years old were lower than those in adults despite a higher mg/kg dose in the children (human PK summary, volume 1, page 148). The lower systemic availability in children was attributed to lower pulmonary deposition. The comparison of plasma levels suggests that humans are less sensitive than rats or dogs to the adverse systemic effects of glucocorticoids. Doses were well tolerated in humans that produced Cmax or AUC values associated with adrenal suppression, lymphoid depletion and other systemic effects in rats

or dogs.

Table 7. Systemic Exposure in Inhalation Toxicology Studies

Study (Report #)	Formulation	Dose (µg/kg)	Day	Cmax (nM) male	Cmax (nM) female	AUC (nmol·hr/L) male	AUC (nmol·hr/L) female
4-week adult rat toxicity (850-RD-0390)	dry powder	3.1	1	1.8	1.7	1.2	1.4
		30	1	16	10	11	22
		3.9	27	2.3	2.1	1.3	1.8
		37.5	27	19	20.5	13	16
6-month rat 850-RD-0403	suspension	7	~90	0.76	ND	1.6	1.2
		211	~90	20	20	34	38
1-month immature rat toxicity (850-RD-0397)	suspension	2.4	1	ND	ND	ND	ND
		8.4	1	1.2	BLQ	8.9	BLQ
		60.5	1	2.6	1.9	27	15
		2.4	22	0.17	0.03	BLQ	BLQ
		9.8	22	2.4	0.15	3.5	BLQ
		32	22	3.8	0.50	4.8	1.9
3-month immature dog toxicity (850-RD-0401)	suspension	1.6	30	0.39	0.33	0.41	0.47
		8	30	1.2	0.90	1.5	0.95
		40	30	3.7	6.9	5.1	5.9
		1.6	86	0.96	0.66	1.2	0.86
		8	86	3.9	2.7	4.0	3.1
		40	86	19	22	15	18
adult dog PK (850-RD-0402)	suspension	3	1	ND	2.8	ND	2.7
	dry powder	40	1	ND	22.5	ND	33.7
children 3-6 yrs (04-3104)		56 / 13 *	1	2.6		4.6	
children 10-13 yrs (H10-0025)		20 / 7 *	1	---		3.5	
adults (SD-004-0017)		28 / 5 *	1	4.2		7.8	
adults (04-3043)		29 / 9 *	1	4.1		10.6	

ND = not determined; BLQ = below level of quantitation. * labeled/delivered dose. Labeled dose was 1 mg in children and 2 mg in adults; delivered dose includes pulmonary and oropharyngeal. Shaded cells indicate significant systemic glucocorticoid effects observed in toxicity studies.

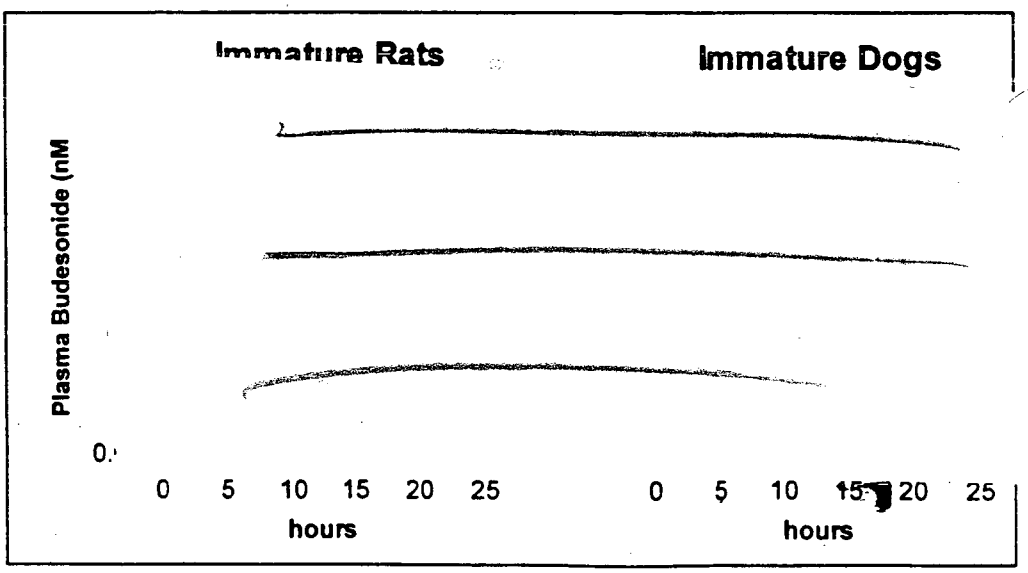

Figure 2. Plasma concentration time curves (males and females combined) from toxicity studies in immature rats (10 days old at start,46 µg/kg) and dogs (41 days old at start 40 mg/kg).

METABOLISM:

Human Liver Budesonide Sulphotransferase is inhibited by Testosterone and Correlates with Testosterone Sulphotransferase.

GM Pacifici et al, Eur. J. Clin. Pharmacol 1994; 46:49-54,. volume 16, page 176

Summary: A radiometric assay for budesonide sulphotransferase is described. Budesonide and testosterone sulphotransferase activity were measured in human liver and lung tissue. Budesonide sulphation rate was 5-8 times greater in liver than in lung; hepatic budesonide sulphation rate was 1.5 times greater in men than in women. Hepatic budesonide sulphotransferase activity was correlated with testosterone sulphotransferase activity (r =0.81) and was inhibited by testosterone. Budesonide has hydroxyl groups at positions 11 and 21, it is not known which is the site of sulphation. The results suggest that budesonide and testosterone are substrates for the same sulphotransferase enzyme.

TOXICOLOGY

General Toxicity of Budesonide given to Young Rats by the Inhalation Route for 1Month

Study 96249-1, (850-TO-0147), volume 11, page 039

Study Dates: Drug treatment began 11 APR 96, report issued 04 JUL 97
Testing Lab: Astra AB, Laboratory of Safety Assessment, Södertälje, Sweden
Test Article: Pulmicort Respules vehicle suspension, batch XA17
Budesonide (99.1% purity) batches DXK1, XA73, VM478
GLP: The study was accompanied by a signed GLP statement.
QA Report: Yes (✓) No ( )

Methods: Wistar rats (10 day old, ♂ 22-29 g, ♀ 20-27 g) were assigned these treatments:

	Vehicle	Budesonide		
Presented dose (µg/kg)	0	2.1	9.7	47
Particle size MMAD (µm)		--	--	
Animals/sex/group	12	12	12	12
PK satellites/sex/group {day 0/day22}	0/6	0/6	12/6	12/6

Treatments were administered as a liquid aerosol by nose-only inhalation. Exposure time was 14-33 minutes; the variation in exposure time was not explained but in other studies the sponsor varied exposure time to correct for daily fluctuations in aerosol concentration. Aerosol concentration and particle size were determined by cascade impactor and chemical assay ——, for budesonide. Presented dose was calculated from aerosol concentration, exposure time, body weight and respiratory minute volume [4.19 × (body weight in g)$^{0.66}$]. Pulmonary deposition was calculated by the sponsor from the fraction of drug or excipient on each stage of the cascade impactor (< 0.3 µm to > 4.6 µm), using a separate deposition factor for each stage; deposition factors were from Raabe et al in WH Walton (ed), Inhaled Particles, New York, Pergamon, 1977, pp 3-21. The overall lung deposition factor was about 10%. Plasma levels of budesonide were measured by a —— method with a limit of quantitation of —— when 1 mL of plasma was analysed. The following observations were made:

Clinical signs ..... daily with twice daily check for moribund or dead animals
Body weight ..... twice weekly in weeks 1 and 2, once in week 3
Food intake ..... once during week 2
Ophthalmology ..... once at 3 weeks
Clinical pathology ..... terminal samples from orbital plexus under enflurane anesthesia
Urinalysis ..... terminal overnight collection
Plasma drug levels ..... days 0 & 22 at end of exposure and 1, 2, 4, 8, and 24 hr after beginning of exposure.
Necropsy ..... terminal
Histopathology ..... comprehensive list of tissues in vehicle and high-dose groups; target tissues (adrenals, lung, liver, lymph nodes, spleen, and thymus) and macroscopic lesions in low- and mid-dose groups; see histopathology inventory.

Results: (summarized in table 8, page 19)

Mortality: None

Clinical Signs: No toxicologically significant treatment-related effects.

Body Weight: Body weight gain was significantly decreased in high-dose males (14%↓) and females (15%↓) and in mid-dose males (8%↓).

Food & Water Intake: Food intake was significantly decreased in high-dose males (15%↓) and females (13%↓) and in mid-dose males (15%↓). Water intake was significantly decreased in high-dose males (13%↓) and females (12%↓).

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Hematology: In high dose males and females there was an increase in red cell number with a corresponding decrease in mean corpuscular volume with no change in hematocrit or blood hemoglobin content. This differs from the typical glucocorticoid effect in older rats, in which erythrocyte count, hematocrit, and hemoglobin content are all slightly increased. An increased number of smaller red cells is typical of an adaptive response to iron deficiency (FDA Staff College Clinical Pathology Course notes). It may be that the younger rats already have a high rate of hemoglobin synthesis as a result of normal rapid growth, and are not able to respond to glucocorticoids with increased hemoglobin content as do the older rats. Although the young rats responded somewhat differently the toxicological significance of this finding is doubtful since normal hemoglobin content and hematocrit were maintained. The rats did not display the decreased lymphocyte count and increased neutrophil count typical of glucocorticoids.

Clinical Chemistry: Serum urea increased 19% in high dose males, probably due to increased protein catabolism, a known glucocorticoid effect. Blood urea nitrogen can increase in humans with Cushing's disease or steroid therapy. Increased urea can occur with decreased renal function but there was no change in serum creatinine (another indicator of renal function) and no microscopic evidence of renal injury.

Table 8. Effects of Inhaled Budesonide for 1-month in 10-38 Day Old Rats

	males				females			
Presented dose (µg/kg)	0	2.1	9.7	47	0	2.1	9.7	47
Body weight gain (g)	101	103	**93**	**87**	87	87	87	**74**
		2%	**-8%**	**-14%**		0%	0%	**-15%**
Food intake (g/day)	12.1	12.6	**10.3**	**10.3**	10.9	11.0	10.7	**9.5**
		4%	**-15%**	**-15%**		1%	-2%	**-13%**
Water intake (g/day)	15.0	14.6	14.4	**13.1**	14.6	13.3	15.3	**12.9**
		-3%	-4%	**-13%**		-9%	5%	**-12%**
Erythrocyte count (10^9/mL)	7.0	7.0	7.4	**7.5**	7.2	7.2	7.3	**7.6**
		0%	6%	**7%**		0%	1%	**6%**
Mean corpuscular volume (fl)	57.5	58	55.5	**55.3**	56.4	56.3	56.1	**52.8**
		1%	-3%	**-4%**		0%	-1%	**-6%**
Thymus weight (mg)	361	386	335	**295**	398	351	**337**	**291**
		7%	-7%	**-18%**		-12%	**-15%**	**-27%**
Lung weight (mg)	823	777	762	**696**	754	706	719	673
		-6%	-7%	**-15%**		-6%	-5%	-11%
Reduced BALT	2/12	2/12	7/12	8/12	5/12	3/12	8/12	9/12
Tracheobr. lymph node missing	2/12	4/12	9/12	8/12	4/12	5/12	4/12	8/12
Toxicokinetics {day1/day22}								
Dose (µg/kg)		2.4/2.4	8.4/9.8	59/32		2.5/2.5	8.4/9.8	62/33
Cmax (nM)		nd/0.17	1.2/2.4	2.6/3.8		nd/0.03	bq/0.15	1.9/0.5
AUC (nmol·hr/mL)		nd/bq	8.9/3.5	27/4.8		nd/bq	bq/bq	15/1.9

Highlighted values differ significantly from vehicle control group. nd = not done; bq = below quantitation level.

Urinalysis: No toxicologically significant treatment-related effects.

Organ Weights: Absolute and relative thymus weight was significantly decreased in mid- and high-dose females and in high-dose males. Absolute weights of several organs, including lung, were decreased in males in proportion to decreased overall body weight gain; relative lung weight was not significantly decreased.

Gross Pathology: No toxicologically significant treatment-related effects.

Histopathology: The number of tracheobronchial lymph nodes was decreased and the amount of bronchus associated lymphoid tissue (BALT) was reduced. Lymphoid atrophy of thymus and spleen were not observed microscopically, nor was there evidence of adrenal cortical atrophy.

Toxicokinetics: The interpretation of the toxicokinetic data is clouded by the finding of detectable plasma budesonide levels (mean = 0.056 nM) in 8/12 samples from control rats on day 22. The level of detection was [redacted] The budesonide in control plasma is probably due to contamination rather than systemic exposure because the levels varied

randomly from 30 to 112 minutes after exposure, rather than following a typical plasma concentration-time curve. It is possible that contamination also affected the reported values for the treated rats. In addition, budesonide levels were often below the level of quantitation in the treated groups, particularly at the low dose. From the available data it appears that plasma levels were lower in females than in males and lower on day 22 than on day 1. The reason for decreased C_{max} levels on day 22 is unclear although it is partly explained by day to day variation in presented dose. The decreased AUC on day 22 may have been due to increased elimination rate. Plasma AUC increased less than proportionally in relation to presented dose.

Key Study Observations:
The NOEL in this study was the low dose of 2.1 µg/kg. The NOAEL can be taken as the mid-dose of 9.7 µg/kg; the only systemic effects at this dose were slightly decreased body weight gain and food consumption in males and decreased thymus weight in females. The reduced lymphoid cellularity of bronchus associated lymphoid tissue and tracheo-broncheolar lymph nodes should probably be considered a desired local airway anti-inflammatory effect. Effects at the high-dose of 47 µg/kg included decreased body weight gain, relatively minor changes in red blood cells, decreased thymus weight, and microscopic evidence of decreased bronchus associated lymphoid tissue and tracheobronchial lymph nodes. There was no evidence of adrenal atrophy or lymphoid atrophy of spleen. Other than effects on the lymphatic tissue the respiratory tract was unaffected. Absolute lung weight was decreased in males but relative lung weight was not affected.

General Toxicity of Budesonide Nebulizing Suspension given to Young Dogs by the Inhalation Route for 3 Months. Study #97058-1, volume 12, page 258

Study Dates: Drug treatment 16 FEB 97 to 28 MAY 97, report issued 14 OCT 97
Testing Lab: Astra AB, Safety Assessment, Södertälje, Sweden
Test Article: Budesonide batch 41-01 99.6% purity
GLP: The study was accompanied by a signed GLP statement.
QA Report: Yes (✓) No ( )

Methods: Beagle dogs (41 days old, 1.4 - 2.8 kg) were assigned these treatments:

	Vehicle	Budesonide		
Budesonide presented dose (µg/kg)	0	1.6	8.0	40
Polysorbate 80 presented dose (µg/kg)	[redacted]			
Budesonide particle size MMAD (µm)	[redacted]			
Budesonide lung deposition (µg/kg)	0	0.22	1.1	5.4
Animals/sex/group	3	3	3	3

Treatments were administered as a liquid aerosol by nose-only face mask. Exposure time was 3-7 minutes until the desired dose had been achieved. Aerosol concentration and particle size were determined by cascade impactor and chemical assay [redacted] for budesonide. Presented dose was calculated by a [redacted] system that simultaneously measured aerosol concentration ([redacted] method) and inhalation

flow. Pulmonary deposition of drug was calculated by the sponsor from the fraction of drug or excipient on each of 8 stages of the cascade impactor (< 0.3 μm to > 5.0 μm), using a separate deposition factor for each stage. The overall lung deposition factor was about 14%. Plasma levels of budesonide were measured by a [redacted] method with a limit of quantitation of [redacted] for 1 mL of plasma. The sponsor did not statistically analyze clinical pathology or organ weight data due to the small number of animals per group. The following observations were made:

Clinical signs ..... daily, with thorough clinical examination weekly
Body weight ..... twice weekly in first 2 weeks, weekly thereafter
Food intake..... not recorded
EKG..... baseline and in third month
Ophthalmology..... prestudy and after approximately 1 and 3 months
Adrenal function..... ACTH stimulated cortisol test at baseline, 3 weeks, and 3 months
Clinical pathology ..... baseline and after approximately 3 and 10 weeks
Urinalysis..... in 10th week
Plasma drug levels..... after 4 weeks at pre-dose and 0, 0.5, 1, 2, 4, & 8 hr post-dose; after 3 months at pre-dose and 0, 0.5, 1, 2, 4, 8, 16, & 24 hr post-dose
Necropsy..... terminal
Histopathology ..... comprehensive list of tissues and macroscopic lesions in all groups; see histopathology inventory.

Results: (Effects were similar in males and females; combined values are shown in table 9, page 22.)

Mortality: One high-dose female in a moribund condition with parvovirus infection was euthanized. The duodenum and jejunum exhibited mucosal erosion, inflammation, and hemorrhage. Immunosuppression by budesonide may have contributed to this outcome.

Clinical Signs: Serous nasal discharge, rattling respiration, snoring, hiccough, licking, salivation, coughing, hyperventilation, subdued behavior, lacrimation, protrusion of the nictitating membrane, and loose stool or diarrhea were observed during the study with no marked differentiation among dose groups. Parvovirus was identified in fecal samples from 3 vehicle, 3 low-dose, 3 mid-dose, and 4 high-dose animals; clinical symptoms (primarily subdued behavior) were observed in 6/13 of the animals.

Body Weight: Body weight gain at the high-dose was 15% less than in vehicle controls.

Food Intake: Not recorded

Ophthalmoscopy: No toxicologically significant treatment-related effects.

EKG: No toxicologically significant treatment-related effects.

Rectal Temperature: No toxicologically significant treatment-related effects.

Adrenal function: Budesonide suppressed plasma cortisol dose dependently; the degree of suppression increased with duration of exposure. See table for details.

Table 9. Budesonide 3-month Inhalation Toxicity in Immature Dogs

	Combined males and females			
Presented dose (µg/kg)	0	1.6	8	40
Body weight gain (kg)	7.4	7.5	7.2	6.3
% change vs vehicle group		1%	-4%	-15%
Plasma Cortisol (nM):				
Baseline - week 3/week 11	12 / 13	15 / 10	11 / 9	18 / 5
% change vs vehicle group		20% / -20%	-5% / -30%	67% / -59%
ACTH stimulated - week 3/ week 11	145 / 169	143 / 158	125 / 88	55 / 6
% change vs vehicle group		-1% / -7%	-14% / -48%	-62% / -96%
Hemoglobin (g/dL)	13.1	14.1	14.2	14.5
% change vs vehicle group		7%	8%	11%
White blood cells (10^9/L)	11.4	9.1	9.3	8.0
% change vs vehicle group		-20%	-17%	-29%
Serum urea (mM)	4.8	4.9	4.5	5.9
% change vs vehicle group		3%	-5%	23%
Total serum protein (g/L)	51.0	52.0	53.0	55.5
% change vs vehicle group		2%	4%	9%
Serum cholesterol (mM)	5.6	6.2	6.8	7.0
% change vs vehicle group		11%	22%	26%
Serum potassium (mEq/L)	4.4	4.7	4.7	5.0
% change vs vehicle group		7%	7%	14%
Thymus weight (g)	32	34	24	8
% change vs vehicle group		8%	-25%	-74%
Adrenal weight (g)	0.92	0.89	0.65	0.52
% change vs vehicle group		-2%	-29%	-44%
Spleen weight (g)	17	21	20	13
% change vs vehicle group		20%	16%	-24%
Liver weight (g)	314	303	314	370
% change vs vehicle group		-3%	0%	18%
Lung weight (g)	77	75	78	52
% change vs vehicle group		-3%	2%	-33%
Thymus involution	1/6	1/6	2/6	6/6
Lymphoid depletion axillary L. node	0/6	0/6	0/6	5/5
Lymphoid depletion mesenteric L. node	0/6	0/6	0/6	3/5
Adrenal cortical atrophy	0/6	0/6	1/6	6/6
Hepatocyte rarefaction	0/6	0/6	0/6	4/5
Cmax (nM) 1month/3month		0.36 / 0.81	1.0 / [redacted]	5.3 / 20.2
AUC (nmol·hr/L) 1-month/3-month		0.44 / 1.0	1.2 / 3.6	5.5 / 16.4

No statistical analyses of these data were performed.

Hematology: Total white blood cell count decreased (62%↓) at the high dose, without a significant change in the ratio of neutrophils to lymphocytes. Hemoglobin, hematocrit, and erythrocyte count increased moderately in treated groups (10-11%↑ at the high dose for all these parameters; for simplicity, only hemoglobin is shown in the table).

Clinical Chemistry: There were dose-related increases for several serum values, which at the high dose were 23%↑ in urea, 9%↑ in total protein, 26%↑ in cholesterol, and 14%↑ in potassium.

Urinalysis: No toxicologically significant treatment-related effects.

Organ Weights: Organ weights were decreased for thymus (25%↓ at mid dose, 74%↓ at high dose), adrenals (29%↓ at mid dose, 44%↓ at high dose), spleen (24%↓ at high dose), and lung (33%↓ at high dose). Relative lung weight decreased by 17% at the high dose. Liver weight increased at the high dose (18%↑).

Gross Pathology: No toxicologically significant treatment-related effects.

Histopathology: Adrenal cortical atrophy (slight to moderate) was seen in all high-dose dogs and in one mid-dose female. Slight hepatocyte rarefaction was seen in 4/5 high-dose dogs. Increased thymic involution and decreased lymphoid cellularity of axillary and mesenteric lymph nodes were seen at the high dose. There was no microscopic correlate for the decreased lung weight.

Toxicokinetics: There were no differences between males and females in plasma concentration vs time curves (data not shown here). C_{max} and AUC increased in proportion to increasing dose. Both C_{max} and AUC were higher (2-3 fold) at 3 months than at 1 month. In view of the relatively short half life of budesonide the increased exposure is unlikely to be due to pharmacokinetic accumulation. More likely the increase represents more efficient deposition in the older dogs as they become acclimatized to the face mask etc.

Key Study Observations:
The NOAEL is the low dose of 1.6 µg/kg. At the next higher dose of 8 µg/kg budesonide suppressed ACTH stimulated cortisol secretion (48%↓), increased serum cholesterol (22%↑), decreased thymus weight (25%↓), and adrenal weight (29%↓) with microscopic evidence of adrenal cortical atrophy (1/6). At the highest dose of 40 µg/kg budesonide caused: decreased body weight gain (15%↓); decreased basal (59%↓) and stimulated (96%↓) cortisol secretion, decreased white blood cell count (29%↓), increased serum urea (23%↑), cholesterol (26%↑), potassium (14%↑), total protein (9%↑), and red cell count, hematocrit, and hemoglobin (11%↑); decreased organ weights of thymus (74%↓), adrenals (44%↓), spleen (24%↓) and lung (33%↓), increased liver weight (18%↑); and microscopic evidence of adrenal cortical atrophy (6/6), thymus involution (6/6), lymphoid depletion of axillary (5/5) and mesenteric (3/5) lymph nodes, and hepatocyte rarefaction (4/5). All of these are considered typical glucocorticoid effects.

General Toxicity Study of Budesonide Nebulizing Suspension and Polysorbate 80 and Potassium Sorbate Given to Young Rats by the Inhalation Route for 6 Months

1/15/98 submission, volume 6.3, study # 97071, report #850-TO-0149 (tox), pg 22, #850-RD-0403 (PK) pg 1

Study Dates: drug treatment 09 FEB 97 to 15 AUG 97, report issued 22 DEC 97

Testing Labs: in vivo studies [redacted]
histology processing - [redacted]
histology reading - [redacted]
toxicokinetics - Astra Draco AB, Lund, Switzerland

Test Article: Aqueous suspension of budesonide (0.02 or 0.5 mg/mL), potassium sorbate, and polysorbate 80. Micronized budesonide batch 41-01, 99.8% purity.

GLP: The study was accompanied by a signed GLP statement.

QA Report: Yes (✓) No ( )

Methods: Wistar rats (25 day old, ♂ 39-58 g, ♀ 39-58 g) were assigned these treatments:

	Air Control	Low Dose Excipients	High Dose Excipients	Low Dose Budesonide	High Dose Budesonide
Presented dose (µg/kg):					
Budesonide	0	0	0	7.3	211
Polysorbate 80	[redacted]				
Potassium sorbate	[redacted]				
Exposure (minutes)	180	60-84	180-252	60-114	60-105
Animals/sex/group					
main study	20	20	20	20	20
PK satellites	2			16	16

Treatments were administered as a liquid aerosol by nose only inhalation. To account for daily fluctuations in aerosol concentration, exposure time was varied to keep presented dose constant. Aerosol concentration and particle size were determined by cascade impactor and chemical assay (HPLC) for budesonide and for potassium sorbate. Average particle size (MMAD) for budesonide and potassium sorbate was close to [redacted] in all treatment groups. The dose of polysorbate 80 was estimated using the ratio of polysorbate 80 and potassium sorbate in the formulation and assuming a particle size distribution similar to potassium sorbate. Presented dose was calculated from aerosol concentration, exposure time, body weight and respiratory minute volume [4.19 × (body weight in g)$^{0.66}$]. Pulmonary deposition of drug was calculated by the sponsor from the fraction of drug or excipient on each of 8 stages of the cascade impactor (< 0.3 µm to > 4.6 µm), using a separate deposition factor for each stage; deposition factors were from Raabe et al in WH Walton (ed), Inhaled Particles, New York, Pergamon, 1977, pp 3-21. The overall lung deposition factor was about 8% for the median particle size ≈ [redacted]. Plasma levels of budesonide were measured by a [redacted] method with a limit of quantitation of [redacted] when 1 mL of plasma was analysed. The following observations were made:

Clinical signs ..... daily with thorough exam twice weekly
Body weight ..... weekly
Food intake ..... weekly per cage of 5
Ophthalmology ..... prestudy and at 6 months
Clinical pathology ..... terminal samples from retro-orbital plexus under ether anesthesia
Urinalysis ..... 18 hr collection before necropsy
Plasma drug levels ..... at 3 months - 0.5, 1, 2, 4, 8, 16, and 24 hr after end of exposure
Necropsy ..... terminal
Histopathology ..... comprehensive list of tissues in air control, high-dose excipient, and high-dose budesonide groups, and spontaneous deaths; target tissues (lungs, adrenals, liver, lymph nodes, spleen and thymus), and macroscopic lesions in low-dose groups; see histopathology inventory.

Results: (summarized in table 10, page 26)

Mortality: Four females (1 air control, 1 low-dose excipient, 2 low-dose budesonide) were found dead in the restraint tube at the end of exposure. These deaths appear to be related to the exposure procedure rather than drug or excipient per se.

Clinical Signs: The only treatment-related clinical sign was alopecia in the head area of all high-dose budesonide males and more generalized alopecia of all high-dose budesonide females. Time of onset was in the third and fourth month.

Body Weight: Final body weight decreased significantly in high-dose budesonide males (21%↓) and females (18%↓).

Food & Water Intake: Food intake decreased in high-dose budesonide males (8%↓); water intake decreased in low-dose (10%↓) and high-dose (12%↓) budesonide males.

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Hematology: Mild hemoconcentration was evidenced by increased red cell count, hemoglobin concentration, and hematocrit in high-dose budesonide males (7%↑) and females (10%↑). In high-dose budesonide males and females the proportion of lymphocytes decreased (13-19%↓) and the proportion of neutrophils increased (65-75%↑). The absolute lymphocyte and neutrophil counts changed in the same directions but the changes were not statistically significant in all cases.

Clinical Chemistry: Plasma glucose increased in all budesonide treated groups, 22-25%↑ at the low dose and 30-38%↑ at the high dose.

Urinalysis: No toxicologically significant treatment-related effects.

Organ Weights: Decreases were seen in thymus (58-65%↓) and spleen (23%↓) weights in high-dose budesonide males and females. Adrenal weight was significantly decreased only in high-dose budesonide males (16%↓).

Gross Pathology: Alopecia, noted above, was the only treatment-related effect.

Table 10. Effects of Excipients and Budesonide in 6 Month Rat Inhalation Toxicity Study

	males					females				
		Excipients		Budesonide			Excipients		Budesonide	
	Air	low	high	low	high	Air	low	high	low	high
Body weight (g)	409	413	413	412	**324**	234	232	240	244	**192**
		1%	1%	1%	**-21%**		-1%	2%	4%	**-18%**
Food intake (g/day)	19.5	19.7	20.0	19.4	**18.0**	13.9	13.8	14.2	14.4	13.7
		1%	3%	-1%	**-8%**		-1%	2%	4%	-1%
Water intake (g/day)	25.1	24.5	25.1	**22.5**	**22.2**	22.3	23.5	25.0	20.0	21.6
		-2%	0%	**-10%**	**-12%**		5%	12%	-10%	-3%
RBC (10^9/mL)	9.5	9.4	9.3	9.5	**10.1**	8.4	8.6	8.4	8.6	**9.3**
		-1%	-2%	0%	**7%**		2%	0%	2%	**10%**
Hb (mmol/L)	10.4	10.6	10.4	10.6	**11.1**	10.1	10.3	9.9	10.2	**11.1**
		2%	0%	2%	**7%**		2%	-2%	1%	**10%**
Neutrophils (%)	23	20	21	18	**38**	16	18	16	15	**28**
		-13%	-9%	-22%	**65%**		13%	0%	-6%	**75%**
Lymphocytes (%)	75	78	77	80	**61**	83	81	82	83	**72**
		4%	3%	7%	**-19%**		-2%	-1%	0%	**-13%**
Glucose (mM)	5.4	5.9	6.0	**6.6**	**7.0**	4.6	5.0	4.6	**5.8**	**6.4**
		9%	11%	**22%**	**30%**		9%	0%	**25%**	**38%**
Thymus weight (mg)	260	240	260	230	**110**	200	210	240	210	**70**
		-8%	0%	-12%	**-58%**		5%	20%	5%	**-65%**
Adrenal weight (mg)	58	56	56	55	**49**	66	70	73	71	60
		-3%	-3%	-5%	**-16%**		6%	11%	8%	-9%
Spleen weight (mg)	820	835	804	863	**634**	571	568	596	566	**441**
		2%	-2%	5%	**-23%**		-1%	4%	-1%	**-23%**
Histopathology										
Thymus:										
cortical atrophy	0	0	0	5/20	19/20	0	0	0	3/20	18/20
medullary atrophy	0	0	0	2/20	20/20	0	0	0	2/20	18/20
lymphocytolysis	0	0	0	4/20	4/20	0	0	0	0	4/20
Lymphoid Depletion:										
spleen	0	0	0	3/20	15/20	1/20	0	1/20	6/20	16/20
mesenteric lymph node	0	0	0	2/20	19/20	0	0	0	1/20	20/20
axillary lymph node	0	0	0	1/20	16/20	0	0	0	1/20	19/20
Skin adnexal atrophy	1/20	0	1/20	0	20/20	0	3/20	2/20	1/20	20/20
Plasma C_{max} (nmol/L)	<0.025			0.76	20.0	<0.025			---*	20.3
AUC (nmol·hr/mL)				1.55	34.3				1.22	38.0

Highlighted values indicate statistically significant difference from air control group.
*C_{max} not captured in females. PK values determined once at 3-months.

Histopathology: Lymphoid depletion or atrophy was noted in thymus, spleen, and lymph nodes of a few animals (1-6/20) in the low-dose budesonide groups and in most animals (15-20/20) in the high-dose budesonide groups. Despite significantly decreased adrenal weight in high-dose budesonide males, adrenal atrophy was not noted microscopically. Histologic skin adnexal atrophy was correlated with the alopecia observed grossly.

Toxicokinetics: Plasma levels of budesonide were similar in males and females. Plasma AUC increased in proportion to dose. The ratio of plasma AUC (nmol·hr/L) over presented dose (mg/kg) was 192 (1.4/0.007) at the low dose and 171 (36/0.21) at the high dose.

Key Study Observations:

No systemic or respiratory tract effects were attributable to the excipients, polysorbate 80, and potassium sorbate. The safety margins for high-dose polysorbate 80 expressed as total dose per kg body or deposited dose per unit of lung are summarized in table 11, below. The human dose of polysorbate 80 assumes the maximum recommended dose of 2 ampules per day, with a polysorbate 80 content of 0.4 mg (0.2 mg/mL) per ampule.

Table 11. Exposure Margin for Polysorbate 80 in 6-month Rat Inhalation Toxicology Study

	Rat	Infant	Rat/Infant	Child	Rat/Child
Allometric Assumptions					
Body weight (kg)	0.33	6	---	20	---
Lung surface area (m^2)	0.6	11	---	37	---
Lung weight (g)	1.26	108	---	342	---
Total Presented Dose					
(µg/kg body wt.)	964	133	7.2	40	24
Calculated Lung Deposition					
(% of presented dose)	8%	20%	---	20%	---
(µg/kg body weight)	81	27	3.0	8	10
(µg/g lung weight)	21	1.5	14.1	0.5	45
(µg/m^2 lung surface)	42	15	2.9	4	9.8

For rats, body and lung weight were measured and lung surface area is from Weibel in De Reuck and Porter (eds), Development of the Lung, Little Brown, 1967, pg 145.

For 6-month old infants and 6-year old children lung weights are from Boyd in Altman & Dittmer (eds), Growth, Including Reproduction and Development, Biological Handbooks, FASEB, 1962, pp 346-348; lung surface area is from Zeltner et al, Respiration Physiology 67:247, 1987.

The toxicities seen with budesonide are characteristic of glucocorticoids as a class. Percent changes are for males and females combined unless otherwise noted. The low dose of budesonide, 7 µg/kg, can be considered a threshold dose for systemic glucocorticoid activity. There were no changes in clinical pathology, adrenal weight, or spleen and thymus weights at that dose but several animals had microscopic evidence of

lymphoid atrophy in thymus, spleen or lymph nodes. At the high dose of 211 µg/kg budesonide caused decreased body weight (20%↓), decreased food intake (8%↓, males only), increased red cell number and hemoglobin (8%↑), increased neutrophil count (70%↑), decreased lymphocyte count (16%↓), decreased weights of thymus (62%↓), spleen (23%↓), and adrenal (16%↓, males only), with microscopic evidence of lymphoid depletion in thymus spleen or lymph nodes in most animals (15-20/20). The alopecia and skin adnexal atrophy observed at 211 µg/kg may correspond to the skin thinning caused by corticosteroids in humans. There was no microscopic evidence of adrenal cortical atrophy in this study.

Overall Toxicology Summary

Three new inhalation toxicology studies were submitted in this application: a 1-month study in immature rats (aged 10 days at start), a 3-month study in immature dogs (aged 41 days at start). And a 6-month study of budesonide plus excipients polysorbate 80 and potassium sorbate. A 1-month inhalation study in adult rats was submitted to IND 44,535 after approval of the previous budesonide NDAs (Reviewed by S. Tripathi, 30 SEP 97).

Adult Rat 1-Month Inhalation Toxicity Study: Budesonide total delivered doses of 0.28, 3.3, and 43 µg/kg were studied. The NOAEL was 3.3 µg/kg. The dose of 43 µg/kg resulted in decreased body weight gain (17-27%↓ in males, 49-55%↓ in females) and decreased relative thymus weights (23%↓ in males, 31%↓ in females). No effects on the adrenals or lymphoid tissue other than thymus were observed.

Immature Rat 1-month Inhalation Toxicity Study: Budesonide total delivered doses of 2.1, 9.7, and 47 µg/kg were studied. The NOAEL was 9.7 µg/kg; minor systemic effects at that dose were 8%↓ body weight gain, and 15%↓ food consumption in males only, and 15%↓ in thymus weight of females only. Reduced bronchus associated lymphoid tissue and tracheobroncheolar lymph nodes should be considered desired local anti-inflammatory effects. Decreased body weight gain (14%↓ in males, 15%↓in females) and decreased thymus weight (18%↓ in males, 27%↓in females) were seen at the high dose; spleen and adrenal weights were not decreased and there was no microscopic evidence of systemic lymphoid depletion or adrenal atrophy. Lung weight was decreased in proportion to the decrease in body weight (15%↓ in males, 11%↓in females). The age of rats used (10 days old at start of study) was within the window of 4 to 14 days in which decreased lung growth has previously been demonstrated for systemic glucocorticoid administration (Massaro et al, 1985; Blanko, 1993). The effects at 47 µg/kg in immature rats are similar to those observed in the previous 1-month dry powder inhalation study in adult rats at 43 µg/kg. Overall average plasma AUC in the young rats at 47 µg/kg (males and females at days 1 and 22 combined = 12.2 nmol·hr/L) was similar to that in the older rats from the previous study (15.0 nmol·hr/L). Thus, the toxicity profile of budesonide in immature rats was similar to that in young adult rats.

Immature Dog 3-month Inhalation Toxicity Study: Budesonide total delivered doses of 1.6, 8, and 40 µg/kg were studied. The NOAEL was 1.6 µg/kg. At 8 µg/kg budesonide partially suppressed cortisol secretion, increased serum cholesterol, and decreased thymus and adrenal weights, with adrenal cortical atrophy microscopically. Additional effects of budesonide at 40 µg/kg were: decreased body weight gain, decreased leukocyte count, increased serum urea, potassium, total protein, and red cell count, hematocrit, and hemoglobin, decreased adrenal, spleen, and lung weights, increased liver weight, and microscopic evidence of adrenal atrophy, lymphoid depletion or atrophy in thymus and lymph nodes, and hepatocyte rarefaction. All of these are known glucocorticoid effects. Unlike the situation in rats, the decrease of lung weight was proportionally greater than the overall decrease of body weight and may represent a specific effect on lung. Decreased absolute and relative lung weights have been seen consistently in several other studies with inhaled corticosteroids in adult dogs. There was no histological correlate for the decreased lung weight but more detailed morphometric studies might be needed to detect subtle treatment-related changes in lung architecture. These immature dogs may have been more sensitive than older dogs to decreased lung weight with budesonide. Previous reviews of budesonide inhalation in dogs for up to one year did not note any decrease in lung weight (preclinical review of NDA 20-233, C. Chen, 26 JUL 93). The decrease in lung weight occurred only at the high dose, at which nearly complete adrenal suppression was observed. These immature dogs also appear to have been more sensitive to the systemic effects of budesonide than older dogs. In the immature dogs dose-related systemic glucocorticoid effects were seen in the range of 8 to 40 µg/kg. In previous 6-week, 6-month, and 1-year studies in adult dogs similar effects were seen in the range of 60 to 200 µg/kg. However, the previous studies used different formulations (metered dose inhaler) and delivery systems which might account for an apparent difference in sensitivity. Toxicokinetics were not measured in the previous studies.

Three issues raised questions about the adequacy of this study. These are, 1) the high incidence of parovirus infection, 2) the stage of lung development in the animals relative to a 6-month human infant, and 3) the GLP status of the Astra, Södertälje, Sweden facility. Each of these issues is discussed below.

Parovirus Infection: Although GLP considerations specify that healthy animals should be used for toxicological studies, canine parovirus (CPV) infection was prevalent (at least 50% incidence) in the animals used in this study. A textbook of veterinary microbiology (Timoney et al 1988) notes that two paroviruses infect dogs. The first, CPV-1, is common in dogs but produces only subclinical infections. Infection with the second type, CPV-2, is subclinical in about 75% of cases but can be associated with severe hemorrhagic enteritis and high mortality in dogs. In addition to intestinal symptoms, CPV-2 infection can also cause leukopenia and, in severe cases, cardiomyopathy. The CPV infection, while not optimal, did not invalidate the results of this study. The only pathological evidence of parovirus infection was the intestinal lesions in the one high-dose female that succumbed to infection. It is likely that this was due to the known immuno-suppressant

effects of corticosteroids. There was no evidence of cardiomyopathy in any of the dogs, and the parovirus infection did not mask or obscure the toxicological profile of budesonide in this study.

Developmental Age: The sponsor had been asked to justify the use of 5-6 week old dogs to support use in [redacted] human infants. The sponsor's justification is based on practical issues (see FAX from Astra dated 9/19/97). The sponsor indicated that they could not find a laboratory that had conducted a 3-month inhalation study with inhaled steroids in 1-2 week old pups and that the laboratories contacted advised against conducting such a study. The sponsor argued: a) that handling of pups at 4-5 days of age is stressful and represents a disease risk; b) that there is no opportunity to acclimate a 2-week old pup to the exposure procedure; c) that their attempts to miniaturize the dosing apparatus to fit 1-2 week old pups have been unsuccessful; and d) that younger pups cannot be immunized against parovirus, potentially increasing problems with infection. The sponsor subsequently found a laboratory which has conducted an inhalation study of budesonide in younger pups but the report of that study is not yet available. The study will be reviewed separately when it is submitted. Growth of lung and body mass in dogs and humans is summarized in figure 3, below.

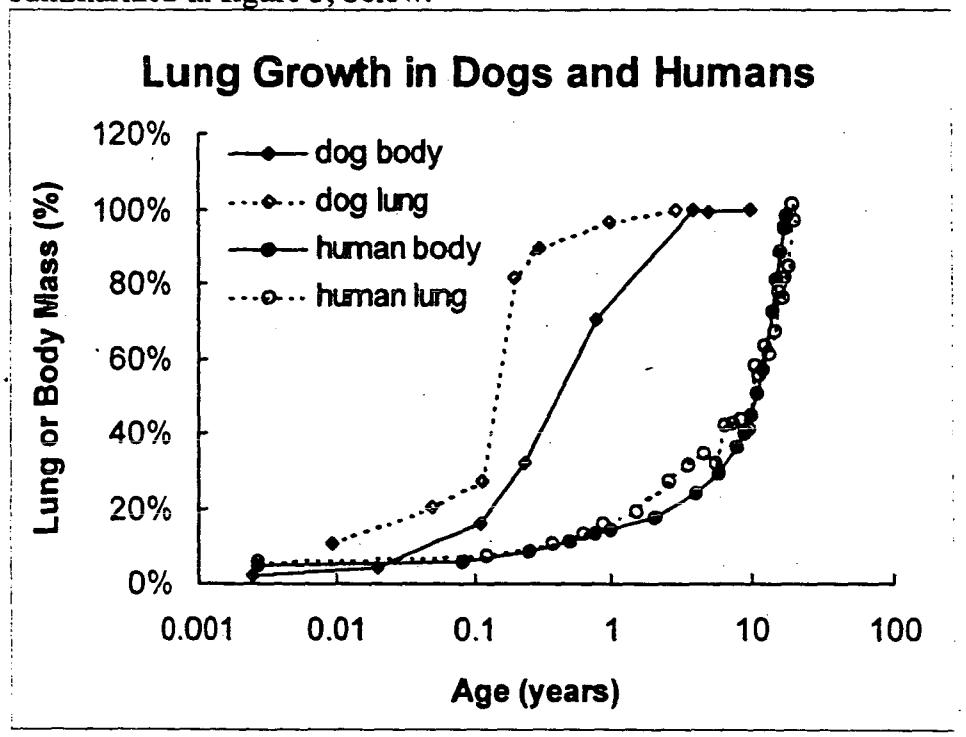

Figure 3. Lung and body growth in dogs and humans.

The overall pattern of growth differs in dogs and humans. In humans there is a slow increase in body and lung mass throughout childhood until a rapid increase in adolescence. Body and lung mass in dogs follow an exponential growth curve. The 41-day old pups used in the 3-month inhalation study are probably developmentally older than a 6 month human infant. At 5-6 weeks, body weight in dogs is ~16% of adult weight

and lung weight is ~20 of adult. Those figures correspond to a human infant of about 1 - 1½ years old.

In terms of lung structure, the final number of airway branches is complete at birth in dogs and humans. In dogs and humans, alveoli are rudimentary at birth; during postnatal development there is an increase in number and surface area of alveoli with a marked thinning of the interalveolar septa and a transformation of the capillary system from a double to a single network structure (Reid, 1967; Thurlbek, 1975; Zeltner and Burri, 1987). Morphologically, dog lungs appear to be less mature at birth than are human lungs (Boyden and Tompsett, 1961), such that a dog lung at 11 days of age is morphologically similar to a human lung at birth. The morphologic and morphometric studies of Zeltner et al (1987) suggest that the most dramatic structural alterations in human children occur between birth and 6 months, followed by a more gradual increase in the number and structural maturity of alveoli. There is considerable debate as to when the process of alveolarization ends; it may end as early as 1½ to 2 years of age (Zeltner et al, 1987) or continue until the end of childhood (Thurlbek, 1975); the rapid lung growth that occurs during adolescence is believed to be by enlargement of already existing lung structures. Some of the morphometric data of Zeltner et al are summarized in table 12, below. Although based on a small sample size, the data suggest that the most dramatic changes are from ≤ 1month to 6 month in wall thickness and wall composition, with more gradual changes from 6 to 64 months, followed by significant further decreases in wall thickness and increase in volume from 64 months to adulthood. Thus, it is likely that the stage of development in the 5-6 week old dogs used in the 3-month dog study corresponds to a period of gradual continued formation and maturation of alveoli that occurs in children from about 1 to 4 years of age. In summary: 5-week old dogs were studied due to practical considerations; dogs of this age are probably comparable to 12-18 month old humans; dramatic changes in lung structure and composition probably do not occur in humans between 6 to 18 months; but studies in younger dogs would give added assurance that budesonide has no detrimental effect on lung development in infants.

Table 12. Composition of Alveolar Septal Wall (from Zeltner et al, 1987)

age		mean thickness	septal volume	Septal tissue components (volume %)				
				epithelium		interstitium		endo-
(mo)	(n)	(μm)	(mL)	type 1	type 2	cells	acellular	thelium
≤ 1	2	5.0	30	9%	7%	40%	29%	15%
6	1	3.1	42	13%	16%	24%	29%	18%
16-18	3	2.8	40	14%	10%	25%	31%	20%
64	1	3.1	78	9%	12%	19%	40%	20%
adult	8	2.2	298	15%	13%	17%	36%	19%

GLP Status: The Astra, Södertälje, Sweden facility underwent a GLP inspection in April 1998. No major GLP deficiencies were identified; studies done at that facility (the 3-month dog study and the 1-month rat study) are reliable from a GLP standpoint.

Rat 6-Month Inhalation Toxicity Study: The 6-month study was primarily for bridging the previous systemic toxicity profile for oral polysorbate 80 to the inhalation route. Several approved oral and topical products contain polysorbate 80 (also known as Tween 80 or sorbitan mono-oleate) but it is not in any approved inhalation products. A similar surfactant, sorbitan trioleate, is an inactive ingredient in one metered dose inhalation product and one metered nasal inhalation product. There was no evidence of any effect attributable to the excipients polysorbate 80 and potassium sorbate in the 6-month rat inhalation study. (Potassium sorbate was included to qualify its use in another Astra product.) The safety margin for polysorbate 80 in this study is adequate, approximately 7- and 24-fold for infants and children, respectively, when expressed as presented dose in µg/kg, or 3- and 10-fold for infants and children, respectively, when expressed as pulmonary deposition in µg/kg body weight or alveolar surface area. The calculations are conservative in that they do not assume any drug is retained within the nebulizer and tubing. The actual delivery to the patient may be 20 to 40 % of the labeled dose, depending on patient age and the specific nebulizer used. The aqueous budesonide suspension was also tested at 7 and 211 µg/kg in this study. These doses were associated with plasma AUCs of 1.4 and 36 nmol·hr/L, respectively. This compares to AUCs of 4-5 nmol·hr/L for children, and up to 10 nmol·hr/L in adults from human PK studies. No toxicologically significant adverse effects were observed at the low dose; all observed toxicities at the high dose were expected glucocorticoid effects. There is no other 6-month rat inhalation toxicity study for direct comparison, and a previous 1-year rat inhalation study (report # 850-01, T1190, NDA 20-233) used a top dose of only 50 µg/kg. The closest study for comparison is a 3-month dry powder inhalation study in rats at 29, 105, and 428 µg/kg. The toxicities seen at 211 µg/kg in the present 6-month study of the nebulizer solution were seen at either 105 or 428 µg/kg in the 3-month dry powder rat inhalation study. This suggests that the formulations are comparable in systemic glucocorticoid effects; no toxicokinetic data were provided in the earlier study.

OVERALL SUMMARY AND EVALUATION

Budesonide is a glucocorticoid steroid that has been well characterized in previous marketing applications for the approved nasal spray (Rhinocort, NDA 20-233) and dry powder inhaler (Pulmicort Turbuhaler, NDA 20-441). The new studies submitted in the present application include updates of nonclinical pharmacology published since the previous submissions and new pharmacokinetic and toxicology studies to support the aqueous nebulizer formulation and the proposed indication for children [redacted] to 8 years old.

SUMMARY AND EVALUATION OF NEWLY SUBMITTED DATA:

Pharmacology

Budesonide is a racemic mixture of two stereoisomers, both with potent glucocorticoid activity. In terms of receptor binding affinity, the R isomer is about twice as potent as the S isomer; the racemic mixture is about half as potent as fluticasone propionate and 7 times more potent than dexamethasone. For budesonide and other corticosteroids with a free hydroxyl at C21, receptor affinity is closely correlated to lipophilicity. For budesonide and other highly lipophilic corticosteroids topical potency for human skin blanching or inhibition of rat ear edema is proportional to receptor binding affinity. Recent pharmacology publications show that budesonide inhibits production of inflammatory cytokines, growth factors, and chemotactic factors *in vitro*. It also inhibits proliferation, activation, locomotion, cytotoxicity, and survival of inflammatory cells. The relative importance of any one of these actions to the anti-inflammatory efficacy of budesonide is not known. The pharmacological actions of budesonide are typical for its class and do not distinguish it from other glucocorticoids. Inhaled or intratracheal budesonide also inhibited airway inflammation mediated by allergen challenge or other triggers *in vivo* at lung deposited doses of 2 to 40 µg/kg. This is a clinically relevant range, pulmonary deposition of 10% for a 1 mg dose in a 15 kg child would correspond to a lung deposited dose of 7 µg/kg. In the animal models delayed responses to allergen challenge were more effectively inhibited by budesonide than were immediate anaphylactic responses.

Pharmacokinetics/Toxicokinetics

Consistent with its lipophilicity, budesonide had a relatively large volume of distribution (3.7 L/kg in rats, 2.1 L/kg in dogs). Budesonide was rapidly cleared (3.1 L/hr/kg in rats, 1.6 L/hr/kg in dogs) with a relatively short plasma half life (1.8 hr in rat, 2.7 hr in dog). There is substantial systemic absorption from the respiratory tract. Bioavailability after tracheal instillation in rats was 148% relative to intravenous administration. This value was probably artificially high due to the barbiturate anesthesia used during tracheal installation, which could decrease hepatic blood flow and compete for hepatic metabolism. In dogs, bioavailability by the inhalation route (intubated) was 55% for a dry powder formulation and 60% for the aqueous suspension. For comparable doses, dogs had higher AUC values than rats. In young adults, the ratio of AUC (in nmol·hr/L) over dose (in µg/kg) ranged from 0.2 to 0.5 in rats, and from 0.4 to 0.9 in dogs. This is consistent with the more rapid clearance of budesonide in rats. In immature rats elimination of budesonide was more rapid on day 22 than on day 1. In immature dogs there was no substantial change in elimination but both C_{max} and AUC increased (2-4 fold) from 1 month (age 71 days) to 3 months. Human plasma levels (AUC or C_{max}, from the human PK summary in volume 1) in children and adults after nebulization of budesonide suspension were usually below levels associated with adverse systemic glucocorticoid effects in animal toxicity studies, but above plasma levels measured at the

rat or dog NOAELs. Published studies in humans showed that budesonide, like testosterone, is a substrate for sulphation.

Toxicology

All of the toxicities observed after inhaled budesonide treatment in rats and dogs were typical glucocorticoid class effects. Decreased body weight and thymus weight were the only significant systemic effects observed at 43 µg/kg in a 1-month dry powder inhalation study in adults and at 47 µg/kg in a 1-month suspension inhalation study in immature rats (10 days old at start). Systemic glucocorticoid target tissues at 8 or 40 µg/kg in immature dogs (41 days old at start) were: adrenal, thymus, spleen and lymph nodes, liver, leukocytes, erythrocytes, and overall metabolism (increased plasma urea). Decreased absolute and relative lung weights were seen at 40 µg/kg in the immature dogs. This is a known glucocorticoid effect and has been seen in previous inhalation toxicity studies in adult dogs but was not previously noted for budesonide. The delivered dose of 40 µg/kg, at which decreased lung weight was observed, was associated with average AUCs over the 3-months of 11 nmol·hr/L; this is 2-3 times the levels observed in children 3-6 years old (4.6 nmol·hr/L) or 10-13 years old (3.5 nmol·hr/L) after a single ampule of 1 mg. The dose at which lung weight decreased in young dogs was associated with nearly complete suppression of ACTH-stimulated cortisol responses. That level of glucocorticoid effect was not observed in safety studies in children (as summarized in the Clinical Data section in volume 1). Young dogs may be more sensitive than human children to systemic glucocorticoid effects since systemic effects were observed at 8 µg/kg (AUC = 2.4 nmol·hr/L); this is a delivered dose and AUC level that was well tolerated in clinical studies. The age of dogs (41 days) at the start of the 3-month study probably corresponds developmentally to a 1-1½ year old child. Practical considerations kept the sponsor from using younger pups. The sponsor has recently completed a study in younger dogs, about 2 weeks old at start. That study should be reviewed when available to ensure that there were no unexpected findings but is not critical for approval. The 1-month rat and 3-month dog studies in immature animals indicate that the general toxicity profile of budesonide is similar in young and adult animals.

A 6-month inhalation study in rats with inactive ingredients polysorbate 80 (Tween 80, sorbitan mono-oleate) and potassium sorbate (an excipient in a different budesonide product) showed no effects on the respiratory tract attributable to the excipients. Calculations of the dose of polysorbate 80 deposited in the respiratory tract indicate a safety margin over the maximum recommended clinical dose of about 3-fold for infants or 10-fold for children. These estimates are conservative because they assume that all of the labeled dose is delivered to the patient, whereas (depending on the particular delivery system) only 20 to 40% of the dose in the ampule is actually delivered to the patient. Budesonide doses of 7 and 211 µg/kg were also tested in this study; typical glucocorticoid effects were observed at the higher dose, similar to those observed at 105 or 428 µg/kg in a previous 3-month dry powder rat inhalation study. This study adequately bridges the

present aqueous suspension formulation to the well characterized toxicity profiles of oral polysorbate 80 and budesonide dry powder.

SUMMARY OF PREVIOUSLY ESTABLISHED PRECLINICAL PROFILE:

Pharmacokinetics

Systemic absorption of budesonide from the gastrointestinal tract is relatively poor with oral bioavailability ranging from 2% or less in monkeys, 9-20% in dogs, and 30-35% in rats and mice. In isolated perfused rat lungs 45% of budesonide administered via the airways was absorbed within 30 minutes. Budesonide is widely distributed in the body and crosses the placenta; sites of greatest uptake included adipose tissue, adrenal glands, and reproductive organs. After intratracheal administration uptake was noted in the cartilaginous tracheal rings. Plasma protein binding was 85% in rabbits, 86% in mice and humans, 89% in dogs, and 92% in rats. C-21 fatty acid conjugates are formed by lung and liver which may prolong the intracellular retention of budesonide in lung tissue; free budesonide can be released from the fatty acid conjugate by lipase activity. Budesonide is extensively metabolized. In human liver homogenates ring hydroxylations mediated via CYP3A at [redacted] form the most prevalent metabolites. The metabolites have considerably weaker glucocorticoid receptor binding affinity compared to either budesonide epimer. No metabolism of budesonide was found in human lung homogenates. In rats and dogs, after oral, arterial, or inhalation administration, 60-85% of budesonide was excreted as metabolites in the bile and eliminated in the feces; 10 to 30% appeared as metabolites in urine. In rabbits, after i.v. administration, 46% was eliminated in the urine and 39% in feces in 72 hours. Elimination was rapid with a plasma t½ after i.v. administration of 1.1 hr in rats, 1.6 hr in mice, and 3.8 hr in monkeys. Analysis of urine and bile samples revealed only traces of unchanged budesonide.

Acute Toxicity Studies

Acute toxicity was assessed after single inhaled doses of 100 mg/kg in mice, 68 mg/kg in rats, and 1 mg/kg in dogs. Expected glucocorticoid toxicities were observed in all species. One out of ten mice at 100 mg/kg died, possibly as a result of immunosuppression. The following acute toxicity parameters were observed:

Species	Route	minimum lethal dose	median lethal dose
mice	oral	≤ 200 mg/kg	> 800 mg/kg
mice	subcutaneous	25 mg/kg	35 mg/kg
rats	oral	< 100 mg/kg	400 mg/kg
rats	subcutaneous	7 mg/kg	15 mg/kg

In mice the median s.c. lethal dose was between 32 and 64 mg/kg for the R epimer and ~280 mg/kg for the S epimer, this correlates with the greater glucocorticoid potency of the R epimer.

Repeated Dose Toxicity Studies

Inhalation and Intranasal Studies: Rat nose-only inhalation studies with an MDI formulation were carried out for 3 months at 29, 105, and 428 µg/kg, and for 12 months at 6, 9, and 50 µg/kg. Typical systemic glucocorticoid effects were observed. In the 12-month study respiratory tract effects were accumulation of alveolar macrophages, pulmonary perivascular lymphocyte infiltration and increased mucus production. Dog nose-only inhalation studies with the MDI formulation were carried out for 6-weeks at 20, 60, and 200 µg/kg, and for 6 and 12 months at 200, 600, and 2000 µg/kg. Typical systemic glucocorticoid effects with no local respiratory tract toxicity were observed. No nasal cavity irritation was observed in a 3-month dog nasal study at 200 and 400 µg/kg BID.

Immature Animals: A 3-month study in rats beginning at age 6-7 days was done at s.c. doses of 0.2, 2.0, and 20 µg/kg budesonide or 20 µg/kg triamcinolone acetonide. Typical glucocorticoid effects were seen only at 20 µg/kg of either steroid. The effects of triamcinolone were somewhat greater than those of budesonide at that dose.

Reproductive Toxicity

Budesonide was tested in the following reproductive toxicity studies:

- Rat fertility and general reproduction at 0, 5, 20, and 80 µg/kg s.c.
- Rat teratology at 0, 20, 100, and 500 µg/kg s.c.
- Rabbit teratology at 0, 5, 25, and 125 µg/kg s.c.
- Rat teratology at 0, 24, 64, and 340 µg/kg by nose only inhalation (MDI)
- Rat peri- and post-natal study at 0, 5, 20, and 80 µg/kg s.c.

At 20 µg/kg s.c. in rats there were decreases in maternal body weight gain, prenatal viability and viability of pups at birth and during lactation; these effects were not observed at 5 µg/kg. Budesonide produced fetal loss, decreased pup weight and skeletal abnormalities at s.c. doses of 25 µg/kg in rabbits and 500 µg/kg in rats.

Carcinogenicity and Mutagenicity

There was no evidence of carcinogenicity in mice at oral (drinking water) doses up to 200 µg/kg for 91 weeks. In a 104-week study in rats the incidence of astrocytomas/gliomas was increased in male rats at an oral dose of 50 µg/kg (incidences of 2, 0, 6, and 14%, respectively, at 0, 10, 25, and 50 µg/kg). Increased incidence of hepatocellular neoplasma (neoplastic nodules and carcinomas) were seen in males at 25 and 50 µg/kg. Two additional 104-week oral carcinogenicity studies were done in control rats and in rats treated with 25-50 µg/kg budesonide, 400-600 µg/kg prednisolone or 5-15 µg/kg triamcinolone acetonide (drug dose was titrated in these studies to achieve a decrease in mean body weight of ~10%). Both studies failed to confirm the increased incidence of brain tumors with budesonide. Compared with controls, the incidence of hepatocellular

tumors was increased significantly in all three steroid groups, suggesting that this is a class effect. Budesonide was negative in these six genotoxicity assays: Ames Salmonella, mouse lymphoma, unscheduled DNA synthesis, human lymphocyte chromosome aberration, mouse *in vivo* micronucleus, recessive lethal test in Drosophila.

LABELING REVIEW

There are several preclinical statements in the "Clinical Pharmacology" section of the label [e.g. "In standard in vitro and animal models, budesonide has approximately a 200-fold higher affinity for the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol (rat croton oil ear edema assay)"]. All of these preclinical statements are justifiable by data in the present or previous NDA submissions.

The calculated safety margins in the Carcinogenicity, Pregnancy, and Overdosage sections are based on mg/m² estimates. None of the animal studies reported in these sections had accompanying toxicokinetic data. A review of available pharmacokinetic studies indicates that there are no suitable data for reliable estimation of AUC data for the carcinogenicity, reproductive toxicity, and acute toxicity studies. The sponsor does not specify what assumptions were made in calculating human mg/m² doses. They appear to base their calculations on a labeled dose of 2 mg/day in a 70 kg adult or 10 kg [redacted] child" with an assumption that only 15% of the labeled dose is delivered to the patient. These sections should be revised based on the full labeled dose of 2 mg/day in a 50 kg adult or 10 kg child without corrections for partial delivery of the labeled dose. Other changes should be made for consistency with the most recently approved budesonide label (Nasacort® AQ). The sponsor should be advised to use the labeling recommend in the review of budesonide Rhinocort AQ nasal spray, NDA 20-746 (pharm/tox review by L. Pei and L. Sancilio, 08 OCT 97) with appropriate changes for differences in dose and formulation. The revised sections should read as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility: In a two-year study in Sprague-Dawley rats, budesinode caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 mcg/kg (less than the maximum recommended daily inhalation dose in adults and children on a mcg/m² basis). No tumorigenicity was seen in male and female rats at respective oral doses up to 25 and 50 mcg/kg (less than the maximum recommended daily inhalation dose in children on a mcg/m² basis). In two additional two-year studies in male Fischer and Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 mcg/kg (less than the maximum recommended daily inhalation dose in adults and children on a mcg/m² basis). However, in the male Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 mcg/kg (less than the maximum recommended daily inhalation dose in adults and children on a mcg/m² basis). The concurrent reference steroids (prednisolone and triamcinolone acetonide) in these two studies showed similar findings.

In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 mcg/kg (less than the maximum recommended daily inhalation dose in adults and children on a mcg/m² basis).

Budesonide was not mutagenic or clastogenic in six different test systems: Ames Salmonella/microsome plate test, mouse micronucleus test, mouse lymphoma test, chromosome aberration test in human lymphocytes, sex-linked recessive lethal test in Drosophila melanogaster, and DNA repair analysis in rat hepatocyte culture.

In rats, budesonide [redacted] along with a decrease in maternal body-weight gain, at subcutaneous doses of 20 mcg/kg and above (less than the maximum recommended daily inhalation dose in adults on a mcg/m² basis). No such effects were noted at 5 mcg/kg (less than the maximum recommended daily inhalation dose in adults on a mcg/m² basis).

PREGNANCY:

Teratogenic Effects: Pregnancy Category C: [redacted]. Budesonide produced fetal loss, decreased pup weights and skeletal abnormalities at subcutaneous doses of 25 mcg/kg in rabbits (less than the maximum recommended daily inhalation dose in adults on a mcg/m² basis) and 500 mcg/kg in rats (approximately — times the maximum recommended daily inhalation dose in adults on a mcg/m² basis). In another study in rats, no teratogenic or embryocidal effects were seen at inhalation doses up to 250 mcg/kg (approximately equal to the maximum human daily inhalation dose in adults on a mcg/m² basis).

There are no adequate and well-controlled studies in pregnant women. Budesonide should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Experience with oral glucocorticosteroids since their introduction in pharmacologic, as opposed to physiologic, doses suggests that rodents are more prone to teratogenic effects from glucocorticosteroids than humans. In addition, because there is a natural increase in glucocorticosteroid production during pregnancy, most women will require a lower exogenous glucocorticosteroid dose and many will not need glucocorticosteroid treatment during pregnancy.

OVERDOSAGE

The potential for acute toxic effect following overdose of PULMICORT RESPULES is low. [redacted], systemic corticosteroid effects such as hypercorticism may occur (see PRECAUTIONS). [redacted]

In mice the minimal lethal inhalation dose was 100 mg/kg (approximately ——— times, respectively, the maximum recommended human daily inhalation dose in adults or children on a mcg/m² basis). In rats there were no deaths at an inhalation dose of 68 mg/kg (approximately ——— times, respectively, the maximum recommended human daily inhalation dose in adults or children on a mcg/m² basis). In mice the minimal oral lethal dose was 200 mg/kg (approximately ——— times, respectively, the maximum recommended human daily inhalation dose in adults or children on a mcg/m² basis). In rats the minimal oral lethal dose was less than 100 mg/kg (approximately ——— times, respectively, the maximum recommended human daily inhalation dose in adults or children on a mcg/m² basis).

The calculations used for the safety margins are shown below.

Drug: **Pulmicort Respules (10 kg child - labeled dose)**

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric	1	1	2	2	10	0.20	25	5.00
Adult	>12	1	2	2	50	0.04	37	1.48

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Carcinogenicity:								
mouse	po	0.20	3	0.6	0.41	0.12	1/2	1/8
rat	po	0.05	6	0.3	0.20	0.06	1/5	1/17
rat	po	0.025	6	0.15	0.10	0.03	1/10	1/33
rat	po	0.01	6	0.06	0.04	0.01	1/25	1/83
Reproduction and Fertility:								
rat	sc	0.02	6	0.12	0.08	N/A	1/12	N/A
rat	sc	0.005	6	0.03	0.02	N/A	1/49	N/A
Teratogenicity:								
rabbit	sc	0.025	12	0.3	0.20	N/A	1/5	N/A
rat	sc	0.5	6	3	2.03	N/A	2.0	N/A
rat	inhalation	0.25	6	1.5	1.01	N/A	1	N/A
Overdosage:								
mouse	inhalation	100	3	300	202.70	60.00	200	60
rat	inhalation	68	6	408	275.68	81.60	280	80
mouse	po	200	3	600	405.41	120.00	410	120
rat	po	100	6	600	405.41	120.00	410	120

RECOMMENDATIONS

1. The recommended labeling changes should be communicated to the sponsor.
2. The application is approvable from a preclinical viewpoint, pending the results of the sponsor's completed 3-month study in dogs aged 1-2 weeks.

/S/ 04/28/98

Mark Vogel, Ph.D., Pharmacologist

/S/ April 28, 1998

Original NDA 20-929
c.c. HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/W.M. Vogel
HFD-570/S. Chu
HFD-570/G. Trout

Histopathology Inventory for NDA 20-929 (organ weights obtained for highlighted tissues)

Species	young rat	young dog	rat
Duration	1-month	3-month	6-month
Route	inhalation	inhalation	inhalation
Formulation	suspension	suspension	suspension
Report #	96249-1	97058-1	97071-1
Adrenals	✓	✓	✓
Aorta			✓
Bone Marrow smear	✓	✓	✓
Bone (femur)	✓	✓	
Brain	✓	✓	✓
Colon	✓	✓	✓
Duodenum	✓	✓	✓
Epididymis	✓	✓	✓
Esophagus	✓	✓	✓
Eye	✓	✓	✓
Gall bladder		✓	
Harderian gland			✓
Heart	✓	✓	✓
Ileum	✓	✓	✓
Jejunum	✓	✓	
Kidneys	✓	✓	✓
Lachrymal gland			✓
Larynx	✓	✓	✓
Liver	✓	✓	✓
Lymph node, axillary	✓	✓	✓
Lymph node, mesenteric	✓	✓	✓
Lungs	✓	✓	✓
Mammary Gland	✓	✓	
Nasal cavity	✓	✓	✓
Optic nerves	✓	✓	✓
Ovaries	✓	✓	✓
Pancreas	✓	✓	✓
Parathyroid	✓	✓	✓
Peripheral nerve	✓	✓	✓
Pharynx			✓
Pituitary	✓	✓	✓
Prostate	✓	✓	✓
Salivary gland	✓	✓	✓
Seminal vesicles	✓		✓
Skeletal muscle	✓	✓	✓
Skin	✓	✓	✓
Spinal cord			✓
Spleen	✓	✓	✓
Stomach	✓	✓	✓
Testes	✓	✓	✓
Thymus	✓	✓	✓
Thyroid	✓	✓	✓
Tongue			✓
Trachea	✓	✓	✓
Urinary Bladder	✓	✓	✓
Uterus	✓	✓	✓
Vagina	✓	✓	✓
Preputial/clitoral gland			✓
Other	carina	soft palate	oviduct
Other		carina	
Other		bronchus	
Other		rib	

NDA 21-day Pharmacology fileability check list

NDA No.: 20,929

Date of submission: November 18, 1997

Date of 21-day fileabililty meeting: December 10, 1997

Date of check list: December 08, 1997

(1) On its face, is the pharm/tox section of the NDA organized in a manner to allow substantive review? Yes (x) No ( )

(2) On its face, is the pharm/tox section of the NDA legible for review?
Yes (x) No ( )

(3) Are final reports of all required and requested preclinical studies submitted in this NDA?

	Yes	No	NA
Pharmacology (Summaries provided)	(x)	( )	( )
ADME	(x)	( )	( )
Toxicology (duration, route of administration and species specified)			
acute	( )	( )	(x)
subchronic and chronic studies:			
1-month juvenile rat study	(x)	( )	( )
3-month juvenile dog study (age: 5-6 weeks)*	(x)	( )	( )
6-month rat bridging study (expected to arrive: 1/20/98)	( )	(x)	( )
reproductive studies	( )	( )	(x)
carcinogenicity studies	( )	( )	(x)
mutagenicity studies	( )	( )	(x)
special studies	( )	( )	(x)
others	( )	( )	(x)

** 3-month juvenile dog study (age: 1-2 weeks) will be available in May, 1998*

EA (items 7, 8, 9, 10, 11 and 15)	( )	( )	(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 (x) No ( ) NA ( )

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 (x) No ( )

(5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose mutiples 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 ( )

* *Six month rat bridging study will arrive in January, 1998.*

(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 ( )

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

(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 ( )

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

Per Chemistry Reviewer, no issues have been identified so far.

(10) Are there any outstanding preclinical issues? Yes ( ) No (x)**

If yes, identify those below:

** *Age of dogs for 3-month juvenile dog study is a Review Issue.*

(11) From a preclin. perspective, is NDA fileable? Yes (x) No ( )

If no, state below why it is not.

If yes, should any additional info/data be requested? Yes (x) No ( )
If yes, identify those below.

The applicant should be requested to provide following information:

Justification for conducting 3-month toxicity study in dogs of age group 5 to 6 weeks for the proposed pediatric population of —— of age.

NDA 45-day Planning Timeline

NDA NO:	20-929
Date of 45-day planning meeting:	01/12/98
Date of planning timeline:	01/12/98
User Fee Due Date:	05/20/98
Final review completion date:	03/18/98

	Milestone Date
Pharmacology and ADME	02/06/98
Toxicology	
General toxicity studies	02/27/98
Carcinogenicity studies and mutagenicity studies	N/A
a. Statistical consult request for CA studies	N/A
b. Submission of CA studies for CAC=s concurrence	N/A
Reproductive studies	N/A
Special studies and Others	N/A
Labeling	03/18/98
EA:	Not Needed.

/S/

Satish C. Tripathi, Ph.D.

/S/ Jan. 12, 1998

HFD-570/Division File
HFD-570/~~Tripathi~~
HFD-570/Sun