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APPLICATION NUMBER:

205613Orig1s000

PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205-613
Supporting document/s:	0050
Applicant's letter date:	August 26, 2014
CDER stamp date:	August 26, 2014
Product:	Budesonide (UCERIS) Rectal Foam
Indication:	For the treatment of active mild to moderate distal ulcerative colitis (UC) extending up to 40 cm from the anal verge.
Applicant:	Salix Pharmaceuticals, Inc.
Review Division:	Division of Gastroenterology and Inborn Errors products (DGIEP)
Reviewer:	Dinesh Gautam, Ph.D.
Supervisor/Team Leader:	Sushanta Chakder, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Kelly Richards, MSN, RN

Background:

On August 15, 2014 the Agency requested the Applicant to amend NDA 205-613 (Uceris Rectal Foam) from a 505(b)(1) to a 505(b)(2) application with reference to publically available nonclinical data of budesonide capsules and tablets (Entocort capsules and Uceris tablets) and published literature. On August 26, 2014 the Applicant submitted a scientific justification for the 505(b)(2) application cross-referencing Entocort capsules, Uceris tablets, publically available safety data for budesonide, available published literature, and the nonclinical studies submitted in the application.

Summary and Evaluation:

The Applicant compared the systemic human exposures of Uceris rectal foam from the available published and submitted study reports with the systemic exposure of budesonide in animals. In addition, the Applicant also referred to publically available nonclinical data and submitted published reports of carcinogenicity, mutagenicity, and reproductive and developmental toxicity studies in the Budesonide Rectal Foam application.

The systemic and local toxicity profiles of budesonide rectal foam was assessed in 6-week and 9-month toxicity studies in beagle dogs (study #BUSA0300 and BUSA0301). In both studies, the highest dose tested was 2 mg intra-rectally BID (0.47 mg/kg/day), which provides a human equivalent dose of 15.04 mg/day (>3.7 times higher than the human dose of 2 mg BID). The mean plasma exposures (AUC_{0-24}) of budesonide in the 6-week toxicity study were up to 13.2 ng.h/mL and 10.4 ng/h/mL on Days 1 and 24, respectively, which exceeds the human AUC of 4.3 ng.h/mL following intra-rectal administration of Uceris rectal foam at a dose of 2 mg BID. The mean AUC_{0-24} values in the 9-month intra-rectal toxicity study were 18.4 ng.h/mL on Day 1 and 1.64 ng.h/mL on Day 269. The toxicokinetic profile of budesonide in rats was assessed by cross-referencing to a published study by Chanoine et al (1991, *Drug Metabolism and Disposition*, 19:2, 546-553). As per the published study (Chanoine et al, 1991), the linear interpolation based on the known pharmacokinetics of budesonide in rats predicts the AUC of about 54 ng.h/mL following an 80 µg/kg/day dose, which is about 12.5-fold higher than the estimated human AUC.

Published two-year carcinogenicity studies of budesonide in CD-1 mice and SD rats were (Ryrfeldt et al, *Toxicology pathology*: 1992, 20:1, 115-117) submitted in the NDA. The published studies (Ryrfeldt et al, 1992) are also referenced in the labelling section of the Uceris rectal foam application. In addition, publically available data and published literature (Fujii et al, 1985, *The report No 4*; 19:9, 1-15; Richter et al 1992, *Topical corticosteroids*, Basel, Karger, 1992, 349-369) showed that budesonide was not genotoxic in the Ames test, the mouse lymphoma cell forward gene mutation (TK+/-) test, the human lymphocyte chromosome aberration test, the Drosophila melanogaster sex-linked recessive lethality test, the rat hepatocyte UDS test and the mouse micronucleus test which are reflected in the labeling of Uceris Rectal Foam.

The Applicant also referred to published reproductive and developmental toxicity studies of budesonide in rats and rabbits (Toteno et al, 1985, *The Clinical report # 6*, 85, 19:10; 1-76; Kihlstrom et al, 1987, *Arzneim. Forsh/Drug Res*. 37(1):4346). The findings of the nonclinical studies showed that budesonide was teratogenic and embryocidal in rabbits and rats. However, budesonide had no effect on fertility in rats at subcutaneous doses up to 100 µg/kg/day. It caused a decrease in prenatal viability and viability in pups at birth and during lactation, along with a decrease in maternal body-weight gain, at a subcutaneous dose of 20 µg/kg/day. These findings

are similar to the findings used in the nonclinical labeling sections of Uceris Tablets and Uceris Rectal Foam.

Thus, the Applicant provided adequate information to scientifically justify the bridging of the nonclinical PK, toxicity, genotoxicity, carcinogenicity and reproductive toxicity information for the current NDA to the publicly available information and/ or published literature. The Applicant's approach to bridge publicly available information and published nonclinical data to the Uceris Rectal Foam application (NDA 205-613) appears to be adequate and is acceptable.

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/s/

DINESH C GAUTAM
09/03/2014

SUSHANTA K CHAKDER
09/03/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	205-613
Supporting document/s:	0002
Applicant's letter date:	November 15, 2013
CDER stamp date:	November 15, 2013
Product:	Budesonide (UCERIS) Rectal Foam
Indication:	For the treatment of active mild to moderate distal ulcerative colitis (UC) extending up to 40 cm from the anal verge.
Applicant:	Salix Pharmaceuticals, Inc.
Review Division:	Division of Gastroenterology and Inborn Errors products (DGIEP)
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Disclaimer

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Executive Summary

1.1 Recommendations

1.1.1 Approvability

From a nonclinical standpoint, approval of the NDA application is recommended.

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The draft labeling of UCERIS rectal foam generally conforms to the format specified under 21CFR 201.57(c)(14) Requirements for PLR (Physician's Labeling Rule) Prescription Drug Labeling. However, the following changes are recommended.

8.1 Pregnancy

Sponsor's version:

Evaluation: The text should be modified as proposed below.

Pregnancy Category C

Risk Summary

There are no adequate and well controlled studies with UCERIS in pregnant women. Animal reproduction studies using subcutaneous administration of budesonide were conducted in rats and rabbits. Skeletal abnormalities, fetal loss and decreased pup weight were observed in these studies. UCERIS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. All pregnancies, regardless of drug exposure, have a background rate of 2 to 4 percent for major malformations, and 15 to 20 percent for pregnancy loss.

Animal Data

Budesonide is teratogenic and embryocidal in rabbits and rats. In a subcutaneous embryofetal development studies, fetal loss, decreased pup weights, and skeletal abnormalities were observed at a subcutaneous dose of 25 mcg/kg in rabbits (approximately 0.12 times the recommended human intrarectal dose of 4 mg/day, based on the body surface area) and 500 µg/kg in rats (approximately 1.2 times the recommended human intrarectal dose of 4 mg/day, based on the body surface area).

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Sponsor's version:

Carcinogenicity

Carcinogenicity studies with budesonide were conducted in rats and mice. In a 2-year study in Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 µg/kg. In addition, there were increased incidences of primary hepatocellular tumors in male rats at 25 µg/kg and above. No tumorigenicity was seen in female rats at oral doses up to 50 µg/kg. In an additional two-year study in male Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 µg/kg. However, it caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 µg/kg. The concurrent reference (b) (4) (prednisolone and triamcinolone acetonide) showed similar findings. In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 µg/kg.

Mutagenesis

Budesonide (b) (4) in the Ames test, the mouse lymphoma cell forward gene mutation (TK) test, the human lymphocyte chromosome aberration test, the *Drosophila melanogaster* sex-linked recessive lethality test, the rat hepatocyte UDS test and the mouse micronucleus test.

Impairment of Fertility

In rats, budesonide had no effect on fertility at subcutaneous doses up to 80 µg/kg [redacted (b) (4)]. However, it caused a decrease in prenatal viability and viability in pups at birth and during lactation, along with a decrease in maternal body-weight gain, at subcutaneous doses of 20 mcg/kg [redacted (b) (4)] and above. No such effects were noted at 5 µg/kg.

Evaluation: The text should be modified as proposed below.

Proposed version:

Carcinogenicity

Carcinogenicity studies with budesonide were conducted in rats and mice. In a 2-year study in Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 µg/kg (approximately 0.12 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area). In addition, there were increased incidences of primary hepatocellular tumors in male rats at 25 µg/kg (approximately 0.06 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area) and above. No tumorigenicity was seen in female rats at oral doses up to 50 µg/kg (approximately 0.12 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area).

In an additional 2-year study in male Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 µg/kg (approximately 0.12 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area). However, it caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 µg/kg (approximately 0.12 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area). The concurrent reference glucocorticosteroids (prednisolone and triamcinolone acetonide) showed similar findings. In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 µg/kg (approximately 0.24 times the recommended intrarectal dose of 4 mg/day in humans, based on the body surface area).

Mutagenesis

Budesonide showed no evidence of mutagenic potential in the Ames test, the mouse lymphoma cell forward gene mutation (TK+/-) test, the human lymphocyte chromosome aberration test, the *Drosophila melanogaster* sex-linked recessive lethality test, the rat hepatocyte UDS test or the mouse micronucleus test.

Impairment of Fertility

In rats, budesonide had no effect on fertility at subcutaneous doses up to 80 µg/kg (approximately 0.20 times recommended intrarectal dose of 4 mg/day in humans, based on a body surface area basis). However, it caused a decrease in prenatal viability and viability in pups at birth and during lactation, along with a decrease in maternal body-weight gain, at subcutaneous doses of 20 µg/kg (approximately 0.05 times recommended intrarectal dose of 4 mg/day in humans, based on a body surface area basis) and above. No such effects were noted at 5 µg/kg.

1.2 Brief Discussion of Nonclinical Findings

The applicant provided published literature on the pharmacology, pharmacokinetics, mutagenicity, carcinogenicity and reproductive toxicology studies of budesonide. Study reports of single- and repeated-dose toxicity studies in rats, mice and dogs were also provided. Brief discussion of nonclinical findings of Budesonide is presented below.

Budesonide is a non-halogenated glucocorticosteroid, which is structurally related to hydroxyprednisolone. *In vitro* pharmacology studies showed that it has a high glucocorticoid receptor affinity compared to other corticosteroids (hydrocortisone, prednisolone, and dexamethasone). Following topical administration, budesonide exhibits a high ratio of topical to systemic activity. Budesonide has a pronounced anti-inflammatory effect after both subcutaneous and topical administration in animals. In an *in vitro* hERG assay at concentrations of 4.5, 15, 45 and 150 µM budesonide produced 4, 14, 31 and 58% inhibition of hERG potassium ion current, respectively, with in an IC_{50} value of 106 µM. Safety pharmacology studies of budesonide were conducted in mice, rats, guinea pigs, cats and dogs. These studies showed no pronounced action on the central nervous system, respiratory system, circulatory system, or the autonomic nervous system at doses up to 10.0 mg/kg. No action on the neuromuscular junction or the blood clotting system was observed. Extremely mild acceleration of urinary electrolyte excretion was observed in the rat kidneys at 0.01, 0.1, 1.0, and 10.0 mg/kg budesonide.

The pharmacokinetics of budesonide were studied in various laboratory animals (mice, rats and dogs) following different routes of administration. In published reports, the absorption, distribution and excretion of budesonide were evaluated in mice, rats and dogs following intravenous, subcutaneous, oral, colonic, inhalation and rectal administrations. Budesonide has moderate to high clearance and a high volume of distribution in all species following intravenous administration. Low systemic bioavailability of budesonide was observed following oral and rectal administration, which can be attributable to a high first pass effect. Following repeated rectal administration of budesonide in the dog in a chronic study, it was rapidly absorbed into the systemic circulation.

Acute and repeated dose toxicology studies of budesonide have been conducted in mice, rats, dogs, and monkeys after oral, intravenous, intraperitoneal, subcutaneous and intrarectal administration. In acute toxicity study, deaths occurred mostly in the 2nd week after treatment. In the chronic toxicity studies, budesonide at high doses showed glucocorticoid related activities such as atrophy of the thymus, adrenals and lymph nodes, gastric ulcerations, decreases in white blood cell counts, depression of the hypothalamic pituitary adrenal (HPA) axis, increased liver glycogen, and gastrointestinal hemorrhage. Twice daily rectal administration of budesonide foam in dogs was well tolerated at doses up to 4 mg/day in 6- and 39-week studies.

Budesonide was not genotoxic in the Ames test, the mouse lymphoma cell forward gene mutation ($TK^{+/-}$) test, the human lymphocyte chromosome aberration test, the *Drosophila melanogaster* sex-linked recessive lethality test, the rat hepatocyte UDS test and the mouse micronucleus test.

Carcinogenicity studies with budesonide were conducted in rats and mice. In a two-year study in Sprague-Dawley rats, budesonide caused a statistically significant increase in the incidence of gliomas in male rats at an oral dose of 50 µg/kg. In addition, there were increased incidences of primary hepatocellular tumors in male rats at 25 µg/kg and above. No tumorigenicity was seen in female rats at oral doses up to 50 µg/kg. In an additional two-year study in male Sprague-Dawley rats, budesonide caused no gliomas at an oral dose of 50 µg/kg. However, it caused a statistically significant increase in the incidence of hepatocellular tumors at an oral dose of 50 µg/kg. In a 91-week study in mice, budesonide caused no treatment-related carcinogenicity at oral doses up to 200 µg/kg.

Budesonide was teratogenic and embryocidal in rabbits and rats. Budesonide had no effect on fertility in rats at subcutaneous doses up to 100 µg/kg. However, it caused a decrease in prenatal viability and viability in pups at birth and during lactation, along with a decrease in maternal body-weight gain, at a subcutaneous dose of 20 µg/kg. No such effects were noted at 5 µg/kg.

Topical administration of budesonide in guinea pigs showed no phototoxicity and had no photoallergic effects. Budesonide has no ocular toxicity in rabbits.

2 Drug Information

2.1 **Drug:** Budesonide (UCERIS) Rectal Foam

CAS Registry Number

51333-22-3

Generic Name

Budesonide

Code Name

None

Chemical Name

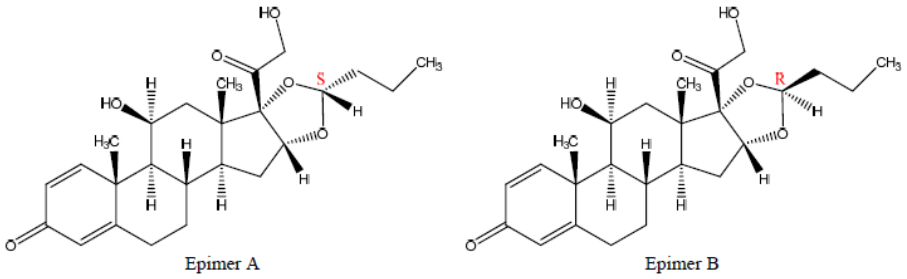
16,17-(butylidenebis(oxy)-11,21-dihydroxy-, (11-β,16-α)-pregna-1,4-diene-3,20-dione.

Molecular Formula/Molecular Weight

$C_{25}H_{34}O_6$ / 430.5

Structure

Budesonide is a mixture of two epimeric forms: epimer A (b) (4) 22S) and epimer B (b) (4) 22R). Chemical structures of epimers are presented below.



Epimer A Epimer B

Pharmacologic class: Glucocorticoid.

2.2 Relevant IND/s, NDA/s, and DMF/s

NDA: 21-324, Entecort®, AstraZeneca, LP, DE.
NDA: 203-634, UCERIS™, Santarus, Inc.
IND: 104,725, Budesonide, Salix Pharmaceticals, Inc.

2.3 Clinical Formulation

2.3.1 Drug Formulation

Budesonide 2 mg rectal foam is a non-sterile emulsion consisting of budesonide, propylene glycol, cetyl alcohol, emulsifying wax, polyoxyl (10) stearyl ether, purified water, edetate disodium, and citric acid monohydrate. The emulsion is filled into a 54

mL, white, aluminum (b) (4) canister (b) (4) Each canister is fitted with a 1 inch metered-valve system consisting of a (b) (4) valve body and stem. A propellant consisting of propane, isobutane, and butane is added to the (b) (4) can before a 1.35 mL dispenser head and a (b) (4) shield are installed. Each multi-dose canister delivers fourteen 1.35 mL doses of the foam product (equivalent to 2 mg budesonide per dose) and will be provided with 14 single use, disposable, white, polyvinyl chloride rectal applicators. Each applicator is pre-coated with paraffin lubricant and stored in a protective, white, (b) (4) tray (7 applicators per tray). The composition of the drug product is shown in the Applicant's Table below.

Table 1: Components and Composition of Budesonide 2 mg Rectal Foam in Metered-dose Canisters

Ingredient	Quality Standard	Function	Quantity per Dose (mg)	Quantity per Can (mg)[a]	Concentration (% w/w)
Drug Product Emulsion					
Budesonide	USP	Active Ingredient	2.0	(b) (4)	
Propylene Glycol	USP	(b) (4)	(b) (4)		
Cetyl Alcohol	NF				
Emulsifying Wax	NF				
Polyoxyl (10) Stearyl Ether	NF				
Purified Water	USP				
Edetate Disodium	USP				
Citric Acid Monohydrate	USP				
(b) (4)					
Total Emulsion Weight:	---	---			
Drug Product Propellant[b]					
Propane	NF	Propellant			
Isobutane	NF	Propellant			
Butane	NF	Propellant			
Total Theoretical Weight:	---	---		(b) (4)	100.0%

Abbreviations: Can = Canister, NA = not applicable, NF = (United States) National Formulary, USP = United States Pharmacopeia

[a] The canister delivers 14 doses (2 mg of budesonide per dose) but is filled with an overfill to ensure the accuracy of each delivered dose.

[b] The drug product propellant, (b) (4) is a mixture of butane, NF, isobutane, NF and propane, NF combined at a molar ratio of (b) (4) and (b) (4) respectively. Each of the components meets its respective NF quality standard.

2.3.2 Comments on Novel Excipients

No novel excipients were used in the formulation of the drug product. However, propane, isobutene and butane were used as propellant. In accordance with CFR 184.1(b)(1), butane, iso-butane and propane are used in food with no limitations other than current good manufacturing practice. The affirmation of these ingredients as generally recognized as safe (GRAS) as direct human food ingredients is based upon

the following current good manufacturing practice conditions. The ingredients (butane, isobutene and propane) are used as propellants, aerating agents, and gases as defined in §170.3(o)(25).

2.3.3 Comments on Impurities/Degradants of Concern

The known impurities that are tested as part of the budesonide drug substance analysis can be classified into three categories: related compounds, inorganic impurities, and residual solvents. The known process related potential impurities and degradation products are presented in the Applicant's Table below.

Table 1: Potential Impurities in Budesonide

Impurity	Source	Structure	Acceptance Criteria
			(b) (4)

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(b) (4)

In the stability studies on the 3 NDA registration batches of Budesonide 2 mg Rectal Foam, the samples were stored in upright and horizontal configurations at 25^0C/60% RH for 12 months and 40^0C/75% RH for 6 months. A summary report is provided in the Applicant's Table below, which also shows the impurities (column 1) identified in the #QCTP-280 (column 3).

Stability Attribute	Test Specification	Stability Method #	Initial	1 Month	3 Month	6 Month	9 Month	12 Month
Appearance	Conforms	QCTP-275	(b) (4)					
Assay per Can Budesonide HPLC	(b) (4) LC	QCTP-276						
(b) (4)	NMT (b) (4)	QCTP-280						
	NMT	QCTP-280						
	NMT	QCTP-280						
	NMT	QCTP-280						
Total Unspecified Impurities	NMT	QCTP-280						
Total Impurities	NMT	QCTP-280						
Foam Actuation Weight	(b) (4)	QCTP-278						
Foam Volume	(b) (4) L/actuation	QCTP-278						
Duration of Foam Expansion	NMT (b) (4) sec	QCTP-278						
pH	(b) (4)	USP <791>						
Canister Weight Loss	NMT (b) (4) %	USP <671>						
Assay Per Actuation	(b) (4) HPLC - 2nd, 7th and 13th Dose	QCTP-279						
Delivered Dose Uniformity over Entire Contents	(b) (4) HPLC - Dose 1, 2, 3, 6, 7, 8, 9, 12, 13, 14	QCTP-279						
Total Aerobic Microbial Count	NMT (b) (4) CFU/g	USP <61>						
Total Yeast and Molds Count	NMT (b) (4) CFU/g	USP <61>						

In the study report, the Applicant stated that two impurities were found at higher concentrations in the drug product in the stability studies. They are (b) (4)

The maximum daily dose of budesonide is not more than 4 mg. These impurities are (b) (4) % in the Budesonide 2 mg Rectal Foam. According to ICH Q3A guidance the qualification threshold is 1.0%. Inorganic impurities are controlled and are not more than (b) (4) % as per the USP <281> residue on ignition test. (b) (4) The applicant stated that the levels of (b) (4) were within the acceptable limits as per the ICH Q3C guidance (the acceptable limits for (b) (4) is NMT (b) (4) ppm and (b) (4) ppm, respectively).

2.4 Proposed Clinical Population and Dosing Regimen

Budesonide Rectal Foam is indicated for the induction of remission in patients with active mild to moderate distal ulcerative colitis. The proposed dosing regimen is 1 metered dose (2 mg budesonide per meter dose) administered twice daily for 2 weeks followed by 1 metered dose (2 mg budesonide per meter dose) administered once daily intrarectally for 4 weeks.

2.5 Regulatory Background

A Type B pre-IND meeting was held on April 30, 2011, and the Agency asked the Applicant to conduct a 3-month intrarectal toxicology study with budesonide rectal foam in a non-rodent species to support a 6-week Phase 3 study. The applicant submitted the final report of a 6-week intrarectal toxicity study with budesonide rectal foam in dogs before initiating the Phase 3 study. A pre-NDA meeting was held on July 23, 2013, and the Agency agreed that no carcinogenicity studies would be required for budesonide.

3 Studies Submitted

Safety pharmacology:

Evaluation of the effects of budesonide on cloned hERG channels expressed in human embryonic kidney (HEK293) cells (Study # BUIV 0101).

Single dose toxicity:

1. Toxicity study of Budesonide (Ito, I., Nakaoka, N., Iwanami, T., Honmura, S., Kohara, M., Fujii, T. and Tensho, A. *The clinical report, report No 1*, 1985; 19:9, 1-30).
2. Acute toxicity study of budesonide by intravenous administration to NMRI mice (Study # BUF 11).
3. Acute toxicity study of (b) (4) by intravenous administration to NMRI mice (Study # BUF 12).

Repeated dose toxicity:

1. 14-day local tolerance study of budenofalk foam by rectal administration to beagle dogs (Study # BUF-1).
2. 14-day local tolerance study of budenofalk foam by rectal administration to beagle dogs (Study # BUF-8).
3. Four-week subchronic toxicity study of budenofalk foam by rectal administration to beagle dogs: comparison of an old batch with a new batch (BUF-14).
4. A six-week rectal toxicity study in beagle dogs (Study # BUSA 0300).
5. A 39-week rectal toxicity study in dogs (Study # BUSA 0301).

Genetic toxicology

1. Toxicity study of Budesonide: Mutagenicity study (Fujii, T., Yamashita, T., Miyamae, Y. and Tensho, A. *The clinical report, [report No 4]*, 1985; 19:9, 1-15).
2. Mutagenicity study of [redacted] (b) (4) in the *Salmonella Typhimurium* (Study # BUF-13).

Reproductive and developmental toxicology:

1. Toxicity study of budesonide: reproductive study (Toteno, T., Furukawa, S., Marihisa, T., Siguro, S., Fujii, T and Tomoie, H. *The Clinical report # 6*, 85, 19:10; 1-76).

Published studies:

1. Toxicity study of the new glucocorticosteroid budesonide in rats (Ekman L, Kihlström I, Ryrfeldt A., Arzneimittelforschung. 1987 Jan;37(1):37-42.).
2. Budesonide-Preferid®- A New, Nonfluorinated Acetal Type of Steroid (Richer, J. R. edited by Maibach H. I. and Surber, C. *Topical corticosteroids*, Basel, Karger, 1992, 349-369).
3. Brief communication: Liver tumors in Male Rats Following Treatment with Glucocorticosteroids (Ryrfeldt, A., Squire, R. A. and Ekman, L. Toxicology pathology: 1992, 20:1, 115-117).
4. Teratogenicity Study of the New Glucocorticosteroid Budesonide in Rabbits (Kihlstrom, I. and Lundberg, C. 1987, *Arzneim. Forsh/Drug Res*. 37(1):4346.

3.1 Studies Reviewed

Relevant published pharmacology and pharmacokinetic studies submitted are reviewed. In addition, single dose and repeated dose toxicity studies, reports of genetic toxicology and reproductive and developmental toxicology studies were also reviewed.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

None

4 Pharmacology

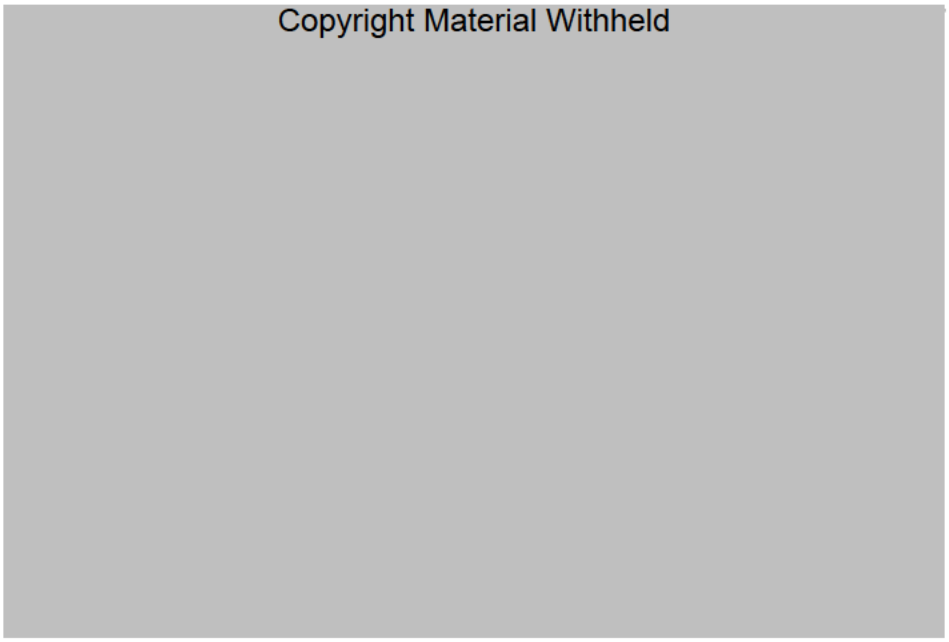
The applicant submitted available published pharmacology studies of budesonide, and relevant studies are reviewed in the following sections:

Pharmacodynamic aspects of glucocorticoid action. (Hochhaus, G., Derendorf, H., Mollmann, H. W., Barth, J. and Hochhaus, R., Proceeding of International Falk

Workshop on Glucocorticoid therapy in Chronic inflammatory bowel disease from basic principles to rational therapy edited by H. W. Mollmann and B. May; *Kluwer Academic Publisher,* 1996; 61-79).

Glucocorticoid levels (B_{max}) and affinities (K_D) vary significantly among patients. Both affinities as well as binding capacity have been correlated to patient response. The binding affinity will define the concentration of steroid necessary to achieve a given effect. Therefore, the receptor binding affinity of several glucocorticoid derivatives is of particular importance for their therapeutic use. In this review article, the authors compared the relative binding affinity of different topical corticosteroids including budesonide. The comparative studies showed that budesonide had a high glucocorticoid receptor affinity compared to several other corticosteroids. The relative binding affinities of different compounds are presented in the Table below.

Table 3 Relative binding affinities of commercially available glucocorticoids for the human glucocorticoid receptor



Effect of local Budesonide treatment in experimental inflammatory bowel disease. (van Rees, E. P., Soesatyo, M., Palmen, M. H. J. H., Pena, A. S. and Meuwissen, S.G.M., Digestive Disease Week and 94th Annual Meeting of the American Gastroenterological Association, May 15-23, Boston, *Gastroenterology Suppl. Vol.* 104:4, 1993, A795).

Trinitrobenzene sulfonic acid (TNBS)-mediated colitis was induced in Wistar rats. The animals were treated with an enema of 0.25 ml budesonide at a dose of 10^{-5}M on Day 1, Day 4 and Day 8 after induction of IBD. Rats were sacrificed at different time points after the last treatment. Control groups received vehicle alone or budesonide after sham-induction of IBD with saline only. To evaluate the effects of budesonide, bowel habits were checked daily, damage scores of the colon were scored from 0-5 scale, and

immunohistochemistry was used to study several subpopulations of macrophages, dendritic cells and lymphocytes. The result showed that macrophages had different distribution patterns in the IBD rats than in control, and the number of dendritic cells was increased in IBD rats, and 90% of the rats had colon damage scores of 3-5. Single treatment of budesonide had no effect on IBD. However, after three subsequent treatment doses, clinical symptoms and damage scores improved significantly. The damage score was 0-1 in 80% of the rats. Immunohistochemistry showed normal number and distribution pattern of macrophages and dendritic cells after three treatments. Major histocompatibility complex (MHC) class II expression was reduced to normal levels after budesonide treatment. Thus, in conclusion, the acute phase of TNBS-induced colitis can be treated with multiple local doses of budesonide.

Plasma Exudation, Hyperemia, and Epithelial Permeability in Rats with Oxazolone-Induced Colitis: Modulatory Effects of Budesonide (Ekstrom, G. M. and Anderson, S. E. *Scand J Gastroenterol* 2000; 35: 190-197).

The effects of budesonide on the inflammatory changes in oxazolone-induced colitis in the rat colon were examined after topical administration of the drug. Colitis was induced by intra-rectal application of oxazolone. The doses of budesonide used were 25.8 and 129 µg/kg. In oxazolone challenged animals, there were mucosal inflammation of the colon with increased plasma exudation, hyperemia and epithelial permeability. Treatment with 25.8 µg/kg budesonide did not cause any attenuation of the absorptive permeability in the colon while the 129 µg/kg dose caused attenuation of the permeability changes. Budesonide (129 µg/kg) caused significant reductions of the plasma exvasation and abolition of the hyperemia in the oxazolone-challenged animals. Thus, in rats with oxazolone-induced colitis, budesonide reduced the inflammation by decreasing permeability, plasma exvasation, and increased blood flow.

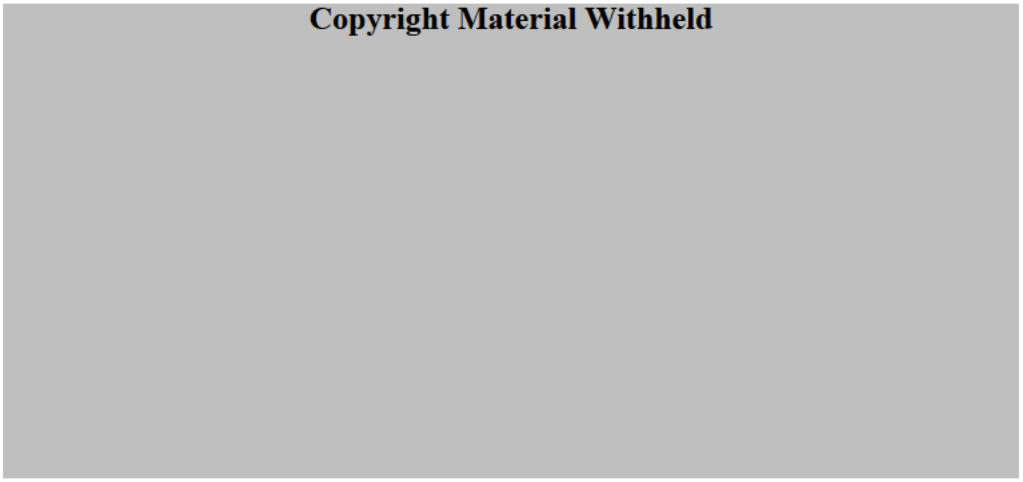
Topical Anticolitic Efficacy and Selectivity of the Glucocorticoid Budesonide in a New Model of Acetic Acid-Induced Acute Colitis in the Rat (Fabia, R., ArRajab, A., Willen, R., Brattsand, R., Erlansson, M. and Svensjo, E. *Aliment Pharmacol Ther* 1994; 8: 433441).

Colitis was induced in Sprague-Dawley rats by intrarectal administration of 4% acetic acid for 15 seconds. The effects of budesonide on acetic acid-induced colitis in the rats were examined after local instillation (10^{-6} or 10^{-8} M) or SC administration (0.5, 0.75 or 1.0 mg/kg) of budesonide. The treatment started on the day after acetic acid instillation and continued for Days 2 and 3. The effect of the drug on the plasma exudation into the colonic lumen was measured using ^{125}I-labeled albumin as a tracer. The animals were sacrificed on the fourth day after colitis induction and the colonic segments were examined microscopically and the tissue myeloperoxidase activity was determined. Acetic acid induced colitis caused significant increases in total morphological scores, tissue myeloperoxidase activity (units/g; one unit was defined as the amount of the enzyme present that produces a change in absorbency at 655 nm per min at 37^0C) and plasma exudation (µL/min/g) into the colonic lumen of the rats as compared with the controls, and treatment with local budesonide caused significant reduction of all the three parameters (total morphological scores: control 1.5±0.2, acetic acid 14.8±0.8,

budesonide 3.5±0.4; myeloperoxidase activity: control 83.8±5.5, acetic acid 258.6±23.4: budesonide 109.1±8.3; plasma exudation: control 0.28±0.05, acetic acid 1.68±0.18, budesonide 0.56±0.08). Subcutaneous budesonide at 0.75 mg/kg caused significant improvements of the morphologic scores (80% reduction). These doses also normalized myeloperoxidase activity and significantly reduced the plasma exudation. The systemic effects of the drug were most pronounced in the group treated with parenteral budesonide.

Anti-inflammatory Effects of Budesonide (Hiroi, J., Fujii, T., Satoh, S., Takehiro, O., Katsumasa, K., Yoshihiko, O., Hachiro, S., Jo, M and Hiroyuki, K., *Folia Pharmacol. Japon*. 1985; 86:1-38).

In this study, the anti-inflammatory effect of budesonide was compared with betamethasone 17-valerate, hydrocortisone-21-acetate, hydrocortisone-17-butyrate and fluocinolone acetonide after systemic and topical administration to several inflammation models of laboratory animals (listed in the table below). Subcutaneous administration of budesonide showed stronger anti-inflammatory action than betamethasone 17-valerate in carrageenan paw edema, cotton pellet granuloma, adjuvant arthritis, croton oil edema, and in contact hypersensitivity in mice. In topical treatment of carrageenan edema, croton oil edema, croton oil skin reaction, cotton pellet granuloma, and contact hypersensitivity, budesonide showed more potent anti-inflammatory action than betamethasone 17-valerate. The anti-inflammatory effect of budesonide after topical administration to rodents was 1.3 to 200 times superior to comparators betamethasone 17-valerate, hydrocortisone-21-acetate, and hydrocortisone-17-butyrate. Only fluocinolone acetonide achieved better anti-inflammatory effects than budesonide. Anti-inflammatory index of budesonide compared to other corticosteroids are shown in the Table below.



Source: Hiroi 1985.
ND = Not Determined

Effects of Plain and Controlled-Ileal-Release Budesonide Formulations in Experimental Ileitis (Boyd, A.J., Sherman, I. A. and Saibil, F. G., *Scand J Gastroenterol* 1995; 30: 974-81).

The effects of plain and controlled-ileal-release (CIR) budesonide were examined on trinitrobenzene sulfonic acid (TNBS)-induced intestinal inflammation in hamsters after oral administration of the drug. The doses used were 200 and 800 µg/kg/day (plain budesonide), or 200 µg/kg/day (CIR budesonide). The pre-treatment groups received the drug or placebo 2 days before the induction of inflammation and the post-treatment groups received the drug from Day 7 after the induction of inflammation. The animals were killed after 2 weeks and inflammation was assessed by histologic examination of the intestine and by measuring mastocytosis and myeloperoxidase activity.

Two weeks after TNBS-treatment, ileal segments showed clear histologic evidence of inflammation and increased levels of tissue myeloperoxidase activity (control segments, 0.300±0.001 units/g; TNBS-treated segments, 9 units/g). Treatment with 200 µg /kg/day of plain budesonide had no effect on the intestinal inflammation as assessed by histological examination. The 800 µg/kg/day dose caused a decrease in the histologic appearance of inflammation in both pre- and post- treatment groups. This dose also caused a significant decrease in the mast cell numbers (decreases 43% and 27% in pre- and post-treatment groups) as compared with controls. Treatment with the CIR formulation also caused an improvement of the histologic appearance of inflammation and decreased the number of mast cells (31.7% and 36% in pre- and post- treatment groups, respectively). Treatment with both formulations of budesonide (800 µg /kg/day plain and 200 µg/kg/day CIR) caused decreases in the myeloperoxidase activities of the inflamed intestines. Treatment with 800 µg/kg/day of plain budesonide caused 87.7% and 62.2% decreases in the myeloperoxidase levels in the pre- and post-treated animals, respectively. The 200 µg /kg/day dose of the CIR formulation caused 77.8% and 67.8% decreases of the enzyme activity, respectively. Thus, oral CIR budesonide was effective in reducing TNBS- induced inflammation in hamsters.

General pharmacology study of budesonide: Nishimura K., Kobayashi F., Nishimura, K., Kobayashi, F., Nishimori, M, Mori, M. and Tomoie, H. *The Clinical Report* 1985; 19(9):109-136).

The effect of budesonide on the mucosa of the gastrointestinal tract was examined in male Wistar rats. In this study, male Wistar rats (6 animals per group) were administered budesonide intravenously at single doses of 0.1, 1.0 and 10.0 mg/kg. Four hours after dosing, the rats were sacrificed and the extent of damage to the internal wall of the stomach and duodenum was observed.

Results showed slight erosion of the glandular stomach in 2 out of 6 rats following a single intravenous dose of 10 mg/kg budesonide. Similarly, stomach erosion was also noticed in 1 out of 6 rats in each dose group of 0.1 and 1.0 mg/kg budesonide and control group. No irritant effect was seen on the duodenal mucosa in any treatment group. Data are shown in the Applicant's Table below.

Table 13 Gastric Irritation Potential of Budesonide in Rats

Test Article	Dose (mg/kg IV)	Stomach			Intestine
		Fore Stomach	Glandular Stomach		Duodenal Mucosa
		Normal	Normal	Slight Erosion	Normal
Control	---	6/6	5/6	1/6	6/6
Budesonide	0.1	6/6	5/6	1/6	6/6
Budesonide	1.0	6/6	5/6	1/6	6/6
Budesonide	10.0	6/6	4/6	2/6	6/6

4.3 Safety Pharmacology

Cardiovascular system:

Evaluation of the effects of budesonide on cloned hERG channels expressed in human embryonic kidney (HEK293) cells (BUIV 0101).

Methods: Budesonide was tested at concentrations of 4.5, 15, 45, and 150 µmol/L. Whole cell patch clamp was used to measure potassium currents from HEK cells stably expressing the hERG channel to determine the effect of extracellular application of budesonide on hERG current amplitude. As a positive control, cisapride was used at a concentration of 0.1 µmol/L. In whole-cell mode, once stable current responses were obtained, HEK293 cells expressing hERG were held at a potential of -80 mV. hERG mediated currents were evoked by application of a depolarizing voltage command step to +40 mV for 2 seconds followed by a repolarizing command step to -50 mV for 1.5 seconds. This voltage command protocol was repeated once every 10 seconds. Peak tail currents from the last 30 seconds of the baseline period were averaged and compared to 30 seconds of data recorded in the presence of the test solutions. Current inhibition was reported in percent according to Inhibition(%) = 100 x (1-[$I_{test}/I_{baseline}$]) where I_{test} was the peak tail current measured in the presence of the test solution, and $I_{baseline}$ was the peak tail current measured prior to exposure to the test solution; each cell served as its own control. IC_{50} or IC_{20} values were calculated.

Results: The result showed that budesonide produced concentration-dependent inhibition of mean hERG-mediated potassium currents of approximately 4, 14, 31, and 58%, respectively, resulting in approximate IC_{20} and IC_{50} values of 23 µM and 106 µM, respectively. The positive control article (cisapride) produced approximately 84% inhibition of hERG channel-mediated potassium currents. The effects of budesonide on tail peak currents in hERG transfected HEK cells are shown in the Table below.

Summary of hERG Current Inhibition, %			
Concentration	Inhibition, % Mean	SEM	N
0 µM (PSS + 0.3% DMSO)	1.40	1.243	3
4.5 µM Budesonide	4.44	0.825	3
15 µM Budesonide	13.81	1.921	3
45 µM Budesonide	30.61	2.729	4
150 µM Budesonide	58.37	0.046	3
0.1 µM Cisapride	83.91	0.143	3

Central Nervous System:

General pharmacology study of budesonide (Nishimura, K., Kobayashi, F., Nishimori, M., Hoken, K. and Kenkyu, S. 1985, *The Clinical Report* 19(9):1-75).

Effect s of budesonide on body temperature was determined after IV administration to the mice and rabbits. Budesonide was administered intravenously at doses of 0.1, 1.0, and 10 mg/kg body weight. In mice, rectal temperature was measured prior to administration of budesonide and 10, 20, 30, 45, 60 and 90 minutes post-dose. Likewise, in rabbits, rectal temperature was measured pre-dose and 15, 30, 60, 120 and 240 minutes post-dose. Changes in body temperature after drug administration were compared with the results before administration and with the control group. There was no effect on body temperature after IV administration of budesonide in both mice and rabbits.

Effects of budesonide on muscle coordination were determined in male mice after IV administration of the drug at doses of 0.1, 1.0, and 10 mg/kg body weight or vehicle control. Mice were tested for muscle coordination prior to dosing and 10, 20, 30, 45 and 60 minutes post-dose using the horizontal wire test. Animals were made to grasp a 1 mm diameter wire suspended horizontally at a height of 30 cm with their forelegs. Animals that failed to reach the wire with their hind legs within 10 seconds for all 3 assessments at a single time point were deemed positive for ataxia (muscle-relaxing action). The result showed that budesonide had no effects on muscle coordination in mice.

Prolongation of anesthesia, analgesic effects, pain and seizure effects of budesonide were determined in mice after IV administration of drug at doses of 0.1, 1.0, and 10 mg/kg body weight or vehicle control. Ten minutes after budesonide administration mice were administered thiopental sodium (30 mg/kg, IV) and acetic acid (0.1mL/10 g 0.6%, IP) and pentetrazole (115 mg/kg, SC), respectively. In order to determine the pain and seizure effects, an arterial clamp was applied to the root of the tail to determine biting and squeaking 10 min after budesonide injection. Budesonide had no effects on prolongation of thiopental sodium-induced anesthesia duration, acetic acid-induced

analgesic effects and pentetrazole-induced seizures. Similarly, no inhibition of pain response was observed at any dose of budesonide.

Respiratory and circulatory system:

General pharmacology study of budesonide (Nishimura, K., Kobayashi, F., Nishimori, M., Hoken, K. and Kenkyu, S. 1985, *The Clinical Report* 19(9):1-75).

Cannulas were inserted into the femoral artery of male beagle dogs to measure blood pressure. Budesonide was administered intravenously to male beagle dogs at doses of 0.1, 1.0 and 10 mg/kg. Respiratory rate, blood pressure, heart rate and ECG were measured until 60 minutes post-dosing in unanesthetized, unrestrained animals. One animal in the control group and one animal in each budesonide treated group showed increased respiratory rate and fine tremor of the hind legs. None of the budesonide-treated animals showed any marked changes in blood pressure, heart rate or ECG compared to vehicle control. Thus, budesonide had no effects on respiratory rate, blood pressure, heart rate and ECG.

Gastrointestinal system:

General pharmacology study of budesonide (Nishimura, K., Kobayashi, F., Nishimori, M., Hoken, K. and Kenkyu, S. 1985, *The Clinical Report* 19(9):1-75).

Effects of budesonide on gastric transit were determined in mice. Mice were intravenously dosed with 0.1, 1.0 or 10.0 mg/kg budesonide or vehicle control. Ten minutes after drug administration, 0.1 mL charcoal (suspension of 10% gum arabic and 5% charcoal) was administered orally. The mice were sacrificed 30 minutes after charcoal administration, and migration rate of the charcoal along the entire length of the small intestine was measured. The result showed that there was a mild inhibition of charcoal transport rate at 0.1 mg/kg budesonide. However, this action was not seen at the 1.0 or 10.0 mg/kg doses.

Effects of budesonide on gastric secretion were determined in rats. Male Wistar rats were fasted for 24 hours, pyloric region of abdomen was ligated under ether anesthesia and budesonide was intravenously administered immediately at doses of 0.1, 1.0 or 10.0 mg/kg. After 4 hours of budesonide administration, the rats were sacrificed and samples of gastric juices were collected. The gastric juices were centrifuged and the liquid volume, pH and general acidity of the resulting supernatant were measured. The result showed that 0.1 mg/kg budesonide had no effects. However, at 1.0 and 10 mg/kg budesonide, a significant elevation in pH was observed compared to control. At 10.0 mg/kg budesonide, a significant decrease in general acidity compared to control was observed. The effects of budesonide in gastric volume, acidity and pH are presented in the Table below.

Study Drug	N	Dose (mg/kg IV)	Gastric Volume (mL)	Total Acidity (mEq/L)	pH
Control	9	---	2.81 ± 1.26	62.2 ± 12.1	1.85 ± 0.349
Budesonide	8	0.1	2.27 ± 0.867	53.0 ± 7.90	1.89 ± 0.206
	8	1.0	2.44 ± 0.899	55.7 ± 11.6	2.25 ± 0.338*
	8	10.0	2.27 ± 0.793	45.6 ± 12.9*	2.45 ± 0.586*

Values are expressed as mean ± SD
Abbreviations: IV = intravenous; N = number of animals; SD = standard deviation
*Significantly different from control ($p < 0.05$).

Effects of budesonide on bile secretion were determined in Wistar rats. Polyethylene tube was inserted into the common bile ducts of male Wistar rats under urethane anesthesia. Rats were intravenously dosed with 0.1 mg/kg, 1.0 mg/kg or 10.0 mg/kg budesonide or vehicle control. Bile samples were collected prior to budesonide administration and at 30, 60, and 90 and 120 min post-dose. The weights of the samples were measured. The result showed that 30 min after budesonide administration, bile secretion was reduced to 60-77% in control, 0.1 and 10 mg/kg dose groups, when compared to pre-dose. At 1.0 mg/kg dose group, the decrease in bile volume 30 minutes after administration was very small compared to the other groups. No significant changes in the bile volume were observed at 60 to 120 min post-dose at any dose levels and control. The effects of budesonide on bile secretion is presented in the figure below.

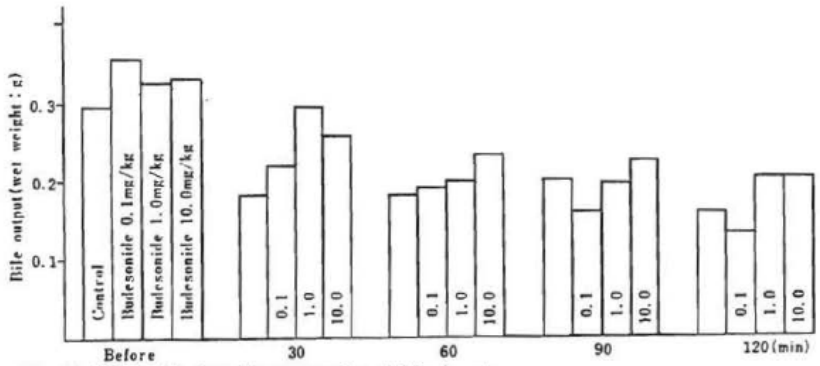


Fig. 14 Effect of budesonide on excretion of bile in rats

Renal system:

General pharmacology study of budesonide (Nishimura, K., Kobayashi, F., Nishimori M., Hoken, K. and Kenkyu, S. 1985, *The Clinical Report* 19(9):1-75).

Renal function of budesonide was determined in male Wistar rats. Budesonide was administered intravenously to male rats at doses of 0.003, 0.01, 0.03, 0.1, 1.0 or 10.0 mg/kg. Another group of male rats received IV injection of betamethasone 17-velarate at doses of 10.0 or 100.0 mg/kg, and a control group received the vehicle. Immediately following drug administration, physiological saline solution (2.5 mL/100 g of body weight) was administered orally. Then the animals were placed in individual urine collection cages, and urine volume and urinary electrolyte (sodium ion, potassium ion, and chloride ion) were determined for 6 hours following dosing. The result showed that

no changes in urine volume or electrolyte were observed in animals dosed with 0.003 mg/kg budesonide. Significant increases in K^+ excretion were observed in the 0.01mg/kg dose group. Dose-dependent increases in urine volume and electrolyte excretion were observed in the 0.03, 0.1, 1.0, and 10.0 mg/kg dose groups. Increases in K^+ excretion were observed with 10.0 mg/kg betamethasone 17-valerate. Significant decreases in urine volume and electrolyte secretion were observed at 100.0 mg/kg betamethasone 17-valerate. The urine parameters are presented in the Table below.

Study Drug	N	Dose (mg/kg IV)	Urine Volume (mL/100 mg)	Na^+ (µEq/100 g)	K^+ (µEq/100 g)	Na^+/K^+	Cl^- (µEq/100 g)
Control	21	---	1.43 ± 0.457	235 ± 65.1	257.4 ± 61.6	0.9 ± 0.229	325 ± 72.0
Budesonide	7	0.003	1.42 ± 0.717	258 ± 89.2	260.6 ± 51.3	1.0 ± 0.287	362 ± 105
	7	0.01	1.67 ± 0.550	278 ± 49.9	313.2 ± 76.3*	0.9 ± 0.141	384 ± 77.3
	7	0.03	1.66 ± 0.652	292 ± 40.2*	343.8 ± 64.8**	0.9 ± 0.127	389 ± 63.6*
	7	0.1	3.53 ± 0.910**	487 ± 91.0**	439.0 ± 39.6**	1.1 ± 0.163*	563 ± 82.8**
	7	1.0	4.72 ± 0.584**	601 ± 86.5**	520.2 ± 36.8**	1.1 ± 0.0976*	638 ± 57.6**
	7	10.0	4.69 ± 0.534**	657 ± 98.5**	564.2 ± 78.5**	1.2 ± 0.121**	712 ± 78.9**
Betamethasone 17-Valerate	7	10.0	1.70 ± 0.852	259 ± 77.9	325.4 ± 46.6*	0.8 ± 0.146	333 ± 91.6
	7	100.0	0.65 ± 0.507**	60 ± 62.7**	162.9 ± 88.9**	0.3 ± 0.207**	130 ± 91.0**

Values are expressed as mean ± SD
Abbreviations: Cl^- = chloride; IV = intravenous; K^+ = potassium; N = number of animals; Na^+ = sodium; SD = standard deviation
*Significantly different from control ($p < 0.05$).
**Significantly different from control ($p < 0.001$).

5 Pharmacokinetics/ADME/Toxicokinetics

The applicant did not submit any pharmacokinetic studies with budesonide. However, the applicant submitted available published pharmacokinetic studies of budesonide, which are reviewed below.

Plasma concentrations and therapeutic effects of budesonide in dogs with inflammatory bowel disease (Pietra, M., Fracassi, F., Diana, A., Gazzotti, T., Bettini, G., Peli, A., Morini, M., Pagliuca, G and Roncada, P. *Am Jour Vet Rer*, 2013; 74(1): 78-83).

The pharmacokinetics of budesonide was assessed in male and female dogs with inflammatory bowel disease (IBD). Eleven dogs (6 males and 5 females) with histopathologically confirmed moderate to severe inflammatory bowel disease (IBD) were treated with oral controlled-release budesonide (3 mg/m^2 once daily) for 30 days. Plasma and urine samples were assessed for budesonide and 16-α-hydroxyprednisolone (a budesonide metabolite) concentrations on days 1 and 8. Blood samples were collected pre-dose, 0.5, 1, 2, 4, and 7 hours following administration of budesonide. A single urine sample was collected from the dogs (n=9) after the final blood sample collection on Days 1 and 8. Budesonide was rapidly absorbed in dogs, with the highest plasma concentrations after 1 hour on Day 1. Mean C_{max} values of budesonide were 0.46±0.52 ng/mL on Day 1, and 0.56±0.37 ng/mL on Day 8. Budesonide was metabolized to 16-α-hydroxyprednisolone, and both compounds were excreted into urine. Data are presented in the Table below.

Table 1—Mean ± SD plasma concentrations of budesonide and 16-α-hydroxyprednisolone in samples obtained from 11 dogs with IBD before the start of budesonide administration (3 mg/m², PO, q 24 h for 30 days) and 0.5, 1, 2, 4, and 7 hours after drug administration on days 1 and 8 of treatment.

Tissue distribution and fate of budesonide in the mouse (Andersson, P., Appelgren, L. E. and Ryrfeldt, A., *Acta Pharmacol Toxicol (Copenh).* 1986;59(5):392-402.).

The tissue distribution and pharmacokinetics of ^{3}H-budesonide were studied in the mouse after intravenous administration via the caudal vein at a dose of 50.4 µg/kg. The drug was rapidly (distribution $t_{1/2}$ = 0.062 hours) distributed into the tissues and organs (Vss=9.4 l/kg). The short elimination half-life ($t_{1/2}$ = 1.55 hours) and high clearance (CL=9.04 L/h/kg) demonstrated a rapid elimination of budesonide from the body. Whole body autoradiography showed very high levels of radioactivity in the liver and kidney, as well as in the lung and lymphatic tissues. The adrenal cortex also showed high levels of radioactivity. Radioactivity passed the blood-placenta and blood-brain barriers. Based on liquid chromatography analyses, radioactivity in the lung, spleen, and brain was primarily unchanged budesonide. In the kidney and liver, radioactivity was identified as polar metabolites of budesonide.

Pharmacokinetics of butixocort 21-propionate, budesonide, and beclomethasone dipropionate in the rat after intratracheal, intravenous, and oral treatments. (Chanoine, F., Grenot, C., Heidmann, P. and Junien, J. L., *Drug Metab Dispos.* 1991; 19(2):546-53).

The pharmacokinetics of ^{3}H-budesonide and other glucocorticoids (butixocort 21-propionate and beclomethasone dipropionate) were studied following intravenous, oral, and intratracheal administration in male rats. Budesonide exposure was measured by whole body autoradiography, HPLC with UV detection and liquid scintillation counting. Budesonide was rapidly absorbed from lung to blood following intratracheal administration at a dose of 50 µCi, with the maximum concentration observed 3 minutes after administration. The last detectable concentration of budesonide was 4 hours after dosing. Only unchanged budesonide was measured in rat lungs following intratracheal administration, indicating that budesonide was not metabolized in the lung. The plasma AUC of unchanged budesonide accounted for 61% of the radioactivity. One metabolite (metabolite 1) was identified in plasma following aerosol administration. Following administration of an IV dose at 1.5 mg/kg, the AUC of budesonide was 2350 nM.h/L, with a mean half-life of 3.4 hours. The total body clearance was 1.5 L/h/kg, and volume of distribution was 7.9 L/kg. After oral administration of budesonide (1.5 mg/kg), detectable concentrations were observed for 3 hours. The oral bioavailability of budesonide in the rat was estimated to be approximately 15%.

Pharmacokinetic studies of a potent glucocorticoid (budesonide) in dogs by high-performance liquid chromatography (Ryrfeldt, A., Tonnesson, M., Nilsson, E. and Wikby, A., *J Steroid Biochem*. 1979;10(3):317-24).

The pharmacokinetics of ^{3}H-budesonide was measured in female beagle dogs following oral and intravenous administration at doses of 10µg/kg and 100µg/kg. Following oral administration, peak plasma concentrations of unchanged budesonide of approximately 0.5 nmol/L in the 10 µg/kg dose group and 50 nmol/L in the 100µg/kg dose group were observed within 30 minutes of administration. By 72 hours after oral dosing, approximately 78% of total radioactivity was eliminated (~59% in feces). Following intravenous administration, the plasma elimination half-life of unchanged drug was approximately 2 hours after the low dose (10µg/kg) and approximately 2.4 hours after the high dose (100µg/kg). Bioavailability ranged from 9.2% to 16.2% in low dose and 18.3% to 18.8% in high dose.

Budesonide is Metabolized by Cytochrome P450 3A (CYP3A) Enzymes in Human Liver (Jonsson, G., Astrom, A. and Andersson, P. *Drug Metab Disp* 1995; 23(1): 137-42).

To identify the isoform of cytochrome P450 involved in the metabolism of budesonide, budesonide was incubated with microsomes from ten different human liver samples. A strong correlation between the formation of the two budesonide metabolites, 16α-hydroxy prednisolone and 6β-hydroxybudesonide, and testosterone 6β-hydroxylation (a marker of CYP3A) was observed (R=0.98 and 0.95). When budesonide was incubated with the human liver microsomes in the presence of compounds that interact with different isoforms of CYP, ketokonazole was found to be the strongest inhibitor of budesonide metabolism (IC_{50} ~ 1 µM) followed by troleandomycin (IC_{50}~1 µM), erythromycin and cyclosporine, all substances known to be metabolized by CYP3A. Substances known to be metabolized by CYP2C (sulfaphenazole, mephenytoin and tolbutamide and CYP2D6 [Quinidine]) did not specifically inhibit the metabolism of budesonide. The formation of the budesonide metabolites was also inhibited by CYP3A antibody but not by CYPIA antibody or control immunoglobulin G. Thus, budesonide is metabolized by CYP3A subfamily of enzymes.

Pharmacokinetics of budesonide and its major ester metabolite after inhalation and intravenous administration of budesonide in the rat (Jendbro, M., Johansson, C. J., Strandberg, P., Falk-Nilsson, H. and Edsbacker, S. *Drug Metabolism and Disposition*, 2001: 2905-769–776).

Pharmacokinetics of budesonide and budesonide-fatty acid esters (oleates) was examined following both inhalation and IV administration to the rat. For inhalation administration of budesonide, micronized dry powder was administered by inhalation to rats using an aerosol delivery system adapted for small animals. The inhaled dose was 210 nmol/kg. The rats were put in restraining tubes connected to the inhalation chamber and exposed to budesonide for approximately 10 min. Groups of 3 rats were sacrificed

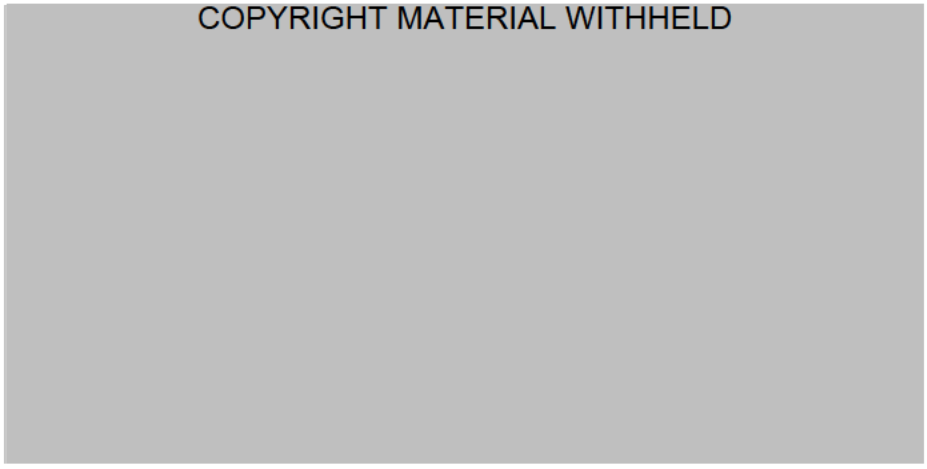
at 15, 30, 45, 60, and 90 min and 2, 4, 8, 12, and 24 h after start of the inhalation. Plasma, lungs, and trachea were collected at each time points.

Male Sprague-Dawley rats were administered [^{3}H]-budesonide intravenously (tail vein) at an approximate dose of 7 µg/kg (16 nmol/kg). Groups of 3 animals were sacrificed at each of the following time points and plasma samples were collected at 5, 15, 30, 45, and 60 minutes, 1.5, 2, 4, 8, 12 and 24 h after budesonide administration. Samples of lung, trachea and muscle tissues were collected for radioactivity analysis of [^{3}H]-budesonide. Levels of both [^{3}H]-budesonide and [^{3}H]-budesonide-oleate were determined in both plasma and tissues.

After inhalation, budesonide half-life was longer (8.2 h) in trachea than in plasma (3.7 h), with similar findings after intravenous dosing. Results are presented in the Table below. Following IV dosing, a half-life of 4.7 hours and $AUC_{(0\text{-inf})}$ of 1.59 µg.h/L were obtained for budesonide, and budesonide-oleate exhibited a half-life of 5.4 hours and an $AUC_{(0\text{-inf})}$ of 0.56 µg.h/L. A plasma clearance of 4.4 L/h/kg was determined for budesonide. In the tissues tested for radioactivity, the exposure to the tissues was notably higher than that of the plasma. For comparison, the AUC values, indicative of total exposure over time, for the lung, trachea and muscle were 14.7, 10.1 and 6.06 µg.h/L, respectively, compared to the value of 1.59 µg.h/L obtained for plasma following IV administration. Formation of budesonide-oleate occurred in all tissues, and concentrations of the oleate form of the compound decayed in a bi-phasic pattern over time. This study confirmed that fatty ester esterification represents a common mechanism for metabolism and ultimately storage of steroid hormones. Pharmacokinetic data of budesonide following inhalation and IV doses is presented in the Table below.

TABLE 1

Noncompartmental pharmacokinetic analysis of BUD and BUD-oleate after administration of BUD to rats; intravenous administration (16 nmol/kg) and inhalation (210 nmol/kg)



PXR-mediated induction of human CYP3A4 and mouse CYP3 all by the glucocorticoid budesonide. (Zimmermann, C., van Waterschoot, R. A., Harmsen, S., Maier, A., Gutmann, H. and Schinkel, A. H. *Eur. J. Pharm. Sci,* 2009; 36: 565-571).

Budesonide is metabolized by cytochrome P450 3A4 (CYP3A4). Inhibition or induction of CYP3A4 could cause drug-drug interactions. Thus, CYP3A4 activity was assessed by the metabolism of a luminogenic substrate (luciferin-benzylether) using a recombinant human CYP3A4 protein. There was no inhibition of the metabolism in the presence of budesonide at concentrations up to 25 µM. Induction experiments in human LS180 colon carcinoma cells showed an increased expression of CYP3A4 mRNA after budesonide treatment. Transactivation assays revealed that budesonide activates the CYP3A4 promoter via the pregnane X receptor (PXR). In mice, oral budesonide administration (25 mg/kg) for 4 days induced 3-fold murine homolog CYP3a11 in the intestine, whereas liver expression was markedly less influenced. In knockout mice devoid of PXR, budesonide-mediated inductions were significantly reduced compared to wild-type mice. In conclusion, budesonide is not an efficient inhibitor but rather an inducer of CYP3A via a PXR-mediated mechanism. However, oral administration of budesonide to mice showed only modest gene induction, which occurred mainly in the intestine. Thus, the risk for budesonide-mediated drug interactions seems to be low but cannot be ruled out entirely.

6 General Toxicology

6.1 Single-Dose Toxicity

Toxicity study of Budesonide (Ito, I., Nakaoka, N., Iwanami, T., Honmura, S., Kohara, M., Fujii, T. and Tensho, A. *The clinical report, [report No 1]*, 1985; 19:9, 1-30).

In this is a non-GLP study, the acute toxicity study of budesonide was investigated in mice and rats after oral, IV, IP and SC routes of administration, and in dogs after SC administration. Different routes of administration and doses for different species are shown below.

Mouse:	Oral:		0, 100, 320, 1000, 3200, and 10000 mg/kg
		IV:	32 – 320 mg/kg.
		IP:	10-1000 mg/kg.
		SC:	0, 18, 32, 56, 100, 180, and 320 mg/kg
Rat:	Oral:		0, 32, 100, 320, 1000 and 3200 mg/kg
		IV:	10 – 320 mg/kg.
		IP:	10-1800 mg/kg.
		SC:	0, 10, 18, 32, 56, 100 and 320 mg/kg
Dog:	SC:		0, 100, 320 and 100 mg/kg

Mortality:

Mice: Deaths occurred in the male mice treated with 130 mg/kg or more and the female mice treated with 56 mg/kg or more IV doses of budesonide. Deaths were also noticed in males treated with 100 mg/kg or more and females treated with 320 mg/kg or more IP doses of budesonide. Likewise, deaths were also observed in both male and female mice at 32 mg/kg or more SC doses of budesonide. Deaths also occurred among the males that received 320 mg/kg or more and the females that received 1,000 mg/kg or more oral doses of budesonide.

Rats: Deaths were noted among the males that received 56 mg/kg or more and the females that received 100 mg/kg or more IV administration of budesonide. The males and females that received 100 mg/kg or more IP dose of budesonide and males that received 32 mg/kg or more and females that received 56 mg/kg or more SC dose of budesonide also showed mortalities. Also, some of the females that received 1,000 mg/kg or more oral dose of budesonide died. However, there was no death among the male rats even at highest oral dose of budesonide (3,200 mg/kg).

Dogs: Some of the dogs treated subcutaneously with 100 mg/kg or more, and all of the dogs that received the 320 mg/kg SC dose of budesonide died.

Clinical signs: Mice and rats showed emaciation and associated weakness up to Day 10 following administration of budesonide, and a number of late deaths were recorded. Before death, both mice and rats showed suppressed spontaneous motility with prone position. The majority of surviving mice and rats recovered during the 21-day follow-up period. Reduced motility and clonic seizures were observed in rats and mice receiving IV doses of budesonide. Reduced motility was also observed in rats receiving higher doses of IP budesonide and a transient increase in sensitivity to pain was observed in both rats and mice following IP administration. Almost all dogs appeared to be emaciated from Day 10 of the follow-up period. The dogs that died showed reduced motility prior to death.

Body weights: Dose-related inhibition of weight gain was observed in both mice and rats following all routes of budesonide administration. Similarly, dogs treated with budesonide SC showed reduced body weight.

Gross pathology and histology: In mice, following findings were recorded during macroscopic and histopathological investigations of animals who died or were sacrificed: nodules in the gastric mucosa, peritonitis and/or pleurisy and abscesses and/or grayish white nodules under the skin of the neck, back and other regions, necrosis in the muscle tissue, grayish white nodules or foci in the myocardium, liver, and kidneys, and elevated extramedullary hematopoiesis in the spleen. In rats, atrophy of the adrenal cortex, ulcers in the glandular section of the stomach, adhesion of abdominal organs, peritonitis associated with ulcers, pyelonephritis and necrotic foci in the liver were observed. In dogs, localized indurations were seen at the injection site after SC administration. There was histological evidence of atrophy of the spleen and

thymus and interstitial edema in the thymus. There was also evidence of hypertrophy of the liver cells and vacuolization of the fasciculated cells of the adrenal glands. Pulmonary hemorrhages or ascites, ulcers in the gastrointestinal tract and gastrointestinal hemorrhages were found in approximately 50% of the animals. Edema and bacterial colony formation were observed at the injection site.

Study: Acute toxicity study of budesonide by intravenous administration to NMRI mice (Study # BUF-11).

A single dose of Budesonide was administered to NMRI mice via the intravenous route at doses of 31.6, 68.1, 147, 316, and 681 mg/kg. Eight out of ten total mice died within 6 hours to 14 days at 316 mg/kg. At 681 mg/kg, all males and females died. However, there was no mortality in 31.6, 68.1 and 147 mg/kg dose groups.

All animals in the 316 mg/kg group showed slight to severe ataxia, dyspnoea, and reduction in motility within 0.5 to 30 minutes.

Body weights were recorded before administration of test article and thereafter in weekly intervals up to the end of the study. A reduction of body weight of female animals was observed at 147 and 316 mg/kg doses during both weeks. In addition, the body weight gain was negative during the first week at 68.1 mg/kg. A reduced body weight gain was noticed in 147 and 316 mg/kg dose groups. Data is presented in the Table below.

Parameters	Control		31.6 mg/kg		68.1 mg/kg		147 mg/kg		316 mg/kg		681 mg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
BW (g) Test Day 0	22.2	18	22.4	18.2	21.8	18.6	21.4	18.4	21.4	19	20.8	19.6
BW (g) Test Day 8	28.2	22	25.6	18.6	24.4	17.6	21	17	23	17.3	Died	Died
BW (g) Test Day 15	31.4	23.2	28.4	21	28.4	19	24.6	17.4	22.7	18.3	Died	Died
BW gain (%) Test day8	27	22.2	14.3	2.2	11.9	-5.4	-1.9	-7.6	7.5	-8.8		
BW gain (%) Test day15	41.4	28.9	26.8	15.4	30.3	2.2	15	-5.4	5.9	-3.5		

Gross pathology: At the end of the experiments all surviving animals were sacrificed, dissected and inspected macroscopically.

No macroscopical changes were noticed at necropsy at any dose level.

Study: Acute toxicity study of (b) (4) by intravenous administration to NMRI mice (Study # BUF-12).

A single dose of (b) (4) was administered to NMRI mice via intravenous route at dose levels of 31.6, 68.1, 147 and 316 mg/kg. All animals were observed for clinical signs before, immediately after dosing, and at 5, 15 and 30 minutes and 1, 3, 6, and 24 hours following administration of the test article. All surviving animals were observed for a period of 14 days. One male and two female mice died within 15 minutes to 4 days at the 147 mg/kg dose. All animals in the high dose group (316 mg/kg) died within 5-15 minutes after administration of the test article. No clinical signs were observed in the 31.6 mg/kg dose group. However, at a dose level of 68.1 mg/kg, all animals showed slight to moderate ataxia, dyspnoea, and reduction in motility within 0.5 to 30 minutes.

Body weights were recorded before administration of the test article and thereafter in weekly intervals up to the end of the study. The body weight gain of the animals was within normal range. At the end of the dosing, all surviving animals were sacrificed, dissected, and inspected macroscopically. No macroscopic changes were noticed at necropsy at any dose level.

6.2 Repeat-Dose Toxicity

Toxicity study of the new glucocorticosteroid budesonide in rats (Ekman L, Kihlström I, Ryrfeldt A. *Arzneimittelforschung.* 1987 Jan;37(1):37-42.).

Budesonide was administered subcutaneously to male and female Wistar rats at doses of 0.01, 0.1 and 5 µg/kg/day (10 animals/group), and 5, 20 and 80 µg /kg/day (15 animals/group) for 26 weeks in two separate studies. A dose-dependent decrease in body weight gain was noticed in the groups that received 5, 20 and 80 µg/kg/day of budesonide compared with the control group, as well as a dose-related reduction in food intake was observed in males in the 20 and 80 µg/kg/day. Increased values for packed cell volume, hemoglobin and erythrocyte counts were observed for both sexes at the dose levels of 20 and 80 µg/kg/day. Marked decrease in the number of lymphocytes was observed in both sexes at 80 µg/kg/day, and in females also at 20 µg/kg/day. Pathological changes associated with treatment with budesonide were found in the liver, which showed panacinar hepatocytic fine vacuolation in females in the 80 µg/kg/day dose group; cervical lymph nodes showed low numbers of small lymphocytes in both males and females in the 20 and 80 µg/kg/day dose groups; mesenteric lymph nodes showed low numbers of small lymphocytes in females in the 20 and 80 µg/kg/day dose groups; thymus showed low numbers of small lymphocytes in females at the 80 µg/kg/day; mammary glands showed acinar hyperplasia in both sexes in the 20 and 80 µg/kg/day dose groups; uterus showed dilation of the lumen in females in the 20 and 80 µg /kg/day dose groups. Treatment with budesonide at doses of 5 µg/kg/day and below had no effects.

Study title: 14-day local tolerance study of budenofalk foam by rectal administration to beagle dogs (BUF-1 and BUF-8).

Study no.:	BUF-1 and BUF-8
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 8, 1996 and October 14, 1998.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	95039-1/4-2.5 and 70221, purity- Not indicated.

Key Study Findings

The objective of this study was to evaluate the local tolerance of Budenofalk foam following daily rectal administration for 14 days to Beagle dogs. Approximately 1g budenofalk foam (about 15 mL) was administered twice daily by rectal application to female beagle dogs for a period of 14 days. This was a preliminary study, and only female animals were used in this study. No treatment-related adverse effects on clinical signs were observed following 14 days of intra-rectal administration of budenofalk foam to dogs.

Methods

Doses:	approx 1g (~15 mL).
Frequency of dosing:	BID
Route of administration:	Rectal application
Dose volume:	~15 mL
Formulation/Vehicle:	White to greyish white foam, creamy firm with disodium acetate, sorbic acid in pressurized can fitted with a 1mL valve (pump head).
Species/Strain:	Beagle dogs
Number/Sex/Group:	6 females/dose group
Age:	18-27 months
Weight:	8.3 to 13.1kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

All dogs were observed at least once daily for mortality or moribundity.

There was no mortality. All animals survived until the end of the study.

Clinical Signs

All dogs were observed for clinical signs prior to and 24 hours after the first application of each day. General behavior, external appearance, appearance of anal region and anal sphincter, and fecal observation were carried out.

No treatment-related clinical signs were observed.

Body Weights

Body weights were measured before application of the test article and thereafter in weekly intervals.

A 3 to 4% reduction of body weight was observed in the treatment as well as the control group. The applicant indicated that the reduction of body weight was due to stress associated with technical handling of animals during rectal application twice daily for a period of 14 days. Body weight differences are presented in Table below.

Parameters	Control	Treatment (Budenofalk foam g application)
BW (kg) Day 0	14.37	14.87
BW (kg) Day 7	13.93	13.6
BW (kg) Day 14	13.73	14.27
BW gain (%) on Day 7	-3.1	-8.6
BW gain (%) on Day 14	-4.5	-4.1

Feed Consumption

Food consumption was measured daily starting on Day 1 of test article administration, and calculated as weekly mean values.

Food was offered to the dogs based on body weight (50 g/kg b.w./day). There was significant decrease in food consumption in the budesonide treated groups compared to the control group. The effect on food consumption might be associated with stress. In week 1, dogs consumed only 29% of the offered food and in week 2 they consumed 39% of the offered food.

Gross Pathology

Entire lower bowel was removed to observe for any signs of irritation, injury to the epithelial layer of tissue, and necrosis.

The examination of the anal region, anal sphincter did not show any sign of discharge, erythema, and irritation. No pathological findings were noticed in the bowel.

Organ Weights

Organs weights were not measured.

Histopathology

Histological Findings

The rectum and distal portion of the large bowel of all animals was fixed in 10% buffered formalin and examined histologically.

The distal portion of the bowel did not show any histopathological changes.

Study title: Four-week subchronic toxicity study of budenofalk foam by rectal administration to beagle dogs: comparison of an old batch with a new batch (Study # BUF 14).

Study no.:	BUF-14
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4) .
Date of study initiation:	May 7, 2003.
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	70221, 3042301 and 2071001, purity-Not indicated.

Key Study Findings

The objective of this study was to evaluate the toxicity of an old batch of budenofalk foam (batch No. 70221) containing some decay products of active ingredient of budesonide compared to a new batch of budenofalk foam (batch No. 3042301) in beagle dogs. Approximately 1g budenofalk foam (about 15mL) was administered twice daily intra-rectally to beagle dogs for a period of 4 weeks. No signs of systemic toxicity were noticed during the study period. Body weight and food intake were unaffected. Necropsy findings were negative. Histopathological examination of the distal part of the colon and rectum revealed no sign of any local irritation or inflammation.

Methods

Doses:	approx 1g (~15mL).
Frequency of dosing:	BID
Route of administration:	Intra-rectal application

Dose volume:	~15mL
Formulation/Vehicle:	White to greyish white foam, creamy firm with disodium acetate, sorbic acid in pressurized can fitted with a 1 mL valve (pump head).
Species/Strain:	Beagle dogs
Number/Sex/Group:	3 females/dose group
Age:	9-25 months
Weight:	12.4-14.9kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

All dogs were observed at least once daily for mortality or moribundity.

There were no deaths related to the test article throughout the study period.

Clinical Signs

All animals were observed throughout the working day from 7.00 a.m. to 4.00 p.m. During weekends (Saturdays and Sundays), animals were checked from 8.00 a.m. to 12.00 noon and final observation was performed at 4.00 p.m.

Rectal administration of budenofalk foam from both batches (new and old) caused no clinical signs of systemic toxicity.

Body Weights

Body weights were measured before application of the test article and thereafter in weekly intervals.

No test item-related effect was noted on the body weights. However, a significant decrease in body weight was detected in the group of animals treated with the new batch of budenofalk foam at weeks 2 to 4. The applicant stated that these changes are within the normal variability. Body weight differences are presented in the Table below.

Parameters	Control	Treatment Old-Batch (Budenofalk foam 1g application)	Treatment New-Batch (Budenofalk foam 1g application)
BW (kg) Week 0	13.43 (100%)	13.73 (100%)	13.47 (100%)
BW (kg) Week 1	13.03 (-3%)	13.17 (-4.1%)	12.90 (-4.3%)
BW (kg) Week 2	13.27 (-1.2%)	12.63 (-8.1%)	12.43 (-7.8)

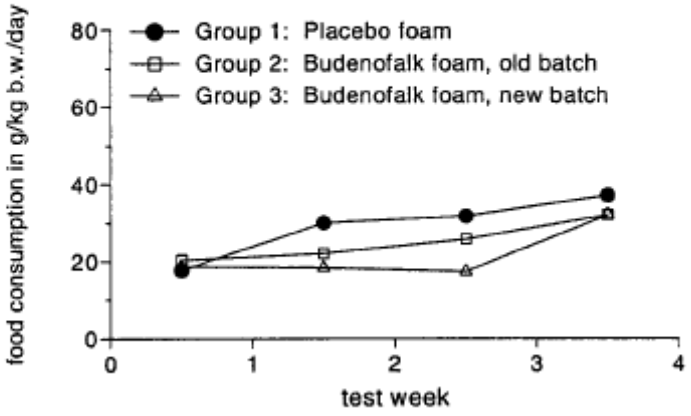
BW (kg) Week 3	13.10 (-2.5%)	12.70 (-7.6%)	11.87 (-11.9)
BW (kg) Week 4	13.70 (+2.0%)	13.10 (-4.6%)	12.50 (-7.8%)

Feed Consumption

The quantity of food left by individual animals was recorded on a daily basis throughout the experimental period. The food intake was calculated as weekly mean values.

Food intake was not affected. However, there was a trend to eat less food in the treatment groups but it failed to show statistical significance. The food intake for different groups is presented in the figure below.

Figure 2 Food consumption of female animals
weekly mean values per group



Gross Pathology

On Day 29, gross pathology examinations of the animals were performed. Endoscopic examination of the recto-sigmoid region of the bowel was also carried out. Entire lower bowel was removed to observe for any signs of irritation, injury to the epithelial layer of tissue, and necrosis. Urinary blabber, lungs, pleura, and cecum were also examined for pathological changes.

No test item-related gross pathological changes were noticed in the colon and rectum of the dogs from any group. A slight reddened mucosa of the rectum was noticed in one dog treated with the new batch of budenofalk foam and this was also observed in one dog in the control group. These findings were considered as technical problem at the time of application. Lungs of the control and treated animals showed reddish, bluish, or brownish discoloration. Discoloration of the thymus and liver, and redness of the upper GI tract was observed in the control group and in dogs treated with new batch of budenofalk foam.

Organ Weights

The weights of the following organs of all animals were determined: liver, ovary, thyroid, brain, lungs, pituitary, parathyroids, heart, cervical and mesenteric lymph nodes, spleen, kidneys, and thymus.

Organ weights were not affected by the treatment.

Histopathology

Adequate Battery: No

Peer Review: No

Histological Findings

The rectum and distal portion of the large bowel of all animals were fixed in 10% buffered formalin and examined histologically. Following organs were preserved in neutral buffered 10% formalin for possible future use. The eyes were preserved in Davidson's solution.

adrenal (2)
aorta abdominalis
bone (*os femoris* with joint)
bone marrow (*os femoris*)
brain *(cerebrum, cerebellum,* brain stem*, hippocampus,* paraventricular parts*)*
caecum
eye with optic nerve (2)
gall bladder
gross lesions
heart (left and right ventricle*, septum)*
intestine, small (*duodenum, jejunum, ileum*)
intestine, large (colon, rectum)
kidney (and ureter) (2)
lacrimal gland (2)
liver
lungs (with mainstem bronchi and bronchioles)
lymph node ((1), cervical),
lymph node ((1), mesenteric)
mammary gland
muscle (skeletal, leg)
nerve (*sciatic*)
oesophagus
ovary (2)
pancreas
pituitary
salivary glands (mandibular, sub-lingual and parotid gland)
skin (left flank)
spinal cord (3 sections)
spleen
stomach
thymus
thyroid (2) (incl. parathyroids)
tissue masses or tumours (incl. regional lymph nodes)
tongue (incl. base)
trachea (incl. larynx)
urinary bladder
uterus (incl. cervix and oviducts)
vagina

The rectum and colon did not show any histopathological changes.

Study title: Budesonide: A six-week rectal toxicity study in beagle dogs.

Study no.:	BUSA0300
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 2, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lots # BAL-C, BEL-C and BEM-C; Purity- Not indicated

Key Study Findings

Beagle dogs were administered budesonide foam intra-rectally at dose levels of 0, 0.6, and 2 mg/25 mL twice daily for 6 weeks. All dogs survived until the end of the study. Mean food consumption was significantly higher in the 2 mg/25 mL in males and females compared to controls. Hematology observation showed an absolute reduction in reticulocytes, lymphocytes, and eosinophils in the 0.6 and 2 mg/25 mL in both male and female dogs. At the end of the study, both male and female dogs showed an increased urine volume with reduced specific gravity in the 2 mg/25 mL dose level. Necropsy finding showed test article-related atrophy of adrenal and thymus glands of males and females. An increased absolute and relative liver weight in males and females were seen at both dose levels. Histopathological examination revealed adrenal cortical atrophy, panlobular hepatocellular hypertrophy, and lymphoid depletion in various lymph nodes, spleen, and thymus gland, decreased cellularity within the bone marrow with increased adipocytes. Skin of the dogs also showed dermal alopecia associated with reduced numbers and size of hair shafts and hair follicles. The target organs of toxicity were the adrenal glands, liver and lymphoid system in males and females, and the skin and bone marrow (femur, rib, and sternum, decreased cellularity) in females.

Methods

Doses:	0, 0.6 and 2 mg/25 mL.
Frequency of dosing:	BID
Route of administration:	Rectal application
Dose volume:	25 mL
Formulation/Vehicle:	N/A
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/dose group
Age:	6.5 to 7 months
Weight:	Males: 7.95 to 10.49 kg and Females: 7.05 to 9.19 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

Animals were checked twice daily for mortality.

No drug-related mortality was observed.

Clinical Signs

All animals were observed twice daily throughout the duration of the study. A detailed clinical examination of each animal was performed prior to randomization, and then weekly during the study period.

No test article-related clinical signs were observed during the study period. Lacrimation was observed in 2 females in the 0 mg/25 mL (control group), 3 males and 2 females in the 0.6 mg/25 mL and 2 females in the 2 mg/25 mL dose groups in all weeks.

Body Weights

Body weights of the animals were measured once during the acclimation period, prior to randomization and thereafter in weekly intervals.

No test item-related effects on the body weights were noted. Body weight data are presented in the Table below.

Mean Body Weights, kg

	Males			Females		
Dose Level	Pretest	Week 6	(%)	Pretest	Week 6	(%)
0 mg/25 mL	8.958	9.445	(+5.4)	7.925	8.348	(+5.3)
0.6 mg/25 mL	8.693	9.050	(+4.1)	7.843	8.333	(+6.2)
2 mg/25 mL	9.355	10.208	(+9.1)	7.778	8.303	(+6.7)

(%) – Percent difference from Pretest

Feed Consumption

Food consumption was measured daily at the end of each feeding period during the study.

Mean food consumption was slightly lower in males and slightly higher in females in the 0.6 mg/25 mL dose group, and was significantly higher in the 2 mg/25 mL in males and females compared to controls. The mean food intake is shown in the Table below.

Mean Food Consumption, g				
	Males		Females	
Dose Level	Consumption	(%)	Consumption	(%)
0 mg/25 mL	285.6	NA	250.8	NA
0.6 mg/25 mL	264.1	(-7.5)	263.3	(+5.0)
2 mg/25 mL	344.0	(+20.4)	331.1	(+32.0)
NA – Not Applicable (%) – Percent difference from control				

Ophthalmoscopy

Ophthalmoscopic examinations were conducted on all animals prior to terminal necropsy.

No ophthalmoscopic abnormalities were detected in any animal during the pretest and during terminal examinations. One dog in the 2 mg/25 mL dose group showed clear ocular discharge from the left eye at the terminal examination. This minor finding was considered incidental.

ECG

All animals received an electrocardiographic examination prior to the initiation of dosing, and 1 to 2 hours post-dose on Day 1 and during the last week of dosing (Day 39). Standard ECGs (10 Lead) were recorded at 50 mm/sec. The RR, PR, and QT intervals and QRS duration were measured and recorded.

No ECG related abnormalities were observed.

Hematology

Blood samples were collected from all animals pretest and prior to terminal necropsy. Blood samples were collected from the jugular vein. All animals were fasted overnight prior to sample collection. Samples were collected at 1, 2, 3, 4, 6 hours prior to the second daily dose, and 7, 8, 9, 10, 12, and 24 hours post-dose

Absolute reticulocytes, lymphocytes, and eosinophils were lower compared to controls in the 0.6 and 2 mg/25 mL in males and females with some of the mean values being statistically significant. All other values are within the normal range when compared with controls. The coagulation values were not affected with test article. Data are presented in the Table below.

Table: Hematological values in Males and Females dogs

	0 mg/25 mL				0.6 mg/25 mL				2 mg/25 mL			
	Male		Female		Male		Female		Male		Female	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Leukocyte (10^3/µl)	11.7±2.4	11.2±1.5	10.2±2	10±3.1	11.8±1.9	9.2±2.2	10.6±1.6	8.2±0.3	13.8±4.5	9.2±1.3	11.6±1.6	9.8±1.4
Erythrocyte (10^6/µl)	7.0±1	6.4±0.5	6.9±0.1	7.0±0.6	7.0±0.2	6.8±0.2	7.2±0.6	6.9±0.3	7.2±0.7	6.5±0.2	6.9±0.6	6.3±0.7
Hb (g/dL)	15.8±2.1	14.3±0.8	15.9±0.2	16.2±1.9	15.2±0.8	15.1±0.2	15.4±0.9	15.5±1.0	15.4±1.6	14.4±0.9	15.5±1.1	14.5±0.9
Hematocrit (%)	45.5±5.6	41.8±2.6	45.7±0.2	47.2±4.7	44.6±1.9	43.9±0.7	45.3±2.4	45±2.2	45.3±3.6	42.1±1.6	45±2.7	42.5±2.8
MCV (fL)	64.4±2.4	64.9±1.5	65.6±1.1	66.4±1.3	63.0±3.0	64.1±2.1	62.6±4.5	65±2.6	62.9±5.3	64.2±3.1	64.9±2.5	67.6±3.8
MCH (pg)	22.3±0.9	22.3±0.6	22.8±0.4	22.9±0.8	21.5±1.0	22.0±0.8	21.4±2.1	22.5±1.4	21.5±2.5	22.0±1.8	22.2±0.8	23.1±1.2
MCHC (g/dL)	34.6±0.5	34.3±0.3	34.7±0.6	34.4±1.0	34.1±0.6	34.3±0.1	34.2±1.0	34.5±0.8	34.0±1.3	34.3±1.2	34.3±0.5	34.2±0.2
Platelets (10^3/µl)	334±75	302±74	389±97	347±66	342±76	285±20	371±65	282±55	343±50	278±40	436±52	375±121
Absolute Reticulocyte (10^3/µl)	58.6±30	32.9±15	49.4±33	35.4±22.9	67.5±9.4	20.5±6.6	45.7±16	13.5±5	60.5±20	13.6±4.3	42.7±5.2	**21.5±9.8**
Neutrophils (10^3/µl)	6.2±1.5	6.5±1.0	6.0±1.2	5.5±1.1	6.5±1.2	6.1±1.3	6.0±1.4	5.0±0.5	9.0±3.8	6.1±1.8	6.7±1.1	6.9±1.2
Lymphocytes (10^3/µl)	4.1±0.5	3.8±0.5	3.2±0.7	3.5±1.6	4.0±0.4	3.2±0.2	3.6±0.7	2.6±0.7	3.5±1.1	2.4±0.3	3.8±0.7	2.2±0.4
Monocytes (10^3/µl)	0.6±0.5	1.5±0.03	0.6±0.2	0.5±0.2	0.8±0.2	0.4±0.1	0.5±0.1	0.3±0.07	0.7±0.06	0.4±0.1	0.6±0.1	0.5±0.2
Eosinophils (10^3/µl)	0.4±0.3	0.1±0.06	0.1±0.07	0.2±0.07	0.2±0.1	0.08±0.03	0.2±0.04	0.04±0.01	0.22±0.1	0.03±0.01	0.17±0.07	0.028±0.009
Basophils (10^3/µl)	0.1±0.06	0.04±.009	0.06±0.02	0.06±0.04	0.07±.008	0.04±0.04	0.07±0.02	0.05±0.01	0.06±0.04	0.04±0.01	0.08±0.01	0.03±0.01

Clinical Chemistry

A number of minor clinical chemistry alterations were noticed in males and females at both dose levels. Most of these values were within normal range, the change was not adverse, and considered to not be test article-related. The changes seen are presented in the Table below:

Table: Clinical Chemistry values in Males and Females dogs

Parameters	Dose							
	0.6 mg/25 mL				2.0 mg/25 mL			
	Male		Female		Male		Female	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Sodium	146.5±1.2	148.8±0.5	146.3±0.5	147.8±0.9	145.3±0.9	149±2.4	145.8±1.7	147.5±1.0
Chloride	111.8±2.0	113.3±1.5	110.5±1.2	111±2.4	108.5±0.5	110±1.4	111±0.8	112.3±3.7
GGT	4.5±0.5	6.8±0.5	3.8±0.96	7±1.6	4.3±1.2	7.8±1.8	4.3±0.5	8.3±1.89
AST	25.8±2.9	22±3.1	26.3±3.2	19.8±3.8	27.8±2.7	17±2.9	33.3±10.6	24.8±9.1
Sorbitol dehydrogenase	6.9±1.11	8.1±2.3	5.8±1.3	9.3±3.3	7.55±1.1	14.5±3.3	8.1±2.2	17.3±6.2
Urea Nitrogen	14±1.8	16.8±2.0	14.8±3.8	17±4.3	14±0.8	21.3±1.8	14±6.3	18.5±7.3
Albumin	3.05±0.2	3.43±0.1	3.28±0.09	3.73±0.2	3.1±0.1	3.55±0.1	3.08±0.1	3.45±0.2

A/G Ratio	1.1±0.09	1.3±0.08	1.4±0.1	1.4±0.1	1.18±0.05	1.28±0.05	1.2±0.2	1.3±0.1
Triglycerides	44.5±5.9	41.3±4.5	31±12.6	54±19	37.5±12.8	67.8±13.3	39.3±5.5	61.8±13.8
Alkaline phosphate	93±33.1	69±16.39	80.3±29.7	88.3±34.6	67.8±17.3	85.3±23.7	80.5±9.8	121.3±49.7
Cholesterol	146.8±34.5	113.3±14.5	127.8±17.3	124±23	147.3±10.9	166.8±22.8	131.5±21.2	136.5±34.5

There was a significant intra-group variability of cortisol levels. There was a tendency for decreased cortisol levels with time as the study progressed. Cortisol levels are presented in the Applicant's Table below.

Table 8 **Summary of Specialized Chemisty Values - MALE**

		0 mg/25 mL			0.6 mg/25 mL			2 mg/25 mL		
Endpoint	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N
Cortisol ng/mL	Day -2#	5.47	0.053	2	4.47	1.656	3	3.89	2.460	3
	Day -1	11.65	2.874	4	12.16	6.510	4	56.26	90.928	4
	Day 1	2.38	NA	1	2.78	0.360	2	3.78	0.922	4
	Terminal#	4.04	2.708	2	NA	NA	0	NA	NA	0

Table 8 **Summary of Specialized Chemisty Values - FEMALE**

		0 mg/25 mL			0.6 mg/25 mL			2 mg/25 mL		
Endpoint	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N
Cortisol ng/mL	Day -2#	4.66	1.787	4	13.60	10.900	2	9.61	3.282	2
	Day -1	8.71	8.436	4	6.71#	6.312	2	8.68	4.882	4
	Day 1	3.50	NA	1	5.32	2.275	3	2.33	0.327	2
	Week 3#	6.74	6.654	2	NA	NA	0	NA	NA	0
	Terminal#	1.95	0.311	2	NA	NA	0	NA	NA	0

Urinalysis

Sixteen-hour urine collection was performed at the end of the study to determine urine volume, specific gravity, and pH.

Urinary volume was significantly higher, and specific gravity was lower in males and females in the 2 mg/25 mL dose group at the terminal evaluation. The female values were statistically significant. The other urinary values in the treated groups were comparable to controls. Summary of urine analysis data are presented in the Applicant's Tables below.

Table 9 Summary of Urinalysis Values - MALE

Endpoint	Study Interval	0 mg/25 mL Mean	SD	N	0.6 mg/25 mL Mean	SD	N	2 mg/25 mL Mean	SD	N
Volume										
mL	Pretest	137.5	124.80	4	152.5	82.71	4	153.8	33.26	4
	Terminal	321.3	232.57	4	216.3	101.36	4	720.0	489.03	4
Specific Gravity										
	Pretest	1.0318	0.01735	4	1.0345	0.01957	4	1.0333	0.00772	4
	Terminal	1.0303	0.01638	4	1.0300	0.01768	4	1.0128	0.00263	4
pH										
	Pretest	7.50	0.913	4	7.25	0.289	4	7.38	0.479	4
	Terminal	7.25	0.500	4	7.50	0.707	4	7.75	0.500	4

Table 9 Summary of Urinalysis Values - FEMALE

Endpoint	Study Interval	0 mg/25 mL Mean	SD	N	0.6 mg/25 mL Mean	SD	N	2 mg/25 mL Mean	SD	N
Volume										
mL	Pretest	253.8	72.73	4	135.0	64.68	4	381.3	233.50	4
	Terminal	363.8	244.89	4	377.5	220.62	4	1008.8[b]	44.23	4
Specific Gravity										
	Pretest	1.0278	0.01223	4	1.0363	0.01994	4	1.0175	0.00904	4
	Terminal	1.0253	0.01438	4	1.0268	0.01443	4	1.0070[b]	0.00216	4
pH										
	Pretest	7.13	0.250	4	7.25	0.289	4	7.13	0.250	4
	Terminal	7.88	0.750	4	7.88	0.750	4	8.50	0.000	4

Gross Pathology

Necropsy examinations were performed on all animals at the end of the study period. The animals were examined for external abnormalities including skin, and the abdominal, thoracic, and cranial cavities.

Mild reductions in thymus and adrenal gland size were observed in 1 of 4 males in the 2 mg/25 mL dose group. Mild reduction of thymus size was also noticed in 2 of 4 females in the 2 mg/25 mL dose group. One female in the 0.6 mg/25 mL dose group showed decreased adrenal gland size with adrenal cortical atrophy.

Organ Weights

The weights of the following organs were determined: brain, adrenal gland, heart, kidneys, liver, lung, pituitary, mandibular gland, spleen, testis, thymus, thyroid, parathyroid, ovaries, uterus and cervix. The weights of the organs were also expressed relative to the body weight.

Test article-related organ weight effects were observed in the adrenal glands, livers, and thymus of males and females. Changes in organ weight are presented in the Applicant's Table below.

Organ Weight Changes (% differences compared to controls) Terminal Necropsy				
Dose level:	0.6 mg/25mL		2 mg/25 mL	
Sex:	Male	Female	Male	Female
Number Examined:	4	4	4	4
Adrenal gland (g)	↓35[b]	↓42[b]	↓50[b]	↓48[b]
Adrenal gland/BWt %	↓34[a]	↓42[b]	↓55[b]	↓50[b]
Adrenal gland/BrWt ratio	↓35[a]	↓42[b]	↓48[b]	↓42[b]
Liver (g)	↑12	↑12	↑42[b]	↑34
Liver/BWt %	↑16	↑13	↑29[b]	↑29[a]
Liver/BrWt ratio	↑13	↑11	↑47[b]	↑51
Thymus (g)	↓9	↓47	↓53[a]	↓50
Thymus/BWt %	↓4	↓44	↓57[a]	↓51
Thymus/BrWt ratio	↓8	↓47	↓52[a]	↓43

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)
↑ - Increased ↓ - Decreased; BWt - Body Weight; BrWt - Brain Weight

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

The tissues/organs examined are listed in the Applicant's Table below. Harvested tissues were processed for staining with hematoxylin and eosin and examined by light microscopy. All designated tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using modified Davidson's fixative.

- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [rib]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)
- Eye including optic nerve (2)
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach [cardia, fundus, and pylorus]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Pituitary*
- Prostate
- Salivary gland, mandibular [2 collected; 1 examined][b]*
- Salivary gland, parotid [2 collected; 1 examined]
- Salivary gland, sublingual [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix*
- Vagina

[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]Only the right mandibular salivary gland weight was obtained.

(2) Paired organ
*Weighed organ

Intra-rectal budesonide caused histopathological changes in the adrenal glands, liver and lymphoid system (lymph nodes, thymus, spleen), and in the bone marrow and skin of male and female dogs in the 0.6 mg/25mL dose group. The skin and bone marrow (rib, sternum, and femur) of males in the 2 mg/25mL dose group also showed changes. Atrophy of the adrenal gland with thinning of the cortex was noticed. Liver cells showed panlobular hepatocellular hypertrophy with diffuse enlargement of the hepatocytes. Periodic Acid-Schiff (PAS) staining confirmed presence of cytoplasmic glycogen deposition within the hepatocytes. An overall reduction in the number of lymphocytes was observed throughout the lymphoid organ. Decreased cellularity within the bone marrow (femur, rib, and sternum) was characterized by an absolute reduction in the number of bone marrow cells with increased adipocytes. Dermal alopecia was noticed with reduced numbers and size of hair shafts and hair follicles. Data are provided in the Table below.

	Severity	0 mg/25 mL		0.6 mg/25 mL		2 mg/25 mL	
		Male	Female	Male	Female	Male	Female
Adrenal gland		(4)	(4)	(4)	(4)	(4)	(4)
Atrophy, cortical	Mild	0	0	1	0	0	0
	Moderate	0	0	3	4	1	3
	Severe	0	0	0	0	3	1
Vacuolation							
	Minimal	0	0	1	2	0	1
	Mild	0	0	1	2	1	1
	Moderate	0	0	1	0	3	1
Bone marrow, femur		(4)	(4)	(4)	(4)	(4)	(4)
Cellularity, decreased	Minimal	0	0	0	1	2	3
Bone marrow, rib		(4)	(4)	(4)	(4)	(4)	(4)
Cellularity, decreased	Minimal	0	0	0	1	4	4
Bone marrow, sternum		(4)	(4)	(4)	(4)	(4)	(4)
Cellularity, decreased	Minimal	0	0	0	1	4	3
Gall bladder		(4)	(4)	(4)	(4)	(4)	(4)
Infiltration, lymphocytic	Minimal	4	1	3	0	3	0
Joint, tibiofemoral		(4)	(4)	(4)	(4)	(4)	(4)
Hyperplasia/hypertrophy, synovial		2	3	2	0	2	1
Liver		(4)	(4)	(4)	(4)	(4)	(4)
Hypertrophy, hepatocyte, panlobular		0	0	3	4	4	4
	Minimal	-	0	-	0	-	1
	Mild	0	0	3	2	0	0
	Moderate	0	0	0	2	4	3
Infiltration, mononuclear cell	Minimal	1		1	1	0	0
Lymph node, mandibular		(4)	(4)	(4)	(4)	(4)	(4)
Depletion, lymphoid, generalized	Minimal	0	0	0	3	0	1
	Mild	0	0	2	1	3	3
Macrophages, pigmented	Minimal	0	0	0	1	0	0
	Mild	1	0	0	0	0	0
Lymph node, mesenteric		(4)	(4)	(4)	(4)	(4)	(4)
Depletion, lymphoid, generalized		0	0	2	2	4	4
	Minimal	0	0	1	0	0	2
	Mild	0	0	1	2	4	2
Erythrocytesis/erythrophagocytosis, sinus	Minimal	2	1	2	2	3	3
	Mild	0	1	2	2	0	1
	Moderate	0	0	0	0	1	0
Skin		(4)	(4)	(4)	(4)	(4)	(4)
Alopecia/hypotrichosis	Minimal	0	0	0	1	4	4
Inflammation, hair follicle/epidermis	Minimal	0	0	0	1	0	0
Spleen		(4)	(4)	(4)	(4)	(4)	(4)
Depletion, lymphoid, generalized	Minimal	0	0	1	4	0	1
	Mild	0	0	2	0	3	3
Thymus		(4)	(4)	(4)	(4)	(4)	(4)
Cyst	Minimal	0		1		0	
Depletion, lymphoid, cortex		4		4		4	
	Minimal	4	1	1	0	0	0
	Mild	0	0	0	1	0	0
	Moderate	0	0	3	3	0	1
	Severe	0	0	0	0	4	3

Study title: A 39-week Rectal Toxicity Study in Dogs.

Study no.:	BUSA0301
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 03, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Lot # BGE-C, CAL-C, CAK-C and CAI-cC and purity >98%

Key Study Findings

Budesonide foam was administered intra-rectally BID to Beagle dogs (4/sex/group) at dose levels of 0, 0.2, 0.6 and 2 mg/25 mL for 39 weeks. There were two unscheduled deaths. One male (0.4 mg/day) and a female (4 mg/day) were euthanized *in extremis* on Day 204 in response to significant weight loss as well as severe preputial ulceration/inflammation in the male and severe generalized demodicosis in the female. The deaths were not considered directly related to budesonide administration; however, the non-resolving preputial infection and demodicosis were considered secondary to immune system compromise (i.e. immunosuppression). All other dogs survived until the end of the study. No adverse effect of treatment was seen on clinical signs, body weights, hematology, coagulation, clinical chemistry, urinalysis, ophthalmoscopic, and electrocardiographic examinations. Weight of adrenal gland and spleen were reduced in both males and females at all dose levels. Necropsy finding showed test article-related atrophy of adrenal glands in females in the 4 mg/day dose group. Histopathological examination revealed adrenal cortical atrophy in both sexes at all dose levels. Test article related microscopic findings were limited to the adrenal glands, liver, skin and lymphoid system (including spleen, lymph nodes, thymus and bone marrow). An increased incidence and/or severity of increased cytoplasmic glycogen were observed in the liver of both sexes at all dose levels. Atrophy of the skin was observed in males at dosages of ≥0.4 mg/day and in females at dosages of ≥1.2 mg/day. Generalized lymphoid depletion was observed throughout the lymphoid system including the lymph nodes, spleen and thymus gland of males and females in the 4 mg/day dose group. The target organs of toxicity were the adrenal glands, liver, lymphoid system and skin in males and females.

Methods

Doses:	0, 0.2, 0.6 and 2 mg/dose.
Frequency of dosing:	Twice daily (6 hours apart)
Route of administration:	Intra rectal
Dose volume:	25 mL burst of foam (1 burst)
Formulation/Vehicle:	N/A
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/dose group
Age:	6-7 months
Weight:	Males – 8.3 to 9.95 kg and females – 7.05 to

	8.15 kg
Satellite groups:	No
Unique study design:	None
Deviation from study protocol:	None

Observations and Results

Mortality

Animals were checked twice daily for morbidity, mortality and injury.

There were two unscheduled deaths; a male (animal number 815) in the 0.4 mg/day dose group and a female (animal number 826) in the 4 mg/day dose group were euthanized *in extremis* on Day 204 in response to significant weight loss as well as severe preputial ulceration/inflammation in the male and severe generalized demodicosis in the female. The deaths were not considered directly related to budesonide administration; however, the non-resolving preputial infection and demodicosis were considered secondary to immune system compromise (i.e. immunosuppression). All other animals survived at the end of the study.

Clinical Signs

Cage-side observations of clinical signs were performed twice daily throughout the study. A detailed clinical examination was performed on each animal once upon arrival, prior to randomization, and once weekly starting one week prior to initiation of treatment.

There were no treatment-related clinical signs during the study.

Body Weights

Body weights were recorded for all animals after arrival, Day -6, prior to randomization and once a week during the study.

There was no effect on body weight changes, considered related to the treatment with the budesonide. However, the mean body weights were lower compared to controls in females in the 0.4 mg/day dose group and in males and females in the 1.2 mg/day dose group. Mean body weights were comparable to controls in males and females in the 4 mg/day dose group. Body weight was slightly higher in males in the 0.4 mg/day dose group, compared to controls. The mean body weights at pretest and Week 39 are presented in the Applicant's Table below.

Mean Body Weights, kg						
Dose Level	Males			Females		
(mg/day)	Pretest	Week 39	(%)	Pretest	Week 39	(%)
0	9.150	10.400	(NA)	7.588	8.713	(NA)
0.4	9.163	10.917	(+5.0)	7.438	8.188	(-6.0)
1.2	9.325	9.800	(-5.8)	7.500	7.588	(-12.9)
4	9.138	10.288	(-1.1)	7.513	8.500	(-2.4)
(%) – Percent difference from control NA – Not applicable						

Feed Consumption

Food intake was determined daily.

The mean food consumption in males in the 4 mg/day dose group and in females in the 1.2 mg/day dose group was higher than controls. Females in the 4 mg/day dose group had reduced food consumption compared to control. There was considerable week to week variation in food consumption and occasional significantly higher food consumption was observed in males in the 4 mg/day dose group. The mean food consumption in males in the 0.4 and 1.2 mg/day dose groups and in females in the 0.4 mg/day dose group was comparable to controls.

Ophthalmoscopy

Ophthalmoscopic examinations were performed on both eyes of all animals, following the application of a mydriatic agent, once prior to the initiation of treatment, and prior to terminal necropsy.

Budesonide did not cause any adverse ocular effects.

ECG

Electrocardiograms (leads I, II and III, and augmented leads aVR, aVL and aVF) were determined in all animals once during the pre-treatment period and once during the last week of dosing. Using an appropriate lead, the RR, PR, and QT intervals, and QRS duration were measured, and heart rate was determined.

There was no effect of the rectal administration of budesonide on ECG parameters.

Hematology

Blood samples for hematology were collected from all animals before test, at week 13, and prior to terminal necropsy.

No adverse hematology alterations were observed during the study. Lymphocytes and eosinophils counts were lower compared to controls in males and females in the 1.2 and 4 mg/day dose groups. Most of these values were not statistically significant and were not biologically relevant but were typical of administered corticosteroid changes. Results are presented in the Applicant's Table below.

Summary of Hematology Values - MALE

Endpoint	Study Interval	0 mg/day Mean	0 mg/day SD	0 mg/day N	0.4 mg/day Mean	0.4 mg/day SD	0.4 mg/day N	1.2 mg/day Mean	1.2 mg/day SD	1.2 mg/day N	4 mg/day Mean	4 mg/day SD	4 mg/day N
Eosinophils $10^3/\mu L$	Pretest	0.200	0.1615	4	0.268	0.0741	4	0.300	0.1225	4	0.180	0.0821	4
	Week 13	0.223	0.0330	4	0.208	0.1118	4	0.153	0.0512	4	0.083[b]	0.0340	4
	Terminal	0.270	0.0648	4	0.323	0.1380	3	0.265	0.0988	4	0.218	0.0377	4
Lymphocytes $10^3/\mu L$	Pretest	3.143	0.4835	4	3.933	1.0659	4	3.608	0.0660	4	4.083	0.5662	4
	Week 13	3.410	0.4566	4	3.225	0.6504	4	2.775	0.4734	4	2.553	0.3640	4
	Terminal	3.168	0.7074	4	2.967	0.6172	3	2.553	0.4705	4	2.585	0.4778	4

Summary of Hematology Values - FEMALE

Endpoint	Study Interval	0 mg/day Mean	0 mg/day SD	0 mg/day N	0.4 mg/day Mean	0.4 mg/day SD	0.4 mg/day N	1.2 mg/day Mean	1.2 mg/day SD	1.2 mg/day N	4 mg/day Mean	4 mg/day SD	4 mg/day N
Eosinophils $10^3/\mu L$	Pretest	0.165	0.0451	4	0.205	0.0926	4	0.230	0.1378	4	0.235	0.0191	4
	Week 13	0.205	0.0289	4	0.220	0.1010	4	0.048[b]	0.0096	4	0.043[b]	0.0206	4
	Terminal	0.220	0.0216	4	0.340	0.2177	4	0.195	0.0933	4	0.157	0.0603	3
Lymphocytes $10^3/\mu L$	Pretest	3.698	0.6259	4	3.573	1.1007	4	4.115	1.0139	4	3.243	0.9140	4
	Week 13	3.500	0.9405	4	3.288	0.8215	4	2.253	0.3504	4	2.223[a]	0.4308	4
	Terminal	3.015	0.6801	4	3.023	1.0433	4	2.640	0.4907	4	2.017	0.2542	3

No treatment-related effects were observed in coagulation parameters in males and females at the Week 13 or terminal evaluation.

Clinical Chemistry

Blood samples for clinical chemistry analysis were collected from all animals pretest, at week 13, and prior to terminal necropsy.

Cortisol levels were determined in both male and female dogs prior to administration of test article and prior to terminal necropsy.

No test article-related clinical chemistry alterations were seen in males or females at Week 13 or terminal evaluation.

Regarding cortisol analysis, cortisol values were variable in males and females and were the expected response of corticosteroid administration. Results are presented in the Applicant's Table below.

Summary of Specialized Chemistry Values - MALE

Endpoint	Study Interval	0 mg/day Mean	SD	N	0.4 mg/day Mean	SD	N	1.2 mg/day Mean	SD	N	4 mg/day Mean	SD	N
Cortisol ng/mL	Day -2	6.78	4.105	3	5.93[#]	2.029	2	7.15[#]	1.665	2	6.26	3.252	4
	Day -1	11.62	7.951	3	10.27	6.230	3	21.61[#]	NA	1	8.97[#]	1.915	2
	Day 1[A]	12.00	9.321	3	6.84	4.435	3	6.87[#]	NA	1	13.30[#]	2.033	2
	Week 13[#]	1.71	NA	1	2.09	NA	1	NA	NA	0	NA	NA	0
	Terminal[#]	2.07	0.898	3	NA	NA	0	NA	NA	0	NA	NA	0

Summary of Specialized Chemistry Values - FEMALE

Endpoint	Study Interval	0 mg/day Mean	SD	N	0.4 mg/day Mean	SD	N	1.2 mg/day Mean	SD	N	4 mg/day Mean	SD	N
Cortisol ng/mL	Day -2	6.12	2.393	3	5.99	3.047	3	7.45	5.666	3	4.36[#]	2.624	2
	Day -1[#]	2.52	NA	1	17.45	19.076	2	4.30	0.052	2	NA	NA	0
	Day 1[A,#]	4.88	NA	1	35.86	NA	1	8.61	2.835	2	3.48	NA	1
	Week 13[#]	1.89	0.408	2	NA	NA	0	NA	NA	0	NA	NA	0
	Terminal[#]	NA	NA	0	1.78	1.064	3	NA	NA	0	NA	NA	0

Urinalysis

Urine analysis was conducted once during the pre-treatment period, towards the end treatment period). Following parameters were determined.

Parameters Examined

Bilirubin	Protein
Blood	Specific gravity
Color and appearance	Urine sediment
Glucose	Urobilinogen
Ketones	Volume
pH	

No treatment-related adverse effects were noticed on urinalysis parameters in males and females.

Gross Pathology

Necropsy examinations were performed on animals euthanized *in extremis* and all surviving animals at the scheduled necropsy. The animals were examined for external abnormalities including skin, and the abdominal, thoracic, and cranial cavities.

Gross pathological examination revealed a small adrenal gland in female dogs in the 4 mg/day dose group. There were sparse hair observed in one female and one male in the 1.2 mg/day (animal numbers 818 and 822, respectively) and in one female in the 4 mg/day (animal number 826). The moderately sparse hair correlated with microscopic demodectic folliculitis. In addition, moderate skin abrasions/scabs and scaling were observed in the 4 mg/day females, and also correlated with demodectic folliculitis.

Organ Weights

The weights of the following organs were determined: brain, adrenal gland, heart, kidneys, liver, lung, pituitary, mandibular gland, spleen, testis, thymus, thyroid, parathyroid, ovaries, uterus and cervix. The weights of the organs were also expressed relative to the body weight.

Budesonide-related organ weight changes were limited to reductions in adrenal gland weights of males and females at all dose levels and reductions in spleen weights in males and females in the 4 mg/day dose group. Slight reductions in spleen weights were also observed in males and females in the 0.4 and 1.2 mg/day dose groups but were similar in magnitude and/or without dose dependency at these dose levels. Reduced adrenal gland weights correlated with minimal to severe microscopic adrenal cortical atrophy and reduced spleen weights correlated with minimal to mild microscopic generalized lymphoid depletion. Table below shows the test article related changes in the organ weights.

Test Article-related Organ Weight Changes - Terminal
Male and Female (Percent change relative to control)

Dose level: mg/day	0.4		1.2		4	
Sex	M	F	M	F	M	F
Number Examined	3	4	4	4	4	3
Adrenal gland (g)	↓28	↓14	↓48[a]	↓40[a]	↓42[a]	↓51[b]
Adrenal gland/BWt%	↓34	↓9	↓46[a]	↓30	↓43[a]	↓49[a]
Adrenal gland/BrWt ratio	↓28	↓11	↓46[b]	↓38[a]	↓42[a]	↓50[b]
Spleen (g)	↓11	↓22	↓17	↓28	↓33	↓28
Spleen/BWt%	↓17	↓17	↓15	↓17	↓33	↓27
Spleen/BrWt ratio	↓14	↓19	↓17	↓27	↓35	↓28

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)
BWt - Body Weight
BrWt - Brain Weight

↓ - Decreased
M – Male
F - Female

Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings

The tissues/organs examined are listed in the sponsor's Table below. Harvested tissues were processed for staining with hematoxylin and eosin and examined by light microscopy. All designated tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes, which were fixed using modified Davidson's fixative.

- Adrenal (2)*
- Aorta
- Bone with marrow [femur]
- Bone with marrow [rib]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*
- Epididymis (2)
- Eye including optic nerve (2)
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach [cardia, fundus, and pylorus]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2)* with oviduct (2)
  - testis (2)*
- Gross lesions
- Heart*
- Joint, tibiofemoral
- Kidney (2)*
- Larynx
- Liver [3 sections collected; 2 examined]*
- Lung with bronchi [collected whole; 2 sections examined]*
- Lymph nodes: mandibular and mesenteric
- Mammary gland [process females only]
- Pancreas
- Pituitary*
- Prostate
- Salivary gland, mandibular [2 collected; 1 examined]*[b]
- Salivary gland, parotid [2 collected; 1 examined]
- Salivary gland, sublingual [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen*
- Thymus*
- Thyroid/parathyroid (2)*
- Tongue
- Trachea
- Ureters (2)
- Urinary bladder
- Uterus [both horns]/Cervix*
- Vagina

[a]Bone marrow smears were collected at the scheduled necropsy and held.
[b]Only the right mandibular salivary gland weight was obtained.

(2) Paired organ
*Weighed organ

Histopathological examinations showed that budesonide-related microscopic findings were limited to the adrenal glands, liver, skin and lymphoid system (including spleen, lymph nodes, thymus and bone marrow). These microscopic findings are expected as of pharmacologic effects of corticosteroids. Minimal to severe adrenal cortical atrophy was observed in both sexes at all dose levels. An increased incidence and/or severity of increased cytoplasmic glycogen were observed in the liver of both sexes at all dose levels. The hepatocytes had a minimal to moderate swollen appearance with variable clear cytoplasm. Periodic Acid-Schiff (PAS) staining confirmed presence of cytoplasmic glycogen deposition within the hepatocytes. Atrophy of the skin was observed in males at dosages of ≥0.4 mg/day and in females at dosages of ≥1.2 mg/day. Minimal atrophy was characterized by a generalized thinning of the epidermis with reduced numbers and size of hair shafts and/or hair follicles. Generalized lymphoid depletion was observed throughout the lymphoid system including the lymph nodes, spleen and thymus gland of males and females in the 4 mg/day dose group. The lymphoid depletion was characterized by an overall reduction in the number of lymphocytes throughout the lymphoid organ. Variable lymphoid depletion was observed in the thymus glands in all groups (including controls); however, the depletion was observed at increased severity

in a single 4 mg/day male and a female. Decreased cellularity within the bone marrow (femur, rib and sternum) was observed in a single female in the 4 mg/kg and a single male in the 1.2 mg/day. Decreased cellularity was characterized by an absolute reduction in the number of bone marrow cells from all lineages (erythroid, myeloid, lymphoid, and megakaryocytes) with increased adipocytes presence. Data are presented in the Applicant's Table below.

Test Article-related Microscopic Observations - Terminal

Dose level: mg/day	0		0.4		1.2		4	
Sex	M	F	M	F	M	F	M	F
Number Examined	4	4	4	4	4	4	4	4
Adrenal gland								
Atrophy, cortical	0	0	3	2	4	4	4	4
-minimal	0	0	3	2	1	0	0	0
-mild	0	0	0	0	0	4	0	0
-moderate	0	0	0	0	3	0	4	1
-severe	0	0	0	0	0	0	0	3
Bone marrow, femur								
Cellularity, decreased, minimal	0	0	0	0	1	0	0	1
Bone marrow, rib								
Cellularity, decreased, minimal	0	0	0	0	1	0	0	1
Bone marrow, sternum								
Cellularity, decreased, minimal	0	0	0	0	1	0	0	1
Liver								
Glycogen, increased cytoplasmic	0	2	2	3	4	2	4	4
-minimal	0	2	1	1	2	1	1	0
-mild	0	0	1	2	2	0	3	3
-moderate	0	0	0	0	0	1	0	1
Lymph node, mandibular								
Depletion, lymphoid, generalized								
-minimal	0	0	0	0	0	0	1	2
Lymph node, mesenteric								
Depletion, lymphoid, generalized	0	0	1	0	0	0	4	3
-minimal	0	0	1	0	0	0	0	1
-mild	0	0	0	0	0	0	4	2

M - Male
F - Female

Test Article-related Microscopic Observations - Terminal								
Dose level: mg/day	0		0.4		1.2		4	
Sex	M	F	M	F	M	F	M	F
Number Examined	4	4	4	4	4	4	4	4
Skin								
Atrophy, minimal	0	0	2	0	2	3	4	3
Spleen								
Depletion, lymphoid, generalized	0	0	1	0	1	0	4	2
-minimal	0	0	1	0	1	0	3	0
-mild	0	0	0	0	0	0	1	2
Thymus								
Depletion, lymphoid, generalized	4	4	3	4	3	3	4	3
-minimal	4	1	0	2	2	2	2	0
-mild	0	3	1	1	1	1	0	2
-moderate	0	0	2	1	0	0	1	0
-severe	0	0	0	0	0	0	1	1

M - Male
F - Female

Special Evaluation

None

Toxicokinetics

Blood samples (approximately 1.0 mL) were collected from all surviving animals via the jugular vein for determination of the plasma concentrations of the test article. Samples were collected at 1, 2, 3, 4, 6 (immediately prior to the second dose), 7, 8, 9, 10, 12, and 24 hours post-dose, and on Days 1 and 269.

Between Day 1 and Day 269, mean C_{max} and AUC_{0-24} decreased substantially to 11% or less of the Day 1 values. Several plasma samples were below the limit of quantitation or very low on Day 269. For both males and females on both sampling days, C_{max} and AUC increased with dose. On Day 1, the increases in C_{max} were slightly less than dose-proportional, but the increases in AUC_{0-24} were approximately dose proportional. Absorption was rapid, with the median T_{max} of 1 hour after either the first or second dose. On Day 1, there was no apparent sex difference at 0.2 mg BID, but at 0.6 and 2 mg BID, the values of C_{max} and AUC were consistently higher for the female dogs. Toxicokinetic data are presented in the Applicant's Table below.

Table 3. Mean C_{max} Values for Budesonide

Dose: mg/dose BID	Sex	Day	C_{max} (ng/mL) for Whole Day Mean ± SD	C_{max} (ng/mL) for 1st Dose Mean ± SD	C_{max} (ng/mL) for 2nd Dose Mean ± SD
0.2	Male	1	0.226 ± 0.129	0.162 ± 0.174	0.178 ± 0.042
		269	0 [a]	0 [a]	0 [a]
		% Difference	-100%		
0.2	Female	1	0.240 ± 0.086	0.106 ± 0.080	0.240 ± 0.086
		269	0.0161 ± 0.0322	0	0.0161 ± 0.0322
		% Difference	-93%		
0.6	Male	1	0.357 ± 0.103	0.246 ± 0.085	0.290 ± 0.176
		269	0.0394 ± 0.0299	0.0366 ± 0.0295	0.0219 ± 0.0254
		% Difference	-89%		
0.6	Female	1	1.02 ± 0.66	0.485 ± 0.257	0.970 ± 0.701
		269	0.0734 ± 0.0564	0.0523 ± 0.0659	0.0330 ± 0.0409
		% Difference	-93%		
2	Male	1	1.07 ± 0.23	0.592 ± 0.102	1.07 ± 0.23
		269	0.109 ± 0.143	0.0947 ± 0.1522	0.0578 ± 0.0500
		% Difference	-90%		
2	Female	1	2.72 ± 1.36	1.28 ± 1.22	2.72 ± 1.36
		269	0.222 ± 0.209[a]	0.120 ± 0.059[a]	0.210 ± 0.221[a]
		% Difference	-92%		

n = 4, except as noted. Values for SD were not calculated when all values were 0 ng/mL.

[a] n = 3

Table 4. Median T_{max} Values for Budesonide

Dose: mg/dose BID	Sex	Day	T_{max} (hr) for Whole Day Median (Range)	T_{max} (hr) for 1st Dose Median (Range)	T_{max} (hr) for 2nd Dose Median (Range)
0.2	Male	1	7 (1 - 7)	1 (1 - 1)	7 (7 - 7)
		269	na	na	na
0.2	Female	1	7 (7 - 8)	1 (1 - 1)	7 (7 - 8)
		269	7 [a]	na	7 [a]
0.6	Male	1	7 (1 - 7)	1 (1 - 1)	7 (7 - 12)
		269	6 (1 - 7)[c]	1 (1 - 6)[c]	7 (7 - 7)[b]
0.6	Female	1	4 (1 - 8)	1 (1 - 2)	7 (7 - 8)
		269	1 (1 - 7)[c]	1 (1 - 1)[b]	7 (7 - 7)[b]
2	Male	1	7 (7 - 8)	1 (1 - 1)	7 (7 - 8)
		269	1 (1 - 7)[c]	1 (1 - 1)[b]	7 (7 - 7)[c]
2	Female	1	7 (7 - 8)	1 (1 - 1)	7 (7 - 8)
		269	7 (1 - 7)[c]	1 (1 - 1)[c]	7 (7 - 24)[c]

na = not applicable since all concentration values were 0 ng/mL for all animals.

n = 4, except as noted.

[a] n = 1

[b] n = 2

[c] n = 3

Table 5. Mean AUC Values for Budesonide

Dose: mg/dose BID	Sex	Day	AUC_{0-24} (ng•hr/mL) for Whole Day Mean ± SD	AUC_{0-6} (ng•hr/mL) for 1st Dose Mean ± SD	AUC_{6-24} (ng•hr/mL) for 2nd Dose Mean ± SD
0.2	Male	1	1.51 ± 0.91	0.384 ± 0.470	1.13 ± 0.45
		269	0 [a]	0 [a]	0 [a]
		% Difference	-100%		
0.2	Female	1	1.38 ± 0.56	0.252 ± 0.224	1.12 ± 0.34
		269	0.0242 ± 0.0483	0	0.0242 ± 0.0483
		% Difference	-98%		
0.6	Male	1	2.81 ± 1.17	0.651 ± 0.153	2.16 ± 1.21
		269	0.0751 ± 0.0771	0.0366 ± 0.0295	0.0385 ± 0.0507
		% Difference	-97%		
0.6	Female	1	6.15 ± 2.67	1.07 ± 0.57	5.09 ± 2.43
		269	0.175 ± 0.160	0.119 ± 0.171	0.0551 ± 0.0559
		% Difference	-97%		
2	Male	1	8.27 ± 1.40	1.47 ± 0.20	6.80 ± 1.36
		269	0.565 ± 0.944	0.272 ± 0.476	0.292 ± 0.469
		% Difference	-93%		
2	Female	1	18.4 ± 12.8	3.52 ± 3.38	14.8 ± 9.5
		269	1.64 ± 1.50[a]	0.405 ± 0.255[a]	1.24 ± 1.25[a]
		% Difference	-91%		

n = 4, except as noted. Values for SD were not calculated when all values were 0 ng/mL.

[a] n = 3

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Toxicity study of Budesonide: Mutagenicity study (Fujii, T., Yamashita, T., Miyamae, Y. and Tensho, A. *The clinical report, [report No 4]*, 1985; 19:9, 1-15).

Study no.:	Report 4
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9 August, 1985
GLP compliance:	No
QA statement:	Not provided
Drug, lot #, and % purity:	Lot # 28T, Purity-Not provided

Key Study Findings

In reverse- mutation assays in *Salmonella typhimurium* and *Escherichia coli*, budesonide at concentrations of 0.5-5,000 µg/plate did not induce gene mutation with or without metabolic activation.

Methods

Strains:	*Salmonella typhimurium* strains: TA1535, TA1537, TA1538, TA100, TA98 and *E. Coli* WP2uvrA
Concentrations in definitive study:	0. 5, 1, 5, 10, 50, 100, 500, 1,000 and 5,000 µg/plate.
Basis of concentration selection:	The highest dose in the main test was set at 5000 µg/plate. However, the Applicant did not mentioned for the basis of concentration selection or dose-finding assay.
Negative control:	Dimethyl sulfoxide (DMSO)
Positive control:	N-ethyl- N-ethyl-N'-nitro-N-nitrosoguanidine, 9- aminoacridine.HCl.H_2O, 2-nitrofluorene, 2-(2-furyl)-3-(5- nitro-2-furyl) acrylamide and 2-aminoanthracene
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Plates were incubated at 37^0C for approximately 48 h for all strains in the presence or absence of metabolic activation (S9).

Study Validity

The study was validated by the incidence of spontaneous revertants in the solvent controls within the range of historical values, and the assay results were evaluated as positive if the number of revertant colonies in the groups treated with the test substance was at least twice the number in the group treated with the solvent (that is, at least twice the number of spontaneous revertant colonies) and if this increase in the number of revertant colonies was found to be concentration- dependent.

Results

The dose levels tested were 0.5, 1.0, 5.0, 10, 50, 100, 500, 1000 and 5000 µg per plate. In this assay, no positive mutagenic responses were observed with tester strains TA1535, TA1537, TA1538, TA100, TA98 and WP2*uvr*A either in the presence or absence of metabolic (S9) activation.

Budesonide at 500 µg/plate and higher concentrations inhibited the growth of TA1535, TA1537, and TA1538; and at 5,000 µg/plate, it inhibited the growth of TA100 and *E. coli*. The number of revertant colonies in the plates treated with budesonide at concentrations that did not inhibit bacterial growth did not exceed twice the number in the plates treated with DMSO. No appreciable toxicity was observed.

Study title: Mutagenicity study of (b) (4) in the *Salmonella Typhimurium*

Study no.:	BUF-13
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 4, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TARS-264, Purity-97.9%

Key Study Findings

The degradation product of budesonide, (b) (4), was examined in the Ames test, with and without metabolic activation. No mutagenic effect was observed for (b) (4) at 5 concentrations, up to the highest concentration of 5000 µg/plate.

Methods

Strains:	*Salmonella typhimurium* TA 98, TA 100, TA 102 and TA 1535
Concentrations in definitive study:	100, 316, 1000, 3160 and 5000 µg/plate
Basis of concentration selection:	A dose-finding assay of (b) (4) was performed at 0.316, 1.0, 3.16, 10.0, 31.6, 100, 316, 1000, 3160 and 5000 µg/plate in the preliminary test using strain TA 100. Slight cytotoxicity was observed at the highest tested concentration of 5000 µg/plate. Hence, 5000 µg/plate was chosen as the highest concentration for the main study.
Negative control:	DMSO
Positive control:	Sodium azide, 2-nitro-fluorene, 9-amino-acridine, methyl methane sulfonate, 2-anthraceneamide and cyclophosphamide.
Formulation/Vehicle:	DMSO and water
Incubation & sampling time:	Plates were incubated at 37^0C for approximately 48 h for all strains in the presence or absence of metabolic activation (S9).

Study Validity

The study was validated by the incidence of spontaneous revertants in the solvent controls within the range of historical values, and the significant increase in numbers of revertant colonies in the positive control articles. All necessary criteria for a valid study, including appropriate phenotypic confirmations and vehicle/positive control response were fulfilled.

Results

In the initial assay, the maximum dose tested was 5000 µg per plate. The dose levels tested were 0.316, 1.0, 3.16, 10.0, 31.6, 100, 316, 1000, 3160 and 5000 µg per plate. In this assay, slight cytotoxicity responses were observed with tester strain TA100 at concentration of 5000 µg/plate. Data is presented in the applicant's Table below.

TABLE 1 Mutagenicity study of [redacted] (b) (4)

in the Salmonella typhimurium reverse mutation assay (in vitro)

Preliminary cytotoxicity test

Test substance concentration (µg/plate)	Background lawn	Revertants per plate (TA 100) (cytotoxicity)
[redacted] (b) (4)		
		plate 1 / plate 2
5000	normal	89 / 85
3160	normal	98 / 106
1000	normal	102 / 96
316	normal	97 / 100
100	normal	90 / 131
31.6	normal	109 / 113
10.0	normal	122 / 105
3.16	normal	114 / 93
1.0	normal	106 / 97
0.316	normal	115 / 108
Solvent control 100 µl/plate	normal	116 / 94

In main study, five concentrations ranging from 100 to 5000 µg per plate were employed in a plate incorporation test carried out with and without metabolic activation. No mutagenic effect (no increase in revertant colony numbers as compared to control counts) was observed for [redacted] (b) (4) tested at 5 concentrations up to the highest concentration of 5000 µg/plate. Cytotoxicity was observed at a concentration of 5000 µg/plate in the test strain TA 98 without metabolic activation. In the experiment with metabolic activation, no cytotoxicity was observed in any of the tested strains. In summary, no mutagenic effect of [redacted] (b) (4) was detected in the Ames test. A summary of the results is shown in the Applicant's tables below.

TABLE 2 Mutagenicity study of [redacted] (b) (4)
in the Salmonella typhimurium reverse mutation assay (in vitro)

Substance (µg/plate)		Mutagenicity test summarized data - *without* metabolic activation Number of reverted colonies TA 98	TA 100	TA 102	TA 1535	TA 1537
(plate incorporation test) [redacted] (b) (4)						
5000	M	12.7 #	95.3	183.0	25.3	5.7
	±SD	4.2	8.1	44.2	7.0	1.5
3160	M	21.3	97.7	241.3	25.3	5.7
	±SD	5.0	11.6	2.1	5.5	2.1
1000	M	33.3	99.3	261.0	25.7	5.0
	±SD	8.6	9.0	34.6	4.5	1.7
316	M	25.0	111.3	241.0	26.7	3.7
	±SD	6.2	23.1	18.2	4.0	3.1
100	M	26.3	119.0	237.7	30.7	4.0
	±SD	2.1	10.5	46.9	11.6	2.0
Negative control 100 µl/plate	M	22.3	105.7	249.3	26.3	8.7
	±SD	5.7	12.7	3.5	4.2	2.1
Positive control substance		2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	Amino-acridine
Concentration µg/plate		10	10	1300	10	100
	M	717.0	876.7	662.7	502.0	469.3
	±SD	25.5	6.7	8.5	68.6	37.5

TABLE 2 Mutagenicity study of (b) (4)

in the Salmonella typhimurium reverse mutation assay (in vitro)

Substance (µg/plate)		Mutagenicity test summarized data - *w i t h* metabolic activation Number of reverted colonies TA 98	TA 100	TA 102	TA 1535	TA 1537
(plate incorporation test)						
(b) (4)						
5000	M	41.3	92.0	264.0	12.3	10.7
	±SD	3.1	12.5	17.7	1.2	3.5
3160	M	29.0	106.3	245.0	10.3	9.0
	±SD	7.0	13.0	91.3	2.3	2.6
1000	M	36.7	115.3	302.0	13.3	8.7
	±SD	3.5	13.7	27.0	1.5	3.1
316	M	47.3	108.0	294.3	16.3	8.0
	±SD	15.8	0.0	14.3	4.0	5.6
100	M	35.3	100.7	308.0	15.3	9.3
	±SD	9.1	9.1	23.5	0.6	1.2
Negative control 100 µl/plate	M	43.0	106.0	335.3	14.0	8.7
	±SD	3.6	11.0	17.8	4.6	2.1
Positive control substance		2-Anthra-cene amide	Cyclophos-phamide	2-Anthra-cene amide	Cyclophos-phamide	2-Anthra-cene amide
Concentration µg/plate		2	1500	2	1500	2
	M	263.0	795.0	683.0	163.3	447.3
	±SD	110.1	36.2	110.7	17.2	42.4

TABLE 3 Mutagenicity study of [redacted] (b) (4)

in the Salmonella typhimurium reverse mutation assay (in vitro)

Substance (µg/plate)	Mutagenicity test individual data - *without* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	(plate incorporation test)				
[redacted] (b) (4)					
5000	8 #	94	132	26	4
	14 #	104	208	18	6
	16 #	88	209	32	7
3160	26	111	239	19	4
	16	92	243	28	8
	22	90	242	29	5
1000	41	108	221	30	6
	35	100	281	26	3
	24	90	281	21	6
316	18	114	230	26	7
	27	133	262	23	3
	30	87	231	31	1
100	24	120	253	44	6
	28	129	275	25	4
	27	108	185	23	2
Negative control 100 µl/plate	27	101	253	25	7
	16	96	249	31	11
	24	120	246	23	8
Positive control substance	2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	Amino-acridine
Concentration µg/plate	10	10	1300	10	100
	727	881	671	537	492
	688	880	654	546	490
	736	869	663	423	426

TABLE 3 Mutagenicity study of [redacted] (b) (4)
in the Salmonella typhimurium reverse mutation assay (in vitro)

Substance (µg/plate)	Mutagenicity test individual data - *w i t h* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	(plate incorporation test)				
[redacted] (b) (4)					
5000	42	104	248	11	7
	44	93	283	13	11
	38	79	261	13	14
3160	29	93	145	13	10
	22	107	324	9	11
	36	119	266	9	6
1000	37	106	333	12	8
	33	131	284	13	12
	40	109	289	15	6
316	51	108	282	12	3
	30	108	310	17	7
	61	108	291	20	14
100	42	111	319	15	8
	25	94	324	16	10
	39	97	281	15	10
Negative control 100 µl/plate	39	106	316	18	11
	44	117	351	9	7
	46	95	339	15	8
Positive control substance	2-Anthra-cene amide	Cyclo-phosphamide	2-Anthra-cene amide	Cyclo-phosphamide	2-Anthra-cene amide
Concentration µg/plate	2	1500	2	1500	2
	366	799	559	160	496
	147	829	772	182	418
	276	757	718	148	428

7.2 *In Vitro* Assays in Mammalian Cells

No *in vitro* mammalian cells were conducted.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Toxicity study of Budesonide: Mutagenicity study (Fujii, T., Yamashita, T., Miyamae, Y. and Tensho, A. *The clinical report, [report No 4]*, 1985; 19:9, 1-15).

Study no:	Report No. 4
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9 August, 1985
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Lot # 39/78T; Purity-Not mentioned

Key Study Findings

In the *in vivo* micronucleus assay, budesonide was administered intraperitoneally at doses of 33, 100 and 320 mg/kg BID to Jcl:ICR mice. Intraperitoneal administration budesonide did not exhibit any propensity to induce chromosomal aberrations in *in vivo* micronucleus assay.

Methods

Doses in definitive study:	33, 100 and 320 mg/kg
Frequency of dosing:	BID
Route of administration:	Intra peritoneal (IP)
Dose volume:	10 mL/kg
Formulation/Vehicle:	Physiological saline mixed with carboxymethylcellulose
Species/Strain:	Jcl:ICR mice
Number/Sex/Group:	5 males/group
Satellite groups:	None
Basis of dose selection:	The highest dose selected in the main study was 320 mg/kg.
Negative control:	Physiological saline mixed with carboxymethylcellulose
Positive control:	Cyclophosphamide (100 mg/kg)

Study Validity

The negative and positive controls valued for micronucleated polychromatic erythrocytes were within the range of the conducting laboratory, and the study was valid. At each dose, bone marrow was harvested from 5 mice. Micronuclei (polychromatic erythrocytes (PCE), normochromatic erythrocytes (NCE), micronucleated polychromatic erythrocytes (MNPCE), micronucleated normochromatic

erythrocytes (MNNCE)) were evaluated by flow cytometry. Percent PCE from total erythrocytes was determined as an index of target organ toxicity. A decrease in this ratio was used as an indication of altered erythrocyte production. A statistically significant % PCE is suggested to be bone marrow cytotoxicity. A positive result was defined as a treatment-related increase in % MNCPE, a statistically significant trend ($p \leq 0.05$), or at least one or more doses produce a statistically significant increase, compared to vehicle control. A value (% MNCPE) from control animals was compared with historical control data. Data from 5 animals per sex per dose (at least 3 dose levels) were analyzed.

Results

The result showed that the percentage of micronucleated polychromatic erythrocytes (MNPCE) and normochromatic erythrocytes (NCE) count were less in all the budesonide-treated groups and in the negative control group that was treated with carboxymethylcellulose mixed solution with physiological saline when compared to positive control (cyclophosphamide). No significant difference was found between any budesonide-treated group and the negative control group. In contrast, the percent of micronucleated polychromatic erythrocytes (MNPCE) in the group treated with cyclophosphamide, the positive control, was 4.58%, about 46 times that in the saline-treated negative control group. Thus, budesonide is not mutagenic in *in vivo* micronucleus assay in mice. Data of *in vivo* micronucleus assay are presented in the Applicant's table below.

Table 2. Percent of micronucleated erythrocytes in the bone marrow of budesonide-treated mice

投与物質	投与量 (mg/kg)	動物数	1,000個の多染性赤血球中, 小核を有する赤血球数 平均値	1,000個の正染性赤血球中, 小核を有する赤血球数 平均値
カルボキシメチルセルロース混液	—	5	2.8	1.4
Budesonide	33	5	2.4	1.4
	100	5	3.4	2.6
	320	5	4.2	3.0
生理食塩液	—	5	1.0	2.2
Cyclophosphamide	100	5	45.8*	1.2

*: There was a significant difference between this group and the group treated with physiological saline solution ($p < 0.01$).

7.4 Other Genetic Toxicity Studies

Budesonide-Preferid®- A New, Nonfluorinated Acetal Type of Steroid (Richer, J. R. edited by Maibach H. I. and Surber, C. *Topical corticosteroids*, Basel, Karger, 1992, 349-369).

In this review article provided by the Applicant, the author provided only brief information about genetic toxicity of budesonide as follows.

"Budesonide is neither mutagenic nor clastogenic as was verified by means of the Ames Salmonella/microsome plate test, the mouse lymphoma test, the DNA repair analyses in rat hepatocyte culture, the chromosome aberration test in human lymphocytes, the mouse micronucleus test and the sex-linked, recessive lethal test in *Drosophila melanogaster*".

8 Carcinogenicity

Brief communication: Liver tumors in Male Rats Following Treatment with Glucocorticosteroids (Ryrfeldt, A., Squire, R. A. and Ekman, L. *Toxicology pathology*: 1992, 20:1, 115-117).

In this published article, the authors from AB Astra (Astra Zeneca) described the 2-year carcinogenicity studies of budesonide in CD-1 mice and SD rats. No positive findings occurred in mice, but an equivocal increase in liver tumors occurred in male SD rats. In order to evaluate potential hepatotoxicity of budesonide, a second 2-year carcinogenicity study was conducted in male rats with larger groups of animals. In this study, prednisolone and triamcinolone were included as reference glucocorticosteroids. The study was performed at Hazleton Laboratories, Vienna, VA. Male SD rats (7-weeks-old) received oral doses of budesonide (50 µg/kg/day), prednisolone (400 µg/kg/day) and triamcinolone (15 µg/kg/day) in drinking water for a period of 104 weeks. Two control groups were given only the vehicle (ethanol) with drinking water. In each treatment and control groups, there were 100 animals. Water consumption was similar among all groups. The average daily compound consumptions during the study were as follows: budesonide, 46.6 (±3.4) µg/kg, weeks 1-104; prednisolone, 368 (±26) µg/kg, weeks 1-104; triamcinolone, 12.7 (±1.2) µg/kg, weeks 1-9; 9.2 (±0.4) µg/kg, weeks 10-20, and 4.8 (±0.25) µg/kg, weeks 21-104. Moribund animals and those surviving until terminal sacrifice (104 weeks) were anesthetized with pentobarbital and exsanguinated for necropsy. All tissues were fixed in 10% neutral buffered formalin. Three liver lobes plus gross liver lesions were sectioned at 6 microns and stained with hematoxylin and eosin. Histopathological evaluation was limited to livers. The mean body weight was reduced by at least 10% in all drug-treated groups compared to controls. The survival rate was also reduced in all treated groups (controls, 68% and 70%; triamcinolone acetonide, 59%; prednisolone, 53%; and budesonide, 47%).

The incidences and grades of hepatocellular basophilic foci were slightly increased in the drug-treated groups, while there were no apparent effects on eosinophilic foci. Some of the basophilic foci in treated animals were of the diffuse or atypical type. In diffuse basophilic foci, hepatocytes were enlarged with vesiculate nuclei and diffusely basophilic cytoplasm. All drug-treated groups showed reduction in portal inflammation and bile duct proliferation. These effects were also noticed in control groups. Data are presented in the Table below.

TABLE I.—Number of male rats with nonneoplastic liver lesions.

Group	Control	Control	Budesonide	Prednisolone	Triamcinolone acetonide
Number animals examined	100	100	100	100	100
Foci eosinophilic alteration	20	18	14	17	17
Grade 3+ or higher	1.0	3.0	3.0	3.0	5.0
Mean grade	1.2	1.6	1.8	1.8	1.8
Foci basophilic alteration	23	20	26	31	26
Grade 3+ or higher	2.0	3.0	6.0	7.0	10.0
Mean grade	1.4	1.6	2.0	1.9	2.3
Bile duct proliferation	57	57	14	5.0	18
Portal inflammation	60	54	19	1.0	28

The incidences of hepatocellular adenomas and combined adenomas/carcinomas were significantly increased in all treated groups, with the highest total number of tumors occurring in the prednisolone group. Data are presented in the Table below.

TABLE II.—Number of male rats with neoplastic liver lesions.

Group	Control	Control	Budesonide	Prednisolone	Triamcinolone acetonide
Number animals examined	100	100	100	100	100
Hepatocellular adenoma	4.0	3.0	10.0[a]	15.0[b]	8.0
Grade 3+ or higher	2.0	1.0	3.0	6.0	3.0
Mean grade	2.2	2.7	2.2	2.1	2.2
Hepatocellular carcinoma	3.0	1.0	6.0	6.0	5.0
Grade 3+ or higher	3.0	0.0	2.0	2.0	2.0
Mean grade	3.0	2.0	2.3	2.3	2.4
Total animals with tumors	7.0	4.0	16.0[b]	21.0[b]	13.0[a]

One-sided Fisher exact tests, group 3–5 v. combined 1 and 2.
[a] $p = 0.05$.
[b] $p = 0.01$.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Toxicity study of budesonide: reproductive study (Toteno, T., Furukawa, S., Marihisa, T., Siguro, S., Fujii, T and Tomoie, H. *The Clinical report # 6*, 1985, 19:10; 1-76).

Study no.:	Clinical report # 6
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not available
GLP compliance:	Not provided
QA statement:	Not available

Key Study Findings

Male and female Jcl:Sprague-Dawley rats (n=25/dose group) received budesonide subcutaneously at doses of 0, 0.8, 4.0 and 20 µg/kg/day. The males were dosed for 63

days prior to mating to the end of the copulation phase, and females were dosed for 14 days prior to mating period to the 7th day of gestation. Males exhibited dose-related localized intolerance (swelling at injection sites) with scab and ulcer at 4 and 20 µg/kg/day doses. Male and female rats in the 20 µg/kg/day dose group showed reduced food intake and also exhibited reduced body weight. Mating and fertility indices were unaffected. In the 20 µg/kg/day dose group, the number of stillbirths (embryos/fetuses) was increased and the number of live births reduced. Delayed ossification of the 5th sternebral segment was observed in the high dose group.

Methods

Doses:	0, 0.8, 4.0 and 20 µg/kg/day
Frequency of dosing:	Daily
Dose volume:	0.1 mL/100 gm body weight
Route of administration:	SC
Formulation/Vehicle:	Polysorbate 80: 0.04 w/v %; Sodium chloride 0.07 w/v % in distilled water for injection.
Species/Strain:	Jcl:SD rats
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Male and female rats received budesonide SC at doses of 0, 0.8, 4.0 and 20 µg/kg/day. The males were dosed for 63 days prior to mating to the end of the copulation phase, and females for 14 days prior to mating period to the 7th day of gestation.
Deviation from study protocol:	Not available

Observations and Results

Mortality

Early-stage still birth and death were observed daily.

No deaths were observed.

Clinical Signs

The dams were observed for general condition.

Mild swellings at injection sites were noticed in the control and low dose groups. However, mid and high dose groups showed moderate to severe swelling, and some animals showed ulcers and scab formation at injection sites.

Body Weight

For males, body weight was measured before mating and during mating twice weekly. For females, body weights were measured every alternate day before mating and during mating, every day from day 0 to day 8 of gestation, and every other day thereafter until day 20.

There was no effect of budesonide on body weight in the low and mid dose groups compared to control. However, rats in the 20 µg/kg/day dose group showed a significant decrease in body weight, throughout the study period, compared to control. Body weight data are presented in the Applicant's Table below.

Table 1 Fertility study of budesonide in rats by subcutaneous administration
— Body weight (g) of males and females —

Dose (µg/kg)		Control	0.8	4	20
No. of males		25	25	25	25
(Premating period)	Week 0	185.5 ± 6.3	185.6 ± 6.2	185.5 ± 6.0	185.5 ± 5.9
	Week 3	332.1 ± 16.9	326.7 ± 16.8	323.9 ± 18.3	303.0 ± 18.4***
	Week 6	418.4 ± 26.2	411.4 ± 29.4	405.4 ± 33.8	370.7 ± 29.9***
	Week 9	463.8 ± 32.4	463.3 ± 35.4	457.4 ± 44.3	414.0 ± 37.4***
(Mating period)	Week 13	510.0 ± 39.6	515.4 ± 37.9	507.8 ± 53.9	474.1 ± 40.7**
No. of females		25	25	25	25
(Premating period)	Day 0	199.6 ± 6.0	199.8 ± 6.2	199.9 ± 6.1	199.8 ± 6.2
	Day 6	214.4 ± 6.4	215.8 ± 7.6	214.0 ± 8.8	206.4 ± 9.2**
	Day 14	229.8 ± 8.0	230.2 ± 10.5	228.2 ± 10.5	218.3 ± 8.0***
No. of females		22	25	24	23
(Gestation period)	Day 0	237.18 ± 13.21	235.36 ± 10.93	235.29 ± 11.09	225.26 ± 11.56**
	Day 7	268.09 ± 12.47	267.76 ± 13.08	270.12 ± 15.21	259.04 ± 11.47*
	Day 20	386.09 ± 19.86	385.44 ± 21.85	387.79 ± 25.95	372.69 ± 24.88

Mean ± S.D.
*, **, and *** Significant difference from control, at 5%, 0.1% and 0.01%

Feed Consumption

Feed consumption and water consumption were measured on the same day as body weight, except during mating.

The feed consumption in the low dose group was similar to that of control. A moderate decrease in feed consumption was noticed in the 4 µg/kg/day dose group compared to control. However, a significant reduction in food consumption was noticed in the high dose group compared to control.

Toxicokinetics

Toxicokinetic was not determined.

Dosing Solution Analysis

Dosing solution analysis was not conducted.

Necropsy

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Female rats were euthanized on day 20 of gestation and exsanguinated to death by incising the femoral artery. Laparotomies were performed, and the presence or absence of gestation and distribution, and numbers of corpora lutea, early-stage dead embryos and fetuses (implantation scars, placental residue, absorbed embryos), late-stage dead embryos and fetuses (macerated fetuses, dead fetuses), and surviving fetuses were recorded. With respect to the surviving fetuses, sex, individual body weight and placental weights, and the presence or absence of oral cavity or external abnormalities were recorded. In the surviving fetuses which did not show oral cavity or external abnormalities, approximately 2/3 of the fetuses in each litter were randomly selected, and gross observations were conducted, and organs were fixed in 70% ethanol. Skeletal specimens were prepared according to the Dawson method and stained by alizarin red S to detect any skeletal abnormalities. The remaining 1/3 of the fetuses were fixed with Bouin solution, and morphological abnormalities of the organs like head, chest, and abdomen were observed. The males were subjected to autopsies on completion of the 4-week mating period. Pathological gross findings were recorded, the weights of the brain, pituitary, submandibular gland, thymus, heart, lungs, liver, kidneys, spleen, adrenals, testes, epididymis, seminal vesicle, prostate, ovaries, uterus (including fetuses and placenta in pregnant dams) were measured.

There were 100% copulation rate in all control and drug-treated groups with conception rates of 88% in control and 92 to 100% in budesonide-treated groups. The embryonic death and fetal viability was significantly decreased in the 20 µg/kg/day dose group compared to control. Beside these effects, no other significant differences were observed between the control and various budesonide-treated groups in the number of corpora lutea, number of implantations, implantation rate, fetal viability index, sex ratio, fetal body weight, placental weight, or number of immature fetuses. In the gross visceral examination, no abnormalities were seen in any dose groups. External malformations include 1 case (0.2%) of hypoplasia of tail in each of the 4 and 20 µg/kg/day dose groups, and 3 cases of general edema (0.9%) in the 0.8 µg/kg/day dose group. The visceral malformation includes ventricular septal defects in 1 case in the control group (0.9%), 2 cases in the 0.8 µg/kg/day group (1.8%), and 3 cases in the 20 µg/kg/day group (2.8%). Effects on fetuses in visceral observation, the control group and the budesonide treated groups showed thymic cervical residue (5.4-8.5%), excessive opening of the coronary arteries (0-1.8%), insufficient elevation of the kidneys (1.8-2.9%), dilatation of the renal pelvis (9.0-18.0%), dilatation of the renal tubules (0.9-1.9%), dilatation/curvature of the renal tubules (10.7-15.3%), and dilatation of left-side umbilical artery (0-0.9%). No skeletal malformations were observed in any groups. However, significant acceleration of ossification compared to the control group was seen in the base bone of the left phalanx in the 0.8 µg/kg/day group and the 7th cervical vertebral centrum in the 4 µg/kg/day group, but these fluctuations were not dose-

dependent. The effects of budesonide in fertility of rats are presented in the Applicant's Table below.

Table 4 Fertility study of budesonide in rats by subcutaneous administration

Dose (μg/kg)		Control	0.8	4	20
No. of rats (Female/Male)		25/25	25/25	25/25	25/25
Mating and fertility data					
Male No. of copulating / No. of mated	(%)[a] (1st)	25/25 (100.0)	25/25 (100.0)	25/25 (100.0)	25/25 (100.0)
No. of pregnant / No. of copulating	(%)[a] (1st)	22/25 (88.0)	25/25 (100.0)	24/25 (96.0)	23/25 (92.0)
Female No. of copulating / No. of mated	(%)[a] (1st)	25/25 (100.0)	25/25 (100.0)	25/25 (100.0)	25/25 (100.0)
No. of pregnant / No. of copulating	(%)[a] (1st)	22/25 (88.0)	25/25 (100.0)	24/25 (96.0)	23/25 (92.0)
Median day from mating to copulation		2.0	2.0	3.0	3.0
No. of corpora lutea		16.13± 2.62	16.48± 1.93	16.87± 3.21	17.26± 2.26
No. of implantations		14.31± 1.58	14.44± 2.02	11.91± 2.61	15.26± 2.24
Implantation rate	(%)	88.7	87.6	88.3	88.4
No. of live fetuses	Male	6.95± 2.59	6.72± 2.49	6.70± 2.42	7.04± 2.40
	Female	6.77± 2.36	6.56± 2.55	7.29± 2.47	6.60± 2.10
	Total	13.72± 2.18	13.28± 2.50	14.00± 2.62	13.65± 2.97
No. of embryonic and fetal deaths		0.59± 1.05	1.20± 1.44	0.91± 0.92	1.60± 1.61*
Rate of live fetuses	(%)	95.8	91.9	93.8	89.7*
Sex ratio	(Male/Female)	1.02	1.02	0.92	1.06
Fetal weight (g)	Male	3.62± 0.23	3.74± 0.36	3.56± 0.34	3.50± 0.40
	Female	3.47± 0.17	3.50± 0.29	3.31± 0.35	3.21± 0.33
Placental weight	(mg)	487.20± 85.98	480.25± 88.11	480.46± 66.42	472.55± 58.14
No. of immature fetuses/examined	(%)	1/302 (0.3)	0/332 (0.0)	13/336 (3.8)	6/314 (1.9)
No. of changes and external malformations/examined	(%)	0/302 (0.0)	0/332 (0.0)	1[b]/336 (0.2)	3[c]/314 (0.9)
No. of visceral malformations/examined	(%)	1[d]/102 (0.9)	2[e]/109 (1.8)	0/111 (0.0)	3[f]/105 (2.8)
No. of skeletal malformations/examined	(%)	0/200 (0.0)	0/223 (0.0)	0/224 (0.0)	0/206 (0.0)
Skeletal development					
Retarded ossification					
Cervical vertebral centrum	1st (%)	147 (73.5)	149 (66.8)	183 (81.6)	171 (83.0)
Sternebrae	5th (%)	58 (29.5)	71 (31.8)	69 (30.7)	102 (49.5)*
	6th (%)	54 (27.0)	53 (23.7)	97 (43.3)	87 (42.2)
No. of ossification centrum					
Metacarpal bone	Right	3.32± 0.26	3.30± 0.30	3.27± 0.26	3.26± 0.30
	Left	3.32± 0.25	3.31± 0.30	3.27± 0.23	3.25± 0.29
No. of proximal phalanx	Right	0.17± 0.28	0.40± 0.48	0.14± 0.34	0.28± 0.48
	Left	0.16± 0.26	0.42± 0.52*	0.17± 0.34	0.26± 0.45
No. of distal phalanx	Right	5.00± 0.00	5.00± 0.00	4.99± 0.02	4.96± 0.15
	Left	5.00± 0.00	5.00± 0.00	4.99± 0.04	4.96± 0.15
No. of metatarsal bone	Right	4.00± 0.00	3.99± 0.01	3.96± 0.14	3.94± 0.16
	Left	4.00± 0.00	3.99± 0.01	3.96± 0.15	3.93± 0.16
No. of distal phalanx	Right	5.00± 0.00	5.00± 0.00	5.00± 0.00	4.99± 0.02
	Left	5.00± 0.00	5.00± 0.00	5.00± 0.00	5.00± 0.00
No. of coccygeal vertebrae		3.72± 0.40	3.93± 0.40	3.73± 0.82	3.52± 0.78

Mean± S. D.
a): Figures shown in percentages are the copulation index and fertility index
b): Includes one case of twins (one live fetus and one macerated fetus)
c): Hypoplasia of the tail (1), d) General edema (3), e): Ventricular septal defect (1)
f): Ventricular septal defect (2), g): Ventricular septal defect (3)
*: Significant difference from control, $p<0.05$

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The autopsy of the maternal animals conducted at the time of cesarean section showed involution and involution/fatty degeneration of the thymus in the control, 0.8, 4, and 20 μg/kg/day groups in 3, 4, 2, and 4 cases respectively. Congestion of the thymus, dilatation of the renal pelvis, mammary tumors (histopathologically, adenocarcinoma consisting of epithelial cells), and uterine hemorrhage were sporadically observed in a

small number of cases in the control and budesonide treated groups. In male rats, significant changes corresponding to absolute weight and relative weight were seen in the pituitary in the 0.8 µg/kg/day group, and increases in adrenal weight were seen in the 4 µg/kg/day group.

In females, fluctuations in organ weight, corresponding to absolute weight and relative weight were noticed. Adrenals weights were increased in the 4 µg/kg/day dose group and an increased weight of the ovaries were observed in the 20 µg/kg/day dose group. However, these changes were not dose-dependent.

9.2 Embryonic Fetal Development

1. **Study title: Toxicity study of budesonide: reproductive study (Toteno, T., Furukawa, S., Marihisa, T., Siguro, S., Fujii, T and Tomoie, H. *The Clinical report* # 6, 85, 19:10; 1-76).**

Study no.:	Clinical report # 6
Study report location:	Electronic submission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not available
GLP compliance:	Not mentioned
QA statement:	Not available
Drug, lot #, and % purity:	Lot # 28, purity: not provided

Key Study Findings

Four groups of pregnant Jcl:SD female rats (40 females/group) received SC budesonide doses of 0, 4, 20 and 100 µg/kg/day from day 7 to day 17 of gestation. The test substance, budesonide when given SC to pregnant rats showed reduced food intake and inhibited weight gain in dams in the 20 and 100 µg/kg/day dose groups. In the highest dose group, dams which delivered spontaneously showed an extended gravid phase. A dose-related low fetal weight was noticed in the 20 and 100 µg/kg/day dose groups. Reduced number of live fetuses in the high dose group was observed. In the 20 and 100µg/kg/day dose groups, delayed ossification of the sternum was noticed. Delayed ossification of the metacarpal bone and coccyx, and first cervical vertebra and metatarsal bone was noticed in the 100 µg/kg/day dose group.

The birth weight for male neonates (F_1 generation) was reduced in the 20 and 100 µg/kg/day dose groups compared with controls, and weight gain during the lactation period was inhibited. Delayed ossification of the thoracic vertebral arch was observed in the weaned pups of the 100 µg/kg/day dose group. Administration of budesonide did not affect behavior, ability to learn, or reproductive capacity of the F_1 generation nor its offspring (ie, F_2 fetuses).

Methods

Doses:	0, 4, 20 and 100 µg/kg/day
Frequency of dosing:	Daily
Dose volume:	0.1 mL/100 gm body weight
Route of administration:	SC
Formulation/Vehicle:	Polysorbate 80: 0.04 w/v %; Sodium chloride 0.07 w/v % in distilled water for injection.
Species/Strain:	Jcl:SD rats
Number/Sex/Group:	25/sex/group
Satellite groups:	None
Study design:	Four groups of pregnant Jcl:SD female rats (40 females/group) received SC budesonide at doses of 0, 4, 20 and 100 µg/kg/day from day 7 to day 17 of gestation.
Deviation from study protocol:	No deviation from the protocol was mentioned.

Observations and Results

Mortality

The presence or absence of early-stage stillbirth and deaths were observed daily.

In control group, one dam showed vaginal hemorrhage on day 14 of gestation, and another dam died on day 23 of gestation due to complication in late delivery. All other animals survived.

Clinical Signs

Maternal behavior (nesting, lactation, recovery) were observed daily.

No abnormal general conditions were seen in any dose groups including control. Due to abnormal nursing behavior of the dams, entire litters were died from 3 controls and 1 litter from the 4 µg/kg dose group. Those abnormal nursing behavior were seen during early stage of nursing.

Body Weight

Body weights were measured on days 0, 3, and 7- 20 of gestation and on days 0, 4, 7, 14, and 21 after delivery.

In the 4 µg/kg dose group, significant a decrease in body weight was seen on day 20 of gestation compared to control. Dams in the 20 µg/kg dose group showed a significant decrease in body weight from day 12 of gestation to day 4 after delivery compared to control group. Dams in the 100 µg/kg dose group showed a significant decrease from

day 8 of gestation, to day 7 after delivery. Body weight data are presented in the Applicant's Table below.

Table 5 Teratological study of budesonide in rats by subcutaneous administration
—— Body weight (g) of dams ——

Dose (μg/kg)		Control	4	20	100
No. of dams		38	40	39	39
(Gestation period)	Day 7	268.6 ± 12.3	268.2 ± 12.7	269.5 ± 11.0	267.1 ± 13.4
	Day 8	273.6 ± 12.3	272.1 ± 12.6	271.5 ± 12.3	263.8 ± 13.5**
	Day 12	294.0 ± 13.8	291.8 ± 15.8	287.4 ± 14.8*	270.6 ± 17.3***
	Day 18	351.6 ± 20.4	343.8 ± 19.1	335.5 ± 20.7**	302.4 ± 22.6***
	Day 20	382.6 ± 25.0	371.6 ± 23.4*	365.6 ± 24.0**	329.5 ± 25.7***
No. of dams		14[a]	15[b]	15	15
(Lactation period)	Day 0	281.9 ± 12.7	280.2 ± 17.2	264.9 ± 21.9*	266.7 ± 14.9**
	Day 7	323.3 ± 23.3	317.3 ± 13.7	309.1 ± 19.6	304.1 ± 19.8*
	Day 14	341.2 ± 15.5	338.3 ± 16.5	337.6 ± 20.0	330.2 ± 21.0
	Day 21	331.4 ± 16.4	330.8 ± 18.2	331.0 ± 22.3	320.0 ± 16.9

Mean ± S.D.
a) Three dams were sacrificed due to total litter loss on days 2, 3 and 8 post partum respectively
b) One dam was sacrificed due to total litter loss on day 4 post partum
*, **and***: Significant difference from control, $p<0.05$, 0.01 and 0.001

Feed Consumption

Food consumption was measured on days 0, 3, and 7-20 of gestation and on days 0, 4, 7, 14, and 21 after delivery.

Food consumption was slightly decreased in the 4 µg/kg dose group but was not statistically significant. Animals in the 20 µg/kg group showed a significant decrease in food intake compared to the control group on day 16 and day 18 to 20 of gestation. Animals in the 100 µg/kg group showed a significant decrease in food intake compared to the control group on days 10 to 20 of gestation and 15 to 21 after delivery.

Toxicokinetics

No toxicokinetic data were provided.

Necropsy

On Day 20 of gestation, 25 dams from each dose group were euthanized and exsanguinated to death by incising the femoral artery and dams and fetuses were examined. On day 20 of gestation after autopsy, brain, pituitary, submaxillary glands, thymus, heart, lungs, liver, kidneys, adrenals, spleen, ovaries and uterus were weighed. Laparotomies were performed, and distribution and numbers of corpora lutea, early-stage dead embryos and fetuses (implantation scars, placental residue, absorbed embryos), late- stage dead embryos and fetuses (macerated fetuses, dead fetuses), and surviving fetuses were recorded. With respect to the surviving fetuses, sex, individual body weight and placental weights were recorded.

Autopsy result showed that involution and involution/fatty degeneration of the thymus were present in 4 cases in the 20 µg/kg dose group and 23 cases in the 100 µg/kg dose group. Atrophy of the adrenals was observed in 7 cases in the 100 µg/kg dose group. These changes in the thymus and adrenals occurred with high frequency in the 100 µg/kg group. The absolute and relative weight of the pituitary gland was increased in the 100 µg/kg dose group; thymus weight was decreased in the 20 and 100 µg/kg dose groups. In the 100 µg/kg dose group, spleen and ovarian weights were reduced. Absolute weight of adrenal glands was decreased in the 100 µg/kg dose group and the spleen weight was decreased in the 20 µg/kg dose group. Organ weight data on day 20 of gestation is presented in the Applicant's table below.

Table 6 Teratological study of budesonide in rats by subcutaneous administration
—Organ weight of dams on day 20 of gestation—

Dose (µg/kg)		0	4	20	100
No. of dams		23	25	24	21
Brain	(g)	1.94 ± 0.08	1.95 ± 0.07	1.98 ± 0.08	1.96 ± 0.09
	(%)a)	0.61 ± 0.03	0.63 ± 0.04	0.66 ± 0.04***	0.71 ± 0.03***
Pituitary	(mg)	14.8 ± 1.6	15.5 ± 2.2	15.6 ± 1.9	16.1 ± 2.4*
	(%)	4.7 ± 0.5	5.0 ± 0.8	5.2 ± 0.6**	5.8 ± 0.8***
Submaxillary glands	(g)	0.53 ± 0.04	0.54 ± 0.03	0.52 ± 0.04	0.51 ± 0.05
	(%)	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01**
Thymus	(g)	0.26 ± 0.05	0.27 ± 0.05	0.19 ± 0.07**	0.07 ± 0.02***
	(%)	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.02**	0.02 ± 0.008***
Heart	(g)	0.91 ± 0.08	0.90 ± 0.07	0.91 ± 0.12	0.85 ± 0.07*
	(%)	0.28 ± 0.02	0.29 ± 0.02	0.30 ± 0.03	0.30 ± 0.02*
Lungs	(g)	1.17 ± 0.10	1.13 ± 0.14	1.11 ± 0.09*	1.08 ± 0.11**
	(%)	0.37 ± 0.03	0.36 ± 0.04	0.36 ± 0.02	0.39 ± 0.03
Liver	(g)	15.97 ± 1.37	15.35 ± 1.53	15.07 ± 1.50*	13.90 ± 1.39***
	(%)	5.11 ± 0.36	5.02 ± 0.42	5.04 ± 0.34	5.09 ± 0.33
Kidneys	(g)	1.85 ± 0.12	1.86 ± 0.17	1.85 ± 0.19	1.82 ± 0.19
	(%)	0.58 ± 0.04	0.60 ± 0.04	0.61 ± 0.05	0.66 ± 0.04***
Adrenals	(mg)	71.6 ± 11.3	72.0 ± 12.6	70.1 ± 11.9	57.8 ± 14.1***
	(%)	22.9 ± 3.2	23.4 ± 3.7	23.4 ± 3.2	21.0 ± 4.3
Spleen	(g)	0.71 ± 0.08	0.72 ± 0.11	0.65 ± 0.08*	0.53 ± 0.07***
	(%)	0.22 ± 0.02	0.23 ± 0.03	0.21 ± 0.02	0.19 ± 0.02***
Ovaries	(mg)	134.3 ± 14.6	134.9 ± 21.3	137.1 ± 15.7	132.9 ± 20.6
	(%)	43.0 ± 4.8	44.0 ± 5.9	45.8 ± 4.3*	48.6 ± 6.3**
Uterus b)	(g)	75.62 ± 11.91	67.11 ± 14.82*	70.37 ± 12.89	53.44 ± 13.90***
	(%)	24.20 ± 3.31	22.00 ± 4.75	23.58 ± 3.95	19.55 ± 4.93***

Mean ± S. D.
a): Relative organ weight. [Absolute organ weight/(Body weight−Uterine weight)] × 100
b): Figures represent weights of uteri containing fetuses and placentae
*, **and***: Significant difference from control, $p < 0.05$, 0.01 and 0.001

Dose-dependent decreases in fetal weight were observed in the 20 and 100 µg/kg dose groups. Decrease in the number of surviving fetuses and the placental weight were noticed in the 100 µg/kg dose group. Increase in the number of immature fetuses were observed in the 20 and 100 µg/kg dose groups with increased number of dead embryo in the 100 µg/kg dose group only. There were no changes among different dose groups in the number of corpora lutea, number of implantations, implantation rate, and number of surviving male and female fetuses, fetal viability index, or sex ratios. Gross visceral examination showed no abnormalities in all treated groups. The external malformation

revealed 1 case of omphalocele and 1 case of exencephaly in the control group. Fetuses in the 4 µg/kg dose groups showed 1 case of deformity of the limbs. Fetuses in the 20 µg/kg dose group showed 1 case of omphalocele and 1 combined case of general edema, omphalocele and shortness of crown-rump length. Similarly, fetuses in the 100 µg/kg dose group showed 1 case of general edema, 1 case of hypoplasia of the tail, and 1 case of combined hypoplasia of the tail and shortness of crown-rump length. The visceral malformation includes dilatation of the lateral ventricle in 1 case in the 4 µg/kg dose group, and 2 cases of ventricular septal defects in the 20 µg/kg group and 5 cases in the 100 µg/kg group. The visceral examination showed thymic cervical residue (0.8-8.1%), excessive opening of the coronary arteries (0-3.7%), insufficient elevation of the kidneys (1.8- 4.0%), dilatation of the renal pelvis (3.0-10.4%), dilatation of the renal tubules (0-0.9%), dilatation/curvature of the renal tubules (2.5-10. 4%), and dilatation of left-side umbilical artery (0-3.0%) in all dose groups compared to control. The skeletal abnormalities showed fusion of the thoracic centrum in 1 case in the 4 µg/kg group (0.4%). Significant retardation of ossification compared to the control group was seen in the 20 µg/kg dose group in the 5th sternebra, left metacarpal bone, and coccygeal vertebra, and in the 100 µg/kg dose group in the 1st cervical vertebral centrum, the 5th and 6th sternebrae, the metacarpal bone, the metatarsal bone, and the coccygeal vertebral bone. Significant acceleration of ossification was seen in the 4 µg/kg group for the distal phalanx and in the 100 µg/kg group in the distal phalanx of the foot. The effects of budesonide in rats on fetus survival, vitality, implantation, external and visceral malformation and effects in bone ossification are presented in the Applicant's Table below.

Table 8 Teratological study of budesonide in rats by subcutaneous administration
—Cesarean section of dams—

Dose (μg/kg)		0	4	20	100
No. of dams		23	25	24	24
No. of corpora lutea		16.26 ± 2.13	15.92 ± 2.13	16.70 ± 2.15	15.45 ± 2.22
No. of implantations		15.26 ± 1.73	14.68 ± 1.90	15.66 ± 2.16	14.41 ± 2.56
Implantation rate	(%)	93.8	92.2	93.7	93.2
No. of live fetuses	Male	7.47 ± 2.23	6.28 ± 2.31	7.16 ± 2.40	6.33 ± 2.35
	Female	6.60 ± 1.87	6.64 ± 2.25	7.37 ± 2.39	6.04 ± 1.80
	Total	14.08 ± 2.27	12.92 ± 2.99	14.54 ± 2.32	12.37 ± 3.30*
No. of embryonic and fetal deaths		1.17 ± 1.26	1.76 ± 2.43	1.12 ± 1.19	2.04 ± 2.36
Rate of live fetuses	(%)	92.3	88.0	92.8	85.8
Sex ratio	(Male/ Female)	1.13	0.94	0.97	1.04
Fetal weight (g)	Male	3.61 ± 0.28	3.59 ± 0.30	3.38 ± 0.36*	3.21 ± 0.30***
	Female	3.45 ± 0.24	3.38 ± 0.25	3.22 ± 0.33**	3.02 ± 0.26***
Placental weight	(mg)	462.28 ± 50.27	475.24 ± 74.39	446.23 ± 46.02	381.76 ± 56.94***
No. of immature fetuses/examined	(%)	6/324 (1.8)	1/323 (0.3)	21/349 (6.0)	13/297 (4.3)
No. of changes and external malformations / examined	(%)	2[a]/324 (0.6)	2[b]/323 (0.6)	3[c]/349 (0.8)	3[d]/297 (1.0)
No. of visceral malformations/ examined	(%)	0/108 (0.0)	1[e]/105 (0.9)	2[f]/116 (1.7)	5[g]/ 98 (5.1)
No. of skeletal malformations / examined	(%)	0/214 (0.0)	1[h]/217 (0.4)	0/230 (0.0)	0/196 (0.0)
Skeletal development					
Retarded ossification					
Cervical vertebral centrum 1st	(%)	174 (81.3)	175 (80.6)	199 (86.5)	190 (96.9)**
Sternebrae 5th	(%)	73 (34.1)	72 (33.1)	132 (57.3)**	130 (66.3)**
6th	(%)	43 (20.0)	36 (16.5)	65 (28.2)	62 (31.6)*
No. of ossification centrum					
No. of metacarpal bone	Right	3.43 ± 0.30	3.28 ± 0.23	3.23 ± 0.32*	3.09 ± 0.18***
	Left	3.41 ± 0.31	3.27 ± 0.23	3.22 ± 0.32	3.09 ± 0.17***
No. of distal phalanx	Right	4.92 ± 0.09	4.99 ± 0.02**	4.96 ± 0.18	4.88 ± 0.15
	Left	4.89 ± 0.17	4.99 ± 0.03*	4.96 ± 0.18	4.88 ± 0.15
No. of metatarsal bone	Right	3.99 ± 0.04	3.97 ± 0.04	3.92 ± 0.23	3.94 ± 0.06**
	Left	3.99 ± 0.04	3.97 ± 0.04	3.92 ± 0.23	3.94 ± 0.06**
No. of distal phalanx	Right	4.89 ± 0.21	4.97 ± 0.09	4.75 ± 1.01	4.99 ± 0.03*
	Left	4.88 ± 0.22	4.97 ± 0.09	4.75 ± 1.01	4.99 ± 0.03*
No. of coccygeal vertebrae		4.06 ± 0.38	3.88 ± 0.60	3.56 ± 0.90*	3.23 ± 0.53***

Mean ± S. D.
a): Omphalocele(1), Exencephaly(1)
b): Reduction deformity of the limbs(1), Adhesion of placentae(1)
c): Omphalocele(1), General edema(1), Omphalocele, pes valgus and shortness of the crown-rump length(1)
d): General edema(1), Hypoplasia of the tail(1), Hypoplasia of the tail and shortness of the crown-rump length(1)
e): Dilatation of the lateral ventricle
f): Ventricular septal defect(2)
g): Ventricular septal defect(5)
h): Fusion of sternebrae
*, **and ***: Significant difference from control, $p < 0.05$, 0.01 and 0.001

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Fifteen pregnant dams from each group (0, 4, 20 and 100 µg/kg) were allowed to give birth naturally and the delivery status of the animals was observed: the day on which delivery was completed was considered as day 0 of delivery. Pups were weaned after 22 days of delivery. The autopsy was conducted after weaning the pups, and pathological gross findings were recorded. The weights of the brain, pituitary, submandibular gland, thymus, heart, lungs, liver, kidneys, spleen, adrenals, ovaries and uterus were measured absolutely and relative to per 100g of body. The duration of

gestation was recorded, and the birth index was calculated. At the time of weaning on day 22 after birth, all dams were autopsied, and the number of uterine implantation scars was recorded.

The autopsy results showed involution of the thymus in the control, 4, 20, and 100 µg/kg dose groups in 1, 2, 3, and 4 cases respectively. Congestion of the thymus and lungs, cystic kidney, dilatation of the renal pelvis, and diaphragmatic hernia were sporadically observed in a small number of cases in the control and all budesonide-treated groups. The absolute and relative weights of liver was reduced in the 20 µg/kg dose group. Absolute weights of heart, liver and adrenals were reduced in the 100 µg/kg dose group. Absolute weight of kidneys was also reduced in the 20 µg/kg dose group. Organ weights of dams on Day 22 are presented in the Applicant's table below.

Table 7 Teratological study of budesonide in rats by subcutaneous administration
—Organ weight of dams on day 22 post partum—

Dose (µg/kg)		0	4	20	100
No. of dams		11	14	15	15
Brain	(g)	1.93 ± 0.06	1.95 ± 0.12	1.92 ± 0.06	1.95 ± 0.07
	(%)a)	0.58 ± 0.03	0.59 ± 0.04	0.58 ± 0.03	0.61 ± 0.03
Pituitary	(mg)	15.0 ± 2.3	15.2 ± 1.3	15.0 ± 2.3	15.0 ± 2.8
	(%)	4.5 ± 0.8	4.6 ± 0.4	4.5 ± 0.6	4.7 ± 0.8
Submaxillary glands	(g)	0.61 ± 0.06	0.57 ± 0.04	0.57 ± 0.05	0.56 ± 0.04
	(%)	0.18 ± 0.02	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Thymus	(g)	0.18 ± 0.05	0.17 ± 0.05	0.19 ± 0.09	0.19 ± 0.07
	(%)	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.05 ± 0.02
Heart	(g)	1.08 ± 0.04	1.05 ± 0.11	1.04 ± 0.14	1.02 ± 0.07*
	(%)	0.32 ± 0.02	0.32 ± 0.02	0.31 ± 0.03	0.32 ± 0.01
Lungs	(g)	1.18 ± 0.13	1.18 ± 0.17	1.18 ± 0.16	1.17 ± 0.09
	(%)	0.35 ± 0.03	0.36 ± 0.05	0.36 ± 0.04	0.36 ± 0.03
Liver	(g)	16.52 ± 1.22	15.57 ± 1.87	15.25 ± 1.57*	14.74 ± 1.78**
	(%)	5.04 ± 0.37	4.79 ± 0.48	4.69 ± 0.41*	4.68 ± 0.55
Kidneys	(g)	2.41 ± 0.16	2.36 ± 0.23	2.24 ± 0.18*	2.26 ± 0.20
	(%)	0.73 ± 0.05	0.72 ± 0.05	0.68 ± 0.05	0.71 ± 0.05
Adrenals	(mg)	81.4 ± 8.0	76.2 ± 10.7	76.4 ± 9.7	75.2 ± 7.1*
	(%)	24.8 ± 2.5	23.4 ± 3.0	23.5 ± 2.8	23.8 ± 2.0
Spleen	(g)	0.63 ± 0.09	0.57 ± 0.05	0.57 ± 0.08	0.60 ± 0.06
	(%)	0.19 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	0.18 ± 0.01
Ovaries	(mg)	100.1 ± 11.9	98.0 ± 10.9	92.6 ± 19.0	95.8 ± 15.3
	(%)	30.5 ± 3.7	30.1 ± 3.2	28.5 ± 5.8	30.6 ± 3.8
Uterus	(g)	0.38 ± 0.07	0.38 ± 0.13	0.36 ± 0.12	0.36 ± 0.14
	(%)	0.11 ± 0.02	0.11 ± 0.03	0.10 ± 0.03	0.11 ± 0.05

Mean ± S. D.
a): Relative organ weight. (Absolute organ weight/Body weight) × 100
* and **: Significant difference from control. $p < 0.05$ and 0.01

F_1 generation: Immediately after birth, the number of pups, sex, mortality and the presence or absence of abnormalities of the oral cavity and external appearance were recorded. During nursing period, mortality was observed daily and body weight was measured on days 0, 4, 7, 14, and 21 after birth. On day 4 after birth, 4 males and 4 females from each litter were randomly selected, and viability indexes of day 4 were calculated. At the time of the weaning on day 22, 2 males and 2 females were randomly selected, and weaning indexes were calculated. Pups were examined for auricular development, abdominal fur growth, incisor eruption, and palpebral opening on days 4,

8, and 11 after birth, respectively. On days 7, 16, 18, 19 and 20 after birth, pups were tested for any abnormal behaviors. The remaining pups were euthanized, examined and skeletal abnormalities were observed.

The result showed no significant differences between the control and various budesonide-treated groups for birth index, number of implantation scars, number of surviving fetuses, number of dead fetuses in the perinatal period, total number of fetuses born, birth index, mortality, body weight of newborn females, or sex ratios. One case of hypoplasia of the tail and the internal malformation of horseshoe kidney was observed in a fetus in the 100 µg/kg dose group. The viability index of pups was 81.0% in the control and 87.3-87.7% in the various budesonide-treated groups. The weaning index was 97.7% in the control and 97.9-98.2% in the budesonide-treated groups. Body weight showed a significant reduction in males in the 20 µg/kg dose group on days 7 and 14 after birth. Males in the 100 µg/kg dose group showed significantly reduced body weight on days 0, 7, 14 and 21 after birth. Females in the different doses groups and males in the 4 µg/kg dose group had no effect in body weight.

Age of eruption of incisors was significantly reduced in the 100 µg/kg dose group when compared to the control. Budesonide had no effects on auricular development, abdominal fur growth, and palpebral opening at any dose levels. The autopsy examination on the young showed no changes in any of the groups including control. The reflex functions of the pups were unaffected by budesonide. Skeletal malformation showed combined case of nodules and fusion of the ribs seen in 1 animal in the 100 µg/kg group (2.2%). The thoracic vertebral arch in the 100 µg/kg group showed significant retardation of ossification compared to the control group. The data are presented in the Applicant’s table below.

Table 9 Teratological study of budesonide in rats by subcutaneous administration
—Parturition and lactation—

Dose (μg/kg)		Control	4	20	100
No. of pregnant dams		15	15	15	15
No. of dams delivering live newborns		14[a]	15	15	15
Gestation index	(%)	93.3	100	100	100
Duration of gestation	(days)	22.10 ± 0.28	22.03 ± 0.12	22.23 ± 0.41	22.56 ± 0.45**
No. of implantations		14.64 ± 1.94	14.40 ± 2.61	15.46 ± 1.95	14.93 ± 2.52
No. of live newborns	Male	6.35 ± 1.69	6.33 ± 2.16	6.93 ± 2.05	5.33 ± 2.52
	Female	6.85 ± 2.21	7.06 ± 2.18	7.26 ± 2.68	6.60 ± 2.97
	Total	13.21 ± 2.69	13.40 ± 2.87	14.20 ± 1.78	11.93 ± 4.60
No. of perinatal deaths		0.21 ± 0.42	0.06 ± 0.25	0.40 ± 0.91	0.46 ± 0.91[b]
Total newborns		13.42 ± 2.53	13.46 ± 2.82	14.60 ± 2.09	12.40 ± 4.65
Birth index	(%)	90.2	93.0	91.8	79.9
Mortality	(%)	1.5	0.4	2.7	3.7
Sex ratio	(Male/Female)	0.92	0.89	0.95	0.80
No. of external malformations/ examined	(%)	0/185 (0)	0/201 (0)	0/213 (0)	0/179 (0)
Viability index	(%)	81.0	87.5	87.3	87.7
Weaning index	(%)	97.7	98.1	98.2	97.9
Body weight (g) of young					
Day 0	Male	6.17 ± 0.43	6.01 ± 0.41	5.91 ± 0.57	5.68 ± 0.45**
	Female	5.87 ± 0.39	5.60 ± 0.42	5.62 ± 0.62	5.48 ± 0.61
Day 7	Male	15.67 ± 1.89	14.42 ± 2.22	13.53 ± 3.24*	13.63 ± 2.20*
	Female	13.69 ± 3.49	13.66 ± 2.65	12.92 ± 2.88	13.35 ± 3.10
Day 21	Male	54.72 ± 5.47	50.42 ± 5.46	50.35 ± 6.66	47.45 ± 10.20*
	Female	51.78 ± 5.14	48.32 ± 6.79	48.58 ± 5.56	47.20 ± 11.50
Mean ages of developmental stages of offspring					
Auricular standing	(days)	4.20 ± 0.58	4.00 ± 0.02	4.02 ± 0.07	4.00 ± 0.00
Full coat growth	(days)	10.96 ± 0.55	11.16 ± 0.79	11.48 ± 1.24	11.04 ± 1.01
Incisor eruption	(days)	11.46 ± 0.79	11.42 ± 0.54	11.15 ± 0.91	10.40 ± 0.67**
Eye opening	(days)	14.15 ± 0.59	14.33 ± 0.57	14.46 ± 0.67	13.80 ± 0.66
Reflex functions of offspring					
Positive offspring/ offspring examined	(%)				
Righting reflex on surface		89/89 (100.0)	108/109 (99.0)	112/112 (100.0)	99/99 (100.0)
Righting reflex in midair		88/88 (100.0)	109/109 (100.0)	110/111 (99.0)	98/98 (100.0)
Vibrissa placing response		88/88 (100.0)	109/109 (100.0)	111/111 (100.0)	98/98 (100.0)
Pain reflex		88/88 (100.0)	109/109 (100.0)	111/111 (100.0)	98/98 (100.0)
Corneal reflex		88/88 (100.0)	109/109 (100.0)	111/111 (100.0)	98/98 (100.0)
Preyer reflex		88/88 (100.0)	109/109 (100.0)	111/111 (100.0)	98/98 (100.0)

Mean ± S. D.
a) One dam died of a difficult delivery on day 23 of gestation
b) One case of hypoplasia of the tail together with horseshoe kidney was observed in perinatal deaths
*and**: Significant difference from control, $p<0.05$ and 0.01

Table 10 Teratological study of budesonide in rats by subcutaneous administration
—Growth of offspring—

Dose (μg/kg)		Control	1	20	100
No. of skeletal malformations/ weanlings examined	(%)	0/11 (0)	0/53 (0)	0/52 (0)	1*/45 (2.2)
Skeletal development in weanlings					
Retarded ossification					
Thoracic vertebral arch	(%)	22 (50.0)	30 (56.6)	38 (73.0)	36 (80.0)*
No. of ossification centrum					
No. of coccygeal vertebrae		28.49 ± 0.35	28.29 ± 0.50	28.37 ± 0.43	28.21 ± 0.36

Reproductive performance of F_1 generation and fetuses of F_2 generation: In this study, 1 male and 1 female from each litter from each dose group were selected randomly at the age of 10 weeks, and male and female were paired randomly for the breeding. Body weight and feed consumption of dams (F_1) were monitored on days 0, 4, 8, 12, 16 and 20 of gestation. Sexual maturation was determined. After weaning on days 22 and 30, animals were tested for motor coordination by rota rod test. On week 4, open field test was conducted to measure emotional response. On week 5, water-filled multiple T-Maze was conducted to assess the learning ability of animals. Laparotomies were conducted on all of the dams, and the dams and fetuses (F_2) were examined. Laparotomy examination includes distribution and numbers of corpora lutea, early-stage dead embryos and fetuses (implantation scars, placental residue, absorbed embryos), late-stage dead embryos and fetuses (macerated fetuses, dead fetuses), and surviving fetuses. Observations included examination of the abnormalities in the oral cavity and external appearance, and gross observations of the internal organs.

No specific abnormalities in clinical signs were noticed in any dose group except 1 case of retarded growth in the 100 μg/kg dose group. There was no change in food consumption at any dose levels. Body weight of males was significantly reduced in the 4, 20 and 100 μg/kg dose groups on week 3 compared to control. On week 3, females showed significantly reduced body weight in the 4 and 100 μg/kg dose groups. No significant differences were observed between the control and budesonide-treated groups. In the rota rod test, open field test, and water-filled multiple T-Maze test, no dose-dependent effects were observed. The copulation rate was 92.8% in in the 4 μg/kg dose group, and 100% in the control, 20, and 100 μg/kg dose groups. However, after 2nd mating, the copulation rate reached 100% in the 4 μg/kg dose group. Laparotomy examination showed reduced number of surviving male fetuses in the 20 and 100 μg/kg dose groups compared to control. Budesonide treatment did not show any abnormalities l in number of corpora lutea, number of implantations, implantation rate, number of surviving female fetuses, total number of male and female surviving fetuses, number of dead embryos, fetal viability index, sex ratio, fetal body weight, placental weight, or number of immature fetuses. The external malformations of fetus showed 1 case of hypoplasia of the tail (0.5%) in the 20 μg/kg dose group, and 1 case of general edema (0.8%) in the 100 μg/kg dose group. However, the frequency of incidence of these malformations showed no significant difference compared to the control group. In the gross visceral examination, no abnormalities were observed in any dose groups of

budesonide. Data of reproductive performance of F_1 generation and effects of budesonide on fetuses of F_2 generation are presented in the Applicant's Table below.

Table 10 Teratological study of budesonide in rats by subcutaneous administration
—Growth of offspring—

Dose (μg/kg)			Control	1	20	100
Body weight (g) of offspring	Week 3	Male	55.2 ± 5.9	50.2 ± 6.0**	50.6 ± 7.2*	47.3 ± 10.0**
		Female	52.6 ± 5.5	48.5 ± 7.2*	50.1 ± 6.3	46.9 ± 11.7*
	Week 10	Male	417.5 ± 36.5	407.5 ± 28.9	396.7 ± 44.2	381.9 ± 37.0*
		Female	254.1 ± 18.9	251.7 ± 18.3	249.6 ± 18.0	239.8 ± 30.9
	Week 11	Male	509.7 ± 44.4	487.5 ± 38.3	483.8 ± 47.1	472.3 ± 44.9
Body weight (g) of pregnant offspring	Day 0		261.0 ± 13.8	259.6 ± 10.1	259.2 ± 23.6	258.4 ± 27.3*
	Day 20		400.7 ± 24.4	390.6 ± 41.0	383.9 ± 34.5	360.6 ± 40.5*
Mean sexual maturation ages of offspring						
Descent of testes		(days)	22.59 ± 0.91	22.42 ± 0.61	22.96 ± 1.06	22.66 ± 1.54
Vaginal opening		(days)	31.50 ± 1.20	32.03 ± 1.62	31.60 ± 0.82	32.16 ± 2.33
Mating and fertility data of offspring						
Male No. of copulating/No. of mated (%)[b]		(1st)	11/11 (100.0)	13/14 (92.8)	15/15 (100.0)	12/12 (100.0)
		(2nd)	–	1/1 (100.0)	–	–
No. of pregnant/No. of copulating (%)[b]		(1st)	9/11 (81.8)	13/13 (100.0)	15/15 (100.0)	10/12 (83.3)
		(2nd)	–	1/1 (100.0)	–	–
Female No. of copulating/No. of mated (%)[b]		(1st)	11/11 (100.0)	13/14 (92.8)	15/15 (100.0)	11/15 (100.0)
		(2nd)	–	0/1 (92.8)	–	–
No. of pregnant/No. of copulating (%)[b]		(1st)	9/11 (81.8)	13/13 (100.0)	15/15 (100.0)	11/15 (73.3)
		(2nd)	–	–	–	–
Median day from mating to copulation			2.0	3.0	4.0	2.0
No. of corpora lutea			15.14 ± 1.94	14.00 ± 4.35	15.53 ± 3.27	15.27 ± 4.54
No. of implantations			14.00 ± 3.87	11.61 ± 4.89	13.80 ± 3.23	11.90 ± 4.76
Implantation rate		(%)	90.6	82.9	88.8	77.9
No. of live fetuses		Male	7.77 ± 2.58	5.53 ± 2.72	5.85 ± 1.76*	5.18 ± 2.31*
		Female	5.33 ± 3.50	5.23 ± 2.83	6.60 ± 2.19	5.72 ± 3.19
		Total	13.11 ± 4.34	10.76 ± 4.28	12.46 ± 3.20	10.90 ± 4.27
No. of embryonic and fetal deaths			0.88 ± 1.05	0.84 ± 0.89	1.33 ± 0.72	1.00 ± 1.00
Rate of live fetuses		(%)	93.6	92.7	90.3	91.6
Sex ratio		(Male/Female)	1.45	1.05	0.88	0.90
Fetal weight (g)		Male	3.40 ± 0.34	3.46 ± 0.30	3.47 ± 0.23	3.66 ± 0.37
		Female	3.19 ± 0.28	3.21 ± 0.19	3.27 ± 0.23	3.38 ± 0.28
Placental weight		(mg)	446.19 ± 55.67	514.12 ± 105.30	469.66 ± 56.82	516.55 ± 139.73
No. of immature fetuses/examined		(%)	3/118 (2.5)	0/140 (0.0)	1/187 (0.5)	0/120 (0.0)
No. of changes and external malformations/examined		(%)	0/118 (0.0)	0/140 (0.0)	1[c]/187 (0.5)	1[d]/120 (0.8)

Mean ± S. D

a) Nodule and fusion of ribs

b) Figures shown in percentages are the copulation index and fertility index. The 1st copulation and fertility indexes are the result of the 1st mating. The 2nd copulation and fertility indexes are the overall result of the 1st and 2nd matings.

c) Hypoplasia of the tail. d) General edema

*, **and *** Significant difference from control, $p<0.05$, 0.01 and 0.001

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2. Study title: Teratogenicity Study of the New Glucocorticosteroid Budesonide in Rabbits (Kihlstrom, I. and Lundberg, C. 1987, *Arzneim. Forsh/Drug Res*. 37(1):4346.

Key Study Findings

Gravid New Zealand White rabbits (15 females/ dosing group) were administered SC doses of vehicle or 5, 25, or 125 µg/kg/day budesonide from Day 6 through Day 18 of pregnancy. The animals were killed on Day 29 of pregnancy and fetuses were examined for abnormalities. Two dams in the 25 µg/kg/day treatment group and all dams in the 125 µg/kg/day treatment group aborted at the end of the third or beginning of the fourth week of gravidity. Food consumption decreased in all dose groups and a dose-related inhibition of weight gain was observed. Litter weight and fetal body weight were reduced in the 5 and 25 µg/kg/day dose groups, indicating inhibition of intrauterine growth. In addition, the frequency of fetal abnormalities was significantly increased in the 25 µg/kg/day dose group, particularly in delayed skeletal development. The observed effects primarily included delayed development of the skull and vertebrae.

Methods

Doses:	0, 5, 25 and 125 µg/kg
Frequency of dosing:	Daily from Day 6 through Day 18 of pregnancy.
Dose volume:	0.25 mL/kg body weight.
Route of administration:	SC
Formulation/Vehicle:	Polysorbate (Tween) 80 and 0.5% Methylcellulose
Species/Strain:	New Zealand White rabbit
Number/Sex/Group:	15 females/group

Observations and Results

Clinical Signs

Clinical signs were recorded daily.

One animal in each in the control and 5 µg/kg dose groups showed vaginal bleeding during third and last gestational week, respectively. Two dams in the 25 µg/kg dose group aborted on day 21 and day 23, respectively. All animals in the high dose (125 µg/kg) group aborted at the end of third and at the beginning of the fourth gestational week.

Body Weight

Body weights were recorded regularly during pregnancy.

No differences in body weight gain between any dosed group and the control were seen up to day 10 of gestation. After day I0 of gestation, body weight decreased rapidly in the high dose group. The body weight difference was essentially the same in the 5 and 25 µg/kg dose groups compared to control group up to day 18 of gestation. Thereafter, the body weights decreased compared with the control group.

Feed Consumption

Food consumption was determined weekly, and the mean daily food intake was calculated individually.

There were no obvious intergroup differences in food intake during the first two gestational weeks. During the third gestational week, dams in the high dose group (125 µg/kg) budesonide showed a reduced food intake compared to control group. During the fourth and last gestational week, food consumption was markedly reduced in the low and mid dose groups compared to control. The dams in the 125 µg/kg dose group consumed the same amount of food as the control group during the last gestational week.

Necropsy

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Animals were killed by intravenous injections of a urethane solution (50%) on day 29 of pregnancy. The ovaries and uteri were screened for numbers of corpora lutea and implantations, respectively. Fetal weights were also recorded. All fetuses were examined externally and internally for visible abnormalities.

There was no differences in litter size, and fetal losses were noted in the 5 and 25 µg/kg (0.01 or 0.06.umol/kg) dose groups compared to control. In both groups the mean litter weight and the mean fetal weight were significantly decreased. All dams in the high dose group aborted before day 29 of gestation indicating 100% of fetal loss. Data are presented in the Table below.

Table 2: Litter data.

Group	Compound	Viable young			Resorptions			Abortions	Implantations	Corpora lutea	Preimplantation loss (%)[b]	Fetal loss (%)[b]	Litter weight (g)	Mean fetal weight (g)
		Male	Female	Total	Early	Late	Total							
1	control	–	–	7.7	0.2	1.0	1.2	0.1	8.9	11.0[a]	12.3[a]	17.7	–	–
		4.5	3.8	8.4	0.2	1.1	1.2	–	9.6	11.0	12.3	10.2	367.2	43.9
2	budesonide 0.01 µmol/kg	4.0	3.8	7.8	0.2	0.3	0.5	–	8.3	9.8	16.8	5.4	288.6	37.0
3	budesonide	–	–	7.8	0.2	0.3	0.5	1.2	9.5	10.3[a]	7.1[a]	18.7	–	–
	0.06 µmol/kg	4.2	4.8	9.0	0.2	0.4	0.6	–	9.6	10.3	7.1	6.2	287.2	31.9
4	budesonide	–	–	–	–	–	–	8.9	8.9	a)	a)	100.0	–	–
	0.29 µmol/kg	–	–	–	–	–	–	–	–	–	–	–	–	–
5	fluocinolone acetonide	–	–	–	–	–	–	8.9	8.9	a)	a)	100.0	–	–
	0.28 µmol/kg	–	–	–	–	–	–	–	–	–	–	–	–	–

a) Animal(s) excluded, corpora lutea not discernible. b) Mean value calculated on individual litter basis.

Offspring (Malformations, Variations, etc.)

All fetuses were preserved in 95% ethanol and stained with alizarin red-S for examination of the skeleton to determine any malformation. Skeletons were examined for the following malformations.

Skull
- Degree of ossification;
- presence of sutural bones;
- fusions between bones;
- position and size of lower jaw;
- presence of upper and lower incisors.

Vertebral column
- Number and shape of vertebrae;
- degree of ossification.

Ribs
- Number and shape of ribs.

Sternal centers
- Number and shape of sternebral segments;
- degree of ossification;
- presence of cartilaginous bands.

Limbs including scapula and the pelvic girdle
- Degree of ossification;
- shape of the bones.

The results showed no incidence of external malformation in the control group or the 5 µg/kg dose group. In the 25 µg/kg dose group, one fetus showed brachygnathia superior together with fusion of the frontal and nasal bones. Visceral abnormalities were found in one fetus in the 5 µg/kg dose group and two fetuses in the 25 µg/kg dose group. No obvious differences in the frequency of skeletal anomalies were observed in the 5 µg/kg dose group compared to control group. Fetuses in the 25 µg/kg dose group showed higher frequency of decreased degrees of ossification of the vertebra and the skull compared to control. Malformation and visceral and skeletal anomalies are presented in the Table below.

Table 3: Group mean incidence of gross malformations and visceral and skeletal anomalies.

Group	Compound	Examined	Number of young					
			Gross malformations		Visceral anomalies		Skeletal anomalies	
			Total No.	Mean (%)	Total No.	Mean (%)	Total No.	Mean (%)
1	control	92	0	0	0	0	11	14.3
2	budesonide 0.01 μmol/kg	78	0	0	1	1.0	12	18.3
3	budesonide 0.06 μmol/kg	117	1	0.8	2	1.9	58	52.7

9.3 Prenatal and Postnatal Development

Study title: Toxicity study of budesonide: reproductive study (Toteno, T., Furukawa, S., Marihisa, T., Siguro, S., Fujii, T and Tomoie, H. *The Clinical report # 6*, 85, 19:10; 1-76).

Key Study Findings

Gravid Jcl:SD rats (25 females per treatment group) were administered subcutaneously either vehicle or budesonide (0.8, 4 or 20 µg/kg/day) from Day 17 of gestation until Day 21 after delivery. Localized effects (swelling at the injection site) were observed in all dams at the end of the lactation period. On autopsy, hypertrophy of the subcutaneous tissue and abscesses were observed. General condition, reproduction parameters and fetal development were not impaired. Reduced food consumption and inhibited weight gain were observed in animals in the 4 or 20 µg/kg/day dose groups. On autopsy, atrophy of the thymus was noticed in the 20 µg/kg/day dose group, the pup viability index in budesonide treated groups were 90.2-96.3%, whereas in control it was 96.4%. Budesonide had no effects on clinical signs, food intake and body weight, sexual maturation and neurological functions. The reproductive performances between the control and budesonide-treated groups were similar. For F_2 generation, the numbers of male survival fetuses were higher in thet budesonide treated groups compared to the control group. Body weights of male fetuses were decreased in the 0.8 and 20 µg/kg/day dose groups. An elevated male to female sex ratio was also noticed in the 20 µg/day dose group. No abnormalities were observed on the visceral gross examination of the F_2 pups.

Methods

Doses:	0, 0.8, 4.0 and 20 µg/kg/day
Frequency of dosing:	Daily
Dose volume:	0.1 mL/100 gm body weight
Route of administration:	SC
Formulation/Vehicle:	Polysorbate 80: 0.04 w/v %; Sodium chloride 0.07 w/v % in distilled water for injection.
Species/Strain:	Jcl:SD rats
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	Female pregnant rats received budesonide SC at doses of 0, 0.8, 4.0 and 20 µg/kg/day from day 17 of gestation until day 21 of delivery.
Deviation from study protocol:	Not provided

Observations and Results (Optional Table)

F_0 Dams

Survival: In the initial period of nursing, 1 dam from 0.8 µg/day dose group and 1 dam from 4 µg/day dose group showed abnormal nursing behavior, and the entire litter died from these groups.

Clinical signs: Clinical signs were observed daily. Majority of animals showed swelling at the injection site. No other clinical signs were noticed.

Body weight: Body weight were measured on days 0 and 7 of gestation, and daily from day 14 until delivery, and on days 0, 4, 7, 11, 14, 18 and 21 after delivery. Body weight of females in the 0.8 µg/day dose group was identical to that of control. Females in the 4 µg/day dose group showed similar body weight compared to control during the gestation period, but on day 21 after birth, there was a significant decrease compared to the control group. Females in the 20 µg/day dose group showed a significant decrease in body weight from day 20 of gestation to throughout the nursing period. Body weight data are presented in the Applicant's Table below.

Table 11 Peri-and postnatal study of budesonide in rats by subcutaneous administration
— Body weight (g) of dams —

Dose (µg/kg)		Control	0.8	4	20
No. of dams		25	25	25	25
(Gestation period)	Day 17	344.5 ±14.8	344.8 ± 14.8	343.3 ± 16.1	343.7 ± 14.6
	Day 18	359.9 ±16.6	361.2 ± 16.0	356.8 ± 18.0	353.4 ± 16.2
	Day 20	395.6 ±19.3	396.4 ± 17.4	390.9 ± 20.9	383.2 ± 20.6*
No. of dams		25	25[a]	25[b]	25
(Lactation period)	Day 0	295.3 ±16.7	296.3 ± 18.5	292.5 ± 15.6	283.8 ± 21.8*
	Day 7	330.4 ±15.1	331.0 ± 15.0	327.2 ± 15.6	318.1 ± 16.5**
	Day 11	349.6 ±15.2	347.2 ± 15.5	341.9 ± 13.9	333.1 ± 16.9***
	Day 14	358.9 ±18.7	356.6 ± 16.5	348.8 ± 19.7	343.8 ± 18.4**
	Day 18	363.0 ±16.5	360.4 ± 19.4	355.6 ± 18.1	348.8 ± 17.2**
	Day 21	361.6 ±17.7	355.2 ± 17.5	350.8 ± 13.4*	343.9 ± 15.9***

Mean ± S. D.
a): One dam was sacrificed due to total litter loss on day 3 post partum
b): One dam was sacrificed due to total litter loss on day 6 post partum
*, **and***: Significant difference from control, $p<0.05$, 0.01 and 0.001

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Food Food consumption was measured on days 0 and 7 of gestation, and

consumption:	daily from day 14 until delivery and on days 0, 4, 7, 11, 14, 18 and 21 after delivery. Animals in the 4 µg/day dose group showed a significant decrease in food intake compared to the control group on day 19 of gestation. Animals in the 20 µg/day dose group showed a significant decrease in food consumption compared to the control group from day 18 to day 20 of gestation. However, females in both dose groups showed no changes in the food consumption during the nursing period. Food consumption in the 0.8 µg/day dose group was unchanged compared to control.
Uterine content:	Not provided.
Necropsy observation:	Necropsy was conducted at the time of weaning. Necropsy examination showed involution of the thymus in the control, 0.8, 4, and 20 µg/day dose groups. The frequency of incidence was significantly higher in the 20 µg/day dose group compared to control. In addition, there were sporadic findings in a small number of cases in the control and budesonide-treated groups for insufficient development of the accessory spleen and mammary gland, abnormal lobation of the liver, dilatation of the renal pelvis, and unilateral implantation. However, these findings were not dose-dependent. Swelling at the administration site and hypertrophy of the subcutaneous tissue were observed in 10-13 cases in various dose groups, including the control, and ulcers were observed in one case in the control, 2 cases in the 4 µg/day dose group, and 3 cases in the 20 µg/day dose group.
Toxicokinetics:	None
Other:	The weights of the brain, pituitary, submandibular gland, thymus, heart, lungs, liver, kidneys, spleen, adrenals, ovaries and uterus were measured absolutely and relative to body weight per 100g. The absolute and relative weight of thymus was decreased in the 20 µg/day dose group compared to control. The absolute and relative weights of kidneys were higher in the 20 µg/day dose group compared to control. Organ weights are presented in the Applicant's table below.

Table 12 Peri-and postnatal study of budesonide in rats by subcutaneous administration
—Organ weight of dams on day 22 post partum—

Dose (μg/kg)		Control	0.8	4	20
No. of dams		25	24	24	25
Brain	(g)	1.95 ± 0.08	1.93 ± 0.11	1.97 ± 0.08	1.95 ± 0.08
	(%)[a]	0.55 ± 0.03	0.55 ± 0.04	0.56 ± 0.03	0.56 ± 0.03
Pituitary	(mg)	15.9 ± 2.7	15.7 ± 1.9	15.8 ± 1.9	16.3 ± 2.3
	(%)	4.4 ± 0.7	4.5 ± 0.6	4.5 ± 0.4	4.7 ± 0.6
Submaxillary glands	(g)	0.60 ± 0.05	0.60 ± 0.04	0.61 ± 0.06	0.61 ± 0.05
	(%)	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Thymus	(g)	0.18 ± 0.05	0.20 ± 0.07	0.17 ± 0.06	0.12 ± 0.04***
	(%)	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01**
Heart	(g)	1.13 ± 0.12	1.12 ± 0.08	1.11 ± 0.10	1.13 ± 0.09
	(%)	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.32 ± 0.02
Lung	(g)	1.28 ± 0.11	1.31 ± 0.10	1.29 ± 0.11	1.29 ± 0.12
	(%)	0.36 ± 0.03	0.37 ± 0.03	0.36 ± 0.03	0.37 ± 0.03
Liver	(g)	15.81 ± 1.50	16.26 ± 1.71	16.03 ± 1.33	16.17 ± 1.54
	(%)	4.50 ± 0.25	4.65 ± 0.30	4.59 ± 0.30	4.70 ± 0.38*
Kidneys	(g)	2.41 ± 0.21	2.49 ± 0.30	2.46 ± 0.19	2.56 ± 0.15**
	(%)	0.68 ± 0.05	0.70 ± 0.07	0.70 ± 0.05	0.74 ± 0.04***
Adrenals	(mg)	87.6 ± 11.3	87.5 ± 9.8	82.9 ± 14.1	82.2 ± 11.0
	(%)	25.0 ± 3.7	25.1 ± 3.3	23.7 ± 4.0	23.9 ± 3.0
Spleen	(g)	0.67 ± 0.09	0.65 ± 0.06	0.67 ± 0.10	0.68 ± 0.09
	(%)	0.18 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.02
Ovaries	(mg)	105.6 ± 13.5	105.2 ± 14.2	100.2 ± 13.7	110.9 ± 16.9
	(%)	30.1 ± 3.6	30.1 ± 4.0	28.7 ± 3.9	32.2 ± 4.9
Uterus[a]	(g)	0.39 ± 0.11	0.38 ± 0.15	0.40 ± 0.10	0.35 ± 0.13
	(%)	0.10 ± 0.03	0.10 ± 0.04	0.11 ± 0.03	0.10 ± 0.04

Mean ± S. D.
a): Relative organ weight. (Absolute organ weight/Body weight) × 100
*, **and ***: Significant difference from control, $p < 0.05$, 0.01 and 0.001

F_1 Generation

Survival: There were 13 deaths in the control group and 15-36 deaths in the budesonide-treated groups. The viability index on day 4 was 96.4% in the control group and 90.2-96.3% in the various administration groups.

Clinical signs: No clinical signs were noticed except one animal in the 4 µg/day dose group showed unilateral cataracts.

Body weight: No change in the body weight of the F_1 animals was observed from day 1 to 21. Data are presented in the Applicant's table below.

Dose (µg/kg)			Control	0.8	4	20
Body weight (g) of young	Day 0	Male	6.15 ± 0.39	6.10 ± 0.38	6.20 ± 0.44	6.00 ± 0.50
		Female	5.83 ± 0.46	5.84 ± 0.35	5.89 ± 0.39	5.65 ± 0.60
	Day 7	Male	15.06 ± 1.87	14.62 ± 2.24	15.46 ± 2.29	15.20 ± 2.38
		Female	14.40 ± 1.93	13.90 ± 1.99	15.02 ± 1.46	14.32 ± 2.43
	Day 14	Male	32.54 ± 3.14	32.09 ± 3.82	32.97 ± 4.61	32.68 ± 3.49
		Female	31.52 ± 3.47	31.06 ± 3.45	32.56 ± 2.51	31.51 ± 3.47
	Day 21	Male	52.45 ± 5.25	50.80 ± 6.32	53.46 ± 6.74	54.16 ± 5.60
		Female	50.99 ± 5.65	49.14 ± 5.92	52.59 ± 3.84	51.80 ± 5.55

Feed consumption: Food consumption in males and females showed no significant differences between control and budesonide-treated groups.

Physical development: Retarded growth was noticed both in the control (n=2) and in the 20 µg/day dose group (n=4).

Neurological assessment: Neurological assessments were conducted to determine reflex functions. The reflex functions such as positive rates for righting reflex on a surface, righting reflex in midair, vibrissa placing response, pain reflex, corneal reflex, and Preyer reflex showed no significant differences between the control and treated groups. Findings are presented in the Applicant's Table below. Similarly, no dosage-dependent findings were seen in the rota rod test, the open field test, or the water-filled multiple T-Maze test.

Dose (µg/kg)	Control	0.8	4	20
Reflex functions of offspring				
Positive offspring/offspring examined (%)				
Righting reflex on surface	200/200(100.0)	188/188(100.0)	184/184(100.0)	198/198(100.0)
Righting reflex in midair	200/200(100.0)	188/188(100.0)	183/184(99.4)	197/197(100.0)
Vibrissa placing response	200/200(100.0)	188/188(100.0)	184/184(100.0)	197/197(100.0)
Pain reflex	200/200(100.0)	188/188(100.0)	184/184(100.0)	196/196(100.0)
Corneal reflex	200/200(100.0)	188/188(100.0)	184/184(100.0)	196/196(100.0)
Preyer reflex	200/200(100.0)	188/189(100.0)	184/184(100.0)	196/196(100.0)

Reproduction: No significant differences between the control and drug-treated

groups were observed in the ages of descent of testes or vaginal opening. Copulation rates for the first mating were 100% in the 0.8 and 20 µg/day dose groups, 96.0% in the control group due to failure to achieve copulation by one pair, and 95.8% in the 4 µg/day dose group due to failure to achieve copulation by the females. In the first estrous cycle following mating of the females, no differences were observed among the groups in number of days of copulation and number of days required until copulation was achieved. Data are presented in the Applicant's Table below.

Dose (µg/kg)		Control	0.8	4	20
Mean sexual maturation ages of offspring					
Descent of testes	(days)	22.06 ± 0.21	22.02 ± 0.10	22.08 ± 0.24	22.08 ± 0.23
Vaginal opening	(days)	31.38 ± 1.05	31.36 ± 0.84	31.52 ± 0.84	31.50 ± 1.23
Mating and fertility data of offspring					
Male No. of copulating/ No. of mated	(%) (1st)	24/25 (96.0)	24/24 (100.0)	23/24 (95.8)	25/25 (100.0)
	(2nd)	1/1 (100.0)	—	1/1 (100.0)	—
No. of pregnant/ No. of copulating	(%) (1st)	20/24 (83.3)	23/24 (95.8)	23/23 (100.0)	24/25 (96.0)
	(2nd)	1/1 (84.0)	—	1/1 (100.0)	—
Female No. of copulating/ No. of mated	(%) (1st)	24/25 (96.0)	24/24 (100.0)	22/23 (95.6)	25/25 (100.0)
	(2nd)	1/1 (100.0)	—	0/1 (95.6)	—
No. of pregnant/ No. of copulating	(%) (1st)	20/24 (83.3)	23/24 (95.8)	22/22 (100.0)	24/25 (96.0)
	(2nd)	1/1 (84.0)	—	—	—
Median day from mating to copulation		3.0	2.0	3.0	3.0

Other:

F_2 Generation

Survival: The numbers of surviving male fetuses were higher in different dose groups compared to the control group. Data are presented in the Table below.

Dose (µg/kg)		Control	0.8	4	20
No. of live fetuses	Male	5.57 ± 2.39	7.39 ± 1.67**	6.86 ± 1.69*	8.25 ± 2.23***
	Female	7.04 ± 3.90	7.82 ± 1.99	6.81 ± 2.87	7.29 ± 1.89
	Total	12.61 ± 5.38	15.21 ± 2.06*	13.68 ± 2.74	15.54 ± 3.03*
No. of embrionic and fetal death		0.61 ± 0.74	0.60 ± 0.72	0.63 ± 0.90	0.62 ± 0.92
Rate of live fetuses	(%)	95.3	96.1	95.5	96.1
Sex ratio	(Male/Female)	0.79	0.94	1.00	1.13*
Fetal weight (g)	Male	3.66 ± 0.28	3.47 ± 0.17*	3.53 ± 0.22	3.49 ± 0.12*
	Female	3.44 ± 0.23	3.33 ± 0.15	3.36 ± 0.19	3.32 ± 0.11*

Body weight: Body weights of male fetuses were decreased in the 0.8 and 20 µg/day dose groups. Body weight data are presented in the Table above.

External evaluation: In the gross visceral examination, no abnormalities were seen in any of the treated groups.

Male/Female ratio: An elevated male to female sex ratio was noticed in the 20 µg/day dose group. Data are presented in the Table above.

10 Special Toxicology Studies

Study of local toxicity, phototoxicity, photoallergic properties and antigenicity (Kohara, M., Kimura, M., Yamada, A. and Tensho, A. *Toxicity study of Budesonide, clinical report,* 1085, Vol 19, No. 9; 1-19).

(a) Phototoxicity in Guinea pigs:

The phototoxicity of budesonide was assessed in groups of male Slc:Hartley guinea pigs. The backs of the guinea pigs were shaved and fur was removed. A central line was drawn on the back of the animals after removal of the fur. In each treatment group there were 10 male Slc:Hartley guinea pigs. Animals in each group were either applied with vehicle cream or ointment, 0.05% budesonide cream or ointment (250 mg/site) or active positive control (0.005% 8-methoxypsoralen [8-MOP] in acetone solution [0.05 mL/site]). Treatments were applied to the shaved back of each animal with two doses of the same medication on either side of a central line that was drawn on the backs of the animals. One side of each animal (one treatment) was covered with foil, and the other was irradiated for 3 min from a height of 10 cm with wavelengths of 270-320 nm. After 3 minutes irradiation, the backs were covered with a glass filter and additional irradiation was carried out for 2 hours with wavelength of 320 to 400 nm. The symptoms were recorded at 24, 48 and 72 hours after irradiation at the application site.

The results showed that there was no damage in the irradiated and non-irradiated skin treated with the ointment base, the cream base, the budesonide ointment, or the budesonide cream. In the 8-MOP group (positive control), cutaneous damage was not observed at non-irradiated sites but all of the irradiated sites showed clear cutaneous damage with erythema. Phototoxicity data are presented in the applicant's Table below.

Table 8 Phototoxic Potential of Budesonide in Guinea Pigs

Source: Kohara 1985.

Abbreviations: + = with irradiation; - = without irradiation; 8-MOP = 8-methoxypsoralen

(b) Photoallergy in Guinea pigs:

The photoallergy of budesonide was studied in groups of 10 male Slc:Hartley guinea pigs. Animals were administered intradermal injections of water with Freund's Complete Adjuvant (FCA) into hairless sites on the neck. After injection, the sites were stripped

with cellophane tape. On each site, vehicle cream or ointment, 0.05% budesonide cream or ointment (500 mg/site) or active control (0.1% 3,3,4,5-tetrachlorosalicylanilide [TSCA] in acetone solution [0.2 mL/site]) were applied. Thirty minutes after application, sites were irradiated for 120 min from a distance of 10 cm with wavelengths of 270 to 400 nM. Irradiation was conducted on alternate days for 5 sensitization cycles. Two weeks after the final sensitization, sites separated from the original sensitization site were selected and the drugs were reapplied. The sites were irradiated for 100 minutes. At 24 and 48 hours after irradiation, the sites were evaluated for red spots or swelling.

Both the budesonide ointment and the budesonide cream sensitization groups had no cutaneous irritation at either the irradiated or non-irradiated sites. In the TCSA group (positive control), skin irritation accompanying erythema was observed primarily at the irradiated sites with a positive incidence rate of 80% and 90% after 24 and 48 hours, respectively. Photoallergy test result for guinea pigs is presented in the Applicant's Table below.

Table 9 Phototoallergic Potential of Budesonide in Guinea Pigs

Source: Kohara 1985.

Abbreviations: + = with irradiation; - = without irradiation; TCSA = 3,3,4,5-tetrachlorosalicylanilide

(c) Ocular irritation in Rabbits:

The effects of budesonide on ocular irritation were assessed in male New Zealand White rabbits. In this study, ointment base, budesonide ointment, cream base or budesonide cream were applied in the left conjunctival sac at a dose of 10 or 100 mg/eye. The damage to the cornea, iris and conjunctiva were evaluated at 2, 24, 48 and 72 hours after administration by a slit lamp.

No damage was noticed in the cornea, iris or conjunctiva in the budesonide 10 mg ointment and 10 mg base ointment groups. However, in the budesonide 100 mg ointment and 100 mg base ointment groups, mild swelling of the conjunctiva was observed in 1 of 5 animals after 2 (both groups) and 24 hours (budesonide treated group only). Similarly, no damage was observed in the budesonide 10 mg cream group. In the 10 mg base cream group, mild swelling of the conjunctiva was observed in 1 of 5 animals after 2 and 24 hours after application. In both the budesonide 100 mg cream

and 100 mg base cream groups, symptoms such as mild reddening, swelling and discharge were observed in the conjunctiva in 4 of 5 animals per group after 2 hours and in 1-2 animals per group after 24 hours. All symptoms were resolved over time and all animals in all groups showed complete recovery by 48 hours post-administration. Ocular effects of budesonide are presented in the Applicant's Table below.

Table 10 Study of the Ocular Mucous Membrane Irritation Potential of Budesonide in Rabbits

Test Agent	Dose (mg/eye)	No. Animals	Observation Time	Score: Mean ± SE (Number of Cases of Damage)			
				Cornea	Iris	Conjunctiva	Overall
Ointment Base	10	5 M	2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	100	5 M	2	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Budesonide Ointment	10	5 M	2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	100	5 M	2	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			24	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Cream Base	10	5 M	2	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			24	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	100	5 M	2	0 ± 0	0 ± 0	2.8 ± 0.8 (4)	2.8 ± 0.8 (4)
			24	0 ± 0	0 ± 0	0.8 ± 0.5 (2)	0.8 ± 0.5 (2)
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Budesonide Cream	10	5 M	2	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			24	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	100	5 M	2	0 ± 0	0 ± 0	1.6 ± 0.4 (4)	1.6 ± 0.4 (4)
			24	0 ± 0	0 ± 0	0.4 ± 0.4 (1)	0.4 ± 0.4 (1)
			48	0 ± 0	0 ± 0	0 ± 0	0 ± 0
			72	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Safety Assessment of the Extractables of the Container-closure system of Budesonide 2 mg rectal foam (Study # ASRPT-246):

An extractable study was performed to assess the possible extractables released from the container closure system (canister and closing valves) of budesonide rectal foam. Two solvents (57% propylene glycol and 30% ethanol) were used for the sonication extraction instead of using long term storage under different room temperatures and relative humidity. For each solvent, two separate sets of components were used for the sonication extraction. For Set A, one canister for each solvent was half-filled with 30 mL of the appropriate solvent and capped with the metering valve. For Set B, 30 mL of the appropriate solvent and enough glass beads to cause the solvent to completely fill the

container were added to the canister so that the extraction solvent would be in contact with the interior of the metering device during the extraction process; then, the canister was capped with the metering device. The samples were sonicated for 30 minutes. Two control solutions (one for Set A and one for Set B) for each solvent were prepared in glass jars in the same manner as the sample preparations to ensure that there was no contamination from the labware or reagents. Glass beads were added to the control solutions for Set B. Two test methods were used in these studies. They are Gas chromatography with a mass spectrometer (GC/MS) and Liquid chromatography with a mass spectrometer (LC/MS).

No semi-volatile organic compounds of the container closure system were detected above the reporting limits by the GC/MS methods when compared to controls. The extractable data for the container closure system in 57% propylene glycol and 30% ethanol are presented in the Applicant's Table below.

Table 5. Direct Injection Semi-Volatile GC/MS Results for 57% Propylene Glycol Extracts

Sample ID	Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit, signal to noise of (b) (4)

Table 6. Direct Injection Semi-Volatile GC/MS Results for 30% Ethanol Extracts

Sample ID	Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit, signal to noise of (b) (4)

No volatile organic compounds of the container closure system were detected above the reporting limits by the GC/MS methods when compared to controls as shown in the Applicant's Table below.

Table 10. Headspace Volatile GC/MS Results for 57% Propylene Glycol Extracts

Sample ID	Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit, signal to noise of (b) (4)

Table 11. Headspace Volatile GC/MS Results for 30% Ethanol Extracts

Sample ID	Retention Time (Minutes)	Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit, signal to noise of (b) (4)

Similarly, no compounds were detected above the reporting limits by the LC/MS analysis compared to controls as shown in the Applicant's Table below.

Table 14. LC/MS MM^+ Results for 57% Propylene Glycol Extracts

Sample ID	Retention Time (Minutes)	MM^+ Major Ions (m/z) Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

Table 15. LC/MS MM^+ Results for 30% Ethanol Extracts

Sample ID	Retention Time (Minutes)	MM^+ Major Ions (m/z) Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

Table 16. LC/MS MM Results for 57% Propylene Glycol Extracts

Sample ID	Retention Time (Minutes)	MM Major Ions (m/z) Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

Table 17. LC/MS MM Results for 30% Ethanol Extracts

Sample ID	Retention Time (Minutes)	MM Major Ions (m/z) Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

Table 18. HPLC/UV Results for 57% Propylene Glycol Extracts

Sample ID	Retention Time (Minutes)	UV Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

Table 19. HPLC/UV Results for 30% Ethanol Extracts

Sample ID	Retention Time (Minutes)	UV Tentative Identification	Est. Conc. (µg/mL)
Canister and Metering Valve Set A – Half Full	NA	NA	< RL
Canister and Metering Valve Set B – Full	NA	NA	< RL

NA = Not applicable; RL = Reporting limit of (b) (4) µg/mL

To determine the metals present in the extractable emulsions (57% propylene glycol and 30 % ethanol), 6 mL of each sample solution was evaporated to near dryness and then reconstituted with an equivalent volume of 5% nitric acid or 5% hydrochloric acid. A blank solution of 5% nitric acid or 5% hydrochloric acid was used to ensure that there was no contamination from the labware or reagents. The metals present in the extractable solvents were assessed by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The Applicant's Table below shows the metals extracted from canister and metering valve when sonicated for 30 minutes in the presence of 57% propylene glycol and 30% ethanol.

Table 21 ICP-OES Results for 57% Propylene Glycol Extracts

(b) (4)

In the 57% propylene glycol extracts, no reportable metals were detected in set A-half full. In set B-full, (b) (4) were detected above the reporting limit and were not associated with the control.

Table 22 ICP-OES Results for 30% Ethanol Extracts

(b) (4)

In the 30% ethanol extracts, no reportable metals were detected in set A-half full. In set B-full, (b) (4) were detected above the reporting limit and were not associated with the control.

As per CFR 184, (b) (4) were considered to be generally recognized as safe (GRAS). (b) (4)

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The anticipated exposures of the (b) (4) (b) (4) from budesonide rectal foam are several fold lower than the recommended daily intake for these elements.

11 Integrated Summary and Safety Evaluation

In the current submission, the applicant is seeking approval of budesonide (UCERIS) Rectal Foam for the treatment of active mild to moderate distal ulcerative colitis (UC) extending up to 40 cm from the anal verge. Budesonide 2 mg Rectal Foam is an aerosol foam delivered by disposable, (b) (4), dose-metering, multi-dose canister. The recommended dosing regimen is 1 metered dose (2 mg budesonide per meter dose) to be administered twice daily for 2 weeks followed by 1 metered dose (2 mg budesonide per meter dose) administered once daily intrarectally for 4 weeks.

Budesonide is a synthetic glucocorticoid with topical anti-inflammatory activity, with limited systemic adverse effects. Budesonide has been shown to exhibit significant anti-inflammatory activity in the lower GI tract. Budesonide has strong glucocorticoid receptor (GCR) affinity and has an effective first pass metabolism by the liver with a short half-life.

This NDA is supported by published nonclinical studies and additional toxicological studies conducted by the Applicant. As per the Agency's recommendation, a 6-week repeated dose intra rectal toxicity study in dogs was conducted and the final study report was submitted in the NDA. In addition, the applicant has submitted the final report of the 39-week toxicology study with budesonide rectal foam in dogs. The applicant also provided available published nonclinical studies of budesonide. The published studies

showed that budesonide is an effective anti-inflammatory agent and not mutagenic. In a subcutaneous embryofetal development studies, fetal loss, decreased pup weights, and skeletal abnormalities were observed at a subcutaneous dose of 25 µg/kg in rabbits.

A Six-week and a 39-week repeated intra rectal dose toxicity study of budesonide was conducted in Beagle dogs. In both studies, Beagle dogs were administered budesonide foam intra-rectally at 0, 0.6, and 2 mg/25 mL doses twice daily for 6 weeks and 39 weeks, respectively. Twice-daily rectal administration of budesonide foam was well tolerated at doses up to 4 mg/day for 6- and 39-weeks in dogs.

In conclusion, budesonide has a long history of use in humans. Budesonide (Entocort®) was approved for marketing in 2001 for the treatment of mild to moderate CD involving the ileum and/or the ascending colon. In 2013, UCERIS (budesonide) extended release tablets were approved for the treatment of mild to moderate ulcerative colitis (UC). For safety of this NDA, the applicant relied upon the published literatures and toxicology studies conducted in dogs (6- and 39 weeks repeated dose intrarectal toxicity studies). Six-week and 39-week intra rectal toxicology studies in dogs showed that budesonide foam was well tolerated at doses up to 4 mg/day in dogs. Thus, there are no safety concerns for the proposed dose of budesonide in the proposed patient population in the proposed dosage form.

12 Appendix/Attachments: None

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/s/

DINESH C GAUTAM
08/25/2014

SUSHANTA K CHAKDER
08/25/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 205-613 **Applicant: Salix Pharmaceuticals, Inc.** **Stamp Date: November 15, 2013**

Drug Name: Budesonide Rectal Foam **NDA Type: 505(b)(2)**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	Yes		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	Yes		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	Yes		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	Yes		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	Yes		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Yes		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	Yes		Division requested (April 30, 2009) a 3-month intra rectal toxicity study in a non-rodent species. The applicant has submitted

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
				a 6-week and a 39-week intra rectal toxicity study in dogs.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	Yes		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	Yes		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __ YES___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Dinesh Gautam, Ph.D

Reviewing Pharmacologist Date

Sushanta Chakder, Ph.D

Team Leader/Supervisor Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

DINESH C GAUTAM
01/08/2014

SUSHANTA K CHAKDER
01/08/2014