CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20762

PHARMACOLOGY REVIEW(S)

AUG 19 1997

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

NDA Number: 20-762

Supplement Number: N(BB)

Date of Submission: 12/3/1996

Information to be Conveyed to Sponsor: Yes ( ), No (x)

Reviewer: T. Tom Du, Ph.D.

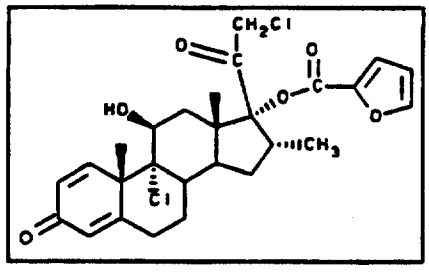
Date Review Completed: 8/19/1997

Sponsor: Schering Corporation
2000 Galloping Hill Road,
Kenilworth, NJ 07033

Drug Name: NASONEX™ Nasal Spray
(SCH 32088; Mometasone furoate monohydrate)

CAS Number: 83919-23-7

Molecular Formula and Structure: $C_{27}H_{30}Cl_2O_6H_2O$



Molecular Weight and Formula: 539.458

Class: Anti-inflammatory steroid

Indication: Prophylaxis and treatment of seasonal allergic rhinitis/Perennial rhinitis

Route of Administration: Intranasal

Study Reviewed in this Amendment:

1. In vitro metabolism of SCH 32088 across species by liver, lung and intestinal tissue preparation (P-6376; 6/93-11/96)

REVIEW OF STUDIES

1. IN VITRO METABOLISM OF SCH 32088 ACROSS SPECIES BY LIVER, LUNG AND INTESTINAL TISSUE PREPARATIONS (P-6376; 6/93-11/96)

Test Lab: Schering-Plough, Kenilworth, NJ
Study Number: 92347 GLP: No
Batch Number of Test Article: 30329-46-10, 36711-94-5, 32613-27-23, 35490-10-3, 50492-059 and 23047-131

This study was to compare the metabolite profiles and metabolites of SCH 32088 in the livers, lungs, and intestines of various species (rat, mouse, dog and human).

Methods: In this study, tissues were harvested from Crl:CD(SD)BR rats, CD-1 mice and beagle dogs, which are the same animal species and strains used in the toxicology and carcinogenicity studies. In vitro incubations of SCH 32088 were performed with the following tissue preparations:

1. Rat, mouse, dog and human liver slices. (Evaluated by LC/MS, LC/MS/MS and 2D-TLC.)
2. Rat, mouse, dog and human liver microsomal preparations. (Evaluated by HPLC, LC/MS, LC/MS/MS.)
3. Human lung slices; rat, mouse, dog and human lung microsomal preparations. (The techniques used for the evaluation were not defined.)

4. Rat, mouse and dog everted intestines.(Evaluated by HPLC.)
5. Rat and mouse freshly isolated hepatocytes and commercially available rat and dog primary cultured hepatocytes. (Evaluated by HPLC.)

After the incubations, drug-derived radioactivity was analyzed using HPLC, HPLC/MS, HPLC/MS/MS, NMR or two dimensional thin layer chromatographic (2D-TLC) techniques.

Results: Following in vitro incubations of SCH 32088 at a concentration of 0.3 µg/g liver, rat, mouse, dog and human liver slices produced highly polar, polar, moderately polar, mometasone-like polarity and non-polar metabolites across all species. (See table below.) Non-polar products were mainly degradation products, including a 9,11-epoxide, a spirodihydrofuranone and a spirodihydrofuranone-9,11-epoxide. The 9,11-epoxide has been tested previously in an in vitro chromosomal aberration study using CHO cells.

In vitro Metabolism of SCH 32088 at 0.3µg/g liver

Sources of Liver Slices[a,b,c]	MF Concentration (µg/g tissues)	% Applied Radioactivity					
		Very Polar	Polar	Moderated Polarity[a]	MF*-like Polarity	No-polar Decomposition Compounds	-Total
Mouse	0.30	5	7	18	22	47	99
Rat	0.30	5	67	1	28	0	101
Dog	0.30	26	29	4	39	1	99
Human	0.30	21	16	25	26	10	97

a: 6β-Hydroxy-SCH 32088 was the only in vitro metabolite obtained from incubations of the unlabeled SCH 32088 (0.1 µmol to 1.0 µmol) with liver microsomes of mouse, rat, dog and human. This metabolite was found to be identical to SCH 47165 by physical-chemical analysis (HPLC retention time, NMR and mass spectra).

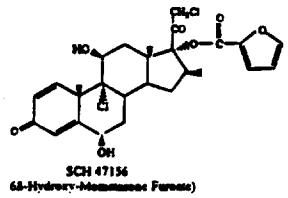
b: Moderately polar metabolites featuring the 6β-Hydroxy-SCH 32088 were found to be the dominant metabolite patterns obtained from in vitro incubations of high drug concentrations (13.3 µg SCH 32088/g liver to 133 µg SCH 32088/g liver) with sliced liver of either mouse, rat, dog or human.

c. 2D-TLC metabolite profiles from liver slice incubations were qualitatively similar across species.

* MF: Mometasone Furoate.

When liver slices were incubated with SCH 32088 at different concentrations, it was noted that in vitro metabolism of SCH 32088 was concentration dependent; hepatic tissues produced large amounts of moderately polar metabolites across all species. The major moderately polar metabolite was 6β-hydroxy-SCH 32088 (SCH 47156; see the structure below). After rat hepatic slices were treated with SCH 32088 at the concentrations of 13.3 and 133 µg/g, liver slices yielded exclusively SCH 47156. (See table below.)

Table 2. Radioactive Profile of Metabolites at High Drug to Liver Tissues Ratio in Rat, Mouse, Dog and Human.



SCH 47156
6β-Hydroxy-Mometasone Furoate)

Species	µg SCH 32088/g liver	Major Metabolite(s)
Rat	13.3 (Pre-Hydrolysis) 133 (Pre-Hydrolysis)	SCH 47156[a] SCH 47156[a]
Mouse	6.7 (Pre & Post Hydrolysis) 13.3 (Pre & Post Hydrolysis) 40, 80, 120 (Pre & Post Hydrolysis) 133	SCH 47156 Including Polar & Moderately Polar metabolites SCH 47156 (Higher Level) Including Polar & Moderately Polar metabolites Moderately Polar metabolites[b] Metabolism Inhibited
Dog	26.6, 39.9 & 79.8 (Pre-Hydrolysis)	SCH 47156[c] & Moderately Polar metabolites
Human	6.7 (Pre & Post Hydrolysis) 13.3 (Pre & Post Hydrolysis) 80, 100, 120, 133	SCH 47156[d] & Moderately Polar metabolites SCH 47156[d] & Moderately Polar metabolites Metabolism Inhibited

a: In the rat the presence of SCH 47156 was supported by both HPLC (co-elution with reference standard) and by mass spectral analyses. In all other species the presence of SCH 47156 was detected solely by HPLC.
b: Scaling up the drug to tissue ratios to 40 µg/g, 80 µg/g and 120 µg/g liver promoted the formation of higher amounts of moderately polar metabolites.
c: The presence of a radioactive metabolite, which co-eluted with SCH 47156 reference standard, was detected in all incubations with dog liver slices at high drug to tissue ratios.
d: Additional SCH 47156 is released following β-glucuronidase/sulfatase hydrolysis.

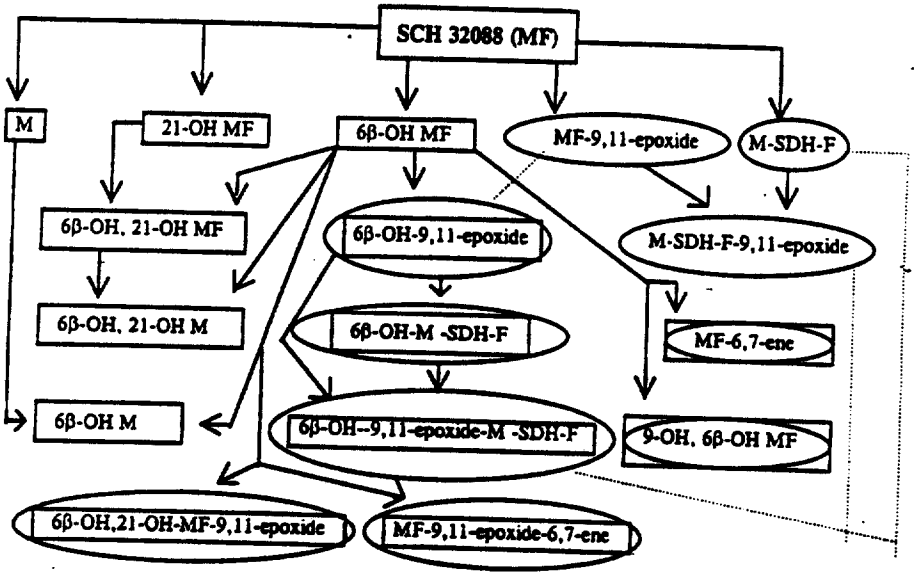
Following incubation, rat, mouse, dog and human liver microsomes produced exculsively SCH 47156 across all species. SCH 47156 was the major metabolite following in vitro incubation of SCH 32088 with rat, mouse and dog everted intestines. Extensive in vitro metabolisms of SCH 32088 were also noted in isolated rat, mouse and dog hepatocytes. However, no metabolism was observed when SCH 32088 was incubated with lung microsomes or human lung slices. (See table below.) The lung perfusion study was not provided in this submission.

Table 3. *In Vitro* Metabolism of SCH 32088 - A Consolidated Table.

Species	Liver Slices (High Conc.)	Liver Slices (Physiol. Conc.)	Liver Microsomes	Hepatocytes[c]	Intestinal Tract	Lung Perfusion	Lung Microsomes	Lung Slices
Rat	SCH 47165[a]	Extensive metabolism	SCH 47165[b]	Extensive metabolism	SCH 47165	SCH 47165	No Metabolism Observed	Not Tested
Mouse	Moderate polarity & SCH 47165	Extensive metabolism	SCH 47165[b]	Extensive metabolism	SCH 47165	Not Tested	No Metabolism Observed	Not Tested
Dog	Moderate polarity & SCH 47165	Extensive metabolism	SCH 47165[b]	Extensive metabolism	SCH 47165	Not Tested	No Metabolism Observed	Not Tested
Human	Moderate polarity & SCH 47165	Extensive metabolism	SCH 47165[b]	Not Tested	Not Tested[d]	Not Tested	No Metabolism Observed	Tissue Non-Reactive

a: SCH 47165 is 6β-hydroxy-SCH 32088.
b: The chemical structure of this metabolite, including its absolute configuration at C(6), was confirmed by both NMR and mass spectral techniques and by direct LC/NMR analyses.
c: *In vitro* drug metabolism studies revealed qualitative differences between freshly isolated hepatocytes (rat and mouse) prepared on-site and the commercially available primary cultured hepatocytes (rat and dog, Cedra Corp.). The overall clearance of SCH 32088 to metabolites was significantly greater in the freshly isolated hepatocytes prepared on-site. The ineffectiveness of Cedra's primary cultured hepatocytes in SCH 32088 metabolism is probably due to a decrease in cytochrome P-450 activity with time.
d: No access to human everted intestines.

Since the 2D-TLC may provide a better separation of SCH 32088 metabolites than the HPLC assay, 2D-TLC was used for the analysis. The results showed that polar and non-polar radioactivity images exhibited similar patterns across all species. This suggests that the metabolic profiles of SCH 32088 were qualitatively similar across all species. Based on the results of 2D-TLC, in vitro metabolism and degradation pathway of SCH 32088 in rat hepatocytes is proposed as the follwing:



In conclusion. SCH 32088 was metabolized extensively in the liver and minimally in the lung; metabolic profiles in all species studied were qualitatively similar but quantitatively different. 6β-hydroxy-SCH 32088 (SCH 47156) was one of the major metabolites following the incubations of various tissue conditions. Since the in vitro and in vivo metabolisms of SCH 32088 were not compared in this study, metabolic profile of SCH 47156 in vivo for the humans treated by intranasal administration of SCH 32088 cannot be provided from this study. Lung tissue metabolized the compound only in the pulmonary perfusion study. The study was requested from the sponsor on August 19, 1997 to allow for full evaluation of this data.

Tao Tom Du, Ph.D.
Pharmacologist/Toxicologist

DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original, Review No. 1

NDA Number: 20-762

Serial Number: 001

Date of Submission: October 3, 1996

Information to be Conveyed to Sponsor: Yes (x), No ( )

Reviewer: T. Tom Du, Ph.D.

Date Review Completed: August 7, 1997

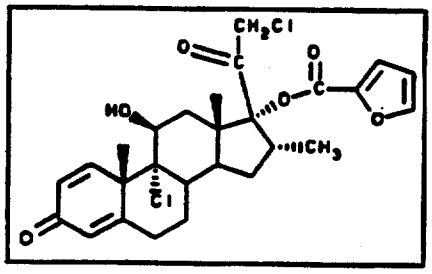
Sponsor: Schering Corporation
2000 Galloping Hill Road,
Kenilworth, NJ 07033

Drug Name: NASONEX™ Nasal Spray
(SCH 32088; Mometasone furoate monohydrate)

Chemical Name: 9,21-Dichloro-11b,17-dihydroxy-1ba-methylpregna-1,4-diene-3,20-dione 17-(2-fluroate) mometasone

CAS Number: 83919-23-7

Molecular Formula and Structure: $C_{27}H_{30}Cl_2O_6H_2O$



Molecular Weight and Formula: 539.458

Related INDs/NDAs/DMFs (if applicable):

NDA 19-543 Elocon (Mometasone Furoate) Ointment (Schering, approved 04/30/87)*
NDA 19-625 Elocon (Mometasone Furoate) Emulsion, Cream (Schering approved 05/06/87)*
NDA 19-796 Elocon (Mometasone Furoate) Lotion (Schering, approved 03/30/89)*

*Note: These products are for dermal topical application.

Class: Anti-inflammatory steroid

Indication: Prophylaxis and treatment of seasonal allergic rhinitis/Perennial rhinitis

Clinical Formulation: Mometasone furoate monohydrate is clinically used as a nasal spray. The ingredients in the drug product are listed in the following table. All inactive ingredients are used in various approved nasal or inhalation drug products in similar quantities (Inactive Ingredient Guide, Jan. 1996).

Ingredient	mg/g in drug products
Mometasone Furoate Monohydrate Micronized (Inhalation Grade) Microcrystalline Cellulose and Carboxymethylcellulose Sodium NF 65 cps Glycerin USP Citric Acid USP Monohydrate Sodium Citrate USP Dihydrate Polysorbate 80 NF Benzalkonium Chloride Solution NF (17%, without alcohol) Phenylethyl Alcohol USP Purified Water USP qs ad	a

[a]Equivalent to 0.515 mg/g of Mometasone Furoate Anhydrous. A 3% manufacturing overcharge is included for Mometasone Furoate Monohydrate.
[b]Equivalent to 0.204 mg/g of Benzalkonium Chloride. A 2% manufacturing overcharge is included for Benzalkonium Chloride.

Route of Administration: Intranasal

Proposed Clinical Protocol: For adults and adolescents 12 years of age or older, the usual

recommended dose for prophylaxis and treatment is 2 sprays (50 μg/per spray) in each nostril once daily (200 μg/day). The daily dose is equivalent to 4 μg/kg on the basis of body weight (50 kg) or 125 $\mu g/m^2$ on the basis of body surface area.

Studies Reviewed in this NDA:

PHARMACOLOGY

1. Effects on pulmonary inflammation and cytokines in an allergic mice (D-27191)
2. Effects on pulmonary inflammation in guinea pigs (D-24250)
3. Effects on cytokine production (Vol. 19)
4. Effects on leukotriene production (D-27220)
5. Anti-inflammation activity of oral dosed SCH 32088 (D-23879 and D-26685)
6. Topical anti-inflammatory activity of SCH 32088 (P-4809)
7. Effect of topically used SCH 32088 (P-4809)
8. Progestational and androgenic activities of SCH 32088 (D-17420)
9. Effect on endocrine profiles (D-27252)
10. Effects on electrolyte levels and hepatic glycogen deposition (D24422)
11. Safety pharmacology studies reviewed previously

TOXICOLOGY

1. Acute inhalation toxicology studies (D-22795, D-22742 & P-5948)
2. Acute oral (PO) and subcutaneous (SC) toxicology study (P-4865)
3. Three-day nasal screening study in dogs (D-22324)
4. One-week nasal irritation study in dogs (P-5995)
5. One-month nasal irritation study in dogs (P-5336)
6. One-month nasal irritation study in dogs (P-5474)
7. Six-month intranasal toxicity study in rats (P-6117)
8. Six-month intranasal toxicity study in dogs (P-6118)
9. One-year intranasal toxicity study in dogs (P-6116)
10. A 26-week oral inhalation toxicity study in dogs (P-5991)
11. A 26-week oral inhalation toxicity study in rats (P-5598)
12. Three-month inhalation study in beagle dogs (D-22796)
13. Three-month inhalation study in rats (D-22797)
14. Three-month inhalation study in rats (P-5736)
15. Two week inhalation study in beagle dogs (D-22607)
16. Two week inhalation study in rats (D-22680)
17. Other inhalation studies
18. Subchronic oral toxicity studies

19. One-month nose-only inhalation study in pediatric rats (P-5980)
20. A 7-week oral inhalation study in pediatric dogs (P-5981)
21. Other studies using pediatric animals

REPRODUCTIVE TOXICOLOGY

1. A pilot oral teratology (Segment II) study in rats (D-26738)
2. Oral teratology (Segment II) study in rabbits (P-5991)
3. Subcutaneous teratology (Segment II) study in rats (P-5543)
4. Single dose pharmacokinetic studies in pregnant female rats (P-6084)
5. Multiple dose pharmacokinetic study in female rats (P-6105)
6. Subcutaneous teratology (Segment II) study in mice (P-5578)
7. Dermal teratogenicity study (Segment II) in rats (P-5054)
8. Dermal teratogenicity study (Segment II) in rabbits (P-5066)
9. Subcutaneous fertility and general reproduction study (Segment I) in rats (P-5174)
10. Perinatal and postnatal reproduction study (Segment III) in rats (P-5164)

GENETIC TOXICOLOGY

1. Ames tests (P-4988 and P-5969)
2. In vitro L5178Y $TK^{+/-}$ -, $TK^{-/-}$ mouse lymphoma cell assay (P-5011)
3. In vivo mouse bone marrow micronucleus assay (P-5050)
4. In vivo hepatocyte UDS assay (P-6017)
5. Chromosomal aberration in CHO cells (D-20741)
6. Chromosomal aberration test in cultured CHL cells (D-23296)
7. In vivo chromosome aberration in rat bone marrow cells (D-23508)
8. Chromosomal aberration assay in CHO cells cultured with SCH 32088 and its degradation product (D-23579)
9. Chromosome aberration in mouse spermatogonial cells (D-23580)
10. Single dose toxicokinetic study in mice (P-5486)

PHARMACOKINETIC STUDIES

1. Single dose oral bioavailability study in male mice (P-6111)
2. Single intranasal dose study in rats (P-5352)
3. Single oral and intravenous dose studies (P-5941 and P-6368)
4. Disposition studies in rat and dog following a single IV or PO dose (P-5313)

5. One-month nose-only inhalation study in rats (P-6137)
6. One-month nose-only inhalation pharmacokinetic study in mice (P-6122)
7. A 28-day oral inhalation pharmacokinetic study in dogs (P-6096)
8. Three-month nose-only inhalational studies in 2 species (P-5836 & P-5837)
9. Three-month oral studies in 3 species (P-6104, P-6138, P-6007)
10. Tissue distribution studies in rats (D-24338, D-24339 and P-5367)
11. A 21-day tissue distribution and excretion study in rats (P-5976)
12. Distribution and excretion study rat (P-6000)
13. Excretion study in rat milk following a single dose administration (P-6010)
14. Biliary excretion and enterohepatic circulation in rats (P-6009)
15. In vitro protein binding in rat, mouse, rabbit, dog and human plasma (P-6004)
16. In vitro metabolism in pulmonary and hepatic tissues (P-5642)
17. Other studies

CARCINOGENICITY STUDIES

1. Two-year nose-only inhalation carcinogenicity study in rats (P-6005)
2. Two-year nose-only inhalation carcinogenicity study in mice (P-6006)

APPEARS THIS WAY

REVIEW OF STUDIES

PHARMACOLOGY

Inhalation Studies

1. Effects on pulmonary inflammation and cytokine levels in an allergic mouse model (D-27191, Vol. 19)

Ovalbumin (OA) sensitized and challenged male B6D2F1/J mice were used in these studies. Pretreatment with inhaled SCH 32088 at 13 -33 μg/kg significantly decreased eosinophil numbers in bronchoalveolar lavage (BAL) fluid, and also reduced the density of eosinophils in the peribronchial and peribronchiolar regions of the lung tissues. Pretreatment of SCH 32088 also decreased the concentrations of $Thy1^+$ T cells* and T-helper (Th) cells in the BAL fluid.

In comparison to the untreated control mice, SCH 32088 at 33 mg/kg significantly reduced the percentage of Th cells expressing CD44 which is a cell surface molecule on activated/memory cells. For the untreated control mice, OA challenge increased levels of steady-state mRNA for IL-4, IL-5 and IFN-γ in the lung tissues. However, pretreatment with SCH 32088 at 3 - 33 μg/kg reduced mRNA levels of all three cytokines.

In conclusion, SCH 32088 has potent anti-inflammatory activity in allergen-induced pulmonary inflammation in mice.

* Thy1 is a glycoprotein found on the membrane of T cells as well as other cells.

2. Effects on pulmonary inflammation in guinea pigs (D-24250, Vol. 19)

In sensitized and allergen challenged male guinea pigs, pretreatment with aerosolized SCH 32088 (5611 μg/kg/day) or SCH 18020 (beclomethasone diproprionate, 1184 μg/kg/day) reduced eosinophil numbers in BAL fluid. SCH 18020 at 3457 μg/kg/day and SCH 32088 at 5611 μg/kg/day inhibited eosinophil infiltration in the airways and alveoli.

In vitro studies

3. Effects on cytokine production (Vol. 19)

By using anti-CD3 and CD28 antibody stimulated human $CD4^+$ T-cells, effects of SCH 32088 on the cytokine production were compared with betamethasone (BETA), fluticasone propionate

(FP), budesonide (BU), triamcinolone acetonide (TA) and beclomethasone dipropionate (BDP).

SCH 32088 inhibited the production of both IL-4 and IL-5 ($IC_{50} = 0.27 \pm 0.15$ nM). The potency of SCH 32088 on the IL-4 or IL-5 release was much greater than other glucocorticoids used in this study. However, SCH 32088 and other glucocorticoids had similar potency on the inhibition of IFN-γ production. (This study was published in J Allergy Clin Immunol. 97, 288, 1996).

SCH 10745 (betamethasone valerate), SCH 11460 (betamethasone diproprionate), SCH 32088 and BDP were also tested for their effects on IL-5 production in mouse D10.G4.1 cells (D-25067). The results showed that SCH 32088 was the most potent inhibitor for IL-5 production among all 4 steroids. (See table below.)

Inhibition of IL-5 production: IC_{50} (nM)	
SCH 32088	0.12+0.11
Betamethasone Diproprionate	4.2+2.8
Betamethasone Valerate	0.75+0.25
Beclomethasone Diproprionate	1.5+0.87

Inhibitory effects of SCH 32088 on IL-1, IL-6 and TNF-α productions were also compared with BETA, hydrocortisone, dexamethasone, and beclomethasone by using murine WEHI-265.1 cells (P-5558). It was demonstrated that SCH 32088 was the most potent corticosteroid for the inhibition of IL-1 ($IC_{50} = 0.1$ nM), IL-6 ($IC_{50} = 0.15$ nM) and TNF-α ($IC_{50} = 0.25$ nM) production. The potencies of other steroidal drugs for the inhibition of IL-1, IL-6 and T.F.-α production are summarized in the following table.

Inhibition of IL-1, IL-6 and TNF-α productions by other steroidal drugs: IC_{50} (nM)			
Drug Name	IL-1	IL-6	T.F.-α
Betamethasone Alcohol	1.9	–	–
Betamethasone Diproprionate	1.2	7	250
Betamethasone Phosphate	>100	300	2000
Betamethasone Valerate	0.82	2	4
Beclomethasone Diproprionate	–	1.8	2000
Dexamethasone Alcohol	4	40	250
Hydrocortisone	100	290	>10000

4. Effects on leukotriene production (D-27220, Vol. 19)

In this study, peripheral leukocytes from atopic patients were pretreated for 18 hr with either SCH 32088 or beclomethasone dipropionate (BDP). After the cells were stimulated by allergen, anti-IgE, IL-3, calcium ionophore or media, production of total leukotrienes (LTs) was quantified by using an ELISA.

The study displayed that both SCH 32088 and BDP were able to reduce allergen- or calcium ionophore-induced LTs production. However, anti-IgE-induced LTs production was more effectively inhibited by SCH 32088 ($IC_{50} \leq 0.01$ nM) than BDP ($IC_{50} = 6$ nM), suggesting that SCH 32088 may more effectively attenuate leukotriene secretion during the allergic responses.

Other Studies

5. Anti-inflammation activity of oral dosed SCH 32088 (D-23879 and D-26685, Vol. 19)

In the following 2 studies, anti-inflammation activity of orally dosed SCH 32088 was determined by the efficacy of treating Reverse-Passive-Arthus-Reaction (RPAR).

1. In the first study (D-23879), efficacy of SCH 32088 (25 mg/kg) was compared with betamethasone (BM: 0.5 mg/kg), corticosterone (CS: 25 mg/kg) and hydrocortisone (HC: 25 mg/kg). The drugs were given orally at 30 min before RPAR was induced in rat pleural cavities by using bovine serum albumin (BSA) and rabbit anti-BSA. Neutrophil numbers in pleural cavities were counted to determine the activity of the drugs. Results showed that the rank order of therapeutic activity was presented as BM > CS > HC > SCH 32088. Therefore, anti-inflammation activity of orally dosed SCH 32088 was lower than other steroids used in this study.

2. In the second study (D-26685), rats were pretreated orally with SCH 32088 (7.5 to 60 mg/kg), fluticasone propionate (FP: 3.75 to 30 mg/kg), budesonide (BU: 0.5 to 4 mg/kg) and triamcinolone acetonide (TA: 0.125 to 1 mg/kg) at 30 min before RPAR was induced in rat pleural cavities. Inhibition of neutrophil infiltration and edema in pleural cavities were applied to determine the efficacy of the drugs. (See table below). This study demonstrated the oral anti-inflammatory potencies of SCH 32088 and FP was statistically similar, but they were less potent than BU and TA.

Compound	ED_{50}	
	Edema	Neutrophils
TA	0.5 (0.3-0.7)	0.3 (0.2-0.5)
BU	2 (1.4-3.1)	1.4 (0.8-2.8)
FP	33 (24-54)	20 (14-32)
SCH 32088	38 (29-55)	28 (12-31)

In conclusion, systemic anti-inflammatory activity of SCH 32088 was lower than most steroids used in the above studies.

6. Topical anti-inflammatory activity of SCH 32088 (P-4809, Vol. 19)

Therapeutic efficacy of topically used SCH 32088 or betamethasone valerate (BV) was evaluated in several experiments. Results are presented as the following:

1. SCH 32088 and BV had equal potency in the inhibition of croton-oil-produced-acute inflammation on mouse ears (ED_{50} = 0.02μg/ear).

2. For croton oil induced subchronic inflammation on mouse ears, SCH 32088 (ED_{50} = 0.002 μg/ear/day) was 7.7 times as potent as BV (ED_{50} = 0.014 μg/ear/day).

3. For M. Ovalis-induced epidermal acanthosis on the ears of guinea pigs, a 14-day treatment with SCH 32088 had similar potency to BV.

Based on the above studies, efficacy of topically used SCH 32088 was greater than BV for the inhibition of croton oil induced acute and subchronic dermal inflammation. However, SCH 32088 had similar potency to BV for the treatment of M. Ovalis-induced acanthosis.

Safety Pharmacology studies

7. Effect of topically used SCH 32088 (P-4809, Vol. 19)

SCH 32088 and betamethasone valerate (BV) produced side-effects were evaluated in mice by measuring hypothalamic-pituitary-adrenal (HPA) axis suppression, thymolysis and skin atrophy.

1. To evaluate the effect of SCH 32088 on the HPA axis, adrenal response to ether stress was measured by determining plasma corticosterone levels after topical application of SCH 32088 on mouse ears. It was found that a 5-daily application of SCH 32088 (ED_{50} = 5.3 μg/ear/day) was less potent than BV (ED_{50} = 3.1 μg/ear/day) in the suppression of the HPA axis. However, this potency difference was not statistically significant.

2. Thymolysis is a systemic effect of corticosteroids. After mice were treated topically for 5 days, thymus weights were used to determine the potency of SCH 32088 or BV. The results showed that SCH 32088 (ED_{50} = 26.6 μg/ear/day) was 2.2 times as potent as BV (ED_{50} = 51.6 μg/ear/day). However, SCH 32088 (ED_{50} = 11.2 μg/mouse) was 5.6 times more potent than BV (ED_{50} = 59.8 μg/mouse) after both drugs were dosed subcutaneously for 5 days. It is suggested that systemically administered SCH 32088 was more potent than topically used SCH 32088 for the induction of thymolysis.

3. Skin atrophy is a common side-effect caused by chronically used topical corticosteroids. In two individual experiments, mice were treated topically with SCH 32088 and BV, and then sacrificed. Skin tissues were prepared for observation. In the first experiment, SCH 32088 was 8.7 times more potent than BV. In the second experiment, SCH 32088 was approximately 3 times more potent than BV. In conclusion, SCH 32088 was more potent in causing skin atrophy.

Therefore, in comparison with BV, SCH 32088 had less potency for suppressing the HPA axis, but had greater potency for the induction of thymolysis and skin atrophy.

8. Progestational and androgenic activities of SCH 32088 (D-17420, Vol. 19)

Progestational and androgenic activities of SCH 32088 were evaluated in estrogen-primed immature New Zealand white female rabbits and immature male CD rats, respectively.

Progestational activity: After the rabbits were injected subcutaneously with 5 μg of β-estradiol for 5 days, animals were treated subcutaneously for 5 days with various dose levels of progesterone, clobetasol propionate, betamethasone valerate and SCH 32088. Twenty-four hours after the last dose, the uterus was removed and prepared for histological evaluation. The degree of progestational activity was measured by the scoring of endometrial proliferation. The study demonstrated that the progestational potency of SCH 32088 is 20.5 times higher than progesterone. Clobetasol propionate (12 times) and betamethasone valerate (5.3 times) were also more potent than progesterone.

Androgenic activity: Twenty-four hours after immature male rats received 7 daily subcutaneous doses of testosterone (0.05 to 5.0 mg/kg), progesterone (0.2 to 25 mg/kg) and SCH 32088 (0.2 to 25 mg/kg), animals were sacrificed. Seminal vesicles and ventral prostates were removed and

weighed. This study demonstrated SCH 32088 did not increase the weight of the male accessory sexual organs.

In conclusion, SCH 32088 has progestational activity in rabbit, but has no androgenic effects in male rats.

9. Effect on endocrine profiles (D-27252, Vol. 20)

In a group of studies, SCH 32088 was compared with Fluticasone Propionate (FP), Triamcinolone Acetonide (TA) and Budesonide (BU) for androgenic, antiandrogenic, estrogenic, antiestrogenic activities in rats. All drugs were also evaluated for their effects on sexual maturation of newborn female rats. All drugs were administered orally at 56 and 280 mg/kg. which were 2-fold and 10-fold their oral anti-inflammatory ED_{50} to the reverse passive Arthus reaction, respectively.

The results are presented in the following:

1) SCH 32088 and other drugs had no androgenic activity.

2) SCH 32088 did not display any antiandrogenic effect on the prostate and seminal vesicles. BU at the highest dose had some effects on the inhibition of prostate growth, but had no effect on the seminal vesicles.

3) Neither SCH 32088 nor other drugs show estrogenic activity.

4) When estradiol benzoate treated immature rats were dosed with different corticoids, SCH 32088 (56 and 280 mg/kg) and FP had some antiuterotrophic activities, but TA and BU had less activity.

5) For immature female rats, the effect of SCH 32088 and other corticosteroids on sexual maturation was determined by the time of vaginal opening. It showed that all steroids significantly delayed vaginal opening and all steroid-treated animals failed to gain weight compared to placebo-treated controls. (Vaginal opening is commonly used to define sexual maturation.)

The results of the above studies suggested that SCH 32088 had significant effects on female sexual maturation, and had some antiuterotrophic activity. However, SCH 32088 had no androgenic, antiandrogenic and estrogenic activity.

10. Effects on electrolyte levels and hepatic glycogen deposition (D24422, Vol. 20)

To compare the effects of SCH 32088, SCH 2509 (Hydrocortisone) and SCH 7302 (Triamcinolone) on electrolyte levels and hepatic glycogen deposition, adrenalectomized male rats were treated with various single subcutaneous or topical doses. (See table below.) Blood samples were collected for electrolyte measurements. Histology and microscopic examination were performed to determine hepatic glycogen deposition.

Test Articles	Subcutaneous Doses (mg/kg)	Topical Doses (gm)
SCH 2509	0.6, 6 and 60	0.1, 0.25 and 0.5 (1% Hytone TH)
SCH 7302	0.06, 0.6 and 6	0.1, 0.25 and 0.5 (0.1% Kenalog TH)
SCH 32088	0.06, 0.6 and 6	0.1, 0.25 and 0.5 (0.1% Elocon TH)

The results showed that serum electrolyte levels were not changed by the treatment of the test articles. After subcutaneous doses, only two SCH 2509-treated rats (at dose levels of 6 and 60 mg/kg, respectively) had minimal glycogen deposition, while minimal or mild hepatic glycogen deposition was found in two SCH 7302-treated rat groups (at dose levels of 6 and 60 mg/kg). At 24 hr after topical treatment with SCH 32088, mild hepatic glycogen deposition was only observed in 1 rat in the 0.5 gm group, but not in other groups.

Based on the above results, SCH 32088 did not have mineralocorticoid activity or any apparent effect on liver glycogen deposition. SCH 2509 and SCH 7302 did not have mineralocorticoid activity, although they had minimal or mild hepatic glycogen deposition activity.

11. Safety pharmacology studies reviewed previously

1. Pharmacological effects on the nervous systems (D-25526, a published article: Yokuoshi, K, et al. J. Yonago medical association 40, 1989)

2. Pharmacological effects on the respiratory and cardiovascular systems (D-25527, a published article: Sakonjo H, et al. J. Yonago medical association 40, 1989)

3. Effect of orally administered mometasone furoate and other steroids on circulating lymphocytes in guinea pigs. (P-5472, Vol. 19)

The above studies are briefly summarized for this submission.

In 2 peer-reviewed articles (D25526 and D-25527), general pharmacological effects of SCH 32088 on the central nervous, cardiovascular, and respiratory systems were reported. In vivo and in vitro measurements were performed using mice, rabbits, guinea pigs, cats, or dogs. Central nervous and autonomic nervous systems were not affected by subcutaneous dose of SCH 32088 at 100, 200, 500 or 1000 mg/kg. SCH 32088 did not affect either biliary secretion or gastric acid and pepsin secretion. After either an intravenous dose at 10 mg/kg or a subcutaneous dose at 200 mg/kg, there were no treatment-related effects on the respiratory and cardiovascular system (blood pressure, EKG and heart rate). However, as shown in the following table, subcutaneous injections of SCH 32088 increased urine volume, creatinine release, accumulation of hepatic glycogen, and decreased levels of ICG (an indicator for hepatic function).

Subcutaneous Dose of SCH 32088 (mg/kg)	Species			
	Rats		Rabbits	Mice
	Urine Volume*	Creatinine*	ICG*	Hepatic Glycogen*
200	40%↑	11%↑	58%↓	54%↑
500	48%↑	**	75%↓	137%↑

* Percentage changes from the control values.
**There was no increase.

Results from another study (P-5472) showed that oral administration of SCH 32088 at 13.3 to 150 mg/kg reduced circulating lymphocyte numbers by 13 to 25% in guinea pigs. However, SCH 32088-produced lymphocyte depression was neither statistically significant nor dose-related. In comparison with other drugs, SCH 32088 caused less immunosuppression than beclomethasone dipropionate and betamethasone.

TOXICOLOGY

Single Dose Toxicity Studies

1. Acute inhalation toxicology studies (D-22795, D-22742 & P-5948; Vol. 23)

Test Lab: 1) D-22795
2) D-22742
3) P-5948:

Acute inhalation toxicities of SCH 32088 were evaluated in mic, rats and dogs. Study designs and mortality of the testing animals are summarized in the following table:

	n/sex	Batch #	Concentration	Exposure time (min)	Observation time	Mortality
Mouse	5	87-MMF-X-2	3.16 mg/L	240	5-week	1♂/1♀
Rat	5	87-MMF-X-2	3.31 mg/L	240	5-week	0
Rat	5	Unknown	5 mg/L	30	3-week	0
Dog	2	93-MMF-DDPX-01	♂: 139.5µg/L ♀:121.5µg/L	60	2-weeks	0

Decreased bodyweight was observed in rodents. Food consumption was only slightly reduced in dogs. After the sacrifice, small spleens were found in rats; discoloration of lungs, livers, kidneys and skins were seen in both rodent species.

2. Acute oral (PO) and subcutaneous (SC) toxicology study (P-4865, 12/82; Vol. 23)

Test Lab: Schering Corporation (GLP: no)

To determine the acute toxicity, rats (10/sex/group) and mice (15/sex/group) were treated orally or subcutaneously with SCH 32088 (Batch #: 14623-034) at 0 (vehicle), 20, 200 or 2000 mg/kg. After treatment, animals were observed for 35 days and then necropsied.

For rats dosed subcutaneously, deaths were found in 3/10 males and 5/10 females in the 2000 mg/kg group. Clinical signs were mainly related to local irritation at the site of injection (hair loss, swelling and scab formation, and/or necrosis). At the end of the study, dose-related reductions in body weight gains appeared in all groups. No abnormal necropsy findings were seen in the 20 and 200 mg/kg groups. However, visceral adhesions were noted in 1/10 female and 2/10 female rats given 2000 mg/kg.

After the mice were injected subcutaneously, deaths were seen in 14 rats/sex of the 2000 mg/kg group. A dose-related decrease in body weight gains appeared in the 20 and 200 mg/kg groups.

Major necropsy findings in the 2000 mg/kg groups were intestinal flatulence/distension of the stomach with food (4 ♂ and 2♀) and white raised areas on the kidneys (4 ♂ and 1♀) or enlarged kidneys (2 ♂ and 1♀). Enlarged kidneys were also found in the 200 mg/kg rats (1 ♂ and ♀). There were no abnormal necropsy findings in the 20 mg/kg group.

Following oral administration, death, abnormal clinical signs and necropsy lesions were not observed in either species throughout the observation period. Results from all animal groups are summarized in the following table:

Species	Route	LD50 (mg/kg)	Non-lethal dose (mg/kg)	No-effect dose (mg/kg)
Rats	PO	>2000	>2000	>2000
Mice	PO	>2000	>2000	>2000
Rats	SC	♂>2000 ♀= 2000	200	<20
Mice	SC	♂>200 ♀>200	20	<20

Multiple Dose Intranasal Toxicity Studies:

3. Three-day nasal screening study in dogs (D-22324; 3/88; Vol. 24)

Test Lab: Schering Company, Lafeyette, NJ (GLP: No; Study #: 88025)
Animal: 4 groups of Beagle dogs (2/sex/group; Mean bodyweight: ♂ = 10.7 kg; ♀ = 8.2.kg)
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml

Three batches of SCH 32088 nasal suspension were given intranasally to the dogs for 3 days. Each dog was examined daily for the changes during the treatment.

After the treatment, no drug-related changes were observed in a nasal examination. The only reaction in the BA 20709-121 group (1/3 dogs) was mild sneezing on Day 2 and moderate sneezing on Day 3. There were no significant treatment-related changes in the body weight, food consumption, clinical pathology parameters, macroscopic and microscopic findings. (See table below.)

Group	Daily dose (mg/day)*	Daily Dose (♂/♀: mg/kg/day)#	Clinical signs
Saline	0	0/0	none
BA. 20709-116	4.3	0.4/0.52	none
BA. 20709-119	4.3	0.4/0.52	none
BA. 20709-121	4.2	0.39/0.51	mild and moderate sneezing

* one-half this amount per nostril; # calculated by using mean body weights.

4. One-week nasal irritation study in dogs (P-5995; 6/94-10/95; Vol. 24)

Test Lab: Schering Company, Lafayette, NJ (GLP: Yes; Study #: 93227)
Animal: 4 groups of Beagle dogs (3/sex/group) with mean bodyweights of 10.7 kg in males and 8.2 kg in females.
Formulation: SCH 32088 nasal suspension; Concentration = 1 mg/ml (Batch # MSMPX05)

Methods: 4 groups were treated once daily for 7 to 10 consecutive days with SCH 32088 at the following dose levels:

Group	Daily dose (mg/day)	Dose volume (ml)	Daily dose (♂/♀: mg/kg/day)*
Vehicle	0	2	0/0
Low-dose	0.5	0.5	0.047/0.061
Mid-dose	1	1	0.093/0.122
High-dose	2	2	0.187/0.244

* Calculated based on the mean bodyweight

Results: Mortality was not seen. There were no dose-related changes in clinical signs, bodyweight, food consumption, clinical pathology parameters and histopathology findings. Nasal irritation was also not noted in the veterinary examinations.

5. One-month nasal irritation study in dogs (P-5336; 7/88-3/89; Vol. 25)

Test Lab: Schering Company, Lafayette, NJ (GLP: Yes; Study #: 88011)
Animal: 3 groups of Beagle dogs (3/sex/group) with mean bodyweight of 11.1 kg in males and 9.1 kg in females.
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml (Batch # 22023-061)

Methods: Dogs were dosed intranasally for 28 days with SCH 32088 at the following dose levels:

Group	Daily dose (mg/day)	Dose volume (ml)*	Doses / day	Daily dose (♂/♀: mg/kg/day)#
Vehicle	0	2	4	0/0
Low-dose	2	2	2	0.18/0.22
High-dose	4	2	4	0.36/0.44

* 1 ml nostril
# Calculated based on the mean bodyweight.

Results:

Mortality (Daily): No death was noted in any group.

Clinical signs (Daily): No treatment-related clinical signs were noted in any group.

Nasal examination (twice daily): Daily nasal examinations did not reveal any nasal irritation.

Bodyweight (Weekly): No remarkable changes were observed.

Food consumption (Daily): No remarkable changes were observed.

Necropsy (Week 5): No gross lesions were observed.

Histopathology (Week 5): Focal polymorphonuclear leukocyte infiltrations were seen in the respiratory mucosa and submucosa of the control (♂: 1/3) and high-dose groups (♂: 2/3; ♀: 1/3). However, this morphological alteration was not observed in the low-dose group.

Based on the results of this study, the NOEL dose is 0.18 mg/kg/day for male dogs and 0.22 mg/kg/day for female dogs.

6. One-month nasal irritation study in dogs (P-5474; 2/90-9/90; Vol. 25)

Test Lab: Schering Company, Lafayette, NJ (GLP: Yes; Study #: 89085)
Animal: 3 groups of Beagle dogs (3/sex/group) with mean bodyweights of 9.4 kg in males and 7.9 kg in females.
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml (Batch # 23605-152)

Methods: 3 groups were treated for one month with SCH 32088 at the following dose levels:

Group	Daily dose (mg/day)	Dose volume (ml)	Daily dose (♂/♀: mg/kg/day)*
Vehicle	0	2	0/0
Low-dose	2	2	0.21/0.25
High-dose	4	2	0.43/0.51

* Calculated based on the mean bodyweight

Results:

Mortality (Daily): No death was noted in any group.

Clinical signs (Daily): No dose-related clinical signs were noted. Red-colored saliva was seen in all of high-dose females, but not in other groups.

Bodyweight (Weekly): No remarkable changes were observed.

Food consumption (Daily): No remarkable changes were seen.

Necropsy (Week 5): No gross lesions were reported.

Histopathology (Week 5): Focal neutrophilic inflammation was observed in the nasal cavity of the 1/3 control, 1/6 of the low-dose and 1/2 of the high-dose animals. In the lungs, chronic interstitial inflammation was seen in 1/3 of the control, 1/3 of the low-dose and all of the high-dose dogs. No other dose-related pathological alterations were observed.

Since the incidences of pathological change in the low-dose group were similar to the controls, the NOAEL dose was defined at 0.21 mg/kg/day for the males and at 0.25 mg/kg/day for the females.

7. Six-month intranasal toxicity in rats (P-6117; 9/94-2/96; Vol. 95)

Test Lab:
Animal: 6 groups of Sprague Dawley rats (25/sex/group; Mean bodyweight: ♂= 162g; ♀=153g)
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml (Batch # 33208-013)

Methods: Six rat groups were dosed intranasally for 6 mouths with SCH 32088 at 0 (non-dosed), 0 (vehicle), 0.017, 0.05, 0.15 and 0.6 mg/kg/day.

Results:

Mortality (Daily): One male in the vehicle group and one male in the 0.17 mg/kg group died on days 37 and 49, respectively. The causes of death were attributed to the dosing procedure. On Day 156, one 0.15mg/kg male died due to a metastatic yolk sac carcinoma. SCH 32088-related mortality was not found.

Clinical signs (Weekly): Dorsal alopecia was found in one 0.05 mg/kg male, one 0.15 mg/kg female, 5 males and 17 females in the 0.6 mg/kg groups. No other dose-related clinical signs were found.

Bodyweight (Weekly): Significant reductions in the bodyweight gain were found in the 0.15 mg/kg females between 21 to 25 weeks postdosing (11 to 14%↓). However, except in the 0.6

mg/kg group, no constant bodyweight reductions were noted in other groups. At the end of the study, both bodyweights (♂: 13%↓; ♀: 12%↓) and bodyweight gains (♂: 13%↓; ♀:26%↓) were statistically lower in the 0.6 mg/kg group when compared with the vehicle treated controls.

Food consumption (Daily): There was no significant decrease in food consumption.

Ophthalmoscopy (Before dosing and at week 26): No treatment-related effects were noted.

Hematology (Weeks 13 and 27): No treatment-related effect were noted.

Biochemistry (Weeks 13 and 27): Compared with the vehicle treated controls, serum cholesterol was statistically increased in the 0.15 (24-53%) and 0.6 mg/kg (16-31%) males, but not in the females. The values of triglyceride were slightly decreased in the 0.017 (15%), 0.15 (15%) and 0.6 mg/kg (20%) females, but not in the males. Serum corticosterone levels were highly variable, but were not changed with dose increases. No drug-related significant changes were seen in other parameters.

Urinalysis (Weeks 13 and 27): There was no treatment-related change in urine.

Pharmacokinetics (Day 1, 30 and 183): Blood samples were collected from 4 rats/sex/timepoint at 0.25, 0.5, 1, 2, 4, 8 and 24 hr on Days 1 and 30, and then collected from 5 rats/sex/timepoint at 0.25, 1 and 4 hr on Day 183. Plasma was analyzed by using HPLC-APCI-MS/MS (level of quantifiable value: LOQ = 50 ng/ml). There were no apparent gender-related differences in pharmacokinetic parameters. Except at a few sporadic time points, plasma SCH 32088 levels of 0.017 mg/kg group were generally below the LOQ of the assay. (See table below.)

Parameter	Day	Dose (mg/kg)			
		0.017	0.050	0.150	0.600
Cmax (pg/ml)	1	--[a]	59.5	127	413
	30	--[a]	115	236	645
	183	--[a]	34[c]	198[c]	460[c]
Tmax (hr)	1	--[a]	1.0	2.0	4.0
	30	--[a]	4.0	2.0	1.0
	183	--[a]	0.25	1.0	1.0
AUC(tf)[b] (pg/hr/ml)	1	--[a]	137	487	2471
	30	--[a]	322	772	2585
	183	--[a]	--[a]	--[a]	--[a]

a: Could not be determined due to insufficient data points.
b: tf was 4 hr for the 0.05 mg/kg dose group and 8 hr for the 0.15 and 0.60 mg/kg dose groups, respectively. AUC(0-24 hr) and AUC(I) could not be determined due to insufficient data points in the terminal phase.
c: Apparent Cmax based on 3 sample time-points.

Organ weights (Week 27): There were no drug-related changes.

Necropsy (Week 27): Except for alopecia, there were no other drug-related macroscopic changes.

Histopathology (Week 27): Skin hypotrichosis was observed in the 0.6 mg/kg group (♂: 6/25; ♀: 11/25). Dose-related morphological alterations were not seen in the nasal cavities. No dose-related morphological changes were reported in the liver, nasal cavity and other organs. Since only 1/25 high-dose males died due to a metastatic yolk sac carcinoma, this tumor finding may be not important.

Based on the results of this study, the NOAEL dose was 0.05 mg/kg/day for the rats. Since no major pathological alteration was found in the rats treated at 0.15 mg/kg/day, except alopecia, this dose can be considered a tolerated dose with mild glucocorticoid effects. A target organ of systemic toxicity was not identified in this study.

8. Six-month intranasal toxicity in dogs (P-6118; 10/94-4/96; Vol. 101)

Test Lab:
Animal: 6 groups of Beagle dogs (5/sex/group; Mean bodyweight: ♂=8.4kg; ♀=8.4kg)
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml (Batch # 33208-013)

Methods: Six groups were dosed intranasally for 6 mouths with SCH 32088 at 0 (untreated), 0 (vehicle), 0.0075, 0.015, 0.045 and 0.15 mg/kg/day.

Results:

Mortality (2 times/day): Mortality was not found in any group.

Clinical signs (2 times/day): Dose-related clinical signs were not noted.

Nasal irritation examination (Before dosing and at weeks 1, 9, 17 and 25): SCH 32088 did not cause any nasal irritation.

Bodyweight (Weekly) and *Food consumption (4 days/week*): The mean values of bodyweight and food intake were comparable between the control and treated groups.

Ophthalmoscopy (Before dosing and at Months 3 and 6): There were no dose-related changes.

Hematology (Before dosing and at weeks 4, 13 and 26): After the treatment, eosinophil counts in the 0.15 mg/kg group were lower (at Week 26, ♂: 55↓; ♀: 68%↓) than the vehicle-treated

controls. However, the total leukocyte and lymphocyte counts were comparable among the groups.

Biochemistry (Before dosing and at weeks 4, 13 and 26): Increased serum cholesterol levels were seen in the 0.15 mg/kg females (31 to 33%↑) at Week 13, but not at Week 26. Plasma cortisol levels in the 0.15 mg/day group were generally lower than the vehicle-treated controls. After animals were treated for 26 weeks, serum cortisol concentration in the 0.045 mg/kg group was also decreased. However, post-ACTH cortisol response value in the 0.045 mg/day group was similar to the control group. (See table below.) No drug-related significant changes were seen in other parameters.

Sex	Treatment (mg/day)	Serum Cortisol (µg/dL)					
		Week 4		Week 13 (µg/dL)		Week 26 (µg/dL)	
		Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH
Males	0 (Undosed)	1.09	15.75	0.64	13.97	0.44	13.44
	0 (Vehicle)	0.92	16.02	0.86	13.12	0.37	13.1
	0.0075	3.04	14.13	1.38	12.77	0.93	13.18
	0.015	1.69	13.34	0.59	6.25	0.55	12.42
	0.045	2.01	13.9	1.44	11.64	0.7	11.41
	0.15	1.03	10.91	0.46	8.86	0.73	9.03
Females	0 (Undosed)	2.09	17.25	1.09	14.28	0.62	11.54
	0 (Vehicle)	2.68	16.85	1.98	14.91	1.6	11.97
	0.0075	2.1	15.56	1.22	17.35	1.16	11.83
	0.015	1.83	14.49	1.88	14.74	1.42	10.84
	0.045	2.82	13.46	1.2	11.69	0.57	10.75
	0.15	1.97	7.68	0.71	7.18	0.12	0.92

Heart rates, blood pressure and EKG (Before dosing and at weeks 9, 17 and 25): There were no significant dose-related effects on the heart rate, blood pressure and EKG results.

Urinalysis (Before dosing and at weeks 3, 13 and 26): There were no treatment-related changes noted in the in urialysis.

Pharmacokinetics: On Days 1, 30 and 180, plasma samples were collected at 1, 2, 6 and 24 hr postdosing. By using a HPLC-APCI-MS/MS technique (LOQ = 50 ng/ml), plasma SCH 32088 level was only quantifiable in the 0.15 mg/kg group. Serum drug concentrations in the 0.0075, 0.015 and 0.045 mg/kg groups were below the LOQ of the assay. Based on the available data, gender differences in pharmacokinetic parameters were not found in the 0.15 mg/kg dogs.

Mean (%CV) Pharmacokinetic Parameters in Male and Female Beagle Dogs (Combined)				
Dose (mg/kg/day)	Exposure Day	Cmax (pg/ml)	Tmax (hr)	AUC(tf) (pg • hr/ml)
0.15	1	80.4 (85)	1.00	64 (125)
0.15	30	151 (45)	1.20	444 (112)
0.15	180	114 (60)	1.13	246 (98)
(%CV) = Coefficient of variation expressed as a percent (n=10, 5 males and 5 females)				

Organ weights (Week 27): Adrenal weights were decreased in the SCH 32088 treated groups (17% - 27%). Liver and lung weights were only slightly reduced. (See table below.)

Percentage change from the vehicle-treated controls

Treatment (mg/kg)	Males		Females	
	Adrenal	Lungs	Adrenal	Liver
0.0075	17%↓	–#	–	–
0.015	16%↓	–	–	9 %↓
0.045	12 %↓	%	22%↓	–
0.15	27%↓	9.5%↓	23%↓	7 %↓

– indicates that the change from the control value was either < 10 %. or none.

Necropsy (Week 27): There were no dose-related macroscopic findings.

Histopathology (Week 27): Morphological findings occurred sporadically and were distributed in all groups, including the controls. No dose-related pathologic alterations were observed in any organ.

Based on the results of this study, the NOEL dose was 0.015 mg/kg/day for the dog. Since post-ACTH cortisol response in the 0.045 mg/day group was similar to the control group, it can be considered a tolerated dose with mild glucocorticoid effects. Target dose toxicity was not clearly defined by this study.

9. One-year intranasal toxicity in dogs (P-6116; 7/94-7/96; Vol. 104)

Test Lab: Schering-Plough Research Institute, Lafayette, NJ (GLP: Yes; Study #: 93196)
Animal: 6 groups of Beagle dogs (5/sex/group; mean body weight: ♂ =10 kg; ♀ =8.4 kg)
Formulation: SCH 32088 nasal suspension; Concentration = 0.5 mg/ml (Batch # 33208-013)

Methods: Beagle dogs in each group were treated daily for 12 mouths by intranasal administration of SCH 32088 at the following dose levels:

Treatment of SCH 32088 (mg/day)	Estimated dose (mg/kg/day)* ♂ / ♀
0 (untreated)	0 / 0
0 (vehicle)	0 / 0
0.1	0.0075 / 0.0089
0.2	0.015 / 0.018
0.6	0.045 / 0.054
2.0	0.15 / 0.179

* Based on the mean bodyweight and 75% absorption.

Results:

Mortality (Daily): Mortality was not found in any group.

Clinical signs (Daily): During treatment, alopecia was mainly seen in the 2 mg/day group, but was also found incidentally in other groups. At the end of the study, occurrences of alopecia from the control to the high-dose (2 mg/day) groups were 0/5, 0/5, 1/5, 0/5, 1/5 and 3/5 in the males, and 0/5, 1/5, 0/5, 0/5, 0/5 and 3/5 in the females. No nasal irritation or other dose-related clinical signs were observed.

Bodyweight (Weekly): There were no dose-related bodyweight changes.

Food consumption (Daily): There were no significant decreases in food consumption.

Ophthalmoscopy (Before dosing and at Weeks 12, 24, 36 and 48): No treatment-related effects were noted.

Physical Examination(Before dosing and at weeks 7, 16, 23, 31, 40 and 47): SCH 32088 did not significantly affect the body temperature, respiratory rate, heart rate, blood pressure and EKG results.

Hematology (Before dosing and at Weeks 4, 14, 27 and 52): At the end of the study, total leukocyte counts in all treated males were lower than the vehicle-treated controls. Percentages of lymphocytes (Lym%) and eosinophils (Eos%) were generally decreased after the treatment of SCH 32088. (See table below.) No other dose-related effects were noted.

Percentage change from the vehicle-treated controls

Treatment (mg/day)	Males			Females		
	WBC	Lym%	Eos%	WBC	Lym%	Eos%
0.01	-#	17 %↓	22%↓	-	-	51 %↓
0.02	20 %↓	-	22%↓	-	14 %↓	36 %↓
0.06	15 %↓	12 %↓	31%↓	-	15 %↓	49 %↓
2.0	28 %↓	39 %↓	75 %↓	7 %↓	22 %↓	92 %↓

– indicates that the change from the control value was either < 10 %↓ or none.

Biochemistry (Before dosing and at Weeks 4, 14, 27 and 52): The treatment of SCH 32088 severely affected the serum cortisol levels of the 2 mg/day group. For this group, baseline cortisol was significantly decreased in Week 4, and was undetectable from Week 14 to 52. The post-ACTH cortisol response was also lower in the 2 mg/day group when compared with the vehicle-treated controls. At the end of the study, post-ACTH cortisol response values in the 2 mg/day group were below the quantifiable limits. Two 0.6 mg/day males had undetectable pre-ACTH values and normal post-ACTH cortisol responses. Adrenal cortex atrophy was noted in one of the dogs. Except for the 2 mg/day group, the mean values of plasma cortisol in the 0.1, 0.2 and 0.6 mg/day groups were comparable to control groups. (See table below.) Dose-related effects were not seen in other parameters.

Sex	Treatment (mg/day)	Serum Cortisol (µg/dL)					
		Week 4		Week 27 (µg/dL)		Week 52 (µg/dL)	
		Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH	Pre-ACTH	Post-ACTH
Males	0 (Untreated)	2.98	13.46	1.54	11.42	2.03	11.18
	0 (Vehicle)	1.04	12.18	1.14	10.94	0.96	9.74
	0.1	1.9	10.1	1.38	11.98	1.56	10.9
	0.2	1.44	10.4	0.98	9.74	0.93	9.96
	0.6	1.46	10.74	1.3	8.36	1.15	8.98
	2	0.75	4.3	0	2.35	0	0
Females	0 (Untreated)	2	12.5	1.08	10.66	0.86	11.38
	0 (Vehicle)	2.54	11.76	1.92	10.54	1.24	11.34
	0.1	2.44	12.24	2.46	12.16	1.7	11.76
	0.2	2.86	10.68	2	10.84	1.5	11.04
	0.6	1.9	9.88	1.76	9.82	1.46	9.08
	2	0.8	2.23	0	1.8	0	0

Urinalysis (Before dosing and at Weeks 4, 14, 27 and 52): There were no treatment-related findings in urine.

Pharmacokinetics (Days 1, 30 and 363): Plasma samples were collected at 1, 3, 6 and 24 hr postdosing. By using an HPLC-MS/MS (LOQ = 50 ng/ml) technique, SCH 32088 concentration was only quantifiable in the 2 mg/day group, but not in other groups. Gender differences were not found. When the AUC and Cmax levels obtained on Day 30 and 363 were compared, no drug accumulation was found in the 2 mg/kg group. Plasma SCH 32088 concentrations in the 2 mg/day group are presented in the following table:

Dose (mg/day)	Study Day	Mean (%CV)		
		Cmax (pg/ml)	Tmax (hr)	AUC(tf) (pg*hr/ml)
2.0	1	NC[a]	NC	NC
	-30	447 (61)	4.29[b] (203)	3836 (85)
	363	278 (20)	1.4 (60)	2756 (29)

a: Not calculated; data not amenable to rigorous pharmacokinetic analysis.
b: Tmax observed at 1 hr for 6/7 animals; 7th value of 24 hr skewed mean value.

Organ weights (Week 53/54): Organ weight changes were mainly observed in the 2 mg/day group. Thyroid and thymus weights were also altered in other treated groups.

Percentage change from the vehicle-treated controls

Treatment (mg/day)	Males				Females				
	Thyroid	Thymus	Adrenal	Testes	Thyroid	Thymus	Spleen	Adrenal	Ovary
0.1	25 %↓	36%↑	--#	--	--	16%↓	--	--	--
0.2	22 %↓	33%↑	--	--	16%↓	--	-	-	-
0.6	16 %↓	21%↑	--	--	--	--	--	--	15%↑
2	33 %↓	50%↑	42 %↓	25 %↓	17%↓	39%↓	20%↑	63%↓	19%↑

-- indicates that the change from the control value was either < 10 %↓ or none.

Necropsy (Week 53/54): Small adrenal glands were observed in 4/5 males and 5/5 females of the 2 mg/kg group. Except skin alopecia, there were no other drug-related macroscopic changes.

Histopathology (Week 53/54): A few small lymphoid aggregates (1-6) were observed in the nasal turbinates of untreated control dogs. After treatment with vehicle or 0.1 mg/kg, lymphoid aggregates in the nasal turbinates were smaller or fewer than the untreated controls. Absence of lymphoid aggregates was mainly seen in the 0.6 and 2 mg/kg dogs. No nasal irritation or inflammation was seen in all dosed groups. Therefore, the changes of the lymphoid aggregates can be attributed to a local response to corticosteroids, but not a toxic effect.

Dose-related thymus atrophy was observed in all groups, however, it was mainly present in the 0.6 and 2 mg/kg groups. Lung granuloma was found in all groups, including the control group. Although the occurrences of lung granuloma were increased with the doses administered, lung granuloma was also seen in the untreated- and vehicle-treated controls. Therefore, lung granuloma was not considered a drug-related change. Adrenal cortex atrophy and epidermal atrophy were only found in the 0.6 and 2 mg/day groups. Dose-related histological alterations were not reported on other organs. (See table below.)

Pathological Alterations	SCH 32088 Treatment (mg/day) to Males						SCH 32088 Treatment (mg/day) to Females					
	0	V*	0.1	0.2	0.6	2	0	V*	0.1	0.2	0.6	2
Absence of lymphoid aggregates #	0/5	0/5	0/5	1/5	3/5	5/5	0/5	1/5	0/5	0/5	2/5	4/5
Thymus atrophy	0/5	1/5	2/5	2/5	1/5	4/5	2/5	1/5	3/5	2/5	4/5	4/5
Lung granuloma	0/5	1/5	1/5	1/5	3/5	3/5	1/5	1/5	0/5	0/5	1/5	2/5
Adrenal cortex atrophy	0/5	0/5	0/5	0/5	1/5	5/5	0/5	0/5	0/5	0/5	0/5	5/5
Epidermal atrophy	0/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5	0/5	0/5	3/5

* Vehicle treated-group; ; # At the level 4 location (3rd premolar tooth)

In summary, intranasal administration of SCH 32088 did not produce nasal irritation in beagle dogs. The changes in the nasal lymphoid aggregates were considered an effect of corticosteroids. Systemic effects were seen mainly in the adrenal gland, thyroid, thymus and skin. These changes can be attributed to a longtime corticosteroid administration. The NOAEL dose is 0.1 mg/day. Intranasal administration at 0.2 mg/day can be considered a tolerated dose with mild glucocorticoid effects. Since plasma drug concentration was only measurable in the 2 mg/day group, systemic exposure of the SCH 32088 was not clearly determined in the animals treated at 0.1 or 0.2 mg/day. Although adrenal cortex atrophy and undetectable pre-ACTH values were found in one 0.6 mg/day male, average cortisol levels were comparable between the 0.6 mg/day and control groups. Based on the pathological findings, target organs of systemic toxicity were thymus, skin and adrenal gland.

Subchronic Toxicity Studies in Adult Animals:

10. A 26-week oral inhalation toxicity study in dogs (P-5991; 10/88-6/91; Vol. 100)

Test Lab:
Animal: 4 groups of Beagle dogs (4/sex/group)
Formulation: SCH 32088 inhaler (Batch # 22023-074)

Study Design: Four groups were treated for 26 weeks by oral inhalation at the following doses:

Group	Target dose (µg/kg/day)	Achieved dose (µg/kg/day)*
Control	0 (Vehicle)	0
Low-dose	20	21
Mid-dose	40	37
High-dose	80	74

* Calculation was based on total deposition.

Results:

Mortality (2 times/day): Mortality was not found in any group.

Clinical signs (2 times/day): Vomiting, loose feces, ocular discharge and estrous were seen in many groups. However, these clinical signs were not dose-related. Redness of skin and a pinna mass were also observed incidentally.

Bodyweight (Weekly): Body weights of most test groups were similar to the controls. At the end of the study, the mean bodyweight of high-dose females was slightly, but not statistically lower than the controls (14%↓);

Food consumption (Weekly): Food consumption in the high-dose males was statistically decreased (16% - 22%↓; $p<0.05$) at Weeks 16, 23, 24 and 26. Food consumption was not significantly changed in other test groups.

Ophthalmoscopy (During the treatment period and 13th and 26th treatment week): No treatment-related effects were noted.

EKG and blood pressure (Before and at Weeks 13 and 26): No treatment-related effects were noted.

Hematology (Before and at Weeks 13 and 26): In this study, total leukocyte counts were comparable among the groups. However, leukocyte differential counts were not performed in this study. Therefore, the suppression of lymphocytes or other leukocytes could not be evaluated. No other dose-related changes were found during treatment.

Biochemistry (Before and at Weeks 13 and 26): During Week 13, serum sodium and potassium levels, particularly in the males, were increased from the control values. At the end of the study, blood concentrations of sodium and potassium were not much higher than the controls. Serum

GTP levels were generally increased in the males and decreased in the females. However, the changes of GTP did not reach statistically significant levels. Therefore, the changes in the serum sodium, potassium and GTP levels may not be biologically significant.

Circulating cortisol levels were variable among the animals. However, the mean concentrations of blood cortisol in the high-dose group were much lower than the controls. This finding appeared to be a SCH 32088-related change. (See table below.) Drug-related changes were not seen in other parameters.

Percentage change from the control values

Sex	Group	Week 13				Week 26 (Terminal)			
		Na	K	Cortisol	GPT	Na	K	Cortisol	GPT
Males	Low-dose	1.0%↑*	13%↑*	17%↓	1.5%↑	0.7%↑	9.3%↑	12%↓	0%↑
	Mid-dose	1.1%↑*	15%↑*	42%↓	16%↑	1.1%↑	7.1%↑	0%↓	15%↑
	High-dose	1.0%↑*	15%↑*	83%↓*	11%↑	1.0%↑	13%↑	82%↓	21%↑
Females	Low-dose	0.2 %↑	10%↑	33%↓	13%↓	0.7%↑	6.8%↑	54%↓	5%↑
	Mid-dose	2.6 %↑*	6%↑	4.8%↑	16%↓	0.8%↑	6.8%↑	60%↓	27%↓
	High-dose	2.2 %↑	8%↑	76%↓	22%↓	2.3%↑*	13%↑	77%↓	28%↓

* Statistically different from the control values ($p < 0.05$)

Urinalysis (Before and at Weeks 13 and 26): There were no treatment-related changes in urinalysis.

Organ weights (Week 27): Adrenal weights in the high-dose males were statistically reduced (48%↓) from the control values. However, significant adrenal weight reduction was not observed in the drug-treated females. Drug-related changes were not noted in other organs.

Necropsy (Week 27): There were no drug-related macroscopic changes.

Histopathology (Week 27): Cortical atrophy of the adrenals was observed in 2/4 males of the mid-dose group, and 3/4 males and 4/4 females of high-dose groups. No other dose-related changes were seen.

Based on the results of this study, the inhalation dose of 21 μg/kg/day is a tolerated dose with mild glucocorticoid effects. Target organ toxicity was noted in the adrenal glands in this study.

11. A 26-week nose-only inhalation study in rats (P-5598; 10/88-3/91; Vol. 93)

Test Lab:
Animal: 4 groups of Sprague Dawley rats (20/sex/group; Mean bodyweights: ♂ = 325g; ♀ = 222g)
Formulation: SCH 32088 oral inhaler (Batch # 22023-074; MDI)

In this study, rats were exposed to SCH 32088 for 26 weeks by nose-only inhalation at the following doses:

Group	Target dose (µg/kg/day)	Achieved dose (µg/kg/day) for ♂/♀*
Control	0 (Vehicle)	0/0
Low-dose	40	50/55
Mid-dose	80	93/102
High-dose	160	214/234

* Calculation based on total deposition.

Mortality and Clinical signs (2 times/day): After the treatment, the animals had dose-related alopecia (5%, 74%, 89% and 93% of male rats in the control to high-dose group; 10%, 76%, 78% and 100% of female rats in the control to high-dose group) and scabbing of muzzle, neck and other skin regions (5%, 37%, 67% and 60% of male rats in the control to high-dose group; 5%, 65%, 56% and 53% of female rats in the control to high-dose group). Between Weeks 12 and 26, 16 animals were sacrificed due to progressive respiratory abnormalities (wheezing, gasping and labored breathing) and the number of animals increased with dose level (1, 4 and 11 rats in the low-, mid- and high-dose group, respectively). Histopathological results characterized the findings as focal acute necrotizing tracheitis caused by a fungal infection.

Labored breathing was also observed in 1 control male. This rat was sacrificed in Week 14. Histopathological examination showed glomerulonephritis and liver necrosis, but no remarkable findings were seen in the lung tissues.

Additionally, 1 low-dose male and 1 high-dose female rats were found dead during the study. These animals were also necropsied. Reduced sizes of thymus, spleen and lymph nodes were observed in these rats.

Bodyweight (Weekly): In comparison with the controls, dose-related statistically significant body weight decreases were present in all treated groups throughout the study. (See table below.)

Sex	Time	Treatment		
		Low-dose	Mid-dose	High-dose
Males	Week 1	7 %↓*	9 %↓*	11 %↓*
	Week 26	28 %↓*	34 %↓*	46 %↓*
Females	Week 1	5 %↓*	10 %↓*	9 %↓*
	Week 26	15 %↓*	27 %↓*	36 %↓*

* P< 0.01

Food consumption (Weekly): Reduced food consumption was also seen in all dosed groups. (See table below.)

Sex	Time	Treatment		
		Low-dose	Mid-dose	High-dose
Males	Week 1	11 %↓*	10 %↓*	17 %↓*
	Week 25	12 %↓*	14 %↓*	22 %↓*
Females	Week 1	34 %↓	38 %↓*	40 %↓*
	Week 25	28 %↓	34 %↓	32 %↓

* P< 0.01

Ophthalmoscopy (Before the treatment and at Week 25): No treatment-related effects were noticed.

Hematology (Before and at Week 14 and 26): Hematological examination revealed dose-related increases in neutrophils and decreases in lymphocytes and total leukocyte counts in all treated groups at Weeks 14 and 26.

Biochemistry (Before and at Week 14 and 26): Blood biochemical analysis revealed treatment-related changes primarily in the males at all dose levels and included increases in total protein and/or albumin, increases in cholesterol (also seen in females); and decreases in LDH, (also see in high dose females), alkaline phosphatase, GOT, phosphate and corticosterone levels at Week 14 and/or 26.

Urinalysis (Before and at Weeks 13 and 26): There were no obvious treatment-related changes in urinalysis, although potassium levels were decreased in the high dose females at Week 26.

Necropsy and Organ weights (Week 27): Small organs were observed in all treated groups. In comparison with the control values, the mean weights of many organs were decreased,

particularly in the spleen, thymus, uterus and adrenal glands. (See table below.) This changes were also seen in the relative organ weights.

Sex	Dose group	Percentage decreases in organ weight (%)								
		Liver	Spleen	Heart	Lungs	Thyroid	Adrenal	kidney	Prostate/Uterus	Thymus
Male	Low-dose	13%↓	28%↓	15%↓	20%↓	12%↓	16%↓	8.4%↓	8.1%↓	46%↓
	Mid-dose	11%↓	39%↓	15%↓	23%↓	8%↓	20%↓	7.8%↓	6.7%↓	56%↓
	High-dose	17%↓	45%↓	21%↓	22%↓	16%↓	50%↓	14%↓	18%↓	62%↓
Females	Low-dose	–*	10%↓	1.5%↓	14%↓	22%↓	17%↓	–*	25%↓	47%↓
	Mid-dose	–*	18%↓	6.4%↓	15%↓	17%↓	22%↓	1.7%↓	36%↓	65%↓
	High-dose	–*	32%↓	11%↓	14%↓	11%↓	37%↓	2.4%↓	46%↓	71%↓

* indicating that organ weights were slightly increased or not changed.

Histopathology (Week 27): Generally, adrenal, spleen, thymus and lymph nodes were atrophied at all dose levels. A secondary lesion in several animals of all treated groups consisted of focal acute necrotizing tracheitis caused by a fungal infection (2/40, 4/40 and 10/40 in the low- to high-dose groups). This infection was considered to be a reflection of SCH 32088-induced immusuppression. (See table below.)

	Treatment (µg/kg) for males				Treatment (µg/kg) for females			
	0	50	93	214	0	55	102	234
Adrenal Atrophy	0/20	0/20	0/20	7/20	0/20	0/20	0/20	3/20
Spleen Atrophy	1/20	2/20	7/20	18/20	1/20	1/20	3/20	13/20
Thymus Atrophy	3/20	16/20	19/19	17/18	7/20	14/18	16/16	17/17

Finally, testes, prostate gland, epididymides and mammary gland in the control and high-dose males; and ovaries, uterus, vagina and mammary gland of all females were re-examined by a reproductive endocrinologist. He reported that SCH 32088 caused a subtle perturbation of the estrous cycle and enhanced mammary gland lobuloalveolar development in all dose groups.

Based on the above results, a tolerated dose with mild glucocorticoid effects was not established in this study. Major target organ toxicity were seen in the liver, spleen, lungs, thymus, heart, kidney, uterus, thyroid, adrenal and mammary glands.

The following toxicity studies were reviewed previously. These studies are re-evaluated and summarized for this submission.

12. Three-month inhalation study in beagle dogs (D-22796, 1988, Vol. 60)

This study was conducted by . In the study, beagle dogs (4/sex/group) were treated inhalationally for 13 weeks with SCH 32088 (Batch #: 21108-059, oral inhaler) at 0 (placebo), 44 (low-dose), 79 (mid-dose) and 158 (high-dose) µg/kg/day. Plasma SCH 32088 concentration was not measured in this study.

After treatment, 1 low-dose female died due to bronchopneumonia, it was considered to be unrelated to the treatment. SCH 32088 did not induce dose-related clinical signs, ophthalmic alterations or EKG changes. Drug related reductions in body weight and food consumption were not significant. In Weeks 4 and 13, significant reductions in the leukocyte counts were found in the mid- and high dose females, but not in the males.

Decreased levels of BUN were noted at mid- and high-dose males. At the end of this study, cortisol levels in all treated groups were lower than controls, particularly in the mid- and high-dose groups.

Adrenal and thymus weights were reduced after treatment. Liver weights were increased in a dose related manner. The histopathology evaluation showed that liver glycogen accumulation was found in all high-dose dogs and about 50% mid-dose and low-dose dogs. Dose related changes in the zona glomerulose of adrenal glands was also observed.

Based on the results of this study, a no effect dose was not established. Target organs of systemic toxicity were liver, thymus and adrenal glands.

13. Three-month inhalation study in rats (D-22797, 10/88; Vol. 57)

This study was conducted by In the study, rats (15/sex/group) were treated inhalationally with SCH 32088 (Batch #: 21108-052 & 21108-058; oral inhaler) at 0 (placebo), 80 (low-dose), 160 (mid-dose) and 320 (high-dose) µg/kg/day for 13 weeks. Estimated achieved doses for the rat groups were 0, 48, 102 and 273 µg/kg/day, respectively. Pharmacokinetic parameters were not evaluated.

After the treatment, no mortality was noted. Alopecia was observed in all treated groups in a dose-dependent manner. Dose-related reductions in body weights and food consumption were also seen, but were higher in the mid- and high-dose groups. Leukocyte and lymphocyte counts were significantly decreased in the mid- and high-dose groups. Increases in plasma cholesterol, glucose and reduction in cortisol were observed in the mid-dose and high-dose groups, but not in

the low-dose group.

Reduced spleen, adrenal and thymus weights were associated with morphological alterations in the mid- and high-dose groups.

In conclusion, inhalation dose at 80 µg/kg targeted dose (48 µg/kg achieved dose) was considered as the NOAEL in rats. Target organs of systemic toxicity were defined as the thymus, spleen and adrenal gland.

14. Three-month nose-only inhalation study in rats. (P-5736, 2/94; Vol. 54)

This study was conducted by
In this study, SD rats were treated with SCH 32088 (Batch #: 26951-133; MDI) by nose-only inhalation at 0 (vehicle), 0.25, 0.5, 1, 2 and 4 µg/L. The target doses were 8, 17, 34, 67 and 134 µg/kg/day for the males, and were 8, 18, 37, 73 and 146 µg/kg/day for the females.

There was no death in any group. Dose-related clinical signs were not observed. When compared with the vehicle group, terminal body weights were statistically lower in the 1, 2, and 4 µg/L males (13%↓, 19%↓ and 31%↓, respectively), and the 2 and 4 µg/L females (12%↓ and 22%↓, respectively). Body weights in the 0.25 and 0.5 µg/L were slightly, but not significantly decreased from the controls. Lung weight was significantly reduced in the 4 µg/L group. Significant reductions in spleen weights were found in the 2 and 4 µg/L groups. Histologically, there were treatment-related decreases in tracheal globule cells. Reduced uterine granulocytic leukocytes was present in a dose-related fashion, but was also observed in the control group. No dose-related pathological changes were reported in the liver, spleen and lungs.

Pathological Alterations	SCH 32088 Treatment (µg/L) to the Males						SCH 32088 Treatment (µg/L) to the Females					
	0	0.25	0.5	1	2	4	0	0.25	0.5	1	2	4
Decreased tracheal globule cells	0/10	10/10	10/10	10/10	10/10	10/10	0/10	10/10	10/10	10/10	10/10	10/10
Decreased uterine granulocytic leukocytes	--	--	--	--	--	--	1/10	2/10	2/10	3/10	6/10	9/10

Systemic toxicity was not obviously found in the 1 µg/L rats (or ♂: 34 µg/kg; ♀: 37 µg/kg). Decreased tracheal globule cells was found in all treated animals, but not in any of the controls which suggests that SCH 32088 may cause degranulation of tracheal globule cells (Breeze RG and Wheeldon EB (1977) Am Rev Respir Dis. 116: 705). Therefore, a NOEL dose was not established in this study.

15. Two-week inhalation study in beagle dogs (D-22607, 5/88; Vol. 59)

This study was conducted by In this study, dogs (3/sex/group) were exposed to SCH 32088 aerosols for 2 weeks at 0, 80 (low-dose), 240 (mid-dose) and 800 (high-dose) µg/kg/day. The achieved doses were 0, 80, 240 and 810 µg/kg/day, respectively.

During exposure, no mortality or dose-related clinical signs were observed. Treatment did not affect body weight change, food consumption, hematological parameters and blood chemistry. Plasma cortisol levels were not measured in this study. Liver weight was increased in the high-dose group. Adrenal weights were reduced in the mid- and high-dose groups, but not in the low-dose group. Histopathological changes in liver, adrenal cortex, lymph nodes, mammary gland and thymus were mainly observed in the mid- and high-dose group. Adrenal gland atrophy was also seen in the 80 µg/kg group (♂: 1/3; ♀: 1/3).

Since no other obvious abnormalities were seen in the low-dose group, except adrenal atrophy, the inhalation dose of 80 µg/kg/day was considered a tolerated dose with mild glucocorticoid effects in dogs.

16. Two-week inhalation study in rats (D-22680, 5/88; Vol. 52)

This study was conducted by Rats (10/sex/group) were treated inhalationally with SCH 32088 (Batch #: 20431-040 & 20211-151; oral inhaler) at 0, 80 (low-dose), 240 (mid-dose) and 800 (high-dose) µg/kg/day. Achieved doses were 0, 68, 239 and 636 µg/kg/day for males and 0, 76, 268 and 710 µg/kg/day for the females.

For the mid- and high-dose groups, there were reductions in body weight, food consumption, WBC and lymphocyte count and GPT, GOT and alkaline phosphatase levels. Increased RBC counts and hemoglobin concentrations were found in all dosed groups.

Decreased weights of spleen, thymus, adrenal glands were found in the mid- and high-dose groups. Adrenal and thymus atrophy were mainly seen in the mid- and high-dose group. Adrenal gland atrophy was also found in the 80 µg/kg group (♂: 0/10; ♀: 1/10).

In conclusion, inhalation dose at 80 µg/kg for 2 weeks is considered as a tolerated dose with mild glucocorticoid effects in rats, although changes of adrenal cortical atrophy were seen.

17. Other Inhalation Studies

In addition to the above inhalation studies, SCH 32088 was also administered inhalationally to rats, dogs and mice at various dose levels and in different formulations. As listed in the following

table, major target organs of toxicity in these studies were generally defined in the adrenal, thymus, lymph tissue, liver, spleen, skin, bone marrow and mammary gland regardless of the species. No effect doses were not clearly determined in the listed inhalation studies. It is presumably due to relatively higher serum drug concentrations, although valid pharmacokinetic data were not provided in these studies. In comparison to the toxicology studies reviewed in this submission, the target organs of toxicity were generally similar in all studies.

Study	Report No. (#/sex/group; Batch #)	SCH32088 Daily Dose (µg/kg)	Target Organs of Toxicity
Rat: 2-wk nose-only inhalation (powder)	P-5834 (16; 92-MMF-DDPX-01)	♂: 12, 27, 48 ♀: 19, 38, 89	adrenal, thymus, lung, spleen, trachea, bone marrow, reproductive tracts (♀)
Rat: 2-wk nose-only inhalation (dry powder/ lactose)	P-6121 (16; 25887-023)	♂: 6.9, 23.4, 67.9; ♀: 4.5, 16.1, 57.5	thymus, lymph organ, trachea, bone marrow, mammary gland (♀)
Rat: 3-mon nose-only inhalation (powder)	P-5836 (25; 92-MMF-DDPX-01)	♂:3.4, 13, 56 ♀:4.5, 17, 70	adrenal, thymus, liver, lymph organ, lung, kidney, trachea, mammary gland
Dog: 2-wk nose-only inhalation (powder)	P-5835 (3; 92-MMF-DDPX-01)	♂:95, 141, 636 ♀:39, 144, 557	adrenal, thymus, liver, lymph node, lung, spleen, trachea, bone marrow
Dog: 2-wk oral inhalation (MDI)	D-22607 (3; 0431-040)	80, 240, 800	adrenal, lymph nodes, liver, thymus, mammary gland
Dog: 4-wk oral inhalation (DI)	D-24448 (4; 20432-040)	80	adrenal, thymus, spleen
Dog: 3-mon nose-only inhalation (powder)	P-5837 5(92-MMF-DDPX-01)	♂:35, 93, 192; ♀:57, 161, 250	adrenal, lymph organ, liver trachea, bone marrow, reproductive systems (♂ & ♀)
Mouse: 1-mon nose-only inhalation (MDI)	P-5739 (10; 26951-110)	♂: 102, 407 ♀: 80, 320	thymus, spleen, bodyweight gain↓ (LD: 50%; HD 100%)
Mouse: 3-mon nose-only inhalation (MDI)	P-5737 (10; 26951-133)	♂:27,82,159,313,648 ♀:26.64,152,238.601	Liver, lymph organ, adrenal, skin

18. Subchronic Oral Toxicity Studies

Oral doses of SCH 32088 were also given to rats, dogs and mice. After oral administration, major target organs of toxicity in the animals were the adrenal, thymus, lymph tissue, liver, spleen, skin, bone marrow and mammary gland. Based on the results of the 3-month studies, the NOAEL doses in rat, dog and mouse were 1.25, 10 and 50 µg/kg, respectively.

Oral Toxicity Study	Report No. (#/sex/group; Batch #)	Daily dose (µg/kg)	Observation
Rat: 2-week study	P-5424 (10; 8-MMF-X-6003)	200, 600, 2000	NOAEL = 200 µg/kg Target organ: adrenal, thymus, lymph tissues
Rat: 3-month study	P-5946 (15 or 25; 92-MMF-DDPX-01)	1.25, 50, 150, 450, 600	NOAEL = 1.25 Target organ: adrenal, lymph tissue, stomach, cecum, mammary gland (♀), thymus, spleen, reproductive tract (♀)
Rat: 3-month study	P-6138 (15 or 25; (92-MMF-DDPX-01)	50, 150, 450, 600	NOAEL = 50 ug/kg Target organ: lymph tissue, spleen, adrenal, mammary gland (♀), reproductive tract (♀)
Dog: 2-week study	P-5416 (3; 8-MMF-X-6003)	200, 600, 2000	NOAEL was not established. Target organ: adrenal, lymph tissue, liver, thymus
Dog: 3-month study	P-5917 (6; 92-MMF-DDPX-01)	50, 150, 600	NOAEL was not established. Target organ: adrenal, lymph tissue, liver, skin, skeletal muscle
Dog: 3-month study	P-6007 (6; 92-MMF-DDPX-01)	10, 150, 600	NOAEL = 10 µg/kg Target organ: adrenal, lymph tissue, liver, skin
Mouse: 3-month study	P-5947 (15 or 25; 92-MMF-DDPX-01)	2.5, 50, 150, 450, 600	NOAEL =50 ug/kg Target organ: adrenal, thymus, spleen, lymph tissues
Mouse: 3-month study	P-6140 (15 or 17; 92-MMF-DDPX-01)	50, 150, 450, 600	NOAEL =150 ug/kg Target organ: adrenal, thymus, spleen

Subchronic Toxicity Studies in Pediatric Animals:

19. One-month nose-only inhalation study in pediatric rats (P-5980; 1/94-2/95; Vol. 82)

Test Lab:
Animal: 2 weeks old Sprague-Dawley rats
Formulation: Micronized SCH 32088 powder (Batch # 92-MMF-DDPX-01)

Study Design: The rats were exposed to micronized SCH 32088 powder by nose-only inhalation

at 0 (filtered air), 0.01, 0.05, 0.25 or 1 µg/L for 1 month. The study design consisted of a main study group, a single-dose pharmacokinetic study group and a 1-month pharmacokinetic study group. (See table below.) Toxic effects of SCH 32088 were observed in the main study group.

Group Name	SCH 32088 concentration (µg/L)	Estimated dose (µg/kg/day)* ♂/♀	Rats (No./sex/group)			
			Main Study	Single-dose PK study	1-month PK study	Total
Control	0 (air)	0 / 0	24	12	8	44
Group 1	0.01	0.2 / 0.2	24	48	24	96
Group 2	0.05	1.1 / 1.1	24	48	24	96
Group 3	0.25	4.9 / 4.5	24	48	24	96
Group 4	1	17.7 / 21.7	24	48	24	96

* During Week 5.

Results:

Mortality (2 times/day): In the main study, no treatment-related deaths were observed. One control male and 1 control female died accidentally. Two males were mis-sexed and were excluded from this study.

Clinical signs (Weekly): No drug-related clinical signs were reported.

Bodyweight (Weekly): After a 1-month treatment, statistically reduced body weight gain was only seen in the 1 µg/L group. (See table below.)

SCH 32088 concentration (µg/L)	Percentage difference in mean bodyweights (MBW)@ ♂ / ♀	Percentage difference in mean bodyweight gains (MBWG)# ♂ / ♀
0 (air)	-/-	-/-
0.01	8.5% / 3%	6.1% / -0.3%
0.05	7.1% / 5.8%	-0.6% / -3.3%
0.25	5.6 % / 5.6%	-1.8 % / -3.8%
1	-0.5 % / -1.8%	-14.8 % * / -19.2%*

* $p < 0.01$;

@Percentage difference in MBW = $\frac{\text{(MBW of test groups - MBW of control group)} \times 100}{\text{MBW of control group}}$

#Percentage difference in MBWG = $\frac{\text{(MBWG of test groups - MBWG of control group)} \times 100}{\text{MBWG of control group}}$

Food consumption: This parameter was not reported.

Ophthalmoscopy (During Week 4): No treatment-related effects were noted.

Pulmonary Functions (During Week 4): There were no dose-related changes in the respiration rate and minute volume.

Hematology (Prior to necropsy): In the 1 µg/L group, compared with the control values, statistically significant changes were observed in erythrocytes (♂: 15%↑; ♀: 9.6%↑), hemoglobin (♂: 11%↑; ♀: 7%↑), and the counts of leukocytes (♀: 24%↓), lymphocytes (♀: 33%↓) and monocytes (♀: 58%↓). However, dose-related hematological changes were not seen in other treated groups.

Biochemistry (Prior to necropsy): The serum potassium (14%↑), albumin (6%↑), globulin (14%↑) and total protein levels (9.3%↑) in the 1 µg/L males were statistically greater than the control males. However, these changes were not significantly present in other treated groups. In comparison with the controls, serum corticosterone levels were statistically higher in the treated males, but not in the treated females. (See table below.)

One-Month Nose-Only Inhalation Study of SCH 32088 Powder in Pediatric Rats– Serum Corticosterone at Terminal Sacrifice (Mean ± SD; N=12)

SCH 32088 Target Exposure Concentration (µg/L)	Serum Corticosterone (ng/mL)	
	Males	Females
0[a]	112 ± 102[b]	270 ± 225
0.01[b]	269 ± 136[†]	105 ± 77
0.05	203 ± 166	106 ± 120[b]
0.25	296 ± 121*	324 ± 244
1.0	440 ± 101*	240 ± 248

[a]Filtered Air Control
[b]N = 11
[†]p≤0.05
*p≤0.01

Urinalysis (During Week 4): There were no treatment-related changes in urinalysis.

Pharmacokinetics (During Weeks 1 and 5): Plasma samples were initially analyzed by the sponsor using EIA technique by Schering Co. The samples collected in Weeks 1 and 5 were then pooled together and reanalyzed using HPLC. Proportions of the plasma collected from 2 sampling days were not defined. Based on the report from DSI, PK data from this study were invalid.

Organ weights (At the end of the study): Liver weights were statistically increased in drug-treated males and females. In comparison with the controls, testis or epididymides weights in the males and adrenal weights in the females were much higher. Lung and thymus weights were statistically lower in the high-dose group when compared with the control values. (See table below.) Since these organ weight changes were generally not dose-dependent and since pathological alterations were not found in these organs, the organ weight changes may be minimal corticosteroid effects.

Percentage change in the organ weights from the controls

Treatment (µg/L)	Males					Females			
	Liver	Lung	Testes	Epididymides	Thymus	Liver	Lung	Adrenal	Thymus
0.01	13 %↑*	–#	–	9 %↑	–	9 %↑*	–	13 %↑	–
0.05	14 %↑	–	20 %↑*	20 %↑*	–	11%↑*	–	23 %↑*	–
0.25	12 %↑*	–	20 %↑*	19 %↑*	–	11%↑*	–	9.7 %↑	–
1	11 %↑*	9 %↓*	26 %↑*	15 %↑*	32 %↓*	6.4 %↑	10 %↓*	19 %↑*	30.1 %↓*

* Statistically significant ($p<0.05$)

\# -- indicates that the change from the control value was either < 10 %↓ or none.

Necropsy (At the end of the study): Dose-related gross lesions were not found.

Histopathology (At the end of the study): Major target organs affected by SCH 32088 were the trachea, nasal cavity, bone marrow and mammary gland. Dose-related decreases in the tracheal globule cells and the appearance of nasal goblet cell hyperplasia were observed in the treated rats. Bone marrow adipose cells appeared in all groups in a dose-related fashion. Enhanced mammary gland secretion and lobuloalveolar development were found in all test group, but not in the control females. (See table below.)

Pulmonary alveolar histiocytic infiltration was found in all SCH 32088-treated males, but not in the control males. For the females, histological features of reproductive organs were within normal limits. No dose-related abnormalities were observed in the liver, thymus, testes,

epididymides and adrenal gland.

Pathological Alterations	SCH 32088 Treatment (µg/L) to the Males					SCH 32088 Treatment (µg/L) to the Females				
	0	0.01	0.05	0.25	1	0	0.01	0.05	0.25	1
Decreased tracheal globule cells	0/23	0/24	0/24	9/24	24/24	1/24	0/23	3/23	24/24	24/24
Nasal goblet cell hyperplasia	0/24	0/24	0/24	1/24	8/24	0/24	0/23	0/23	1/24	8/24
Increased bone marrow adipose cells	6/23	7/24	9/24	11/24	21/24	6/24	9/23	14/23	9/24	23/24
Mammary gland lobuloalveolar development	13/22	10/24	12/22	14/20	19/24	0/24	1/23	2/23	5/24	19/24
Mammary gland secretory activity	11/22	7/24	10/22	12/20	19/24	0/24	1/23	4/23	6/24	20/24
Lung: alveolar histiocytic infiltration	0/23	1/24	1/24	1/24	5/24	0/24	0/23	0/23	0/24	0/24

Based on the results of this study, an inhalation dose of 0.2 µg/kg/day (0.01 µg/L) can be considered a tolerated dose with mild glucocorticoid effects for male rats. However, the NOEL dose for progestational-like activity was not established in the female rats because of the appearance of enhanced mammary gland development in one 0.2 µg/kg female (0.01 µg/L). In this study, the major target organs of toxicity were defined as the trachea, nasal cavity, bone marrow, mammary glands and lungs based on the pathological alterations.

20. A 7-week oral inhalation study in pediatric dogs (P-5981; 12/93-12/94; Vol. 91)

Test Lab.
Animal: 4 groups of 6 weeks old Beagle dogs (5 or 10 /sex/group)
Formulation: Micronized SCH 32088 powder (Batch # 92-MMF-DDPX-01)

Study Design: This study was initially designed as a 3-month inhalation study. Aerosol exposure in this study was performed using a modified mask, which provided mouth-only inhalation and prevented nasal breathing. Four dog groups were exposed to either filtered air (control) or aerosolized SCH 32088 dry powder for 1 hour per day. After 7 weeks of inhalation, 5 dogs/sex/group were sacrificed because the dogs were struggling with their exposure masks and the study was not continued. Five dogs/sex/group in the control and high-dose groups remained on study for an additional 9-week recovery period and then sacrificed. The study design is presented in the following table:

Group	Dogs (No./sex/group)		Aerosol concentration (µg/L)	Estimated dose levels (µg/kg/day)* ♂/♀
	7-week treatment	9-week recovery		
Control	5	5	0 (air)	0
Low-dose	5	0	0.04	1/1
Mid-dose	5	0	0.2	7.3/7.1
High-dose	5	5	1	29.6/24.5

* During Week 5.

Results:

Mortality (2 times/day): One mid-dose male died on Day 35 with obvious abdominal distension. One control male suffered from pulmonary hemorrhage and was sacrificed humanely on Day 47. Both dogs were necropsied.

Clinical signs (Weekly): In this experiment, certain animals in all groups struggled with the exposure masks. During inhalation exposure, 23 dogs experienced syncope or a syncope-like syndrome; some dogs exhibited grey pallor of the tongue and mucus membranes indicative of anoxia; some dogs had distended abdomens and tympany. However, these findings appeared in all groups and were neither dose-related nor duration-related. These abnormalities were attributed to the effects of the exposure masks, and not to SCH 32088. The most frequent clinical signs reported were listless and thin animals. However, the signs did not increase with dose and were also present in the controls. No other dose-related clinical signs were reported.

Bodyweight (Weekly): At the end of Week 7, there were dose-related decreases in the mean body weight gains of the treated groups, particularly in the high-dose females. After the recovery period, the mean body weight gain in the high-dose group was greater than the controls. (See table below.)

Time	Group	Percentage difference in mean bodyweight gains (MBWG)* ♂ / ♀
Week 7	Control	-/-
	Low-dose	10.9% / 0%
	Mid-dose	-2.7% / -1.2%
	High-dose	- 3.9 % / -21%
Week 16	Control	-/-
	High-dose	6.2 % / 23.7

*Percentage difference in MBWG = $\frac{\text{(MBWG of test groups -MBWG of control group) x 100}}{\text{MBWG of control group}}$

Food consumption (Daily): There were no drug-related changes in the food consumption.

Ophthalmoscopy (Before and during the treatment): No treatment-related effects were noted.

EKG, heart rate and blood pressure (Before and at Weeks 1, 4, 7 and 9): No treatment-related effects were noted.

Pulmonary Functions (During Weeks 5): There were no treatment-related changes in the respiration rate, tidal and minute volumes

Hematology (At Weeks 5 and 7, and after Week 9): At Week 7, compared with the control values, mean values of erythrocytes (12%↑) and hemoglobin (13%↑) were slightly increased in the high-dose females; total leukocytes numbers (12%↓) were slightly decreased in the high-dose males. There were no lymphocyte depressions or other dose-related changes. After the recovery period, hematology parameters in the high-dose group were similar to controls.

Biochemistry (At Weeks 5 and 7, and after Week 9): At Week 7, the serum concentrations of potassium (♀:15%), GGT(♀:9.7%), ALT (♀:26%) and ALP (♂:16%; ♀:31%) were obviously increased in the high-dose animals, but not in other treated groups. After the recovery period, these parameters in the high-dose group were similar to the control values.

ACTH response (During Weeks 5 and Week 17): During either Weeks 5 or Week 17, baseline serum cortisol levels in the high-dose group were generally lower than the air-treated group. However, these dogs had normal post-ACTH cortisol values. (See table below.)

SCH 32088 Target Exposure Concentration (µg/L)	Serum Cortisol				
	Exposure Week 5 (µg/dL)		Exposure Week 7 (µg/dL)	Study Week 17 (µg/dL)	
Males	PreACTH	PostACTH	Baseline	PreACTH	PostACTH
0[a,b]	1.82 ± 1.05	13.24 ± 2.83	1.26 ± 0.85[c]	8.41 ± 4.01[d]	16.13 ± 2.05[d]
0.04[d]	1.47 ± 0.30	13.48 ± 1.72	1.94 ± 1.35		
0.2[d]	2.82 ± 1.82	12.72 ± 2.89	1.85 ± 1.09[c]		
1.0[e]	0.88 ± 0.87	10.82 ± 2.11	0.78 ± 0.85	5.80 ± 4.80[d]	17.06 ± 1.85[d]
Females					
0[a,b]	1.14 ± 1.07	14.18 ± 1.44	2.62 ± 2.89	8.86 ± 4.06[d]	17.63 ± 2.79[d]
0.04[d]	2.03 ± 1.38	13.62 ± 2.59	2.42 ± 0.86		
0.2[d]	2.86 ± 0.84	13.71 ± 0.76	2.03 ± 0.84		
1.0[e]	1.71 ± 1.23	11.42 ± 2.86	0.34 ± 0.50	6.12 ± 5.68[d]	16.81 ± 3.99[d]

[a]N = 10
[b]Filtered air control
[c]N = 9
[d]N = 5
[e]N = 4

Urinalysis (At Weeks 5 and 7, and after Week 9): There were no treatment-related changes in the urinalysis.

Pharmacokinetics (During Weeks 1 and 5): Plasma samples were collected at 0, 0.25, 1, 3 and 6 hr postdosing on Day 6, and at 0, 0.25, 1, 3, 6, 12 and 22 hr postdosing on Day 35. The samples were analyzed by using a LC-API-MS/MS procedure (LOQ = 50 pg/ml) at Taylor Technology Inc., NJ. The results showed that the plasma concentrations of SCH 32088 were gender-independent. Serum SCH 32088 was not detectable in the low-dose group during Weeks 1 and 5. For the high-dose males and females, Cmax and AUC levels in Week 5 were higher than the parameters obtained in Week 1. (See table below.) It suggests a possible drug accumulation.

Hour[a]	Target (Actual) Exposure Dose (µg/l)											
	0 (0)			0.04 (0.04 ± 0.01)			0.2 (0.20 ± 0.01)			1.0 (1.0 ± 0.1)		
	Plasma SCH 32088 (pg/ml)											
	Mean	%CV	n	Mean	%CV	n	Mean	%CV	n	Mean	%CV	n
Week 1												
0.25	0	–	20	0	–	10	0	–	10	187	34	20
1	0	–	20	0	–	10	0	–	10	72.2	69	19
3	0	–	20	0	–	10	0	–	10	14.4	198	18
6	0	–	20	0	–	10	0	–	10	0	–	17
Cmax (pg/ml)	NC	NC		NC	NC		NC	NC		189	33	20
AUC(tf) (pg-hr/ml)	NC	NC		NC	NC		NC	NC		227	57	20
Week 5												
0.25	0	–	20	0	–	10	48.3	73	10	420	51	20
1	0	–	20	0	–	10	0	–	10	184	42	19
3	0	–	20	0	–	10	9.26	316	10	55.2	91	20
6	0	–	20	0	–	10	0	–	10	9.06	247	20
12	0	–	20	0	–	10	0	–	10	0	–	19
22	0	–	20	0	–	10	0	–	10	6.22	308	20
Cmax (pg/ml)	NC	NC		NC	NC		74.1	21	7	420	51	20
AUC(tf) (pg-hr/ml)	NC	NC		NC	NC		59.4	68	7	787	62	20

[a] Time after termination of 1 hr exposure
NC Not calculated

Organ weights (After Week 7 and recovery period): Decreased lung and adrenal weight and increased thyroid weight were generally found in all drug-treated dogs. Reduced epididymides and spleen weights were seen in the drug-treated males and females, respectively. Thymus weights were also decreased in the high-dose females.

Lungs (males) and thymus weights (females) in the low-dose group were slightly greater than the

controls. After a 9-week recovery period, organ weights in the high-dose group were similar to the control group except for decreased epididymides weights. (See table below.)

Percentage differences in the organ weights from the controls

Time	Group	Males				Females				
		Lung	Adrenal	Epididymides	Thyroid	Lung	Spleen	Adrenal	Thymus	Thyroid
After Week 7	Low-dose	19%↑	--*	--*	24%↑	--*	16%↓	--*	13%↑	--*
	Mid-dose	16%↓	--*	21%↓	39%↑	--*	--*	--*	--*	26%↑
	High-dose	17%↓	10%↓	18%↓	49%↑	25%↓	28%↓	16%↓	32%↓	19%↑
After recovery										
	High-dose	--*	--*	18%↓	--*	--*	--*	--*	--*	--*

* The change was less than 10%.

Necropsy (After Week 7 and recovery period): After 7 weeks of exposure, hemorrhage was observed in the lung tissues in 15 out of 38 dogs, including the controls. After the recovery period, no abnormal necropsy finding was reported.

Histopathology (After Week 7 and recovery period): After the treatment, no dose-related pathological changes were found in any organ. From the control to high-dose dogs, acute pulmonary hemorrhage was seen in 0/4, 5/5, 2/4 and 0/5 males and 3/5, 3/5, 3/5 and 0/5 females. Perivascular hemorrhage, alveolar edema as well as acute cellular infiltrations were also observed in all groups. These pulmonary abnormalities were also reported in 1 mid-dose and 1 control males that died during the study. After a 9-week recovery period, most pulmonary alterations in the control and high-dose groups were recovered. Following the recovery period, no additional dose-related pathological findings were observed.

This study was designed as an oral inhalation study. Since a dog normally breathes through its nose, it is difficult for a young dog to breathe without proper acclimation to an exposure mask. Clinical signs found in this study were mainly caused by struggling with the face masks. The struggling may be due to the following 3 major possibilities: 1) The face mask was not fitted during the growth period of a young dog; 2) The young dogs have not adapted to the conditions of the face mask and inhalation experiment; 3) The dogs were hurt by the face masks during the experiment. Based on the pharmacokinetic data, systemic drug exposures were achieved in the experimental animals although this inhalation study was not properly conducted due to the problems with the face masks. Based on the results of this study, orally inhaled doses at 7.1 - 7.3 µg/kg/day can be considered as a tolerated dose with mild glucocorticoid effects for pediatric dogs. Target organs of toxicity were present in the adrenal, lungs, epididymides and thyroid.

21. Other Studies using pediatric animals

In addition to the above two inhalation pediatric studies, SCH 32088 were also given to pediatric rats and dogs by oral and intravenous routes of administration. After a 2-week intravenous administration of SCH 32088, the NOEL dose was not established in either rats or dogs. Target organs of toxicity were thymus, lymph tissue and adrenal gland in the rats, and liver, thymus, kidney and adrenal glands in the dogs.

When rats were treated orally at 0.5 to 125 µg/kg, the NOEL dose was determined at 5 µg/kg. However, oral NOEL was not defined in dogs treated with SCH 32088 at 50 to 2500 µg/kg. Target organs of toxicity for these animals were thymus, lymph tissues, liver, adrenal gland and skin.

Study (Animals/sex/group; Report #; Conducting laboratory)	Daily dose: µg/kg (Batch #)	Observation
Young rat: 2-wk IV study (10; P-5440; Schering)	60, 120, 240 (23459-066)	NOEL was not established Target organs: thymus, lymph tissue, adrenal
Young dog: 2-wk IV study (3; D-24315; BioResearch)	100, 300, 1000 (23459-066)	NOEL was not established. Target organs: Liver, adrenal, thymus, kidney
Young rat: 1-mon oral study (16; P-6045; Schering)	0.5, 5, 125 (93-MMF-DDPX-01)	NOEL = 5 µg/kg Target organs: thymus, lymph tissue
Young dog: 1-mon oral study (5; P-6008; Schering)	50, 150, 600,2500 (92-MMF-DDPX-01)	NOEL was not established Target organs: adrenal, liver, thymus, lymph tissue, skin.

APPEARS THIS WAY
ON ORIGINAL

REPRODUCTIVE TOXICOLOGY

1. A pilot oral teratology (Segment II) study in rats (D-26738, 1/95; Vol. 108)

Test Lab: Schering Company, Lafayette, NJ (GLP: No)
Animal: 5 groups of Sprague Dawley female rats (n = 25 /group)
Formulation: **SCH 32088 (Batch # 92-MMF-DDPX-01) in 0.4% aqueous methylcellulose at the concentrations of 0.004 mg/ml to 0.12 mg/ml**

Study Design: The rats were treated orally with SCH 32088 from Day 6 through 15 after mating. (See table below.) After treatment, all rats were sacrificed on Day 21 after mating.

Daily dose (µg/kg)	Dose volume (ml/kg)	Number of Females
0 (Vehicle)	5	8
0.02	5	8
0.1	5	8
0.2	5	8
0.6	5	8

Results: No obvious drug-related clinical signs were observed in any group. Body weights in all rat groups were comparable. No treatment-related differences were found in pregnancy or offspring parameters. There were no gross malformations or variations seen in this study.

Based on this non-GLP study, 0.6 mg/kg/day for pregnant rats can be accepted as a tolerated dose with mild glucocorticoid effects.

2. Oral teratology (Segment II) study in rabbit (P-5991, 6/95; Vol. 109)

Test Lab: Schering Company, Lafayette, NJ (GLP: Yes)
Animal: 4 groups of mated New Zealand White females (n = 18 + 4 /group)
Formulation: SCH 32088 (Batch # 93-MMF-MPX-01) in 0.4% aqueous methylcellulose at the concentrations of 0.5 µg/ml to 100 mg/ml.

Study Design: From Days 6 through 19 after mating, the rabbits were dosed orally with SCH

32088. Four additional rabbits in each group were used to determine plasma drug concentrations at 0.5, 1, 3, 6 and 24 hr postdosing on Day 19. The study design is presented in the following table:

Group	Daily dose (mg/kg)	Females for teratology study	Females for PK study
Control	0 (Vehicle)	18	4
Low-dose	0.14	18	4
Mid-dose	0.7	18	4
High-dose	2.8	18	4

The animals in the teratology study were killed on Day 30 after mating. After sacrifice, all offspring were examined for developmental variations and malformations. Rabbits used for the PK study were killed after the last blood collection on Day 19. The plasma samples were assayed by using a HPLC/MS technique (LOQ = 50 pg/ml).

Results:

Maternal Effects:

Mortalities and Pregnancy (Daily): No death was found in any group. The number of pregnant rabbits were 15/18, 13/18, 16/18 and 14/18 in the control to high-dose group. One mid-dose and 5 high-dose rabbits aborted. Seven high-dose rabbits resorbed all their conceptus. Only 2 high-dose rabbits remained pregnant to Day 30.

Clinical Signs (Daily): No dose-related clinical signs were found in the rabbits.

Bodyweight (At Days 0, 7, 13, 19, 25 and 30 after mating): During the dosing period (Days 7 - 19), mean body weight gains were reduced in a dose-related manner (0.09, 0.01, -0.03, -0.09 in the control, low-, mid- and high-dose groups, respectively). The bodyweight gains in the mid- and high-dose groups were statistically lower than the controls. The reduction of body weight gain remained in the high-dose until the end of the study.

Food consumption (Daily): Food intake in each group was not significantly different from the controls.

Necropsy (Day 30): There were no treatment-related macroscopic findings.

Pharmacokenitics (Day 19): Plasma SCH 32088 concentrations were only measurable in the

high-dose group. In the high-dose group, Tmax was observed at 3 hr postdosing. Cmax and AUC are present in the following table:

Dose (mg/kg/day)	Mean pg SCH 32088/ml plasma[a]					AUC(0.5-24 hr) (pg•hr/ml)
	0.5 hr	1 hr	3 hr	6 hr	24 hr	
0	0.0	0.0	0.0[b] (3)	0.0 (3)	0.0	0.0 (2)
0.14	0.0	0.0	0.0	0.0	0.0	0.0
0.7	0.0	0.0	0.0	0.0	0.0	0.0
2.8	136	109	222	128	24.0	2282

a: Each value is the mean of the data from 4 female rabbits, unless indicated otherwise in parentheses.
b: SCH 32088 was detected in the plasma of one control rabbit at 3 hr post-dose; the concentration was low and inconsistent with pharmacokinetic parameters and was not used in the calculation of the mean plasma concentration.

Litter Findings

Corpora lutea, implantation and resorption: The mean number of corpora lutea and implantation in all groups were comparable. However, the percentage of resorptions was statistically increased in the high-dose group. (See table below.)

Mean±SE	Animal groups			
	Control	Low-dose	Mid-dose	High-dose
Corpora lutea	9.93±0.45	9.38±0.65	10.07±0.56	9.22±0.81
Implantation	8.87±0.46	8.62±0.59	9.67±0.56	9±0.97
Resorption	0.27±0.12	0.62±0.4	1±0.35	8.44±1.16*

* $p<0.01$

Litter and fetuses: Litter size, sex distribution, distribution of fetuses in the left or right horn of the uterus, pup body weights and 24-hour offspring survival were comparable to control in the low- and mid-dose groups. However, two surviving high-dose dams produced only 4 live pups and 1 dead pup.

Malformations: Malformations were seen in all groups. The types of malformation varied in the control and low-dose groups. In the control group, 3/129 fetuses (2.3%) had malformations. One control fetus had a shortened body, absence of gonads, umbilical hernia and agenesis of tail. Another control fetus had hemivertebra. Missing or fused ribs were observed in all 3 control fetuses.

Malformations were found in 5/104 low-dose fetuses (4.8%). One was a conjoined twin. Extra sternebra (2 fetuses) and fused ribs (2 fetuses) were seen in other fetuses.

In the mid-dose group, 9 of 130 fetuses (6.9%) had malformations. In 8 of these, the primary findings were cleft palate and/or head malformations that consisted of hydrocephaly or a domed head. These findings were reported in rabbits treated with corticosteroids during pregnancy(Schardein J., Drugs as Teratogens, 1976, p. 218; Lanhoff L., et al., Anat Rec 193/3:598, 1979; Walker B., Proc Soc Exp Biol Med 125:1281, 1967). In the high-dose group, one of four fetuses (25%) had a cleft palate. However, only 4 fetuses are insufficient for a meaningful evaluation of prenatal development.

Based on the above results, oral administration of SCH 32088 at ≥ 0.7 mg/kg or above caused maternal and fetal toxicities to rabbits. The malformation rate in the 0.14 mg/kg group was considered to be comparable to the control value (2.3%). Since the oral dose at 0.14 mg/kg did not produce significant toxic or teratogenic effects to either the dams or their offspring, it was accepted as a NOAEL.

3. Subcutaneous teratology study in rats (P-5543, 3/91; Vol. 108)

Test Lab: Schering Company, Lafayette, NJ (GLP: Yes)
Animal: 4 groups of Sprague Dawley female rats (n = 25 /group)
Formulation: SCH 32088 (Batch # 9-MMF-X-6002) in 0.4% aqueous methylcellulose at the concentrations of 5 µg/ml to 60 mg/ml.

Study Design: The rats were treated subcutaneously with SCH 32088 from Day 6 through 15 after mating. After treatment, all rats were sacrificed on Day 21. The study design is presented in the following table:

Group	Dose (µg/kg/day)	Dose volume (ml/kg)	Number of Females
Control	0 (Vehicle)	0.5	25
Low-dose	2.5	0.5	25
Mid-dose	15	0.5	25
High-dose	30	0.5	25

Results:

Maternal Effects:

Mortalities and Pregnancy (Daily): All rats survived after the treatment. Pregnancy rates were 24/25, 24/25, 25/25 and 22/25 from the control to high-dose group.

Clinical Signs (Daily): No dose-related clinical sign was seen in the study.

Bodyweight (At Days 0, 6, 9, 12, 15, 18 and 21 after mating): During the dosing period (Days 6-15), mean bodyweights were comparable between the control and low-dose groups. However, bodyweights of the mid- (25%↓) and high-dose (40%↓) groups were statistically lower than the control values.

Food consumption (Days 0-6, 6-10, 10-15 and 15-21 after mating): Food consumption was not significantly different from the controls.

Necropsy (Day 21): Bilateral hydronephrosis was observed in one control rat. However, no treatment-related macroscopic abnormality was found in the study.

Litter Findings

Corpora lutea and implantation: There were no significant differences in the mean numbers of corpora lutea, implantation and resorption in all groups. (See table below.)

Mean±SE	Animal groups			
	Control	Low-dose	Mid-dose	High-dose
Corpora lutea	15.96±0.43	17.17±0.35	17±0.44	16.55±0.47
Implantation	15.38±0.63	16.71±0.32	16.32±0.54	16.09±0.57
Resorption	0.96±0.19	0.71±0.2	0.88±0.19	1.59±0.37

Litter and fetuses: Litter size, sex distribution, offspring delivered as well as 24-hour offspring survival were comparable among all groups. In comparison with the controls, mean fetal body weight was only statistically lower in the mid-dose group. However, this low fetal bodyweight was mainly produced by 1 fetus in the mid-dose group. When this fetus was excluded from the mid-dose group, the means of fetal bodyweights were comparable between the mid-dose and control groups.

Malformations: No drug-related malformations were observed in this study. Incidental malformations were seen in 3/1435 fetuses and were equally distributed in the control, mid- and high dose groups (1 fetus/group). No malformations were observed in the low-dose group. The incidences of skeletal variations, predominantly delayed ossification of the phalanges and sternebrae were slightly increased in the mid- and high-dose groups.

Based on the results of this study, the subcutaneous dose of 2.5 µg/kg/day was the NOEL dose to both dams and fetuses.

4. Single dose pharmacokinetic studies in pregnant female rats (P-6084, 7/95; Vol. 138)

The objective of this study was to determine pharmacokinetic parameters in pregnant rats. Pregnant SD rats (18-day pregnancy; n=3/interval) were treated either subcutaneously (SC) or orally (PO) with a single dose of SCH 32088 suspension (Batch #: 93-MMF-DDPX-01). Blood samples were collected at 0, 1, 2, 3, 4, 8, 12, 24 and 48 hr postdosing and then analyzed using LC-APCI-MS/MS method (LOQ = 50 pg/ml).

As demonstrated in the following table, Tmax was 4 hr in pregnant female rats regardless of administration routes.

Mean Parameters	Subcutaneous Dose (0.03 mg/kg)	Oral Dose (0.6 mg/kg)
Cmax (pg/ml)	517	3381
Tmax (hr)	4	4
$AUC_{0\text{-}24\ hr}$(pg.hr/ml)	6601	16797
$AUC_{0\text{-}48\ hr}$(pg.hr/ml)	8250	17595

Relative bioavailability of PO vs. SC administration was determined using the following formula and the result was approximately 11%.

$$(AUC_{0\text{-}48\ hr}\ PO/AUC_{0\text{-}48\ hr}\ SC) \times (SC\ Dose/PO\ Dose) \approx 11\%$$

Therefore, the drug exposure after SC administration was much higher than that after PO administration.

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5. Multiple dose pharmacokinetic study in female rats (P-6105, 7/95; 146)

To determine pharmacokinetic parameters, female SD rats (n=4 rats/interval) were treated PO or SC with SCH 32088 suspensions for 10 consecutive days at 2.5, 15 or 30 µg/kg/day. On Days 1 and 10, blood samples were collected at different intervals for up to 24 hr postdosing Pharmacokinetic parameters in the plasma determined by a HPLC-APCI-MS/MS assay (LOQ = 50 pg/ml). The data are displayed in the following table:

Parameters	2.5 µg/kg/day				15 µg/kg/day				30 µg/kg/day			
	PO#		SC##		PO		SC		PO		SC	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Cmax(pg/ml)	BQL*	BQL	211	234	120	85	661	794	185	225	903	1360
Tmax (hr)	BQL	BQL	3.0	3.0	2.5	1.5	2.0	2.0	2.0	2.0	3.0	4.0
$AUC_{0\text{-}24\ hr}$ (pg.hr/ml)	BQL	BQL	1248	1457	202	328	7282	9090	613	596	10667	15827
tf	N/A**	N/A	20	14	6	12	24	24	6	12	24	24

* BQL - Below quantifiable limits; ** not applicable

\# Blood samples were collected at pre-dose, and then at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hr after oral administration.

Blood samples were collected at pre-dose, and then at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 14, 20, and 24 hr after subcutaneous administration.

Based on the above table, Cmax and AUC levels in both groups were increased with the dose administered. Cmax and AUC values in SC group were higher than those in PO group.

Plasma drug concentrations were similar following single and multiple oral dose administrations, suggesting that there was no drug accumulation or enzyme induction after oral administrations. Following subcutaneous administration, plasma SCH 32088 levels on Day 10 were normally higher than those on Day 1. It suggests the possibility of a slight drug accumulation.

The above study indicated that subcutaneous administration produced a high systemic exposure in the female rats.

The following 5 reproductive toxicology studies were reviewed previously. These studies have been reevaluated and briefly summarized for this NDA submission.

6. Subcutaneous teratology (Segment II) study in mice (P-5578, 10/91; Vol. 107)

In this study, SCH 32088 (Batch #: 9-MMF-X-6002) was administered subcutaneously to CD-1 mice during days 6-15 of gestation at 20, 60 or 180 µg/kg/day. Loss of body weight (maternal

and offspring) and cleft palate at 60 µg/kg/day (2.9%) and 180 µg/kg/day (42%) were observed. The NOEL was 20 µg/kg/day for both the dams and offspring. Subcutaneous dose at 60 µg/kg/day was the maternally toxic and teratogenic dose in mice.

7. Dermal teratogenicity study (Segment II) in rats (P-5054, 5/85, Vol. 108)

CD (SD) BR rats were treated topically with 0.1% ointment (Batch # 15784-130) at 0 (vehicle), 0.3, 0.6 and 1.2 mg/kg of SCH 32088 from gestation days 6 through 15.

The study showed that the body weight gain of the treated dams was significantly reduced during the treatment. However, after withdrawal of the treatment, the weight gain among treated and untreated rats were comparable. No treatment-related mortality was reported. Pregnancy rate, litter size, distribution of fetuses in the uterus, sex distribution at birth, and body weights of pups at birth were lower than control at all doses. At 0.6 and 1.2 mg/kg, body weights were significantly lower than control.

No treatment related changes were observed in the number of corpora lutea, implantations and resorptions in the treated rats compared with the vehicle treated rats.

Results of the malformation and variation are presented as the following:

1. At 0.3 mg/kg: Dilated renal pelvis, delayed ossification, unossification of cervical vertebra.

2. At 0. 6 mg/kg: Cleft palate, umbilical hernia, delayed ossification.

3. At 1.2 mg/kg: Umbilical hernia, delayed ossification and unossification of cervical vertebra and thoracic vertebra.

8. Dermal teratogenicity study (Segment II) in rabbits (P-5066, 5/85, Vol. 109)

NZ rabbits were treated topically with 0.1% of ointment (Batch # 15784-130) at 0 (vehicle), 150 and 300 mg/kg of SCH 32088 from Days 7 through 19 after mating.

The study showed that dermal application of mometasone produced skin erythema at both dose levels. During treatment at both doses, rabbits lost bodyweight gain significantly. Pregnancy rates among these rabbits were not affected by treatment. Mean offspring weights were reduced significantly at low and high doses.

Incidences of malformations in the dosed groups were significantly higher than the control group. These abnormalities were gallbladder agenesis (absence of the organ) and flexed front paws. The high dose group showed umbilical hernia and cleft palate. The low dose group displayed omphalocele and hydrocephaly. In conclusion, dermal application of mometasone at either 150 or 300 µg/kg was maternal and fetal toxic to rabbits.

9. Subcutaneous fertility and general reproduction study (Segment I) in rats (P-5174, 1/87; Vol. 107)

Sprague Dawley rats were dosed subcutaneously with SCH 32088 (Batch #: 16824-033) at 0 (vehicle), 2.5, 7.5 and 15 µg/kg/day. Male rats were dosed for 63 days before mating and throughout the mating period. Female rats were treated from 14 days before mating until they were sacrificed at either Day 14 after mating or Day 21 after parturition.

The results showed that SCH 32088 did not impair estrous cycles, mating performance and fertility. The pregnancy rate was 96%, 88%, 80% and 92% in the control to high-dose groups. For the F_0 generation males, bodyweight reductions were seen in the 7.5 (15%↓) and 15 µg/kg males (26%↓), but not in the 2.5 µg/kg males. Food intake was only reduced in the 15 µg/kg males.

For the F_0 generation females, bodyweight gains were statistically decreased in the 7.5 and 15 µg/kg females during the premating and gestation period. (See table below.) Food consumption was only reduced in the 15 µg/kg group. Dystocia was also found in the 15 µg/kg groups.

Body weight gains (g) of F_0 generation females

Period	Treatment with SCH 32088			
	0 (Vehicle)	2.5 µg/kg	7.5 µg/kg	15 µg/kg
Pre-mating period (Week 8 - 10)	12.4+2.17	7.8+1.08	0.36+1.24*	-6.24+1.46*
Gestation period (Day 0 -21)	157.57+3.69	153+38+4.06	137.17+5.18*	117.14+7.22*

* P<0.05

Reproduction parameters were comparable among the control, 0.25 and 7.5 µg/kg groups.

Significant reductions in the offspring delivered, litter size and survival rate were noted only after the treatment of 15 µg/kg. Increased resorptions were also seen in the 15 µg/kg group. (See table below.)

Mean±SE	Animal groups (Dose: µg/kg)			
	Control (0)	Low-dose (2.5)	Mid-dose (7.5)	High-dose (15)
Offspring delivered	14.29±0.42	13.85±0.46	12.75±0.99	7.00±2.05*
% of alive offspring on Day 4	57.4	58.8	61.3	30.2
Resorption	0.78±0.17	1.05±0.28	0.67±0.23	0.2±0.2

* $p<0.05$

In conclusion, SCH 32088 at 2.5 µg/kg was the NOEL for the rats. Impairment of fertility in the rat was not produced by subcutaneous dose up to 15 µg/kg/day. Since the subcutaneous dose at 7.5 µg/kg only causes bodyweight reductions and no other effects, 7.5 µg/kg is considered a tolerated dose with mild glucocorticoid effects in the Segment I study based on the effects on the offspring.

10. Subcutaneous perinatal and postnatal reproduction study (Segment III) in rats (5164, 11/86; Vol. 109)

Sprague Dawley rats were treated subcutaneously with SCH 32088 (Batch #: 16824-033) at 0 (vehicle) 2.5, 7.5 and 15 µg/kg from Day 14 of gestation through Day 21 after parturitions. The results showed that SCH 32088 at 15 µg/kg caused bodyweight gain reduction, dystocia, difficulty in labor, reduction in the litter size, and decreased live births. In the 7.5 µg/kg group, prolongation of gestation was also observed when compared to the controls (22.25 days vs. 21.96 days), however, this change was not statistically significant. In conclusion, a subcutaneous dose of 15 µg/kg was maternally toxic in rats. Subcutaneous doses at 2.5 and 7.5 µg/kg were well-tolerated by rats.