CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022522Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-522
Submission date: 8/31/2010 (resubmission in response to CR letter of 5/17/2010)
Drug: roflumilast
Sponsor: Forest Research Institute
Indication: maintenance treatment to reduce exacerbations of chronic obstructive pulmonary disease associated with chronic bronchitis in patients at risk of exacerbations

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Background Comments:

The pharmacology/toxicology reviewer and supervisor in the Division of Pulmonary, Allergy and Rheumatology Products reviewed the nonclinical information for roflumilast during the first review cycle of this NDA and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

An additional pharm/tox review of proposed labeling was completed on 1/26/2011.

Conclusions:

I continue to concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. I have discussed the labeling with the pharm/tox reviewer and supervisor and I concur with the labeling changes suggested in the 1/26/2011 pharm/tox review.

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/s/

PAUL C BROWN
02/24/2011

INTEROFFICE MEMO

TO: NDA 22-522

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology/Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: February 4, 2011

DAXAS (Roflumilast) is a phosphodiesterase-4 inhibitor proposed as a once daily maintenance treatment of chronic obstructive pulmonary disease (COPD) associated with chronic bronchitis in patients at risk of exacerbations.

Dr. Luqi Pei completed a review of nonclinical pharmacology and toxicology studies provided in the original NDA submission on March 19, 2010. Dr. Molly Topper completed a secondary review dated March 31, 2010. Dr. Marcie Wood completed a labeling review on January 26, 2011.

Based on the overall nonclinical NDA evaluation, Dr. Pei concluded that characterization of the toxicity profile of roflumilast was complete from the nonclinical perspective. There are no outstanding pharmacology or toxicology issues to be addressed. Dr. Topper concurred with Dr. Pei's recommendation. Dr. Wood's labeling review provides recommended product labeling for nonclinical sections and detailed explanations of how the labeling was derived from nonclinical pharmacology and toxicology studies. I concur with Dr. Wood's recommendations for the product labeling.

The Pharmacology and Toxicology discipline recommends approval of the application.

There are no outstanding Pharmacology and Toxicology issues.

Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology/Toxicology Team Leader

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/s/

TIMOTHY W ROBISON
02/04/2011

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 22-522
Supporting document/s:	SDN32
Applicant's letter date:	August 30, 2010
CDER stamp date:	August 31, 2010
Product:	Roflumilast 500 mcg tablets
Indication:	COPD
Applicant:	Forest Research Institute
Review Division:	Pulmonary, Allergy and Rheumatology Products
Reviewer:	Marcie Wood, Ph.D.
Supervisor/Team Leader:	Tim Robison, Ph.D., DABT
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Carol Hill

Template Version: December 7, 2009

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1 Executive Summary

1.1.3 Labeling

The following edits to the nonclinical sections of the roflumilast labeling are suggested. See Labeling Review Section of the document for rationales and justifications of the recommendations.

8 Use in Specific Populations

8.1 Pregnancy

Teratogenic effects: Pregnancy Category C÷. There are no adequate and well controlled studies of DAXAS in pregnant women. DAXAS was not teratogenic in mice, rats, or rabbits. DAXAS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

DAXAS induced stillbirth and decreased pup viability in mice at doses corresponding to approximately 16 and 49 times, respectively, the maximum recommended human dose (MRHD) (on a mg/m^2 basis at maternal doses ≥2 mg/kg/day and 6 mg/kg/day, respectively). DAXAS induced post-implantation loss in rats at doses greater than or equal to approximately 10 times the MRHD (on a mg/m^2 basis at maternal doses ≥ 0.6 mg/kg/day). No treatment-related effects on embryofetal development were observed in mice, rats, and rabbits at approximately 12, 3, and 26 times the MRHD, respectively (on a mg/m^2 basis at maternal doses of 1.5, 0.2, and 0.8 mg/kg/day, respectively). (b) (4)

[redacted]

Nonteratogenic effects: DAXAS has been shown to adversely affect pup post-natal development when dams were treated with the drug during pregnancy and lactation periods in mice. These studies found that DAXAS decreased pup rearing frequencies at approximately 49 times the MRHD (on a mg/mg^2 basis at a maternal dose of 6 mg/kg/day) during pregnancy and lactation. DAXAS also decreased survival and forelimb grip reflex and delayed pinna detachment in mouse pups at approximately 97

times the MRHD (on a mg/m^2 basis at a maternal dose of 12 mg/kg/day) during pregnancy and lactation.

8.2 Labor and Delivery

DAXAS should not be used during labor and delivery. There are no human studies that have investigated effects of DAXAS on preterm labor or labor at term; however, animal studies showed that DAXAS disrupted the labor and delivery process in mice. DAXAS induced delivery retardation in pregnant mice at doses greater than or equal to approximately 16 times the MRHD (on a mg/m^2 basis at a maternal dose ≥2 mg/kg/day. (b) (4)

8.3 Nursing Mothers

Roflumilast and/or its metabolites are excreted into the milk of lactating rats. Excretion of roflumilast and/or its metabolites into human milk is probable. There are no human studies that have investigated effects of DAXAS on breast-fed infants. (b) (4)

10 Overdosage

10.1 Human Experience

No case of overdose has been reported in clinical studies with DAXAS. During the Phase I studies of DAXAS the following symptoms were observed at an increased rate after a single oral dose of 2,500 mcg and a single dose of 5,000 mcg: headache, gastrointestinal disorders, dizziness, palpitations, lightheadedness, clamminess and arterial hypotension.

12 Clinical Pharmacology

12.1 Mechanism of Action

(b) (4)

12.2 Pharmacodynamics

(b) (4)

–In COPD patients, 4 week treatment with DAXAS 500 mcg oral once daily (b) (4) sputum neutrophils and eosinophils by 31%, and 42%, respectively. In a (b) (4) pharmacodynamic study in healthy volunteers, DAXAS 500 mcg once daily (b) (4) reduced the number of total cells, neutrophils and eosinophils found in bronchoalveolar lavage fluid following segmental pulmonary LPS challenge by 35%, 38% and 73%, respectively.

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies were conducted in hamsters and mice with roflumilast to evaluate its carcinogenic potential. In 2-year oral gavage carcinogenicity studies, roflumilast treatment resulted in dose-related, statistically significant increases in the incidence of undifferentiated carcinomas of nasal epithelium in hamsters at ≥ 8 mg/kg/day (approximately 11 times the MRHD based on summed AUCs of roflumilast and its metabolites). The tumorigenicity of roflumilast appears to be attributed to a reactive metabolite of 4-amino-3,5-dichloro-pyridine N-oxide (ADCP N-oxide). No evidence of tumorigenicity was observed in mice at roflumilast oral doses up to 12 and 18 mg/kg/day in females and males, respectively (approximately 10 and 15 times the MRHD, respectively, based on summed AUCs of roflumilast and its metabolites).

(b) (4)

Roflumilast tested positive in an *in vivo* mouse micronucleus test, but negative in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosome aberration assay in human lymphocytes, *in vitro* HPRT test with V79 cells, an *in vitro* micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and *in vivo* mouse bone marrow chromosome aberration assay. Roflumilast N-oxide was negative in the Ames test and in vitro micronucleus test with V79 cells.

(b) (4)

(b) (4)

In a human spermatogenesis study, roflumilast 500 mcg had no effects on semen parameters or reproductive hormones during the 3-month treatment period and the following 3-month off-treatment period. In a fertility study, roflumilast decreased fertility rates in male rats at 1.8-mg/kg/day (approximately 29 times the MRHD on a mg/m^2 basis). These rats also showed increases in the incidence of tubular atrophy, degeneration in the testis and spermiogenic granuloma in the epididymides. No effect on male rat fertility rate or reproductive organ morphology was observed at 0.8 mg/kg/day (approximately 13 times the MRHD on a mg/m^2 basis). No effect (b) (4) on female fertility was observed up to the highest roflumilast dose of 1.5 mg/kg/day in rats (approximately 24 (b) (4) times the MRHD on a mg/m^2 basis (b) (4)

(b) (4)

2 Drug Information

2.1 Drug

2.1.2 Generic Name
Roflumilast

2.1.3 Code Name
BY 217, B9302-107, BYK20869

2.1.7 Pharmacologic class
PDE4 inhibitor

Regulatory Background

This NDA is currently in its second review cycle. The application was resubmitted on August 31, 2010 and its PDUFA date is February 28, 2011. Previously, Dr. Luqi Pei completed the nonclinical review of the application on March 19, 2010 during the first review cycle. The application was considered approvable from a nonclinical perspective. Dr. Molly Topper (formerly Shea) completed a Supervisor/Team Leader memorandum on March 31, 2010. A DPAP Advisory Committee meeting was held in Silver Spring, MD on April 7, 2010. The Division issued a complete response memorandum on May 17, 2010 (see letter complete details of deficiencies and recommendations to resolve deficiencies).

The review by Dr. Pei completed on March 19, 2010 did not include a labeling review. This decision was based on the review team's decision that no labeling review was necessary at the time.

LABELING REVIEW

This labeling review was based on the proposed labeling submitted by the Forest Research Institute on August 31, 2010. Nonclinical findings cited in the review were based on the pharmacology and toxicology review by Dr. Luqi Pei completed on March 19, 2010 in the original NDA submission. To simplify the labeling, dose ratios between animals and humans were calculated for the parent drug on a mg/m^2 basis, with the exception of the Carcinogenesis section where dose ratios between animals and humans were calculated for the summed AUCs of roflumilast and its metabolites. Dose ratios in the suggested labeling are rounded to the nearest integer.

8 Use in Specific Populations

Reproductive Toxicology (Sections 8.1-8.3)
As described in the original nonclinical review of the NDA (p52), embryofetal developmental effects of roflumilast were evaluated in mice, rats and rabbits. In embryo-fetal developmental toxicity studies, pregnant females were dosed during the period of gestation days 6 – 18, 6 – 15, 6 – 15 in mice, rats and rabbits, respectively. The roflumilast doses ranged 1.5 – 12, 0.2 - 1.8, and 0.2 - 0.8 mg/kg/day in mice, rats and rabbits, respectively. Roflumilast was not teratogenic in any species; however, increases in the incidence of fetal deaths (stillbirths) and decreased pup viability were observed in mice and increased post-implantation loss and incomplete bone ossification were observed in rats as described below. The fetal deaths in mice observed at ≥ 2 mg/kg/day were apparently attributed to the tocolytic effect of the drug.

Three studies were completed to evaluate the effect of roflumilast on pregnancy and delivery. Report 125/2002 was the first study completed. Pregnant mice (20-23/dose, NMRI) received oral roflumilast at 2, 6 and 12-mg/kg/day during pregnancy (GD 6 – 18) and lactation. Numbers of live births, stillbirths and pup viability and development were evaluated. Roflumilast treatment caused dose-related increases in the number of stillbirths and litter losses and a decrease in pup viability and post-natal development

(Table 1). Statistically significant increases in number of stillbirths ($P < 0.01$) were observed in all treated groups (1.7%, 16.4%, 29.9%, and 75.0%, at 0, 2, 6, and 12 mg/kg/day, respectively). Pups showed decreases in post-natal viability in the mid- and high-dose groups and decreases in forelimb grip reflex and pinna detachment in the high dose groups.

Table 1: Findings of Pre- and Postnatal Developmental Study in Mice (125/2002)

Roflumilast (mg/kg/day)	**0**	**2**	**6**	**12**
F0 Females				
Pregnant #	20	20	21	23
Abnormal parturition - Delivery problems (#dams)	0	1	4	9
F1 litters (pre-weaning)				
No. litters evaluated	19	17	15	7
Mean no. of implantations	12.9	12.4	11.5	11.7
Mean no. pups/litter Day 0	12.4	11.3	8.3**	6.3**
Mean no. live born pups/litter	12.2	10.5	8.1**	2.9**
Stillborn: total #litters	4	7	12	14**
Percentage	1.7%	16.4%**	29.9%**	75.0%**
Postnatal survival to day 4 (%)	95.3	96.6	73.0	35.0
Weight gain(g, from birth to weaning)	11.8	12.5	13.9	15.6
Postnatal survival to weaning (%)	99.5	100.0	92.1	100.0
No. of total litter losses	0	2	6	14**
Slope test (% of pups reaching criteria)	72	76	73	14
Grip reflex forelimb (% reaching criteria)	97	94	96	57
Pinna detachment (% reaching criteria)	93	92	85	57

**, $p < 0.01$.

Report 126/2002 investigated the temporal effect of roflumilast on pregnancy and delivery in mice. Results of the study showed that the fetal effect of roflumilast was mediated through its tocolytic effects. Pregnant mice (10-12/group) were given vehicle (G1) or 12 mg/kg/day roflumilast orally during lactation (lactation day 1 – 20) and at different times of pregnancy: Gestation Days 6 - 16 (G2), 15 – 18 (G3), or 18 (later time of the day, G4). A control group received the vehicle during lactation and GD 6 – 16. Table 3 summarizes the results. Similar to Report 125/2002, the treated dams showed significant difficulty in delivery. Groups 3 and 4 dams showed increases in the incidence of deaths and early sacrifice due to moribund conditions. The death and sacrifice occurred around the time of delivery due to delivery complications. The roflumilast treatment groups showed significant increases in the incidence of stillbirths. Groups 3 and 4 showed decreases in pup viability index. Group 4 pups also showed decreases in forelimb grip reflexes and a delay in pinna detachment.

Table 2: Pregnancy and Offspring Outcome of Report 126/2002

Group	C	G2	G3	G4
Roflumilast dose (mg/kg/day)	0	12	12	12
Treatment Duration (gestation days)	6-18	6-18	16-18	18
Dam data				
Dams with live born #	10	10	6*	6
Dams completing delivery #	10	12	10	6
Dams with stillborn pups [#, (%)]	0 (0)	4 (33.3)	9 (90)**	6 (100)**
Litters with live born but no pus on day 4	0 (0)	1 (10)	4 (66.7)*	1 (16.7)
Litter data				
Pups delivered (total #)	116	93	89	71
Live born	116	75**	39**	39**
Stillborn [#, (%)]	0 (0)	18 (19)**	50 (56)**	32 (45)**
Died in lactation day 1 - 4	1 (0.9)	10 (13)**	31 (80)**	29 (74)**
Viability index (%, day 4)	99.1	86.7**	20.5**	25.6**

*, $p < 0.05$; **, $p < 0.01$.

Report 127/2002 was the last study completed to further investigate effects of roflumilast on pregnancy and post-natal development. Reports 127/2002 and 125/2002 were similar in study designs except the following: Report 127/2002 used a narrow dose range and shorter treatment period during gestation than Report 125/2002. Specifically, the respective roflumilast doses in the LD, MD and HD groups were 2, 6 and 12 mg/kg/day in Report 125/2002 and 1.5, 3 and 6 in Report 127/2002. The treatment duration during pregnancy was gestation days 6 – 18 and 6 – 15 in Reports 125/2002 and 127/2002, respectively. Other differences included the post-natal behavioral developmental parameter (i.e., rearing) evaluated. Table 4 summarizes the results. Dose-related effects on number of stillbirths, live births and litter sizes were observed in the MD and HD groups (Table 3). The prevalence of stillbirths was 0.3%, 0.3%, 3.1% ($P< 0.05$) and 8.8% ($P < 0.01$) at 0, 1.5, 3 and 6 mg/kg/day, respectively.

Table 3: Pregnancy and Offspring Outcome of Report 127/2002

Roflumilast (mg/kg/day)	0	1.5	3	6
F0 Dam data				
No. of Pregnant mice (dams)	29	28	28	28
Duration of gestation (days)	19.0	19.1	19.1	19.4
Dams with stillborn pups [#, (%)]	1 (3.4)	1 (3.6)	3 (11.1)	6 (22.2)
Dams with all stillborn pups [#, (%)]	0 (0)	0 (0)	1 (3.7)	2 (7.4)
Litter data (F1)				
Pups delivered (total #)	366	330	323	271
Live born (total#, %)	365 (99.7)	329 (99.7)	313 (96.9)*	247 (91.1)**
Stillborn (total#, %)	1 (0.3)	1 (0.3)	10 (3.1)*	24 (8.8)**
Litters with live born but no pups on day 4	0 (0)	0 (0)	0 (0)	2 (8)
Mean litter size at birth (#pup/litter)	12.6	11.8	12.0	9.9**
Mean litter size on day 4 (pre-culling)	11.0	11	11	8.2 **
Culled pups (total) on day 4	109	92	80	32
Died in lactation day 5 - 21	4 (1.1)	1 (0.3)*	10 (3.2%)	11 (4.5)*
Pups (total) surviving 21 days	201	208	189	161
Viability index (%, day 4)	87.7	93.9	91.7	82.6

Post-natal development effect				
No. rearing [a]	-	↓4%	↓11.7%	↓30.3%*

a. Calculated from these numbers: the mean number of rearing at 90 minutes was 188.3, 179.9, 166.3 and 131.2 in the 0, 1.5, 3 and 6-mg/kg/day roflumilast groups.
*, $p < 0.05$; **, $p < 0.01$.

The higher rate of stillbirths at 2 mg/kg/day in Report 125/2002 appears to be attributed to the longer treatment duration of the study based on the interpretation of Report 126/2002. Overall, the review concludes that roflumilast treatment induces stillbirths at 2 mg/kg/day or higher doses, but not at 1.5 mg/kg/day. Because the study design of Report 125/2002 does not fit into a typical embryofetal developmental study in mice, the labeling should concentrate on the results of Report 127/2002. The 2-mg/kg/day dose, however, should be mentioned because it reflects the lowest dose that the tocolytic effect was observed. Based on the totality of data from fetal and development studies of roflumilast, the review concludes the following effects in mice: fetal deaths at ≥ 2 mg/kg/day, decreases in pup viability index and the number of rearing at ≥ 6 mg/kg/day, and decreased grip reflex and delayed pinna detachment at 12 mg/kg/day.

In an embryofetal developmental study (Report 8/96), pregnant rats were treated with 0, 0.2, 0.6 and 1.8-mg/kg/day roflumilast during gestation days 6 through 15. Statistically significant increases in the incidence of incomplete ossification of skull bones were observed in the MD and HD groups in rats. The respective incidence in the C, LD, MD and HD was (# fetus/litter) was 53/23, 49/18, 59/22 and 89/29 ($P < 0.05$) in inter-parietal bones and 117/11, 20/11, 34/15 ($P < 0.05$) and 46/23 ($P < 0.05$) in parietal bones. This finding in rats should not, however, be included in the label as it is considered a developmental delay and not a true teratogenic effect. In a fertility study in rats (Report 19/97), the rate of post implantation loss was 4.7%, 5.5%, 14.8% ($P < 0.05$) and 30.7% ($P < 0.05$) in 0, 0.2, 0.6 and 1.8-mg/k/day roflumilast groups when both males and females were treated with the drug prior to, through and until 7 days after mating (Ref., the original NDA review, p51). However, an embryofetal development study that used the same roflumilast doses showed no such effect at any doses (Report 8/96). Neither did embryofetal developmental studies in mice (3 studies at doses up to 36 mg/kg/day) or rabbits (1 study at doses up to 0.8 mg/kg/day).

Rabbits did not show any treatment-related effects on embryofetal development at roflumilast doses up to 0.8 mg/kg/day. The NOAEL for embryo-fetal development was 0.2 and 0.8 mg/kg/day in rats and rabbits, respectively.

8.1 Pregnancy

The applicant proposed the following text for Section 8.1:

(b) (4)

Based on the previous discussions, the review recommends the following for Section 8.1:

Teratogenic effects: Pregnancy Category C. There are no adequate and well controlled studies of DAXAS in pregnant women. DAXAS was not teratogenic in mice, rats, or rabbits. DAXAS should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

DAXAS induced stillbirth and decreased pup viability in mice at doses corresponding to approximately 16 and 49 times, respectively, the maximum recommended human dose (MRHD) (on a mg/m^2 basis at maternal doses ≥2 mg/kg/day and 6 mg/kg/day, respectively). DAXAS induced post-implantation loss in rats at doses greater than or equal to approximately 10 times the MRHD (on a mg/m^2 basis at maternal doses ≥ 0.6 mg/kg/day). No treatment-related effects on embryofetal development were observed in mice, rats, and rabbits at approximately 12, 3, and 26 times the MRHD, respectively (on a mg/m^2 basis at maternal doses of 1.5, 0.2, and 0.8 mg/kg/day, respectively).

Nonteratogenic effects: DAXAS has been shown to adversely affect pup post-natal development when dams were treated with the drug during pregnancy and lactation periods in mice. These studies found that DAXAS decreased pup rearing frequencies at approximately 49 times the MRHD (on a mg/mg^2 basis at a maternal dose of 6 mg/kg/day) during pregnancy and lactation. DAXAS also decreased survival and forelimb grip reflex and delayed pinna detachment in mouse pups at approximately 97 times the MRHD (on a mg/m^2 basis at a maternal dose of 12 mg/kg/day) during pregnancy and lactation.

8.2 Labor and Delivery

As discussed above, mice dosed with ≥ 2mg/kg/day roflumilast during pregnancy, especially near the delivery time, showed dose-related increases in fetal mortality and stillbirths and decreases in postnatal survival. These effects appear to be attributed to the tocolytic effect of the drug. These findings should be described in the labeling as the applicant proposed. The proposed labeling for Section 8.2 is as follows:

(b) (4)

(b) (4)

The review recommends replacing the above text with the following:

> *DAXAS should not be used during labor and delivery. There are no human studies that have investigated effects of DAXAS on preterm labor or labor at term; however, animal studies showed that DAXAS disrupted the labor and delivery process in mice. DAXAS induced delivery retardation in pregnant mice at doses greater than or equal to approximately 16 times the MRHD (on a mg/m^2 basis at a maternal dose ≥2 mg/kg/day).*

8.3 Nursing Mothers

The applicant proposed the following text for Section 8.3:

> "Roflumilast and/or its metabolites are excreted into the milk of lactating rats. Excretion of roflumilast and/or its metabolites into human milk is probable. (b) (4)

The review recommends revising the above text to the following:

> *Roflumilast and/or its metabolites are excreted into the milk of lactating rats. Excretion of roflumilast and/or its metabolites into human milk is probable. There are no human studies that have investigated effects of DAXAS on breast-fed infants.*

12 Clinical Pharmacology

12.1 Mechanism of Action

The applicant proposed the following text for Section 12.1:

(b) (4)

(b) (4)

12.2 Pharmacodynamics

The applicant proposed the following text for Section 12.2:

(b) (4)

The review recommends revising the above text to the following in order to remove references to nonclinical pharmacology data:

> *In COPD patients, 4 week treatment with DAXAS 500 mcg oral once daily reduced sputum neutrophils and eosinophils by 31%, and 42%, respectively. In a* (b) (4) *pharmacodynamic study in healthy volunteers, DAXAS 500 mcg once daily* (b) (4) *reduced the number of total cells, neutrophils and eosinophils found in bronchoalveolar lavage fluid following segmental pulmonary LPS challenge by 35%, 38% and 73%, respectively.*

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

As the original NDA review stated, hamsters, not mice, treated with oral (gavage) roflumilast for 2 years showed dose-dependent and statistically significant increases of nasal tumors ($p < 0.0000$). The prevalence rate of total nasal tumors in hamsters was ≤ 0.8%, 0%, 3.3% and 12.5% in 0, 0.25 – 4, 8, and 16-mg/kg/day roflumilast groups, respectively (p50 of the NDA review), when data in males and females were combined. The prevalence data included both benign (adenoma) and malignant tumors (carcinomas and adenocarcinomas). Among them, the most noticeable tumors were the

undifferentiated carcinomas [incidence males and females combined: 1/300, 0/360, 4/120 and 10/120 in the 0, 0.25-4, 8 and 16 mg/kg/day groups, respectively (Appendix 5 of the original NDA review, p58-60)]. The remaining tumors showed numerical increases, but statistically insignificant increases, in the incidence of adenocarcinoma of Bowman's gland and adenoma of the Steno's gland in the 16-mg/kg/day group (Incidence; 0/60 and ≤3/120 in the cage control and 16-mg/kg/day groups, respectively). The tumorigenicity of roflumilast appears attributed to epoxy-ADCP N-oxide, a reactive intermediate of a roflumilast metabolite, ADCP N-oxide (Figure 1). The role of ADCP N-oxide and its epoxide has been discussed extensively in Pharmacology and Toxicology Review #5 completed on May 25, 2007 in IND 57,883 (Appendix #5 of the original nonclinical NDA review), the memorandum to NDA file by Dr. Luqi Pei on January 25, 2010, and the original nonclinical NDA review completed on March 19, 2010. In rodents, Cytochrome P450 enzyme CYP2G1 in the nasal epithelium converts ADCP to ADCP N-oxide. The same enzyme further converts the latter to ADCP N-oxide epoxide which reacts with the –SH group of protein and eventually results in formations of protein and DNA adducts. Human nasal epithelium does not have active counterparts of CYP2G1. However, ADCP N-oxide has been shown to be present in human blood and urine samples. Also, the enzyme involved in the production of ADCP N-oxide in humans is unknown. Neither is it known whether ADCP N-oxide epoxide is produced in humans. Consequently, the concern about the tumorigenicity of roflumilast focuses on ADCP N-oxide, an up stream intermediate of epoxy-ADCP N-oxide.

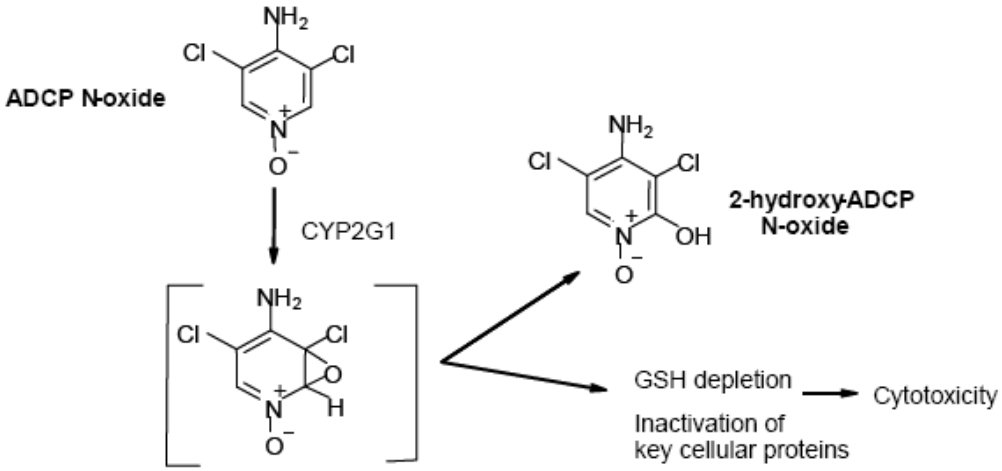

Figure 1: Scheme for the metabolism of ADCP N-oxide by rat and hamster olfactory microsomes with formation of the putative reactive intermediate, epoxy-ADCP N-oxide (source: Report 260/2008, p53).

Because of the special concerns about the ADCP metabolite, the review has considered the best approach to describe the hamster carcinogenicity data in the labeling. Two approaches have been considered. The first approach was to describe the exposure ratios ADCP N-oxide and the remaining compounds separately. Advantage of the approach is that it clearly separates dose ratios based on the level of concerns. A disadvantage is that it will significantly complicate and lengthen the labeling. The other approach is to use the ratio of the summed AUCs of roflumilast and its metabolites. This approach would make the labeling concise and would be appropriate if the ratios based

on the summed AUCs would not underestimate the potential hazards of the intended use of the drug.

The review compared the AUC ratios for individual compounds and combinations when appropriate. Table 4 summarizes these ratios. The exposure ratios of ADCP N-oxide were higher than the rest of the individual compounds or any combination ratios. For example, the ratios for ADCP N-oxide only, the sum of roflumilast + roflumilast N-oxide + ADCP, and the sum of the roflumilast + roflumilast N-oxide + ADCP + ADCP N-oxide at 4-mg/kg/day roflumilast in hamsters to the human clinical dose are 34.7, 3.2, and 4.7, respectively.[1] The review elects to use the ratio of the summed AUCs of roflumilst and its metabolites because it simplifies the nonclinical sections of the roflumilast labeling and does not underestimate the potential risks of intended drug use. However, it would be also acceptable to list the ratios for ADCP N-oxide and the remaining compounds separately if the applicant insists.

Table 4: AUCs and Exposure Ratios for Carcinogenicity Studies in Hamsters and Mice

		Hamster [a]			Mouse [b]		Human
		4	8	16	12 (F)	18 (M)	0.01
Tumor Prevalence (%)		0	3.3	12.5	0	0	-
Plasma AUC (µg.h/L)	Roflumilast	39.8	59.2	519.8	663	961	32.8
	Roflumilast N-Oxide	1075	2806	11335	2145	3736	351
	ADCP [g]	132.0	286	429	134	134	4.1
	ADCP N-oxide	662	1492	1833	1042	1303	19.1 [c]
	Rof + Rof-NO + ACDP	1246	3152	12284	2942	4840	388.3
	Rof + Rof-NO + ACDP + ADCP N-oxide	1909	4644	14116	3984	6143	407
AUC ratio (ani./human)	Roflumilast	1.2	1.8	15.8	20.2	29.3	NA [d]
	Roflumilast N-Oxide	3.1	8.0	32.3	6.1	10.6	NA
	ADCP	32.1	69.9	104.6	32.7	34.9	NA
	ADCP N-oxide [f]	34.7	78.1	96.0	54.6	68.2	
	Rof + Rof N-oxide [e]	4.3	9.8	48.1	27.3	12.2	NA
	Rof + Rof-NO + ACDP	3.2	8.1	31.6	7.6	12.5	NA
	Rof + Rof-NO + ACDP + ADCP N-oxide	4.7	11.4	34.7	9.8	15.1	NA

a. Plasma AUC data in hamsters were based on the following data points: month 24 values for the 4 and 8 mg/kg/day groups and month 3 in the 16-mg/kg/day groups.
b. Plasma AUC data were based on month 24 values.
c. Human plasma ADCP N-oxide AUCs of19.1 µg.h/L the mean of 2 subjects (Patient Nos. 11 and 15, Clinical Report 58/200) on day 12. Data were provided by the clinical PK discipline via email on January 7, 2010.
d. NA, not applicable.
e. These ratios based on the totals of the compounds of interest.
f. The ratios for ADCP N-oxide did not consider its potential accumulation factor in humans. ADCP N-oxide apparently accumulates in hamsters, but its accumulation in humans was unknown. Accumulation of ADCP N-oxide in hamsters is indicated by the mean ADCP N-oxide AUCs over time: the respective mean ADCP N-oxide AUCs at months 1, 3 and 24 of 370, 378 and 662 µg.h/L at 4-mg/kg/day roflumilast and 662, 900 and 1495 µg.h/L at 8-mg/kg/day roflumilast.
g. ADCP, 4-amino-3,5-dichloropyridine; Rof, roflumilast; Rof NO; roflumilast N-oxide.

[1] These AUC ratios would be rounded to 35, 3 and 5 according to the rounding rules.

The applicant proposed the following text for the carcinogenicity section:

The review recommends revising the above text by moving the statement about the hamster tumor findings from the middle of the paragraph to the beginning. Based on the above discussion, the review suggests the following text for the Carcinogenesis section of roflumilast labeling.

> *Long-term studies were conducted in hamsters and mice with roflumilast to evaluate its carcinogenic potential. In 2-year oral gavage carcinogenicity studies, roflumilast treatment resulted in dose-related, statistically significant increases in the incidence of undifferentiated carcinomas of nasal epithelium in hamsters at ≥ 8 mg/kg/day (approximately 11 times the MRHD based on summed AUCs of roflumilast and its metabolites). The tumorigenicity of roflumilast appears to be attributed to a reactive metabolite of 4-amino-3,5-dichloro-pyridine N-oxide (ADCP N-oxide). No evidence of tumorigenicity was observed in mice at roflumilast oral doses up to 12 and 18 mg/kg/day in females and males, respectively (approximately 10 and 15 times the MRHD, respectively, based on summed AUCs of roflumilast and its metabolites).*

Mutagenesis

Genetic toxicity of roflumilast and its metabolites were tested in a number of assays. Roflumilast was positive for induction of micronucleus formation in an *in vivo* mouse micronucleus test, but was negative in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosome aberration assay in human lymphocytes, *in vitro* HPRT test with V79 cells, an *in vitro* micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and *in vivo* mouse bone marrow chromosome aberration assay. In the *in vivo* mouse micronucleus assay, roflumilast induced micronuclei at oral doses of 300 mg/kg at 48 hours and 900 mg/kg 24 and 48 hours. Roflumilast N-oxide was not mutagenic in the Ames and V79 in vitro micronucleus tests. The applicant proposed the following text for the mutagenesis section:

(b) (4)

The review recommends revising the above text by moving the statement about the positive findings from the end of the sentence to the beginning. The revised test would read as:

> *Roflumilast tested positive in an in vivo mouse micronucleus test, but negative in the following assays: Ames test for bacterial gene mutation, in vitro chromosome aberration assay in human lymphocytes, in vitro HPRT test with V79 cells, an in vitro micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and in vivo mouse bone marrow chromosome aberration assay. Roflumilast N-oxide was negative in the Ames test and in vitro micronucleus test with V79 cells.*

Impairment of Fertility

Effects of roflumilast on fertility were evaluated in rats and mice (Reports 19/97, 114/2002 and M33/2002). As described in the nonclinical review of the original NDA, Report 19/97 showed that roflumilast at 1.8-mg/kg/day decreases male fertility rate by 25%. Histology examination showed increased incidence of tubular atrophy, degeneration in the testis and spermiogenic Granuloma in the epididymides (Report 267/2008, p134). However, another study (Report 114/2002) showed no effect on female fertility parameters when treated female rats (at 0, 0.5, 0.8 and 1.5 mg/kg/day from 14 days prior to, through to 7 days after mating) were mated with untreated males. Neither did a mouse study when male mice were treated at up to 36-mg/kg/day roflumilast for 4 months and mated with untreated females (Report M33/2002). Overall, the NOAEL on the male reproductive system was 0.8 mg/kg/day (p90), corresponding to $AUCs_{0-24}$ of 30.9 and 1376.1 μg.h/L for roflumilast and the total of parent and metabolites, respectively (Report 256/2008, p8).

The applicant proposed the following text for the impairment of fertility section:

(b) (4)

(b) (4)

The above text should be revised. The statement about human findings should be moved to the beginning of the section, followed by positive fertility findings in males. The statement about negative fertility findings should be described later. Reference to lack of nonclinical findings in other species should be removed, as this statement is inaccurate (Ref. Tables 38 and 39 and Sections 2.6.6.9.3 and 2.6.6.9.4 of the original NDA review, p89-94). Accordingly, the review recommends replacing the proposed text (above) with the following:

> *In a human spermatogenesis study, roflumilast 500 mcg had no effects on semen parameters or reproductive hormones during the 3-month treatment period and the following 3-month off-treatment period. In a fertility study, roflumilast decreased fertility rates in male rats at 1.8-mg/kg/day (approximately 29 times the MRHD on a mg/m^2 basis). These rats also showed increases in the incidence of tubular atrophy, degeneration in the testis and spermiogenic granuloma in the epididymides. No effect on male rat fertility rate or reproductive organ morphology was observed at 0.8 mg/kg/day (approximately 13 times the MRHD on a mg/m^2 basis). No effect on female fertility was observed up to the highest roflumilast dose of 1.5 mg/kg/day in rats (approximately 24 times the MRHD on a mg/m^2 basis).*

(b) (4)

Animal to human exposure ratios for fertility and reproductive toxicology were calculated on a mg/m^2 comparison basis. (Note: Animal to human exposure ratios for carcinogenicity were calculated on an AUC comparison basis; see Table 4 above). The results of exposure ratio calculations are given in the following table:

Table 5: Animal to human exposure ratios based on mg/m^2 comparisons

Drug: **Roflumilast**

	age	mg/dose	# daily doses	mg/day	kg	mg/kg	factor	mg/m²
Pediatric				0	18	0.0190	25	0.48
Adult	>12	0.5	1	0.5	50	0.0100	37	0.37

	route	mg/kg/d	conv. factor	mg/m²	Dose Ratio Adults	Dose Ratio Children	Rounded Dose Ratio Adults	Rounded Dose Ratio Children
Reproduction and Fertility:								
mouse	p.o	1.5	3	4.5	12.16	N/A	12	N/A
mouse	p.o	2	3	6	16.22	N/A	16	N/A
mouse	p.o	6	3	18	48.65	N/A	49	N/A
mouse	p.o	12	3	36	97.30	N/A	97	N/A
rat	p.o	0.6	6	3.6	9.73	N/A	10	N/A
rat	p.o	0.8	6	4.8	12.97	N/A	13	N/A
rat	p.o	1.5	6	9	24.32	N/A	24	N/A
rat	p.o	1.8	6	10.8	29.19	N/A	29	N/A
Teratogenicity:								
mouse	p.o	1.5	3	4.5	12.16	N/A	12	N/A
rat	p.o	0.2	6	1.2	3.24	N/A	3	N/A
rabbit	p.o	0.8	12	9.6	25.95	N/A	26	N/A
rabbit			12	0	---	N/A	---	N/A
mouse			3	0	---	N/A	---	N/A

Conversion, Correction, and Rounding Factors:

Human Age (yr)	Weight (kg)	Factor (kg/m²)	Species	Factor (kg/m²)	Exposure greater than x-times human	Round to nearest
0	3	25	dog	20	1	1
1	10	25	guinea pig	8	10	5
2	12	25	hamster	4	100	10
4	16	25	monkey	12	1000	100
6	20	25	mouse	3	10000	1000
12	50	37	rabbit	12		
			rat	6		

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/s/

MARCIE L WOOD
01/26/2011

TIMOTHY W ROBISON
01/26/2011

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 22-522
Submission date: July 15, 2009
Drug: roflumilast
Sponsor: Forest Research Institute
Indication: maintenance treatment to reduce exacerbations of chronic obstructive pulmonary disease associated with chronic bronchitis in patients at risk of exacerbations

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Background Comments:

The pharmacology/toxicology reviewer and supervisor in the Division of Pulmonary, Allergy and Rheumatology Products have reviewed the nonclinical information for roflumilast and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Carcinogenicity:

The applicant conducted 2-year carcinogenicity studies in hamsters and mice. The Executive Carcinogenicity Assessment Committee concluded that these studies were adequate. The Committee concluded that there were no drug-related tumors in mice. The Committee concluded that roflumilast cause nasal tumors in male and female hamsters. Initially, these tumors were thought to be due to a rodent specific metabolite produced in the nasal tissues. However, subsequent data showed that the metabolite was also present in humans. The Committee concluded that the metabolite could not be considered rodent specific. The ultimate relevance of the nasal tumors in hamsters to humans is difficult to determine because the human tissues exposed to this metabolite and the levels in these tissues are unknown.

Reproductive and Developmental Toxicity:

Review of the reproductive and developmental toxicity studies revealed dose-related increases in stillbirths, maternal deaths, and decreases in pup viability in mice. Mice also showed decreases in pup viability and muscle strength and delay in pinna detachment when dams were treated with roflumilast during pregnancy and the lactation period. No teratogenic effects were observed in rats and rabbits.

Roflumilast decreased fertility in male rats and produced morphological changes in the reproductive organs in male mice, rats, hamsters and dogs. However, there are clinical data to address concerns about these nonclinical findings.

Conclusions:

I concur with the Division pharmacology/toxicology recommendation that this NDA can be approved. Specific labeling comments will be developed later.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22522	ORIG-1	FOREST RESEARCH INSTITUTE	DAXAS(ROFLUMILAST 500 MCG TABLETS

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/s/

PAUL C BROWN
05/14/2010

INTEROFFICE MEMO

TO: NDA 22-522 Original submission
Daxas Oral Tablets (roflumilast)

FROM: Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: March 31, 2010

Nycomed GmbH submitted their New Drug Application (NDA) 22-522 on July 15, 2009 for Daxas (roflumilast 500 mcg tablets) as a once daily maintenance treatment of chronic obstructive pulmonary disease (COPD) associated with chronic bronchitis in patients at risk of exacerbations. Roflumilast is a selective phosphodiesterase type-4 (PDE4) inhibitor, which is a new molecular entity and new drug class for COPD. On December 4, 2009, this NDA was transferred to the new applicant, Forest Research Institute, who amended their labeling to include a new indication for roflumilast and included new clinical information. No new nonclinical data were submitted at the time of NDA transfer on December 2009. Dr. Luqi Pei reviewed the nonclinical package of the NDA with previous reviews completed for the associated Investigational New Drug (IND), IND 57,883, by Dr. Timothy McGovern on 24-JUL-2000, 29-MAY-2001, and 13-JAN-2003 and by Dr. Luqi Pei on 27-JUN-2007, respectively. Dr. Marcie Wood assisted Dr. Pei in the review of the pharmacology and pharmacokinetic sections of the NDA. Based on the overall nonclinical NDA evaluation, Dr. Pei concluded that characterization of the toxicity profile of roflumilast was complete from the nonclinical perspective. There are no outstanding pharmacology or toxicology issues to be addressed. No recommendations for labeling are currently made to the nonclinical sections; however, review of the labeling with recommended changes is needed prior to concurring with approval from a nonclinical perspective. The following was excerpted from Dr. Pei's NDA review, which provides a brief summary of the relevant nonclinical observations made for roflumilast.

- ***Carcinogenicity:*** Roflumilast caused statistically significant increases in nasal tumors in 2-year oral studies in hamsters at 8 and 16-mg/kg/day. The carcinogenicity of roflumilast appears to be attributed to a metabolite, ADCP N-oxide that is further converted to a reactive intermediate, ADCP N-oxide epoxide in the nasal tissues. Both steps are catalyzed by Cytochrome enzyme P450 CYP 2G1 in rodents. Human nasal tissues appear to lack active enzymes to convert ADCP to ADCP N-oxide, but ADCP N-oxide is found in human plasma and urine. The Agency's Executive Carcinogenicity Assessment Committee (ECAC) reviewed the results and interpretation of the roflumilast carcinogenicity on May 10, 2005 and again on January 19, 2010. Based on the totality of the data, the

ECAC concluded that the ADCP-N-oxide metabolite does not appear to be rodent-specific and the hamster nasal tumor is no longer considered rodent specific. Relevance of the tumor finding to humans is unknown since the tissues and enzymes involved in the production of ADCP N-oxide and its potential down stream metabolite are unknown in humans.

- ***Pregnancy:*** Roflumilast treatment during pregnancy resulted in dose-related increases in stillbirths, maternal deaths, and decreases in pup viability in mice, although the drug is non-teratogenic in rats and rabbits. Fetal and maternal toxicities were related to the tocolytic effect of roflumilast. Roflumilast should be contraindicated for pregnant women unless the concern about its fetal toxicity and maternal health can be adequately addressed by clinical measures.
- ***Post-natal development:*** Mouse pups showed decreases in viability and muscle strength and delay in pinna detachment when dams were treated with roflumilast during pregnancy and the lactation period. Roflumilast should not be used in lactating women.
- ***Male fertility:*** Roflumilast decreased fertility in rats and morphological changes in the reproductive organs in male mice, rats, hamsters and dogs.
- ***Cardiovascular system:*** Male mice treated with ≥12-mg/kg/day roflumilast for 6-months showed moderate peri-arteritis in the heart. Arteritis appears to be a class effect of PDE4 inhibitors and has been observed in multiple organs and species in some drugs. This effect of roflumilast was observed in male mice only.
- ***Gastrointestinal tract:*** Roflumilast treatment-related effects on the gastrointestinal (GI) tract were observed in rats, dogs and monkeys; but not in mice and hamsters. Changes may include intestinal serositis/inflammation, peritonitis, and stomach erosion. The GI effect is also known for PDE4 inhibitors.

_____

Molly E. Shea, Ph.D.
Pharmacology/Toxicology Supervisor

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22522	ORIG-1	FOREST RESEARCH INSTITUTE	DAXAS(ROFLUMILAST 500 MCG TABLETS

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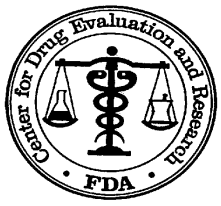
/s/

MOLLY E SHEA
03/31/2010



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-522
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	July 15, 2009
PRODUCT:	Daxas Oral tablets
INTENDED CLINICAL POPULATION:	Maintenance treatment of chronic obstructive pulmonary disease (COPD) associated with chronic bronchitis in patients at risk of exacerbations
SPONSOR:	Forest Research Institute
DOCUMENTS REVIEWED:	Original NDA Submission
REVIEW DIVISION:	Division of Pulmonary, Allergy and Rheumatoid Arthritis Products
PHARM/TOX REVIEWER:	Luqi Pei, Ph.D.
PHARM/TOX SUPERVISOR:	Molly Shea, Ph.D.
DIVISION DIRECTOR:	Badrul Chowdhury, M.D., Ph.D.
PROJECT MANAGER:	Miranda Raggio

Date of review submission to the Document Archiving, Reporting and Regulatory Tracking System (DARRTS): March 19, 2010

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The applicant has submitted adequate nonclinical data to support its approval from the nonclinical perspective. The applicant has submitted all required nonclinical studies to characterize the nonclinical safety profile of the roflumilast. Major findings of the nonclinical studies include: 1) nasal tumors in hamsters, 2) stillbirths and maternal health in pregnant mice, 3) delay in post-natal development in mice, 4) decreases in fertility in male rats, and 5) cardiac and gastrointestinal effects. Nonclinical concerns about the above findings can be addressed through clinical evaluations and labeling. From the nonclinical perspective, approval of the application is recommended if the clinical safety evaluation and overall risk-benefit analysis warrants the approval of the application.

B. Recommendation for nonclinical studies

No additional nonclinical studies are recommended as the applicant has completed the required pharmacology and toxicology studies in need to support their NDA.

C. Recommendations on labeling

No labeling recommendation is available because the labeling review is deferred to a later time.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Roflumilast is a selective phosphodiesterase type-4 (PDE4) inhibitor that possesses bronchodilatory and anti-inflammatory properties. Roflumilast inhibited PDE4 activity and decreased production of inflammatory cytokines in vitro and in vivo. Results of safety pharmacology studies suggest that roflumilast decreases the threshold for penetrazole-induced seizures and coordination capacity in mice, increases tonic convulsions and lethality, and shortened time to tonic convulsions in mice, and. prolongs hexobarbitol-induced loss of righting reflex and increases ethanol-induced sleeping time in rats.

Roflumilast is readily absorbed after oral administration. Maximum plasma concentrations after oral administrations were reached within an hour in mice, rats and rabbits, approximately 4 hours in hamsters and dogs, and 8 hours in humans, respectively. The oral bioavailability of roflumilast was 25%, 11%, 7%, 9%, 19%, 48% and 64% in mice, rats, hamsters, rabbits, dogs, monkeys, and humans, respectively.

Roflumilast in the plasma is present predominantly in bound form (95% - 98.5%). After oral administration, fecal excretion is predominant in rodents and dogs (60% – 90%) while urine excretion is predominant in humans (70%). Roflumilast is metabolized to three major metabolites: roflumilast N-oxide, amino-dichloro-pyridine (ADCP) and ADCP N-oxide. Roflumilast N-oxide is the most abundant among all species and its concentrations are significantly higher than roflumilast.

Results of toxicity studies showed that roflumilast is carcinogenic and tocolytic but is non-genotoxic and non-teratogenic. Roflumilast caused statistically significant increases in nasal tumors in 2-year oral studies in hamsters at 8 and 16-mg/kg/day, but not at 4 mg/kg/day. Mice did not show any evidence of tumorigenicity at $\leqslant$ 18 mg/kg/day. Roflumilast may cause significant harm to fetus and dams when given to pregnant mice: statistically significant increases in the incidence of stillborns were observed at $\leqslant$2 mg/kg/day; statistically significant increases in the incidence of maternal deaths were observed at 12 mg/kg/day. Roflumilast decreased fertility rates in male rats at 1.8 mg/kg/day. Roflumilast was not teratogenic at doses up to 1.8 and 0.8 mg/kg/day in rats and rabbits, respectively, but incomplete bone ossification was observed at $\leqslant$ 0.6 mg/kg/day. Roflumilast at 12 mg/kg/day affected post-natal development in mouse pups when dams were exposed to the drug during pregnancy and lactation.

The target organs of toxicity for roflumilast included the nose and the cardiovascular, gastrointestinal tract and reproductive systems. Significant non-neoplastic nasal lesions (epithelial disorganization, degeneration, necrosis and nerve fiber atrophy of the olfactory area) were limited to the rodent species (mice, rats and hamsters). Cardiovascular changes (focal hemorrhage, myocarditis, or vascularitis) were observed in mice, dogs and monkeys. Changes in the gastrointestinal tract (serositis, inflammation, peritonitis, and stomach erosion) were observed in rats and monkeys. Changes in the male reproductive organs were observed in mice, rats, hamsters and dogs. The affected organs may include the following: the prostate (atrophy), testes (tubular atrophy degeneration and atrophy, spermatogenic disturbances), epididymides (oligospermia and granuloma), and seminal vesicles (atrophy). Changes (uterine and cervical atrophy) in female reproductive organs were observed in mice. Disruption of female reproductive physiology (decreases in estrus events and estradiol levels) were observed in rats and monkeys. The overall NOAEL values in mice, rats, hamsters, dogs and monkeys were 4, 0.8, 4, 0.6 and 0.25 mg/kg/day in nominal doses, respectively, and 153.1, 30.9, 510.1, 253.3 and 32.8 μg.h/L in plasma AUCs, respectively. See Section 2.6.6.1 – Overall Toxicology Summary for detailed descriptions of the dose-response relationship of the findings.

B. Pharmacologic activity

Roflumilast is a selective phosphodiesterase type-4 (PDE4) inhibitor (IC_{50} = 0.3 μg/L) that possesses bronchodilatory and anti-inflammatory properties. Both properties are attributed to its ability to increase the intracellular cAMP, an intracellular messenger that is present in many cells. Roflumilast increases cAMP by preventing its hydrolysis by phosphodiesterases. Increases in cAMP levels in tracheobronchial smooth muscles

result in muscle relaxation and bronchial dilation. The increases of cAMP levels in some inflammatory cells result in functional suppression of the cells.

C. Nonclinical safety issues relevant to clinical use

A number of issues identified in the nonclinical safety evaluation may be relevant to the clinical use of roflumilast. These issues include carcinogenicity, reproductive and developmental toxicity, and roflumilasts effects on pregnancy, the cardiovascular system and the gastrointestinal tract.

Carcinogenicity: Roflumilast caused statistically significant increases in nasal tumors in 2-year oral studies in hamsters at 8 and 16-mg/kg/day, but not at 4 mg/kg/day. The carcinogenicity of roflumilast appears to be attributed to a metabolite, ADCP N-oxide that is further converted to a reactive intermediate, ADCP N-oxide epoxide in the nasal tissues. Both steps are catalyzed by Cytochrome enzyme P450 CYP 2G1 in rodents. The epoxide would result in DNA adduct formation, protein crosslinking and tumor formation. Human nasal tissues appear to lack active enzymes to convert ADCP to ADCP N-oxide, but ADCP N-oxide is found in human plasma and urine. Relevance of the tumor finding to humans is unknown since the tissues and enzymes involved in the production of ADCP N-oxide and its potential down stream metabolite are unknown in humans.

Pregnancy: Roflumilast treatment during pregnancy resulted in dose-related increases in stillbirths, maternal deaths, and decreases in pup viability in mice, although the drug is non-teratogenic in rats and rabbits. Fetal and maternal toxicities were related to the tocolytic effect of roflumilast. Roflumilast should be contraindicated for pregnant women unless the concern about its fetal toxicity and maternal health can be adequately addressed by clinical measures.

Post-natal development: Mouse pups showed decreases in viability and muscle strength and delay in pinna detachment when dams were treated with roflumilast during pregnancy and the lactation period. Roflumilast should not be used in lactating women.

Male fertility: Roflumilast decreased fertility in rats and morphological changes in the reproductive organs in male mice, rats, hamsters and dogs. However, there are clinical data to address concerns about these nonclinical findings.

Cardiovascular system: Male mice treated with ≥12-mg/kg/day roflumilast for 6-months showed moderate peri-arteritis in the heart. Arteritis appears to be a class effect of PDE4 inhibitors and has been observed in multiple organs and species in some drugs. This effect of roflumilast was observed in male mice only.

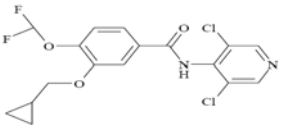
Gastrointestinal tract: Roflumilast treatment-related effects on the gastrointestinal (GI) tract were observed in rats, dogs and monkeys; but not in mice and hamsters. Changes may include intestinal serositis/inflammation, peritonitis, and stomach erosion. The GI effect is also known for PDE4 inhibitors.

Nonclinical data submitted in the application have adequately addressed all of the above nonclinical issues except for the carcinogenicity and male reproductive toxicity. Clinical data appear to have addressed the nonclinical concerns about male

reproductive toxicity of roflumilast. Concerns about carcinogenic potential of roflumilast can be addressed in the clinical risk-benefit analysis of the application.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA Number:	22-522
Review Number :	1
Sequence number/date/submission type:	000/17-JUL-2009/Orginal submission
Information to the Sponsor:	Yes ( ), No (x)
Sponsor/or Agent:	Forest Research Institute, Harborside Financial Center, Plaza Five, Site 1900, Jersey City, NJ 07311
Manufacturer of the Drug Substance:	Nycomed GmbH Production Site Singen Robert-Bosch-Strasse 8, 78224 Singen, Germany
Reviewer Name:	Luqi Pei, Ph.D.
Division Name:	Pulmonary, Allergy and Rheumatoid Products
Review Completion Date:	March 19, 2010 (draft 2)
Drug:	
Trade Name:	Daxas (Roflumilast) oral tablets
Generic Name:	Roflumilast
Code Name:	BY 217, B9302-107, BYK20869
Chemical Name:	3-(cyclopropylmethoxy)-N-(3,5-dichloropyridin-4-yl)-4-(difluoromethoxy)benzamide (IUPAC), or N-(3,5-dichloropyridin-4-yl)-3-cyclopropylmethoxy-4-difluoromethoxybenzamide
Structure:	
CAS Register Number:	162401-32-3
Mole File Number:	N/A
Molecular Form and Weight:	$C_{17}H_{14}Cl_2F_2N_2O_3$, 403.2
Relevant IND/NDAs:	IND 57,883
Drug Class:	Phosphodiesterase (PDE4) inhibitor
Intended clinical population:	Maintenance treatment of chronic obstructive pulmonary disease (COPD)
Route of Administration:	Oral (tablets)

Clinical Formulations: Oral tablets contain 0.5-mg roflumilast, (b) (4) lactose, (b) (4) maize starch, (b) (4) povidone and (b) (4) magnesium stearate. The tablet is coated with a film consisting of (b) (4) hypromellose, (b) (4) Macrogol 4000, (b) (4) titanium dioxide, (b) (4) yellow iron oxide pigment. (b) (4)

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

Studies reviewed within this submission:

Study #	Description	Location
	Primary Pharmacodynamics:	
108/2004	Duration of action of orally administered roflumilast on tumor necrosis factor alpha release in lipopolysaccharide-challenged Sprague-Dawley rats	4.2.1.1
109/2004	Effect of orally administered roflumilast on tumor necrosis factor alpha release in lipopolysaccharide-challenged BALB/c mice	4.2.1.1
113/2004	The effect of i.v. roflumilast N-oxide on serotonin-induced bronchoconstriction, baseline lung mechanics and cardiovascular parameters in anaesthetized, mechanically ventilated Brown-Norway rats	4.2.1.1
130/2000	Inhibition of antigen-induced contraction of guinea-pig trachea in vitro by roflumilast N-oxide	4.2.1.1
136/2004	Effect of roflumilast in an acute and a chronic model of cigarette smoke exposure	4.2.1.1
137/2004	An investigation into the effects of roflumilast, a PDE4 inhibitor, in a subchronic tobacco smoke-induced inflammation model of COPD in guinea pigs	4.2.1.1
172/2000	Inhibition of the luminol-enhanced chemiluminescence response in human eosinophils by roflumilast N-oxide	4.2.1.1
172/2003	Effect of roflumilast and its N-oxide on the activity of phosphodiesterase (PDE)1-11 isoenzymes and various PDE4 splicing variants	4.2.1.1
172/2004	The inhibition by orally administered roflumilast of pulmonary monocyte chemotactic protein and macrophage inflammatory protein-1alpha accumulation induced by intratracheal lipopolysaccharide administration in Brown Norway rats	4.2.1.1
174/2003	Cloning, expression and biochemical characterization of recombinant human phosphodiesterase enzymes	4.2.1.1
175/2000	Effect of orally administered roflumilast on tumor necrosis factor-alpha release in lipopolysaccharide-challenged Sprague Dawley rats	4.2.1.1
182/2000	Effect of orally administered roflumilast N-oxide on tumor necrosis factor-alpha release in lipopolysaccharide-challenged Sprague Dawley rats	4.2.1.1
186/2001	The effect of i.d. roflumilast on serotonin-induced bronchoconstriction, baseline lung mechanics and cardiovascular parameters in anaesthetized, mechanically ventilated Brown-Norway rats	4.2.1.1
189/2005	Further investigation into the effects of roflumilast in a sub chronic TS-induced inflammation model of COPD in guinea pigs	4.2.1.1
20/2005	Effect of roflumilast and its N-oxide on anti-CD3-/anti-CD28- triggered interleukin-2 (IL-2) release from human peripheral blood CD8+ T-cells	4.2.1.1
21/2005	Effect of roflumilast and its N-oxide on anti-CD3-triggered granzyme B release from human peripheral blood CD8+ T-cells	4.2.1.1
216/99	Effect of orally administered roflumilast on tumor necrosis factor-alpha and interleukin-10 cytokine release in lipopolysaccharide-challenged Balb/c mice	4.2.1.1
228/2003	Inhibition by roflumilast of serotonin-induced bronchoconstriction in precision-cut lung slices from Brown-Norway rats	4.2.1.1
229/2000	Inhibition of anti-CD3/CD28 mAb-stimulated human CD4+ T-cell functions by roflumilast N-oxide	4.2.1.1
233/2002	The inhibition by orally administered roflumilast of pulmonary neutrophil accumulation and tumor necrosis factor alpha release induced by intratracheal lipopolysaccharide administration in Brown Norway rats	4.2.1.1
235/99K	Tracheal muscle relaxant effect of roflumilast N-oxide in guinea-pig isolated trachea	4.2.1.1
254/98	Inhibition of the lipopolysaccharide-induced tumor necrosis factor-alpha release in human whole blood by roflumilast N-oxide	4.2.1.1

Study #	Description	Location
277/2008	Effects of roflumilast N-oxide on proliferation, expression of a-smooth muscle actin, connective tissue growth factor and fibronectin of human lung fibroblasts	4.2.1.1
278/2008	Roflumilast N-oxide inhibits expression of ICAM-1 and eotaxin release induced by TNFalpha in human lung fibroblasts in vitro	4.2.1.1
279/2008	Effects of roflumilast on bleomycin-induced lung fibrotic and pulmonary vascular remodeling in mice and rat in comparison to glucocorticoids in a therapeutic vs preventive protocol	4.2.1.1
280/2008	Effects of roflumilast N-oxide on TGFß1-induced epithelial-mesenchymal transition (EMT) of human airway epithelial cells (A549)	4.2.1.1
281/2008	Effects of roflumilast N-oxide on proliferation of human bronchial smooth muscle cells in vitro	4.2.1.1
282/2008	Roflumilast inhibits human lung fibroblast-driven collagen gel contraction and lung fibroblast chemotaxis in vitro	4.2.1.1
296/98	Inhibition of human polymorphonuclear leukocyte functions by roflumilast N-oxide	4.2.1.1
3/2000	Inhibition of the luminol-enhanced chemiluminescence response in human eosinophils by roflumilast	4.2.1.1
315/2006	Effect of roflumilast on airway ciliary beat frequency in proximal and distal airways in rat precision-cut lung slices	4.2.1.1
320/2006	The effect of roflumilast on lipopolysaccharide-induced goblet cell hyperplasia in rats	4.2.1.1
320/2007	Effects of orally and intratracheally administered roflumilast and roflumilast N-oxide on leukocyte accumulation in 16h-bronchoalveolar lavage fluids of lipopolysaccharide aerosol challenged Sprague Dawley rats	4.2.1.1
321/2003	Inhibition by roflumilast N-oxide of serotonin-induced bronchoconstriction in precision-cut lung slices from Brown-Norway rats	4.2.1.1
321/2006	The effect of roflumilast on ovalbumin-induced goblet cell hyperplasia in rats	4.2.1.1
332/2005	The effect of orally administered roflumilast on leukotriene D4-induced bronchoconstriction in anaesthetized, mechanically ventilated guinea pigs	4.2.1.1
388/2005	Effect of roflumilast, roflumilast N-oxide, montelukast and theophylline to inhibit histamine-evoked contraction in isolated guinea-pig trachea	4.2.1.1
389/2005	Effect of roflumilast, roflumilast N-oxide, theophylline and montelukast to inhibit leukotriene LTD4-evoked contraction in isolated guinea-pig trachea	4.2.1.1
42/2000	Inhibition of anti-CD3/CD28 mAb-stimulated human CD4+ T-cell function by roflumilast	4.2.1.1
425/2006	Immunohistochemical investigation of the anti-inflammatory effects of roflumilast on the lungs of mice chronically exposed to cigarette smoke	4.2.1.1
45/99	Inhibition of the lipopolysaccharide-induced tumor necrosis factor-alpha release in human monocytes and monocyte-derived dendritic cells and macrophages by roflumilast N-oxide	4.2.1.1
457/2006	Effects of roflumilast and its N-oxide on fMLP-induced surface CD11b expression on human neutrophils in vitro	4.2.1.1
458/2006	Effect of roflumilast and its N-oxide on leukocyte-endothelial interaction, E-/P-selectin expression, and permeability of human endothelial cells in vitro	4.2.1.1
459/2006	Effects of roflumilast on LPS-induced leukocyte-endothelial interactions in rat mesenteric postcapillary venules in vivo	4.2.1.1
460/2006	Effects of roflumilast on histamine-induced rat mesenteric microvascular permeability in vivo	4.2.1.1
461/2006	Effects of roflumilast on pulmonary vascular remodeling and pulmonary hypertension induced by monocrotaline or chronic hypoxia in rats in vivo	4.2.1.1
462/2006	Effect of roflumilast N-oxide on endothelin-1 release from human pulmonary artery smooth muscle cells in vitro	4.2.1.1
463/2006	Effects of roflumilast on bleomycin-induced lung injury in mice in vivo	4.2.1.1
56/2006	Evaluation of roflumilast in a methacholine induced model of bronchoconstriction	4.2.1.1
57/2006	Evaluation of roflumilast and methylprednisolone in a tobacco smoke-induced inflammation model of COPD in the guinea pig	4.2.1.1

Study #	Description	Location
58/2006	Evaluation of roflumilast and compound L-NIL in a tobacco smoke-induced inflammation model of COPD in C57BL/6 mice	4.2.1.1
79/2007	Effect of roflumilast, rolipram, terbutaline and forskolin on airway ciliary beat frequency in proximal and distal airways in rat precision-cut lung slices	4.2.1.1
99/2001	The inhibition of TNFalpha production in murine macrophage-derived RAW 264.7 cells by roflumilast	4.2.1.1
	Secondary Pharmacodynamics	
105/95K1	The effect of orally administered roflumilast on cell accumulation and protein concentration in BAL of ovalbumin-sensitized Brown-Norway rats	4.2.1.2
111/2002	Time course of cellular, biochemical, and histological changes and the effect of 5 micromol/kg p.o. roflumilast in the ovalbumin-induced lung inflammation model in Brown Norway rats	4.2.1.2
164/99	Investigation on the possible interaction of roflumilast with muscarinic M2-receptors in rat left atrium	4.2.1.2
166/99	Investigation on the possible interaction of roflumilast with smooth muscle muscarinic M2 receptors in rabbit isolated vas deferens	4.2.1.2
176/2006	Differential suppression of inflammatory cytokines by roflumilast and dexamethasone in a mouse model of chronic asthma	4.2.1.2
186/99	Effects of orally administered roflumilast on cell accumulation and protein concentration in 48h-bronchoalveolar lavage fluid of ovalbumin-sensitized/challenged Brown-Norway rats	4.2.1.2
187/99	Effects of orally administered roflumilast N-oxide on cell accumulation and protein concentration in 48h-bronchoalveolar lavage fluid of ovalbumin-sensitized/challenged Brown-Norway rats	4.2.1.2
207/2001	Effects of roflumilast on collagen II arthritis in the rat	4.2.1.2
208/99	Investigation on the possible interaction of roflumilast with muscarinic M1-receptors in rabbit isolated vas deferens	4.2.1.2
218/99	Investigation on the possible interaction of roflumilast N-oxide with muscarinic M3- and histamine H1-receptors in guinea-pig isolated ileum	4.2.1.2
219/99	Investigation on the possible interaction of roflumilast N-oxide with muscarinic M1- and M2-receptors in rabbit isolated vas deferens	4.2.1.2
220/99	Investigation on the possible interaction of roflumilast N-oxide with alpha1A-adrenoceptors in isolated rat vas deferens	4.2.1.2
221/99	Investigation on the possible interaction of roflumilast N-oxide with adenosine A2B-receptors in guinea-pig thoracic aorta	4.2.1.2
227/99	Investigation on the possible interaction of roflumilast N-oxide with adenosine A1-receptors in rabbit isolated vas deferens	4.2.1.2
228/2002	The inhibition by orally administered roflumilast of SRS-A mediated bronchoconstriction and pulmonary leukotriene release in anaesthetized, mechanically ventilated guinea pigs	4.2.1.2
228/99	Investigation on the possible interaction of roflumilast N-oxide with alpha1B-adrenoceptors in guinea-pig spleen strip	4.2.1.2
229/99	Investigation on the possible interaction of roflumilast N-oxide with cardiac muscarinic M2-receptors in guinea-pig left atrium	4.2.1.2
23/99	The inhibition of the SRS-A mediated bronchoconstriction in anaesthetized, mechanically ventilated guinea-pigs by p.o. roflumilast N-oxide	4.2.1.2
230/2001	Investigation into the possible interaction of roflumilast with neuronal dopamine DA2-receptors in the isolated, electrically stimulated mouse vas deferens	4.2.1.2
230/99	Investigation on the possible interaction of roflumilast N-oxide with alpha2-adrenoceptors on adrenergic nerve terminals in rabbit vas deferens and cholinergic nerve terminals in guinea-pig ileum	4.2.1.2
231/99	Investigation on the possible interaction of roflumilast N-oxide with adenosine A2A-receptors in the guinea-pig Langendorff-heart	4.2.1.2

Study #	Description	Location
237/99	Resistance of the tracheal muscle relaxant effect of roflumilast N-oxide in guinea-pig isolated trachea to beta2-adrenoceptor blockade	4.2.1.2
24/2001	The inhibition by p.o. roflumilast of airway hyperresponsiveness to adenosine in ovalbumin-sensitized, anaesthetized, mechanically ventilated Brown-Norway rats 3h after allergen challenge	4.2.1.2
242/99	Resistance of the tracheal muscle relaxant effect of roflumilast in guinea-pig isolated trachea to beta2-adrenoceptor blockade	4.2.1.2
265/99	Effects of orally administered roflumilast on cell accumulation and tumor necrosis factor-alpha concentration in 48h-bronchoalveolar lavage fluid of ovalbumin-sensitized/ challenged Brown-Norway rats	4.2.1.2
39/2001	The inhibition by p.o. roflumilast of airway hyperresponsiveness to acetylcholine in ovalbumin-sensitized, anaesthetized, spontaneously breathing Brown-Norway rats after 48 h after allergen challenge	4.2.1.2
399/2006	Anti-inflammatory effect of roflumilast in a mouse model of an acute exacerbation of chronic asthma	4.2.1.2
53/2005	Study on effects of intragastrically administered roflumilast vs. montelukast on the experimentally allergen-induced lung reaction in Aspergillus fumigatus-sensitized BALB/c mice	4.2.1.2
8/2003	Pharmacologic inhibition of inflammation and remodeling in murine chronic asthma	4.2.1.2
	Safety Pharmacology	
113/99	Effects on renal function and serum glucose after single oral administration of roflumilast N-oxide to rats	4.2.1.3
120/2003	Roflumilast N-oxide: In vitro effect on hERG current (IKr) as expressed in human embryonic kidney (HEK) cells	4.2.1.3
20/2003	Roflumilast: In vitro effect on hERG current (IKr) as expressed in human embryonic kidney (HEK) cells	4.2.1.3
36/2001	Roflumilast: Effects on the corneal reflex in the conscious rabbit (D00.208/3)	4.2.1.3
	Pharmacodynamic Drug Interaction	
110/2007	The effect of i.v. roflumilast in combination with (R,R)-formoterol on serotonin-induced bronchoconstriction, baseline lung mechanics, and cardiovascular parameters in anaesthetized, mechanically ventilated Brown-Norway rats	4.2.1.4
119/2006	Cardiovascular effects after short-time i.v. infusions of roflumilast in combination with roflumilast N-oxide and BYK318597 (sildenafil citrate) in anaesthetized minipigs	4.2.1.4
233/99	Investigation on the possible synergism between roflumilast N-oxide and (-)-isoprenaline at beta2-adrenoceptor-mediated relaxation in guinea-pig trachea	4.2.1.4
254/99	Investigation on the possible synergism between roflumilast N-oxide and (-)-isoprenaline at beta1-adrenoceptor-mediated effects in guinea-pig right atrium	4.2.1.4
295/2004	The inhibition by orally administered roflumilast in combination with ceterizine of ovalbumin-induced bronchoconstriction in anaesthetized, mechanically ventilated guinea pigs	4.2.1.4
33/2003	The effect of i.v. roflumilast in combination with formoterol on serotonin-induced bronchoconstriction, baseline lung mechanics, and cardiovascular parameters in anaesthetized, mechanically ventilated Brown- Norway rats	4.2.1.4
355/2006	Evaluation of a combination of a steroid and a PDE4 inhibitor, roflumilast, in a tobacco smoke-induced inflammation model of COPD in C57BL/6 mice	4.2.1.4
39/2003	The inhibition by orally administered roflumilast in combination with montelukast of SRS-A-mediated bronchoconstriction in anaesthetized, mechanically ventilated guinea pigs	4.2.1.4
396/2005	The inhibition of methacholine-induced bronchoconstriction in anaesthezied, ventilated guinea pigs by roflumilast, revatropate, and the combination of both	4.2.1.4
397/2005	The inhibition of methacholine-induced bronchoconstriction in anaesthezied, ventilated guinea pigs by roflumilast, tiotropium-bromide, and the combination of both	4.2.1.4

Study #	Description	Location
	Absorption	
100/2002	Absorption and absolute bioavailability of roflumilast in hamsters after oral administration	4.2.2.2
102/2002	The disposition of [^{14}C-ADCP]-roflumilast in the Cynomolgus monkey following oral and intravenous administration	4.2.2.2
66/2002	Pharmacokinetics of [^{14}C]-roflumilast in the rabbit: balance excretion, serum kinetics and metabolism after a single intravenous and oral dose	4.2.2.2
99/2002	Absorption and absolute bioavailability of roflumilast in the mouse after oral administration	
	Distribution	
105/2002	Tissue distribution of [^{14}C-carbonyl]-roflumilast in the rat following a single oral dose	4.2.2.3
106/2002	Gender differences in tissue distribution and metabolism following a single oral dose of [^{14}C]-roflumilast to hamsters	4.2.2.3
195/2008	In vitro plasma protein binding of [^{3}H]-roflumilast and [^{3}H]-roflumilast N-oxide in different species	4.2.2.3
285/99	Tissue distribution of [^{14}C]-roflumilast N-oxide in rats following a single intravenous and oral dose	4.2.2.3
85/2001	Placental transfer and mammoglandular passage of [^{14}C]-roflumilast in the rat	4.2.2.3
94/2000	Whole-body autoradiographic study of [^{14}C]-roflumilast N-oxide in the rat following a single intravenous and oral dose	4.2.2.3
96/2002	In vitro plasma/serum protein binding of [^{3}H]-roflumilast and [^{3}H]-roflumilast N-oxide in different species, including human	4.2.2.3
	Metabolism	
107/2002	Metabolism of roflumilast in plasma of the species man, rat, dog, hamster and mouse	4.2.2.4
122E/99	Roflumilast and dichloroaminopyridine. Comparative in vitro metabolism using microsomes prepared from nasal olfactory and respiratory epithelium and liver of rat, mouse, hamster, dog, monkey and human	4.2.2.4
178/99	Pilot study on the in vitro metabolism of roflumilast using rat precision-cut liver slices and rat liver microsomes as compared to related compounds	4.2.2.4
20/2002	Bioactivation of ADCP N-oxide in hamster nasal mucosa	4.2.2.4
21/2002	Assessment of the potential for bioactivation of ADCP in rat nasal mucosa and its relevance for humans	4.2.2.4
210/2002	Summary on distribution and identification of [^{14}C]-roflumilast and its metabolites in mouse, rat, hamster, dog, monkey and human urine	4.2.2.4
213/2002	Metabolite profiling of [^{14}C]-roflumilast in mouse urine	4.2.2.4
214/2002	Metabolite profiling of [^{14}C]-roflumilast in Cynomolgus monkey urine	4.2.2.4
22/2002	Assessment of the potential for bioactivation of ADCP N-oxide in rat nasal mucosa and its relevance for humans	4.2.2.4
254/2002	Metabolite profiling of [^{14}C]-roflumilast in hamster urine	4.2.2.4
265/2002	Metabolite profiling of [^{14}C]-roflumilast in dog urine	4.2.2.4
268/2002	Metabolite profiling of [^{14}C]-roflumilast in rat urine	4.2.2.4
299/2008	Radioprofiling of [^{14}C]-ADCP N-oxide incubations with animal and human CYP1A2 and CYP2G1	4.2.2.4
36/2005	Comparative in vitro metabolism of [^{14}C]-roflumilast in rat, hamster, and human liver S9 fractions and aroclor-induced rat liver S9 fraction	4.2.2.4
48/2000	Study on the in vitro metabolism of roflumilast in hamsters using precision-cut liver slices	4.2.2.4
48/2005	Assessment of ADCP N-oxide metabolism by cytochrome P450 enzymes potentially expressed in human nasal mucosa	4.2.2.4
90E/99	In vitro evaluation of roflumilast as an inducer of microsomal cytochrome P450 expression in rat and human hepatocytes	4.2.2.4

Study #	Description	Location
	Toxicology	
	General toxicity – Repeat Dose	
197/2001	6 month toxicokinetics of roflumilast in the mouse following oral administration at different dose levels	4.2.3.2
232/2001	Combined 4 week/42 week oral (gavage) administration toxicity study in adult cynomolgus monkey with an 8 week treatment-free period	
33/2002	Roflumilast: 26 week repeated dose oral toxicity (gavage) study in the B6C3F1 mouse, including testing on the male fertility	4.2.3.2
34/2002	Comparison of the spermatological data in beagle dogs found in the repeated dose toxicity studies roflumilast (parent compound) and roflumilast N-oxide (metabolite)	4.2.3.2
35/2002	Historical data on the semen quality of beagle dogs bred for experimental studies	4.2.3.2
52/2002	6 month toxicokinetics of roflumilast N-oxide in the mouse following oral administration at different dose levels	4.2.3.2
54/2002	Roflumilast N-oxide: 26 week repeated dose oral toxicity (gavage) study in the B6C3F1 mouse, including testing on the male fertility	4.2.3.2
	Genetic toxicity	
12/2005	Salmonella typhimurium and Escherichia coli reverse mutation assay for azo-dyes with roflumilast	4.2.3.3
225E/99	Reverse mutation assay using bacteria (Salmonella typhimurium) with roflumilast N-oxide	4.2.3.3
67/97	Action of roflumilast on mutations affecting the hypoxanthine-guanine phosphoribosyl transferase locus in V79 cells (HPRT test)	4.2.3.3
135/99	Micronucleus test with V79-cells in vitro with roflumilast N-oxide	4.2.3.3
143/2002	32P-Postlabeling assay for detection of adduct formation by roflumilast in hamster nasal mucosa and liver DNA	4.2.3.3
	Reproductive Toxicology	
114/2002	Toxicity of roflumilast for reproduction. Study for effects on female fertility and early embryonic development to implantation in the rat, p.o.	4.2.3.5
125/2002	Study for effects on pre- and postnatal development with roflumilast in NMRI mice	4.2.3.5.3
126/2002	Study to investigate the effects of roflumilast on delivery in NMRI mice including toxicokinetics in dams and fetuses	4.2.3.5.3
127/2002	Study for effects on pre- and postnatal development with roflumilast in NMRI mice	4.2.3.5.3
	Other Studies	
	Mechanistic studies	
19/2003	Mechanistic studies on the cytochrome P450-dependent metabolism and toxicity of roflumilast and ADCP in the rat olfactory epithelium	4.2.3.7.2
	Other studies	
225/2002	Expert report on roflumilast: Effect on male reproductive organs	4.2.3.7.7
255/2008	Roflumilast: Causal relationship between olfactory metabolism and olfactory toxicity in rodents	4.2.3.7.7
256/2008	Roflumilast: Safety assessment for male reproductive parameters	4.2.3.7.7
259/2008	Assessment of the tumorigenic potential of roflumilast	4.2.3.7.7

Studies not reviewed within this submission (1): The following studies were not reviewed in this NDA submission as they were reviewed previously by Dr. Timothy McGovern on 24-JUL-

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(b) (4)

Drug History:

This new drug application of Roflumilast (NDA 22-522) was developed under IND 57,883 which was opened on February 16, 1999. During the life span of the IND, the sponsor has used several names: Byk Goulden (1999 – 2002), Altana (2002 – 2006), Nycomed GmbH (2006 – 2009) and Forrest Research Institute (since December 4, 2009). Pfizer cosponsored the IND for a brief period of time (2002 – 2005).

Several names and code names were used for the active ingredient. They included roflumilast, Daxas, B9302-107, BYK20889 and APTA-2217. Table 1 lists code names used for roflumilast and its metabolites in the submission. The current review will use the generic names only.

Table 1 Compounds and Their Code Names

Compound name	Compound codes	Chemical name
Roflumilast	B9302-107, BYK20869, APTA-2217	N-(3,5-dichloropyridin-4-yl)-3-cyclopropyl-methoxy-4-difluoromethoxybenzamide
Roflumilast N-oxide	B9502-044, BYK22890	
ADCP	B9202-045, BYK20139	4-Amino-3,5-dichloro-pyridine
ADCP N-oxide	B9502-054, BYK22900	4-Amino-3,5-dichloro-pyridine N-oxide
Roflumilast-acid	B9302-102, BYK20864, Roflumilast-Saeure	3-Cyclopropylmethoxy-4-difluoromethoxy-benzoic acid

DPAP had numerous communications with the sponsor to discuss issues of the drug development program during the IND stage. Major communications included meetings and telephone conferences. Meetings that dealt with nonclinical issues included pre-IND meetings of May 29, 1998 and October 05, 2000, the End-of-Phase 2 meeting of December 06, 2001, issue-driven meeting of March 17, 2003, and the pre-NDA meeting of April 16, 2008. Minutes of these meetings are available. DPAP and the sponsor also held telephone conferences. These conferences were generally issue driven. The conferences were held on March 18, 1999, and June 6, 2001. The June 6, 2001 and January 13, 2010 telecons discussed interpretation of the hamster carcinogenicity studies.

DPAP staff has written four (4) nonclinical reviews in the IND application. These reviews were completed by Dr. Timothy McGovern on July 24, 2000 (Original Review, Review #1), May 29, 2001 (Review #2) and January 30, 2003 (Review #3); and by Dr. Luqi Pei on June 27, 2007 (Review #5), respectively. The Original Review (Review #1 in the current review) was a comprehensive review evaluating relevant issues of the IND in its early stage. Review #2 evaluated the male reproductive toxicity of roflumilast in animals and its relevance to humans. Review #3 evaluated the general toxicity of roflumilast in dogs and further analyzed the male reproductive toxicity of roflumilast. Review #5 evaluated the carcinogenic study results of roflumilast. Other major documents dealing with nonclinical data of the application included the minutes of the Center's ECAC (Executive Carcinogenicity Assessment Committee) meetings

held on 09-FEB-1999, 10-MAY-2005 and 19-FEB-2010. The 09-FEB-1999 meeting discussed the protocols of 2-year oral carcinogenicity studies of roflumilast in hamsters and mice. The 10-MAY-2005 meeting evaluated study reports of the carcinogenicity studies. The 19-FEB-2010 meeting reevaluated the relevance of hamster tumorigenicity data to humans.

The re-evaluation of the relevance of hamster tumorigenicity data to humans was prompted by available new clinical pharmacokinetic data in the NDA submission, which resulted in a significant change in the conclusion of the hamster carcinogenicity study results. The revised ECAC minutes can be found as attachments in relevant reviews.

Under the IND, the sponsor intended to develop the drug for two indications: asthma and COPD. The current NDA is for the COPD indication.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Roflumilast is a selective phosphodiesterase type-4 (PDE4) inhibitor with an $IC_{50} = 0.3$ for PDE4 and $\geq$ 4,000 and $\geqslant$1,700 μg/mL for PDE1-3 and PDE5-11, respectively. Roflumilast possesses bronchodilatory and anti-inflammatory properties. Both properties are attributed to the ability of the drug and one of its metabolite, roflumilast N-oxide, to increase the intracellular cAMP, an intracellular messenger that is present in many cells and is hydrolyzed by phosphodiesterases. Increases in cAMP levels in tracheobronchial smooth muscles result in muscle relaxation and bronchial dilation. The increases of cAMP levels in some inflammatory cells results in functional suppression of the cells.

Pharmacological investigations of roflumilast and roflumilast N-oxide included in vitro and in vivo bronchodilatory effects, effects on reactive or inflammatory intermediates, and effects on lung injury models. Roflumilast dilated constricted airways in vitro and in vivo. In vitro roflumilast inhibited serotonin-induced contractions of lung slices in rats and methacholine-induced contractions of tracheal rings from guinea pigs. In vivo roflumilast inhibited chemically-induced bronchoconstriction in dose-dependent manners in rat and guinea pigs. The drug, however, had no effect on histamine-induced contractions of the guinea pig tracheal rings in vitro and no effect-on LTD4 and methacholine-induced bronchoconstriction in guinea pigs after oral administration.

Roflumilast mediated release of reactive and inflammatory intermediates in vitro and in vivo. Roflumilast N-oxide generally possessed pharmacological activity and potency similar to roflumilast. In vivo, oral roflumilast decreased LPS-induced TNF-α release in mice. Both oral roflumilast and roflumilast N-oxide decreased LPS-induced TNF-α release and LPS-induced neutrophil and leukocyte accumulation in rats. Oral roflumilast also decreased MCP-1, and MIP-1α release, LPS-induced leukocyte-endothelial adhesion, LPS-induced leukocyte-endothelial emigration, and LPS-induced leukocyte-endothelial rolling flux in rats.

Safety pharmacology studies showed that roflumilast at 3 - 100 mg/kg (PO) decreased grip strength by up to 89% in mice. Roflumilast at 3-30 mg/kg (PO) reduced the threshold for penetrazole-induced seizures by 30-93%; the no effect dose was 1 mg/kg. At 30 mg/kg, mice also exhibited increased tonic convulsions and lethality in a potentiation test and shortened time

to tonic convulsions and death. Roflumilast at 3 - 30 mg/kg prolonged hexobarbitol-induced loss of righting reflex (up to 590%) and a dose-dependent increase in ethanol-induced sleeping time (up to 368%) in rats. Roflumilast at 10 mg/kg (PO) caused significant increase in HR (62%) and a decrease in blood pressure (12 - 26 mmHg) in male normotensive rats. A cumulative IV injection in anesthetized male rats, 0.3-20 μmol/kg roflumilast increased systolic arterial pressure (16%) and dP/dt max (9%) at the high dose and decreased diastolic pressure (24-29%, three highest doses). Cats at 0.1-7 mg/kg showed sustained increase in blood pressure (46-63%), heart rate (up to 18%), and cardiac contractility (3-fold). The cats also showed a rise in the ST-phase (two highest doses) and increased breathing rate and respiratory minute volume (33 and 32%). Bolus injections (10^{-11} to 10^{-7} M) increased coronary flow (21-47% at 10^{-8} to 10^{-7} M) in male guinea pig Langendorff hearts.

2.6.2.2 Primary pharmacodynamics

Roflumilast inhibited PDE4 activity, affected production and release of inflammatory intermediates, and dilated airways.

PDE4 inhibition: Roflumilast (also roflumilast N-oxide) selectively inhibited human neutrophil-derived and recombinant PDE4 (Table 2). The respective IC_{50}s of roflumilast and roflumilast N-oxide were 0.3 and 0.8 μg/ml for PDE4 from human neutrophils, and 0.1 - 1.2 μg/ml and 0.3 – 2.9 μg/ml for recombinant PDE4s (i.e., A1, B2, C1 and D4). The IC_{50}s for other PDE isoenzymes were much higher (≥1,700 μg/ml). The isoenzymes included PDE1 from the bovine brain, PDE2 from rat heart, PDE3 and PDE5 from human plates, PDE6 from bovine retina and recombinant PDE7-11. PDE4 is the major PDE isoenzyme in human neutrophilic and eosinophilic leukocytes, B-lymphocytes, and monocytes. PDE4 is important but less prominent compared to the PDE3 isoenzyme in mast cells, T-lymphocytes, dendritic cells, and macrophages.

Table 2 IC50s of Roflumilast and Roflumilast N-oxide on PDE Isoenzymes

		IC_{50}s (μg/L)	
Enzyme	Source	Roflumilast	Roflumilast N-oxide
PDE1	Bovine brain	> 4,000	> 4,200
PDE2	Rat heart	> 4,000	> 4,200
PDE3	Rat heart	> 4,000	> 4,200
PDE4	Human neutrophils	0.3	0.8
PDE4A1	Recombinant	0.3	0.7
PDE4B2	Recombinant	0.1	0.4
PDE4C1	Recombinant	1.2	2.9
PDE4D4	Recombinant	0.1	0.3
PDE5	Human platelets	> 4,000	> 4,200
PDE6	Bovine retina	1,700	2,600
PDE7-11	Recombinant	> 4,000	> 4,200

Bronchodilatory effect: Bronchodilatory effects of roflumilast were investigated in vitro and in vivo. In vitro studies showed that roflumilast dilated tracheobronchial muscles in dose and time-dependent manners (Table 3). Roflumilast inhibited serotonin-induced contractions of lung slices from Brown Norway rats (IC_{50} = 8.1 μg/L) and methacholine-induced contractions of

tracheal rings from guinea pigs (IC_{50} = 0.6 μg/L). The drug, however, had no effect on histamine- and LTD4-induced contractions of the guinea pig tracheal rings (IC_{10} > 1210 μg/L) *in vitro*. Two studies with Wistar rat lung slices showed that roflumilast enhances mucociliary beat frequency.

Table 3 Bronchodilatory and Mucociliary Effect in Vitro

Tissue Model	Action	IC_{50} (μg/L)		Study Report
		Roflumilast	Roflumilast N-oxide	
Lung slices (Brown/ Norway Rat)	↓ Serotonin-induced smooth (SM) muscle contraction	8.1	37	228/2003, 321/2003
Tracheal ring chains (Guinea pig/ Dunkin-Hartley)	↓ Methacholine-induced SM contraction	0.6	-	182/94
	↓ Histamine-induced SM contraction	No effect	No effect	388/2005
	↓ LTD_4-induced SM contraction	No effect	No effect	389/2005
	↓ Spontaneous SM contraction	NA	5.4	235/99
	↓ Antigen (OV)-induced SM contraction	40	~ 19	195/94, 130/2000
Lung slices (Wistar rat)	↑ Ciliary beat frequency	60 (EC50)	NA	315/2006
	↑ Ciliary beat frequency	12.5	NA	79/2007

Table 4 summarizes the bronchodilatory effects of roflumilast in vivo. The ED_{50} of intravenously administered roflumilast at 60 minutes post the inducer exposure was 1.8 mg/kg for serotonin-induced bronchoconstriction in rats, and 2.9 mg/kg for histamine-induced bronchoconstriction in guinea pigs, respectively. The drug, however, showed no efficacy in LTD4- and methacholine-induced bronchoconstriction after oral administration up to 12 mg/kg in guinea pigs.

Table 4 Bronchodilatory Effect of Roflumilast in Vivo

Species	Inducer of Broncho-constriction	ROA	ED_{50} for Bronchodilation (mg/kg)		Report #
			Roflumilast	Roflumilast N-oxide	
Rat	Serotonin	IV	0.04, 1.8 & 4.7 mg/kg at 2, 60 and 120 min.	0.02, 0.5 & 0.6 mg/kg at 2, 30 and 60 min	165/96, 113/2004
		ID	4.8 mg/kg at 60 min	-	186/2001
Guinea pig	LTD4 [a]	OG	No effect at up to 12 mg/kg	-	332/2005
	Methacholine	OG	No effect at 0.4 mg/kg	-	56/2006
	Histamine	IV	2.9 mg/kg at 1 hr	-	209/96
		IV	3.8 and 7.7 mg/kg for expiratory flow & tidal volume	-	3/95

a. These animals were pre-sensitized with ovalbumin.

Anti-inflammatory effect: Roflumilast (also roflumilast N-oxide) decreased formation and/or release of several reactive intermediates. Table 5 summarizes their effects *in vitro*. Roflumilast

decreased the formation of reactive oxygen species and LTB_4 by human polymorphonuclear leukocytes (PMNL). It decreased formation of fMLP- and C5a-stimulated reactive oxygen species by human eosinophils. It decreased TNF-α release from mouse macrophage-derived RAW 264.7 cells and from human blood and monocytes. It attenuated functions of human lymphocytes (CD4+ and CD8+ T cells) and neutrophils. It decreased release of endothelin from arterial smooth muscles. It also decreased interactions between leukocytes and endothelium. Roflumilast N-oxide generally possessed pharmacological activity and potency similar to roflumilast.

Table 5 Effects of Reactive Intermediates, Gene Expression, and Proliferation in vitro

Pharmacologic activity	IC_{50} (μg/L)		Study Report
	Roflumilast	Roflumilast N-oxide	
↓ Formation of reactive oxygen species by human PMNL	1.3	2.5 (IC_{35})	131/94,
↓ LTB4 formation by humans PMNL	0.5	2.1	296/98,
↓ Formation of fMLP-stimulated reactive oxygen species by human eosinophils	2.8 (IC_{35})	8.4 (IC_{35})	3/2000, 172/2000
↓ Formation of C5a-stimulated reactive oxygen species by human eosinophils	4.0 (IC_{35})	17.0 (IC_{35})	3/2000, 172/2000
↓ TNF-α release from human blood cells	20 (IC_{30})	25 (IC_{30})	253/98, 254/98
↓ TNF-α release from human monocytes	2.0 (IC_{30})	4.2 (IC_{30})	37/99, 45/99
↓ TNF-α release from mouse macrophage-derived RAW 264.7 cells	23.0	-	99/2001
↓ Function of human CD4+ T-cells	2.8 (IC_{30})	3.8 (IC_{30})	42/2000, 229/2000
↓ Function of human CD8+ T-cells	3.4 (IC_{30})	1.2 (IC_{30})	20/2005, 21/2005
↓ Function of humans peripheral blood neutrophils	20.5	76.3	457/2006
↓ Endothelin release from human pulmonary artery smooth muscles	-	2.1	462/2006
Leukocyte-Endothelial interaction			
↓ PMNL- human umbilical vein endothelial cell (HUVEC) endothelial adhesion	NA	1.9	458/2006
↓ E-selectin mRNA expression in HUVEC	1.3	1.4	458/2006
↓ IL-1 induced surface P-selectin on HUVEC	NA	3.1	458/2006
↓ thrombin-induced macromolecular permeability in HUVEC	1.3	0.7	458/2006
Gene Expression			
↓ TNF-α-induced ICAM-1 expression and eotaxin release in human fetal fibroblasts	-	0.4 and 0.2	278/2008
↓ TGF-β1-induced α-smooth muscle actin protein and CTGF and fibronectin mRNA	-	0.3 – 0.5	277/2008
Proliferation			
↓ bFGF-induced thymidine incorporation (reflecting proliferation) in adult and fetal human lung fibroblasts	-	0.3 – 0.5	277/2008
↓ Growth medium-induced thymidine incorporation (reflecting proliferation) in human bronchial smooth	1.0	0.6	281/2008

muscle cells

Roflumilast and roflumilast N-oxide effects on in vitro gene and protein expression and cell proliferation are also summarized in Table 6. Roflumilast and roflumilast N-oxide decreased growth medium- and bFGF-induced thymidine incorporation. These observations reflect a decrease in cell proliferation in human bronchial smooth muscle cells, and adult and fetal human lung fibroblasts, respectively. Roflumilast increased TGF-β1-stimulated cyclooxygenase expression (Study 282/2008), and roflumilast N-oxide reduced TGF-β1-induced expression of α-smooth muscle actin protein, CTGF and fibronectin mRNA and ICAM-1 expression. Roflumilast N-oxide also decreased TGF-β1-induced expression of collagen I mRNA and total soluble collagen (Study 280/2008).

Roflumilast and roflumilast N-oxide also decreased formation and/or release of several reactive intermediates *in vivo*. Table 6 summarizes these in vivo anti-inflammatory effects. Oral roflumilast decreased LPS-induced TNF-α release in mice. In a separate study, oral roflumilast also reduced increases in IL-10 release in mice by 79% and 368% at 1 and 10 mg/kg, respectively (Study 216/99). Both oral roflumilast and roflumilast N-oxide decreased LPS-induced TNF-α release and LPS-induced neutrophil and leukocyte accumulation in rats. Oral roflumilast also decreased MCP-1, and MIP-1α release, LPS-induced leukocyte-endothelial adhesion, LPS-induced leukocyte-endothelial emigration, and LPS-induced leukocyte-endothelial rolling flux in rats. Finally, oral roflumilast reduced histamine-induced vascular permeability in mesenteric venules in rats.

Table 6 Effects on Reactive Intermediates In Vivo

Pharmacologic activity	ED_{50} (mg/kg)		Study Report
	Roflumilast	Roflumilast N-oxide	
↓ LPS-induced TNF-α release in mice (PO), 1 hr	2.0		109/2004
↓ LPS-induced TNF-α release in rats (PO)	0.12	0.13	175/2000, 182/2000
↓ LPS-induced TNF-α release in rats (PO)	0.6 – 1.2	-	108/2004
↓ LPS-induced neutrophil & leukocyte accumul. in rats (PO)	0.4 – 0.5 (ID50)	~1.0	233/2002, 320/2007
↓ LPS-induced TNF-α, MCP-1, and MIP-1α release release in rats (PO)	0.09 – 0.78 (ID50)	-	172/2004
↓ LPS-induced leukocyte-endothelial adhesion in rats (PO)	0.2 (ID50)	-	459/2006
↓ LPS-induced leukocyte-endothelial emigration in rats (PO)	0.08 (ID50)	-	459/2006
↓ LPS-induced leukocyte-endothelial rolling flux (PO)	1.0 (ID50)	-	459/2006
↓ Histamine-induced vascular permeability in messent. venules in rats (PO)	0.036	-	460/2006

The effects of roflumilast and roflumilast N-oxide on lung injuries were studied in mice, rats, and guinea pigs (Table 7). The effects of roflumilast on cigarette smoking-induced lung injury were studied in mice and guinea pigs. The smoking duration was up to 7 months in mice (Reports 58/2006, 136/2004 and 425/2006) and 6 – 11 days in guinea pigs (Reports 137/2004, 189/2005 and 57/2006). Roflumilast was given by oral gavage up to 5 mg/kg/day. The daily

roflumilast was given either as one single dose or 2 divided doses. Report 425/2006 showed that roflumilast at 5 mg/kg/day, but not 1 mg/kg/day, prevented the influx of the cells of both the innate and adaptive immune system into the lungs of mice following chronic exposure to cigarette smoke (90 min/day, 5 days/week). It also reduced formation of emphysema associated with the cigarette smoking exposure. Emphysema was determined by the average inter-alveolar distance and the internal surface area and decreased lung desmosine content. Studies in guinea pigs showed that roflumilast at oral doses of 0.004 to 4.0 mg/kg/day given at 1 hr pre-smoke challenge dose-dependently inhibited total and differentiated cells in BALF when measured at one hour and at one day after the last smoke exposure. Roflumilast had no effects on protease, mucin, or MCP-1 in BALF, on mRNA levels of MUC5AC, MUC2, MCP-1, on IL-8 in lung tissue, or on plethysmographic lung function at one hour post-smoke exposure.

Table 7 Effects on Lung Injuries

Model	Pharmacological Activity	Report #
Cigarette smoking (11 day) - induced lung injury in mice	↓ 51 – 81% in cell# in BALF at 2.5 mg/kg, but not at 2 mg/kg twice daily	58/2006
Cigarette smoking (7 mo.) - induced lung injury in mice	↓ formation of emphysema and lung macrophage density at 5 mg/kg, but not at 1 mg/kg	425/2006
Cigarette smoking (11 day) - induced lung injury in guinea pigs	↓ in total and differentiated cells and protein influx in BALF at all doses (0.004, 0.04, and 4.0 mg/kg)	137/2004 189/2005
Cigarette smoking (11 day) - induced lung injury in guinea pigs	Dose-dependent ↓ in total and differentiated cells and protein influx in BALF at 0.004, 0.04, and 0.4 mg/kg	137/2004 189/2005
Cigarette smoking (11 day) - induced lung injury in guinea pigs	Significant ↓ in total and differentiated cells and protein influx in BALF at 0.4 mg/kg	57/2006
Bleomycin (14 day) - induced lung injury in mice	↓ fibrosis, hydroxyproline content, TGF-β1 protein, and TGF-β1, CTGF, and α(I)I collagen mRNA in BAL fluid at 1 and 5 mg/kg; ↓ right ventricular hypertrophy, muscularization of intraacinar pulmonary arterioles, and lung endothelin mRNA at 1 and 5 mg/kg; ↓ in total cells, neutrophils, and protein in BAL, ↓ TNFα mRNA, ↓ lung weight and lung-to-body weight ratio; ↓ MUCA5A protein in BAL fluid and mRNA (lung); ↓ lipid hydroperoxides in BAL fluid; ↓ loss of body weight at 1 and 5 mg/kg	463/2006
Bleomycin- induced lung injury in mice and rats, preventive or therapeutic treatment w/ R	Mice: ↓ lung α(I)I collagen mRNA and ↓ right ventricular hypertrophy at 1 and 5 mg/kg in preventive AND therapeutic protocols. Rats: ↓ lung α(I)I collagen, CTGF, and TGF- β1 mRNA at 1 mg/kg in preventive AND therapeutic protocols	279/2008
Monocrotaline (21 days) - or hypoxia (15 days) - induced remodeling and pulmonary hypertension in rats	Monocrotaline: Dose-dependent ↓ in PAP, right ventricular hypertrophy, and full muscularization of intraacinar vessels at 0.5 and 1.5 mg/kg; ↓ IL-6 and MCP-1 mRNA (lung) at 1.5 mg/kg. Hypoxia: Dose-dependent ↓ in PHT, PAP, right ventricular hypertrophy, and full muscularization of intraacinar lung vessels at 0.5 and 1.5 mg/kg	461/2006
LPS-induced goblet cell hyperplasia (GCH) in rats	Significant inhibition of GCH at Days 7 and 14 post-LPS challenge at 1.2 mg/kg	320/2006
OVA-induced goblet cell hyperplasia (GCH) in rats	Significant inhibition of GCH at Days 3, 7, and 14 by 53%, 89%, and 100% at 1.2 mg/kg	321/2006

The effects of roflumilast on bleomycin-induced lung injury were studied in mice and rats. In study 463/2006, mice received a single intratracheal dose of bleomycin followed by oral

treatment with roflumilast for 14 days, starting the same day of bleomycin treatment. Roflumilast decreased bleomycin-induced lung parenchymal fibrotic and pulmonary vascular remodeling in a dose-dependent manner. Roflumilast reduced lung TGF-β1, CTGF, αI(I) collagen, and ET-1 ("biomarkers of remodeling"), reduced airway inflammatory cell influx, histology, BAL protein accumulation, and lung TNF-α (markers of inflammatory response), and reduced lung wet weight. Roflumilast also reduced lung MUC5AC and lipid hydroperoxides, and mitigated bleomycin-induced body weight loss. In study 279/2008, mice and rats received a bleomycin challenge followed by treatment with roflumilast in preventive (Mice: Days 1-14; Rats: Days 1-21) or therapeutic (Mice: Days 7-14; Rats: Days 10-21) protocols. Both dosing regimens in mice and rats resulted in reduced expression of lung αI(I) collagen, CTGF, and TGF-β1.

The effects of roflumilast on monocrotaline- and hypoxia-induced lung injury were studied in rats (Study 461/2006). Pulmonary hypertension in rats was induced by a 15-day exposure to low oxygen levels (10%) or by a single SC injection of monocrotaline. Rats received oral roflumilast from Days 1-15 of hypoxia or in preventive or therapeutic protocols following monocrotaline treatment (Days 1-21 or Days 21-42, respectively). Roflumilast reduced the development of pulmonary hypertension in response to hypoxia. Roflumilast produced a dose-dependent decrease in pulmonary arterial pressure (PAP), right ventricular hypertrophy, and muscularisation of intraacinar lung arteries. Roflumilast treatment for 1-21 and 21-42 days following monocrotaline treatment also resulted in decreased PAP, right ventricular hypertrophy, and muscularization of lung vessels. Treatment with 1.5 mg/kg roflumilast from days 1-21 also reduced increases in IL-6 and MCP-1 mRNA in lung tissue.

The effects of roflumilast on LPS- and OVA-induced goblet cell hyperplasia (GCH) were also examined in rats (Studies 320/2006 and 321/2006). Rats received roflumilast by gavage at 16 hr and 1 hr pre-LPS challenge and at 24 hrs post-LPS challenge. Roflumilast nearly completely reduced GCH at 1 and 2 weeks post-LPS challenge. Similar effects were seen in rats that were sensitized with SC OVA for 3 weeks followed by a 1 hour OVA challenge. Treatment with roflumilast was the same as the LPS study. Roflumilast treatment reduced GCH by 53%, 89%, and 100% at 72 hrs, 1 week, and 2 weeks post-OVA challenge.

2.6.2.3 Secondary pharmacodynamics

Secondary pharmacology studies evaluated the binding of roflumilast on non-PDE4 enzyme related receptors, and effects of the drug on asthma models and cytokine levels. Receptor binding screen studies in vitro showed that neither roflumilast nor roflumilast N-oxide interacted with muscarinic M_1-, M_2-, or M_3- receptors, histamine H_1-receptors, α_{1A}-, α_{1B}-, or α_2- adrenoceptors, β-adrenoceptors, adenosine A_1- (roflumilast-NO only), A_{2A}-, or A_{2B}-receptors, or Dopamine DA_2-receptors (Roflumilast only) (Study Report #s 208/99, 166/99,219/99, 164/99, 229/99, 197/94, 218/99, 233/94, 220/99, 207/94, 228/99, 209/94, 230/99, 227/99, 231/98 K1, 231/99, 76/95, 221/99, 242/99, 237/99, and 230/2001).

Effects of roflumilast or roflumilast N-oxide in animal models of allergic asthma were examined in mice, rats, or guinea pigs (Table 8). In a mouse model of fungal allergic asthma, roflumilast decreased bronchoconstriction, airway hyperreactivity (AHR), and leukocytes in BALF one day after allergen challenge (53/2005). In chronic models of OVA-induced asthma in mice, mice were treated with 5 mg/kg/day roflumilast for 2 weeks at 0.5 hrs pre-OVA challenge (8/2003, 176/2006). In 8/2003, AHR was slightly reduced. Accumulation of eosinophils and inflammatory

cells, as well as subepithelial collagenation and airway thickening were significantly reduced by roflumilast versus vehicle control. In 176/2006, roflumilast reduced eosinophils, T-cells, and inflammatory cells.

Table 8 Effects in Animal Models of Allergic Asthma

Model	Pharmacological Activity	Report #
Acute antigen-induced airway changes in mice[a]	Dose-dependent ↓ in allergen-induced bronchoconstriction, AHR, and leukocyte accumulation in BALF at 1 and 5 mg/kg R	53/2005
Chronic antigen-induced airway changes in mice[b]	Significant ↓ in eosinophils and chronic inflammatory cells, subepithelial collagenization, and airway epithelium thickening, slight ↓ in AHR at 5 mg/kg R	8/2003
Chronic antigen-induced airway changes in mice[b]	↓ eosinophils, T-cells, and chronic inflammatory cells and ↓ IFNγ, IL-5, and IL-10 mRNA in airways, and ↓ IL-5 and IL-13 release from peribronchial LN at 5 mg/kg R	176/2006
Chronic antigen-induced airway changes in mice[b]	↓ eosinophils and T-cells in airway walls, ↓ inflammatory cytokines in restimulated peribronchial LN in vitro at 5 mg/kg R	399/2006
Cell accumulation and protein influx in BALF (27 hrs) in rats[b]	↓ cell accumulation (ID50s for R = 0.2 mg/kg for total cells and neutrophils, 0.1 mg/kg for eosinophils, 0.4 mg/kg for protein influx)	105/95K 1
Cell accumulation and protein influx in BALF (27 hrs) in rats[b]	↓ cell accumulation (ID50s for R < 0.2 mg/kg for total cells and neutrophils, 0.2 mg/kg for eosinophils, 0.2 mg/kg for protein influx)	104/95
Cell accumulation and protein influx in BALF (48 hrs) in rats[b]	↓ cell accumulation (ID50s for R = 0.7 mg/kg for total cells, 0.6 mg/kg for lymphocytes, 1.1 mg/kg for neutrophils, 1.0 mg/kg for eosinophils, 0.9 mg/kg for protein influx)	186/99
Cell accumulation and protein influx in BALF (48 hrs) in rats[b]	↓ cell accumulation (ID50s for R-NO = 0.8 mg/kg for total cells, 0.5 mg/kg for lymphocytes, 0.6 mg/kg for neutrophils, 1.0 mg/kg for eosinophils, 0.7 mg/kg for protein influx)	187/99
Cell accumulation and TNF-α release in BALF (48 hrs) in rats[b]	↓ cell accumulation and TNF-α release at 4 mg/kg	265/99
Time-dependent cell accumulation and mediator release in BALF in rats[b]	↓ and delay in cell accumulation and inflammatory mediators in BALF, ↓ in inflammatory mediators in lung homogenates, ↓ decreased lung histologic findings at 2 mg/kg	111/2002
AHR to adenosine, neutrophil influx, and TNFα release in BALF (3 hrs) in rats[b]	ID50s for R = 1.5 mg/kg for ↓ AHR to adenosine, 0.9 mg/kg for ↓ neutrophils influx, and 0.4 mg/kg for ↓ TNFα release	24/2001
AHR to acetylcholine (48 hrs) in rats[b]	Dose-dependent ↓ of AHR to Ach at 3 mg/kg	39/2001
SRS-A-mediated bronchoconstriction and LT concentration in BALF in guinea pigs[d]	Dose-dependent ↓ in allergen-induced decrease in compliance and conductance with and ED50s of 0.4 mg/kg for R given at 1 hr pre-challenge; ↓ Cys-LTs and LTB4 release (ED50s for R = 0.12 and 0.04 mg/kg)	228/2002

[a] Aspergillus fumigatus-sensitized and challenged; [b] OVA-sensitized and challenged; [c] OVA-sensitized and pretreated with pyrilamine; [d]OVA-sensitized and pretreated with pyrilamine, propanolol, and indomethacin

Roflumilast also decreased inflammation-related cytokines (IFN-γ, IL-5, and IL-10). In a third chronic study of OVA-induced asthma in mice, mice received a moderate OVA challenge following 4 weeks of low-level OVA exposure. Mice were treated with oral roflumilast at 24 hrs and 2 hrs pre-moderate OVA challenge. Treatment with roflumilast did not affect AHR to methacholine, as measured by whole-body plethysmography; however, roflumilast treatment reduced accumulation of eosinophils and T-cells in airways. Roflumilast also reduced levels of inflammatory cytokines (CD 40 ligand, GM-CSF, IFN-γ, IL-1α, IL-6, and IL-17) in re-stimulated lymph nodes of peribronchial lymph nodes *in vitro*.

Roflumilast effects on cell accumulation and protein concentration in BALF were measured in rats at 27- and 48-hrs post-OVA challenge. Rats were OVA-sensitized for 3 weeks prior to an OVA challenge. At 1 hr pre-OVA challenge, rats received roflumilast by either IV or oral administration (105/95K1 and 104/95, respectively). At 27-hrs post-OVA challenge, BAL was performed. Roflumilast decreased cell accumulation of total cells, neutrophils, eosinophils, and protein influx after both IV and oral administration. In studies 186/99 and 187/99, rats were OVA-sensitized for 4 weeks prior to an OVA challenge. At 1 hr pre-OVA challenge, rats received either roflumilast or roflumilast N-oxide by oral gavage, respectively. At 48-hrs post-OVA challenge, BAL was performed. Roflumilast and roflumilast N-oxide decreased cell accumulation of total cells, lymphocytes, neutrophils, eosinophils, and protein influx with similar ID_{50} values. In a separate study (265/99), rats were OVA sensitized for 4 weeks prior to an OVA challenge. Rats were treated with oral roflumilast at either 18-hrs or 1-hr pre-OVA challenge, or 6-hrs or 24-hrs post-OVA challenge. BAL was performed at 48-hrs post-OVA challenge. All treatments except the 24-hr post-challenge treatment decreased cell accumulation in BALF. TNF-α release was inhibited after all roflumilast treatments. Roflumilast at 1-hr pre-OVA challenge was the optimal treatment time. In a time-course study (111/2002), rats were OVA-sensitized for 4 weeks prior to an OVA challenge. Rats were treated with roflumilast at 1-hr pre-OVA challenge. BAL was performed from 0.5 to 168 hr post-challenge. Roflumilast attenuation of influx of total cells, lymphocytes, neutrophils, eosinophils, and protein was maximal between 24 and 48 hrs. Roflumilast also decreased inflammatory cytokines and chemokines in BALF and lung tissue.

Roflumilast inhibition of AHR to adenosine and acetylcholine was determined in rats (24/2001 and 39/2001). Oral roflumilast administered to OVA-sensitized and challenged rats reduced AHR to both adenosine and acetylcholine. Roflumilast also decreased neutrophils influx and TNF-α release (24/2001).

Roflumilast inhibition of SRS-A-mediated bronchoconstriction was examined in mechanically ventilated guinea pigs (228/2002). Guinea pigs were OVA-sensitized and pretreated with pyrilamine, propanolol, or indomethacin. Roflumilast at doses of 0.04 to 4.0 mg/kg was administered by gavage at 1-hr pre-allergen challenge. Roflumilast produced a dose-dependent inhibition of allergen-induced decrease in airway compliance and conductance. Roflumilast also inhibited Cys-LTs and LTB_4 release.

2.6.2.4 Safety pharmacology

Safety pharmacology studies evaluated effects of Roflumilast on behavior, cardiovascular function, hemodynamics/respiration, neuromuscular function, stomach acid secretion, GI motility, body temperature, locomotor activity, pupil diameter and renal function. Roflumilast at 3 - 100 mg/kg (PO) decreased grip strength by up to 89% in mice. Roflumilast at 3-30 mg/kg (PO) reduced the threshold for penetrazole-induced seizures by 30-93%; the no effect dose was 1 mg/kg. At 30 mg/kg, mice also exhibited increased tonic convulsions and lethality in a potentiation test and shortened time to tonic convulsions and death. Roflumilast at 3 - 30 mg/kg prolonged hexobarbitol-induced loss of righting reflex (up to 590%) and a dose-dependent increase in ethanol-induced sleeping time (up to 368%) in rats. Roflumilast at 10 mg/kg (PO) caused significant increase in HR (62%) and a decrease in blood pressure (12 - 26 mmHg) in male normotensive rats. A cumulative IV injection in anesthetized male rats, 0.3-20 μmol/kg roflumilast increased systolic arterial pressure (16%) and dP/dt max (9%) at the high dose and decreased diastolic pressure (24-29%, three highest doses). Cats at 0.1-7 mg/kg showed sustained increase in blood pressure (46-63%), heart rate (up to 18%), and cardiac contractility (3-fold). The cats also showed a rise in the ST-phase (two highest doses) and increased breathing rate and respiratory minute volume (33 and 32%). Bolus injections (10^{-11} to 10^{-7} M) increased coronary flow (21-47% at 10^{-8} to 10^{-7} M) in male guinea pig Langendorff hearts.

In kidney function studies, male rats received 3 - 10-mg/kg roflumilast N-oxide (PO) showed increases in urine potassium and ketone bodies (~200%) (Reports 98/95 and 113/99). At 10 mg/kg, urine sodium, pH, and osmolality were increased by up to 117% and urine chloride and volume and serum urea were decreased up to 11% (0-6 hrs post-dose).

2.6.2.5 Pharmacodynamic drug interactions

Roflumilast and roflumilast N-oxide drug interactions were assessed in vitro and in vivo in mice, rats, guinea pigs, and minipigs (Table 9). Roflumilast N-oxide had no effect on β_1-adrenoceptor-mediated isoprenaline-induced effects in guinea pig heart atrium (Study 254/99). Roflumilast N-oxide did interact, however, with β_2-adrenoceptor in guinea pig tracheal muscle, as evidenced by a leftward shift in the concentration-response curve for isoprenaline-induced relaxation of guinea pig tracheal muscle (Study 233/99). In rats, IV co-administration of roflumilast and formoterol, a β_2-agonist, increased serotonin-induced bronchospasmolytic activity (Studies 33/2003 and 110/2007). In Study 110/2007, roflumilast also enhanced formoterol-induced decreases in MAP at 60 min post-drug administration. The sponsor notes, though, that drug interactions with respect to CV parameters did not occur when roflumilast was co-administered with salbutamol, formoterol, or theophylline to healthy human volunteers (Studies FHP014, FHP026, CP-059).

In a smoke-induced lung injury model in mice, co-administration of roflumilast and dexamethasone synergistically reduced total cells, eosinophils, and lymphocytes in BALF (Study 355/2006). In guinea pigs, co-administration of roflumilast and montelukast, a LT-receptor antagonist, reduced SRS-A-mediated effects on bronchoconstriction in an additive manner (Study 39/2003). Roflumilast and histamine H_1-receptor antagonist cetirizine co-administration in guinea pigs resulted in a synergistic decrease in allergen-induced bronchoconstriction in guinea pigs (Study 295/2004). Co-administration of roflumilast and either of the muscarinic anticholinergics compounds revatropate or tiotropium resulted in a synergistic decrease in

methacholine-induced bronchoconstriction (Studies 396/2005 and 397/2005). Finally, simultaneous administration of roflumilast, roflumilast N-oxide, and the PDE5 inhibitor sildenafil increased heart rate and decreased blood pressure, left ventricular pressure, and pressure rise to a greater degree than sildenafil alone (Study 119/2006). However, the sponsor states that studies in human patients did not reveal clinically relevant changes in cardiovascular parameters when roflumilast and sildenafil were co-administered at therapeutic doses (Study CP-070).

Table 9 Drug Interactions

Model	Pharmacological Activity	Report #
In vitro β_1-Adrenoceptor synergism	No effect on isoprenaline-induced increase of atrial rate or force following R-NO at 1.3 to 4192 µg/L [a]	254/99
In vitro β_2-Adrenoceptor synergism	↑ in –log EC_{50} of isoprenaline following R-NO at 1.3 to 419 µg/L (additive synergism)	233/99
In vivo glucocorticoid effects; synergism with dexamethasone in mice in a cigarette smoke COPD model	Oral co-administration of R and dexamethasone significantly inhibited total cells, eosinophils, and lymphocytes by 43%, 69%, and 73%	355/2006
β_2-agonist-mediated effects; synergism with formoterol on serotonin-induced bronchoconstriction and CV parameters in rats	IV co-administration of R potentiated the inhibition by formoterol of serotonin-induced bronchoconstriction as evidenced by a significant shift of the R dose-response curve	33/2003
β_2-agonist-mediated effects; synergism with (RR)-formoterol on serotonin-induced bronchoconstriction and CV parameters in rats	IV co-administration of R potentiated the inhibition by formoterol of serotonin-induced bronchoconstriction as evidenced by a significant shift of the R dose-response curve. R also enhanced the formoterol induced ↓ of MAP at 60 min post drug administration	110/2007
CysLT1-receptor antagonism; synergism with montelukast on allergic bronchoconstriction in guinea pigs	Oral co-administration of R potentiated the inhibition by montelukast of SRS-A-induced bronchoconstriction with respect to airway conductance; effects of co-administration on decreased airway compliance were additive	39/2003
Histamine H1-receptor antagonism; synergism with cetirizine on allergic bronchoconstriction in guinea pigs	Co-administration of IV cetirizine potentiated the ↓ in allergen-induced bronchoconstriction by oral R; ID50s ↓ 26- to 40-fold.	295/2004
Muscarinic receptor antagonism; synergism with revatropate on methacholine-induced bronchoconstriction in guinea pigs	R and revatropate inhibited methacoline-induced bronchoconstriction in combination only	396/2005
Muscarinic receptor antagonism; synergism with tiotropium-bromide on methacholine-induced bronchoconstriction in guinea pigs	R and tiotropium-bromide inhibited methacholine-induced bronchoconstriction in combination only	397/2005
PDE5 inhibition; synergism with sildenafil on CV effects in mini pigs	Dose-dependent ↑ in HR with R + R-NO + sildenafil. Dose-dependent ↓ in BP, left ventricular pressure, and pressure rise from R + R-NO + sildenafil.	119/2006

a. Abbreviations: R, roflumilast; R-NO, roflumilast N-oxide; CV, cardiovascular; MAP, mean arterial pressure; IV, introvascular.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Not Applicable.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Roflumilast is readily absorbed after oral administration. Maximum plasma concentrations after oral administrations were reached within an hour in mice, rats and rabbits, approximately 4 hours in hamsters and dogs, and 8 hours in humans, respectively (Table 10). The oral bioavailability of roflumilast was 25%, 11%, 7%, 9%, 19%, 48% and 64% in mice, rats, hamsters, rabbits, dogs, monkeys, and humans, respectively. Roflumilast in the plasma is present predominantly in bound form. The fraction of the free drug was 3.7%, 2.0%, 2.9%, 4.8%, 2.2%, 1.6%, 2.1%, 1.1% and 1.1% in mice, rats, hamsters, guinea pigs, rabbits, dogs, monkeys, mini pigs, and humans, respectively. After oral administration, fecal excretion is predominant in mice and dogs while urine excretion is predominant in humans. Other species were somewhere in between.

Table 10 Pharmacokinetic Profiles in Different Species

	Mouse	Rat	Hamster	Rabbit	Dog	Monkey	Human
Roflumilast (mg/kg, PO) [b]	12	20	1	0.8	2	0.5	0.010
$AUC_{0-\infty}$ (μg.h/L)	1663	643	25	35	1054	648	28
C_{max} (μg/L)	1313	94	1.4	3.1	106	341	8.8
$t_{½}$ (h)	0.7	0.9	4.1	0.8	3.8	4.4	10.9
Clearance (L/h/kg) [a]	1.8	3.9	2.9	3.0	0.4	0.3	0.14
Vd [a]	1.9	5.1	16.9	3.4	2.1	2.1	2.3
Bioavailability (F, %) [a]	25	11	7	9	19	48	64
Report #	99/2002	226/98	100/2002	66/2002	152/95	102/2002	FHP036

a. These values were calculated from studies using different roflumilast doses (extracted from Table 2.4-4 of Section 2.4.3 of the submission).

2.6.4.2 Methods of Analysis

Roflumilast and its metabolite levels in plasma/serum samples were analyzed by high-performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS) with fluorescence detection. ^{14}C-labeled roflumilast and metabolites were measured with radio-thin-layer chromatography (TLC) or radio-HPLC. The lower limits of quantitation (LLOQ) in these assays were 0.1 - 1.0 μg/L roflumilast depending on the sample volume available. Structures of metabolites were elucidated by use of mass spectrometry. Tissue distribution was evaluated using whole body section autoradiography after administration of [^{14}C]-roflumilast in rats. Plasma protein binding of roflumilast and roflumilast N-oxide was determined by equilibrium dialysis.

2.6.4.3 Absorption

Roflumilast is readily absorbed after oral administration. Maximum plasma concentrations after oral administrations were reached within an hour in mice, rats and rabbits, approximately 4 hours in hamsters and dogs, and 8 hours in humans, respectively (Table 10). Plasma roflumilast AUCs, when normalized on a μg/kg dose basis, were 140, 30, 30, 40, 530, 1,300, and 4,500 mg.h/L in mice, rats, hamsters, rabbits, dogs, monkeys, and humans, respectively. The oral bioavailability of roflumilast was 25%, 11%, 7%, 9%, 19%, 48% and 64% in mice, rats, hamsters, rabbits, dogs, monkeys, and humans, respectively. Table 11 presents the steady-state dose-plasma AUCs of roflumilast at various doses in different species. The AUC levels rose proportionally to oral doses at lower doses and supra-proportionally at high doses in animals. For example, the AUC in mice was 71.5, 153.1 and 663.0 and 6113 μg.h/L at oral doses of 2, 4, 12 and 36 mg/kg/day, respectively.

Table 11 Steady State Plasma Roflumilast Levels

Roflumilast	Roflumilast $AUC_{0\text{-}24\ h}$ (μg.h/L) [a]					
(mg/kg/day, PO)	Mouse	Rat	Hamster	Dog	Monkey	Human
0.1	-	-	-	-	80.3	65 [b]
0.2	-	-	-	159.5	-	-
0.25	-	-	-	-	205.2	-
0.50	-	11.7	-	-	556.3	-
0.60	-	-	-	510.1	-	-
1	-	-	-	588.5	-	-
1.5	-	32.4	-	-	-	-
2	71.5	-	-	918.9	-	-
2.5	-	43.5	-	-	-	-
4	153.1	-	39.8	1982.0	-	-
6	-	-	-	-	-	-
8	-	-	59.2	-	-	-
12	663.0	-	-	-	-	-
16	-	-	519.8	-	-	-
18	-	-	-	3458.9	-	-
36	6112.5	-	-	-	-	-

a, Source: Section 2.6.7.3 (p 18) of the submission.
b. AUC in COPD patients at 500-μg oral dose (p42, Report 343/2008)

Figure 1 presents schematically the plasma levels of roflumilast and roflumilast N-oxide, a major metabolite in mice, hamsters, monkeys and humans. The same nominal dose (mg/kg) produced the highest plasma concentrations of roflumilast and roflumilast N-oxide in monkeys.

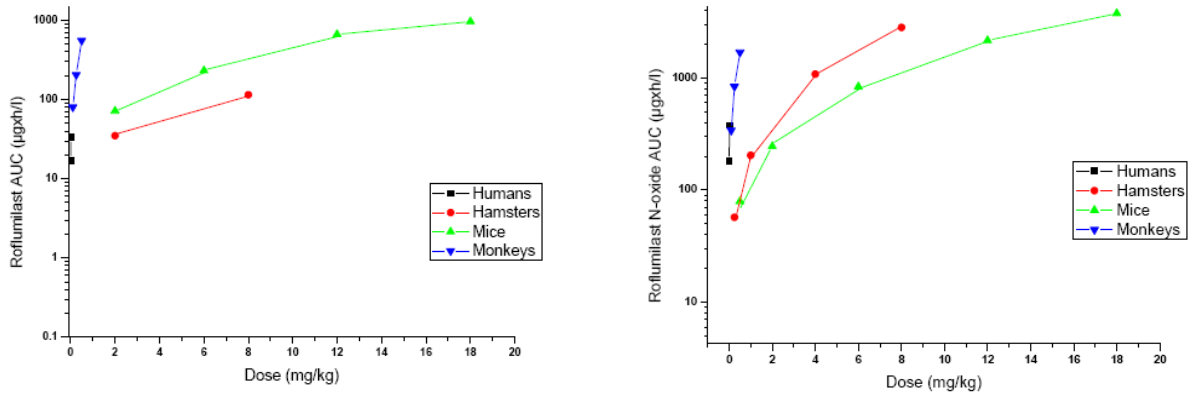


Figure 1 Steady state plasma AUCs of Roflumilast and Roflumilast-N-Oxide levels
(Source: p 22 – 23 of Report 267/2008, Section 2.6.7.3).

2.6.4.4 Distribution

Roflumilast is widely distributed in the body. Tissue distribution was qualitatively analyzed by use of whole body section autoradiography after administration of [^{14}C]-roflumilast in rats and quantitatively by liquid scintillation in mice, rats and hamsters. Highest drug concentrations were found in the liver and adrenals in mice and hamsters, and in the nose and liver in rats (Table). Other organs with relatively high roflumilast concentrations were in a descending order kidney, fat, lung, heart, testes and brain.

Tissue concentrations of roflumilast following administration of a single dose of [ADCP-^{14}C]-roflumilast are given in Table 12. The high nasal concentration of radio-labeled roflumilast in rats was attributed to a roflumilast metabolite. Rats that were given a single-dose of 10 mg/kg of ADCP-^{14}C or carbonyl-^{14}C labeled roflumilast showed strikingly different concentrations of radio-activity. Specifically, the nasal-plasma AUC ratios were 7.92 and 0.24 for ADCP-^{14}C or carbonyl-^{14}C labeled roflumilast, respectively (Table 13).

Table 12 Tissue Roflumilast Concentrations

	Tissue/Plasma AUC Ratio [a]		
Tissue	Mouse	Rat	Hamster
Liver	3.52	4.98	5.32
Kidney	0.91	1.85	1.47
Adrenal	3.26	1.47	0.74
Fat	0.62	0.81	n.a.
Lung	0.62	0.87	0.57
Heart	0.42	0.74	0.42
Nose	0.59	4.69	0.53
Testes	0.27	0.40	0.26
Brain	0.13	0.27	0.14
Report #	133/97	133/95	22/98

a. Roflumilast dose was 1 mg/kg/day (PO) in each species. The plasma roflumilast AUC was 1.59, 5.6 and 2.6 μg h/L in mice, rats and hamsters, respectively. The AUC values were from 0 – 96 hrs in mice and hamsters and 0 – 168 hrs in rats, respectively. [Source: Table 2.4-7 (p 22) of Section 2.4.3.3 of the submission].

The actual difference in the nasal-plasma AUC ratios may be somewhat smaller because of the differences in plasma AUCs between the studies (i.e., 36 and 68 μg.h/L for ACDP-^{14}C or carbonyl-^{14}C labels, respectively). The corrected ratios would be 4.19 [7.92 ÷ (68/38) = 4.19] and 0.24 for ACDP-^{14}C or carbonyl-^{14}C labeled roflumilast, respectively. There was still a 17-fold difference in radio activity concentrations between ACDP-^{14}C and carbonyl-^{14}C labeled roflumilast.

Table 13 Tissue Concentrations of ACDP-^{14}C or ^{14}C-Roflumilast

Roflumilast moiety	Study#	Tissue/Plasma AUC Ratio [a]						
		Liver	Kidney	Adrenal	Lung	Nose	Testes	Brain
ADCP-^{14}C	133/95	4.35	2.21	1.85	0.89	7.92	0.52	0.40
Carbonyl-^{14}C	105/2002	2.08	1.19	0.99	0.52	0.24	0.25	0.06

a. Roflumilast dose was 10 mg/kg/day (PO) in both studies. The plasma roflumilast $AUC_{0-168\ hr}$ was 36 and 68 μg.h/L in Studies 133/95 and 105/2002, respectively. [Source: Table 2.4-7 (p 22) of Section 2.4.3.3 of the submission].

Gender differences is tissue distribution were also analyzed in hamsters following single oral dose administration of [ADCP-^{14}C]-Roflumilast (Study 106/2002). No notable gender differences were observed in plasma, liver, kidneys, bone marrow, brain, or nose.

The tissue distribution of radiolabeled [ADCP-^{14}C]-Roflumilast N-oxide was analyzed in the rat after single oral dose administration. Levels of [ADCP-^{14}C]-Roflumilast N-oxide were qualitatively higher in the nasal mucosa and liver at 96 hrs post-dose (Study 285/99) and quantitatively higher in the liver and nose at 96 hrs post-dose (Study 94/2000).

Roflumilast (probably its metabolites too) readily crosses the placenta to reach the fetus in rats. Table 14 shows roflumilast concentrations in selected tissue in pregnant rats treated with 1-mg/kg/day roflumilast on gestation days 14 and 18 (Study #85/2001). Roflumilast concentrations between dam's plasma and fetal tissues appear similar on both days.

Table 14 Dam and Fetal Tissue Drug Levels in Pregnant Rats

Tissue	Concentration (μg equivalent/g tissue)					
	Gestation Day 14			Gestation Day 18		
	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
Plasma	0.648	0.404	0.083	0.420	0.488	0.098
Lung	0.506	0.311	0.074	0.329	0.437	0.112
Heart	0.706	0.211	0.059	0.290	0.423	0.100
Liver	2.121	0.841	0.360	0.847	1.341	0.355
Adrenal	1.258	0.531	0.227	1.017	0.919	0.368
Kidney	0.582	0.434	0.167	0.436	0.721	0.242
Nasal mucosa	2.094	0.492	1.023	0.331	1.692	2.859
Mammary gland	1.563	0.344	0.054	0.650	0.639	0.143
Amniotic fluid	0.278	0.187	0.016	0.153	0.278	0.066
placenta	0.508	0.285	0.073	0.361	0.590	0.128
Fetus	0.483	0.251	0.039	0.230	0.718	0.078

* Tissue drug levels were measured by the total radioactivity after administered ^{14}C-roflumilast (1 mg/kg/day) orally to pregnant rats on gestation days 14 and 18 [n = 3/time point; Source: Table 2.6.4-2 of Section 2.6.4.7 (p26) the submission, Study # 85/2001].

Plasma roflumilast concentrations in pregnant and non-pregnant rats appear similar. At the 0.5 mg/kg/day dosing, the mean $AUC_{0-8\ hr}$ was 14.0, 15.9 and 9.7 μg.h/L on days 28, 91 and 184,

and 14.4 μg.h/L on (gestation day 13 – 15) in non-pregnant, respectively.[1] The general toxicity study data were the mean of males and females; however, there were no apparent gender differences in the AUC values.

Roflumilast was excreted by milk. Lactating dams (n = 4 – 5) were given intravenously and orally 1-mg/kg roflumilast (radio-labeled) on lactation days 4 or 5 (Study# 85/2001). Roflumilast concentrations in dam milk and the pup liver (n = 10 – 12) was determined at 1, 4, 8 or 24 hrs post dosing. Table 15 summarizes the results. Roflumilast concentrations 8 hour post oral dosing was 0.32 and 0.02 in the milk and pup liver, respectively.

Table 15 Milk and Pup Liver Roflumilast Concentrations in Lactation Period

	Roflumilast Concentration (μg. Equivalent/g, total radio activity) [a]						
	PO			IV			
Time (hr)	1	4	8	1	4	8	24
Milk (dam)	0.055	0.215	0.318	0.094	0.447	0.53	0.18
Pup liver	0.003	0.013	0.020	0.002	0.016	0.039	0.047
Milk/liver ratio	18	17	16	47	28	14	4

a. Total radioactivity (n = 4-5 and 2 – 3/time point in dam and pups, respectively).

Roflumilast in the plasma is predominantly protein-bound. The free drug represents only up to 3.7% of the drug in plasma in animals used in the toxicology program and humans (Table 16). Specifically, the free fraction was 3.7%, 2.0%, 2.9%, 2.2%, 1.6% and 2.1% in mice, rats, hamsters, rabbits, dogs and monkeys, respectively.

Table 16 Plasma/serum Protein Binding of Roflumilast and Roflumilast N-oxide

	Unbound Fraction (%)								
	Mouse	Rat	Hamster	G. pig	Rabbit	Dog	Monkey	Minipig	Human
Roflumilast	3.7	2.0	2.9	4.8	2.2	1.6	2.1	1.1	1.1
Roflumilast N-oxide	12.7	9.5	11.0	7.0	6.4	10.9	12.9	7.6	3.4

Source: Table 2.4-6 (p 21) of Section 2.4.3.3 of the submission.

2.6.4.5 Metabolism

A number of roflumilast metabolites have been identified. Figure 2 presents metabolic pathways of roflumilast in animals and humans. The most abundant metabolites in plasma were roflumilast N-oxide (M06 & M07) in both animals and humans. ADCP N-oxide (M09) is also a major metabolite in rodents. The compound appears responsible for the carcinogenic potential of 2-yr roflumilast carcinogenicity studies in hamsters.

[1] Sources for the AUC data were Table 2.6.6-28 (p 67) of Section 2.6.6.6.5 of the submission for the 28-day data and P/T review #3 completed by Timothy McGovern on January 30, 2003 for days 91 and 184 data.

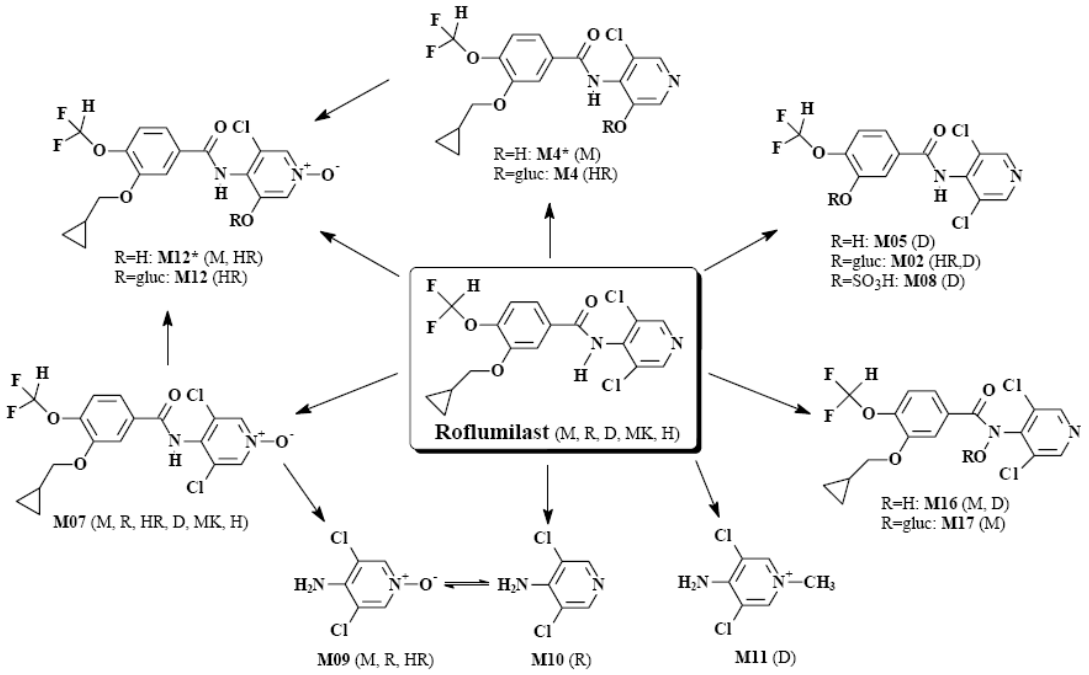


Figure 2 Metabolic pathways of roflumilast in mice, rats, dog, monkeys and humans.

M, mice; R, rats; D, dogs; MK, monkeys; MR, hamsters; and H, humans; M1 & M3, O-dealkylated roflumilast N-oxide, and/or respective – glucuronide; M05, M02 and M8, O-dealkylated roflumilast, and/or respective glucuronide, and/or respective sulfate; M4 & M04*, Mono-dechlorinated hydroxy-roflumilast, and/or respective glucuronide; M09, ADCP N-oxide; M06 and M07, Roflumilast N-oxide, and/or respective glucuronide; M10, ADCP; M11, N-methyl-ADCP; M12 & M012*, Mono-dechlorinated hydroxy-roflumilast Noxide, and/or respective glucuronide; M13, Dealkylated and dechlorinated roflumilast glucuronide; M14, Mono-dechlorinated hydroxy-ADCP sulfate; M15, ADCP N-sulfate; M16 and M17, N-hydroxy roflumilast, and/or respective glucuronide. (Source: Section 2.6.5.11 of the submission)

Roflumilast metabolism was studied in vitro and in vivo. The in vitro studies used both isolated liver microsomes and precision-cut liver slices from mouse, rat, guinea pig, dog and man. Roflumilast was metabolized at a low rate in microsomes from guinea pig and rat. In microsomes from dog and mouse, a limited extent of metabolism was observed. The extent of biotransformation of roflumilast in microsomes from humans was below the lower limit of quantitation (LLOQ). In a subsequent study, the in vitro metabolism of roflumilast by precision-cut liver slices and hepatic microsomes was investigated in the rat (Study 178/99). Incubations with rat microsomes resulted primarily in the formation of roflumilast N-oxide. In an in vitro study using precision cut liver slices from the hamster (Study 48/2000), incubation with roflumilast resulted in the formation of ADCP, roflumilast N-oxide, ADCP N-oxide, and the glucuronide of B9302-077. In vitro biotransformation of [^{14}C] roflumilast was also determined in rat, hamster, and human liver S9 fractions and arochlor-induced rat liver S9 fractions (Study 36/2005). Radiolabeled roflumilast was extensively metabolized by S9 fractions from rats and hamsters. The major metabolites were roflumilast N-oxide and O-dealkylated roflumilast. Other metabolites were also observed, including ADCP and ADCP N-oxide.

The in vitro metabolism of [ADCP-^{14}C]roflumilast and [^{14}C]-ADCP were examined using hepatic, olfactory, and respiratory microsomes from rat, mouse, hamster, dog, monkey, and human pooled tissues (Study 122E/99). ADCP was catabolized extensively and ADCP N-oxide

was formed in nasal microsomes of rat, mouse, hamster, and dog. Rat and dog liver microsomes showed inefficient metabolism of ADCP versus their mouse and hamster counterparts. Only trace ADCP N-oxide was detected in monkey olfactory microsomes, and no ADCP-N-oxide was detected in human olfactory or respiratory microsomes. Roflumilast metabolism to ACDP or ADCP N-oxide was not detected in any species in vitro.

The ability of roflumilast to induce cytochrome P450 (CYP) enzymes was evaluated in rat and human hepatocytes in vitro. Results showed no pronounced interaction of roflumilast with any of the CYPs examined (Study 90E/99).

Roflumilast is metabolized by CYP3A4, CYP1A2 and CYP2G1 to form at least 17 roflumilast metabolites in animals and humans. The metabolites were formed through N-oxidation, O-dealkylation, or oxidative mono-dechlorination followed by conjugation. Figure 2 (previous page) presents structures of the metabolites. Table 17 summarizes proportions of the metabolites in the plasma and urine in animals and humans.

Table 17 Metabolic Profile of Roflumilast in Animals and Humans

Compound	Percentage (%) of Recovered Roflumilast Doses [a]											
	Mouse		Rat		Hamster		Dog		Monkey		Human	
	P [b]	U [b]	P	U	P	U	P	U	P	U	P	U
Roflumilast	**15.0**		**3.7**				**23.8**	**3.2**	**7.9**	**5.3**	**22.7**	
M03 & M1 [c]		10.1		1.3		6.5				30.6		31.1
M05, M02 & M08		45.9		10.9	8.6	32.3	12.9	9.6		26.7		18.3
M04* & M04	1.9	3.3			6.2					6.8		16.4
Roflumilast N-oxide	**30.5**	**1.6**	**61.7**		**45.0**		**19.0**		**86.8**	**11.0**	**64.8**	**1.3**
ADCP N-oxide	**10.3**	**15.0**	**22.2**	**37.7**	**12.6**	**11.8**						**14.9**
ADCP			**2.6**	**19.9**								
N-methyl-ADCP							4.4	60.6				
M12* & M12	3.7	5.5			12.7							
M13						7.4						
M14				11.6								
M15				4.1								
M16 & M17	10.3						27.4					

a. Source: Table 2.6.4 – 3 (p 37) of Section 2.6.4.9.2.8 of the submission.
b. P, plasma; and U, urine.
c. See legend of Figure 1 (previous page) for chemical names for the coded compounds.

Roflumilast N-oxide was the most abundant metabolite in all species, including humans (Table 17). Table 18 summarizes roflumilast N-oxide AUCs obtained from different studies in laboratory animals as well as in humans. These values could be useful in the labeling review of roflumilast if an approval action is taken on the application.

Metabolic pathways leading to ADCP N-oxide formation in rodents were further studied (Studies 20/2002, 21/2002, 22/2002). Results showed that amidase broke roflumilast down to produce ADCP (Figure 3). In rodents, olfactory-specific isoenzyme CYP2G1 oxidized ADCP to ADCP N-oxide, an epoxide and active intermediate that quickly converts to metabolite M1 or depletes cellular glutathione. The lack of glutathione eventually results in neoplastic changes. This pathway was apparently absent in humans due to the lack of corresponding enzymes (Studies 48/2005 and 299/2008).

Table 18 Plasma Roflumilast N-Oxide Levels in Animals and Humans

Roflumilast	Roflumilast N-oxide $AUC_{0\text{-}24\ h}$ (μg.h/L) [a]					
(mg/kg/day, PO)	Mouse	Rat	Hamster	Dog	Monkey	Human
0.1					340.0	517 [b]
0.2				15.1		
0.25			56.9		835.0	
0.50	78.9	246.8			1,682.5	
0.60				65.9		
1			203.8			
1.5		927.5				
2	244.4			53.6		
4	512.5		1,075.2			
6	831.2					
8			2,805.7			
12	2,144.9					
16			11,335.9			
18	3,736.1					
36	15,929					

a. Source P 19 of Section 2.6.7.3 of the submission. The number was lowest one if a cell has more than one number.
b. AUC in COPD patients at 500-μg oral dose (p42, Report 343/2008)

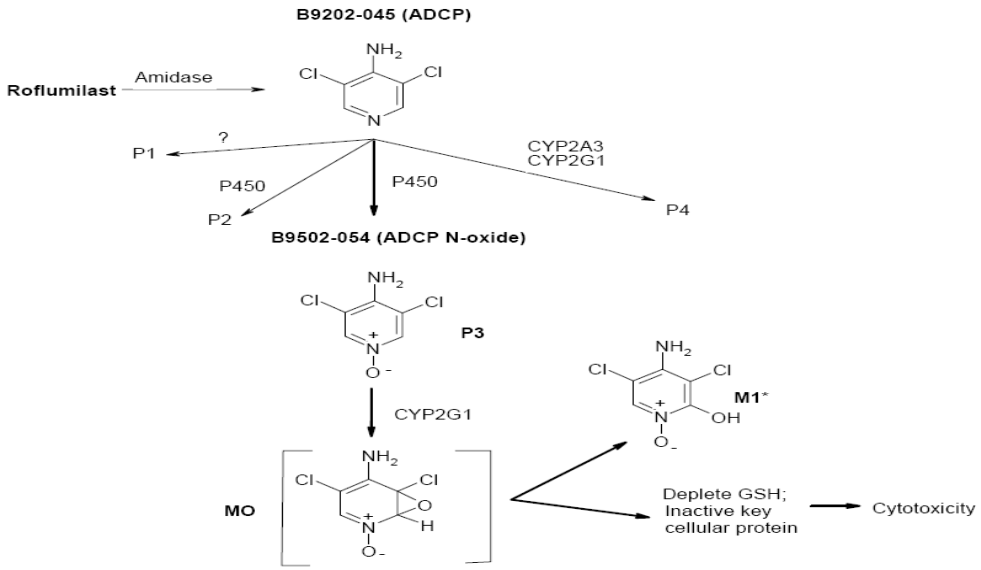


Figure 3 The ADCP pathway in rodents.

2.6.4.6 Excretion

Roflumilast is excreted via feces and urine. The proportion of excretion by each route, however, varied significantly with species and routes of administration. After oral administration, fecal excretion is predominant in mice and dogs while urine excretion is predominant in humans. Other species were somewhere in between. The major route of excretion was fecal in rats and hamsters, and urine in monkeys and rabbits, respectively.

Table 19 Urine and Fecal Excretion of Roflumilast

		Percentage (%) Excreted [a]						
Route	Excreta	Mouse	Rat	Hamster	Rabbit	Dog	Monkey	Human
IV	Urine	30	69	50	84	33	37	71
	Feces	61	31	40	14	58	42	21
Oral	Urine	8	36	24	51	7	42	70
	Feces	60	70	63	34	89	32	20

a. Roflumilast dose range was 0.02 – 1.0 mg/kg/day in animals and 300 µg/human subject by IV route and 0.5 – 10 mg/kg in animals and 500 µg/human subject, respectively. Source: Table 2.4 – 8 (Section 2.4.3.4) of the submission.

2.6.4.7 Pharmacokinetic drug interactions

Not applicable because no data were submitted.

2.6.4.8 Other Pharmacokinetic Studies

Not applicable because no data were submitted.

2.6.4.9 Discussion and Conclusions

Not applicable.

2.6.4.10 Tables and figures to include comparative TK summary

Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Not applicable.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

2.6.6.1.1 Acute Toxicity

The single-dose toxicity of roflumilast has been investigated in mice, rats, and dogs. The minimum lethal dose after oral administration was approximately 1, 100, 400, and 18 mg/kg in mice, rats, and dogs, respectively. The minimum lethal dose after intravenous administration was approximately 600, 100 and > 18 mg/kg in mice, rats and dogs, respectively. The causes of death after administration of lethal doses were not established.

2.6.6.1.2 General Toxicology

Toxicity of orally administered roflumilast was evaluated in studies up to 6, 6, 3, 12, and 9 months in treatment duration in mice, rats, hamsters, dogs, and monkeys, respectively. The target organs of toxicity included the nasal cavity (mice, rats and hamster), the male reproductive system (rats), the gastrointestinal tract (rats and monkeys), and the heart (dogs). The organs in

the reproductive system in rats included the testes and epididymides. The morphological lesions in the reproductive system in rats are accompanied by functional compromises such as decreases in male fertility. See Section 2.6.6.9.1 for discussions on the target organs of roflumilast toxicity.

Mice:

Oral toxicity studies up to 6 months showed that roflumilast affects the nasal cavity and adrenal glands in B6F3F1 mice. Pivotal toxicity studies included 3- and 6-month studies (one each). Roflumilast dose levels were 0, 6, 12 and 18 mg/kg/day in a 3-month study (#216/98) and 0, 4, 12 and 18 mg/kg/day in a 6-month study (#33/2002). Both studies showed dose-dependent lesions in the nasal cavity, the adrenal glands and the female reproductive system. Findings in the nasal cavity included disorganization, degeneration and necrosis of olfactory epithelium, nerve fiber atrophy, respiratory and squamous metaplasia, and Bowman's gland inflammation and hyperplasia. Findings in the adrenals included atrophy and hemorrhage in the cortex, subcapsular cell proliferation. Findings in the female reproductive system included inactivation of the uterus, diminished or lack of corpora lutea in the ovaries, and inflammation/inactivation in the vagina. The NOAEL was unidentified and 4 mg/kg/day in the 3- and 6-month studies, respectively.

In a 3-month GLP non-compliant study (#216/98), mice (10/sex/dose) were given 0, 6, 12 and 18-mg/kg/day roflumilast for 90 days. The MD and HD group males showed slightly lower, mean body weights (-6.3%) compared to controls. Histological evaluation revealed a disorganization (incidence: 3/10-HD Males; 4/10-MD and 5/10-HD in females) and focal necrosis (incidence; 1/10-MD and 2/10-HD in males and one each in MD and HD females) of the olfactory epithelium; severity increased with dose. Atrophy of the cortical x-zone in the adrenal glands was observed in five HD males and all treated female groups (incidence: 0/10-C, 5/10-LD, 8/10-MD, and 8/10-HD. The NOAEL was 6 mg/kg/day based on the lack of adrenal findings in LD males.

In a 6-month study (#33/2002), mice (20/sex/dose) were given roflumilast at doses of 0, 4, 12 and 36 mg/kg/day. The Control and HD groups also included 8 additional mice per sex to study reversibility of lesions after a recovery period of 4 weeks. After 18 weeks of treatment, each male was allowed to mate with an untreated female. The females were sacrificed on gestation day 15 for the evaluation of fertility parameters. The mean body weight gains were decreased in all male groups treated with roflumilast and in the HD female group. (At end of treatment, comparison to controls revealed lower body weight in the LD, MD and HD male groups of -8.8%, -13.6%, -19.8%, and in the HD females of –13.8%, respectively.) Organs with most noticeable findings were the adrenal glands, heart, and stomach in both sexes and the nasal cavity and the reproductive organs in the females. The adrenal glands showed increases in the incidence of cortex hypertrophy in all treatment groups. Respective incidence in C, LD, MD and HD groups was 0/20, 0/20, 6/20, 13/20 and 0/20, 0/20, 9/20 and 11/20. The heart showed increases in the incidence of perivascular inflammation in the MD and HD group in females (incidence 0/20, 0/20, 1/20 and 2/20). The stomach showed increases in the incidence of hyperkeratosis in the HD group. The nasal cavity showed increases in the incidence of nasal lesions in the MD and HD female groups [Ref.: Table 28]. The nasal lesions included olfactory degeneration, nerve fiber atrophy and hypertrophy of the Bowman's gland. No effects on fertility parameters were observed. The findings in the female reproductive organs included the

atrophy of uterus, cervix and vagina in MD and HD groups. The NOAEL was considered to be 4 mg/kg/day based on the lack of adrenal effect in LD males. The AUC at 4 mg/kg/day in the males was 153.1 μg.h/L.

Rats:

Oral toxicity studies up to 6 months of treatment showed that roflumilast affects the nasal cavity, the male reproductive system and the gastrointestinal tract in Sprague-Dawley rats. These studies included 1, 3, and 6-month studies. The respective roflumilast dose levels were 0, 0.5, 2.0, and 8.0 mg/kg/day in a 1-month study (#81/95); 0.02, 0.2, 2.0 mg/kg/day in a 3-month study (#38/98); 0, 0.5, 1.5, and 2.5 in the 1st 6-month study (#62/99); and 0 and 0.8 mg/kg/day in the 2nd 6-month study (#191/2000). Findings in the nasal cavity may include some or all of the following: disorganization, degeneration and necrosis of olfactory epithelium; nerve fiber atrophy; respiratory and squamous metaplasia; and Bowman's gland inflammation and hyperplasia. All but the nasal findings were fully reversible after a 1-month recovery period. Findings in the male reproductive system were present in the prostate, testes, epididymides, and seminal vesicles. Findings in the prostate included atrophy. Testes showed one or more of the following: tubular atrophy, dilation, degeneration and developmental defects and spermiogenic disturbance. Findings in the seminal vesicle included necrosis and atrophy. Findings in the epididymides included oligospermia, spermiogenic granuloma, and spermatocele. The NOAEL in rats was 0.8 mg/kg/day based on the results of Report 19/2000 and was associated with an AUC of 30.9 μg.h/L.

In a 4-week study (81/95), rats (20/sex/dose) were given roflumilast at doses of 0, 0.5, 2 or 8 mg/kg/day. The Control and HD groups also included 8 additional rats per sex to study reversibility of lesions after a recovery period of 4 weeks. Eight HD males and 8 HD females died or were sacrificed in moribund condition during weeks 3 and 4. Reduced body weight gain was noted in HD males and females (-84% and -75%, respectively, versus controls). Histopathological findings in HD animals included gastric erosions, mild degenerative spermiogenic disturbance associated with epididymidal oligospermia and spermiogenic granuloma, splenic follicular atrophy, a slight increase in splenic hemosiderin content, and peritonitis (perisplenitis, ovarian peritonitis). In mid-dose animals, serositis, submucosal inflammation, goblet cell hyperplasia, Paneth cell hyperplasia, vascular dilatation, villous necrosis were all noted in the jejunum and ileum. Mid-dose males also displayed gastric erosions and spermiogenic granuloma formation. In recovery animals, oligospermia, peritonitis, (hepatic serositis, ovarian, perispenitis, purulent lymphadenitis), and jejunum inflammation associated with serositis were noted. Since the nasal cavities, a primary target organ of toxicity in the rat, were not assessed in this study, a NOAEL could not be determined. In addition, findings were noted in high dose males in seminal vesicles, parathyroids and adrenals but those organs were not assessed at the low- and mid-doses. Target organs of toxicity included the heart, adrenals, epididymides, stomach (males), parathyroid, testes, prostate, and seminal vesicles in males and the thymus and spleen in males and females.

In a 4-week/3-month study (38/98), rats (8/sex/dose) were given roflumilast at doses of 0, 0.02, 0.2 or 2 mg/kg/day for 4- or 12 weeks. The Control and HD groups also included 8 additional rat per sex to study reversibility of lesions after a recovery period of 8 weeks following 3 months of dosing. In HD females, a lower number of estrus events was noted during weeks 1-4. Slight increases in absolute testes (24.3% at 3-months) and liver weights (females only; 4-weeks and 3-

months) were noted in the HD group. Gross findings in the HD group included head nodules of the epididymides, discoloration of the glandular stomach (males and females), enlarged testes and discoloration of thyroids (males) and diminished thyroids (females). Thyroid findings were not fully reversible. After both 4 and 12-weeks, a non-reversible disorganization of the olfactory epithelium including basal cell hyperplasia and a loss of PAS staining of Bowman's glands was noted in all HD animals. Additionally, a focal atrophy of the germinative testicular epithelium, formation of giant cells, and epididymal oligo- and aspermia and formation of spermatoceles were observed at the HD after 3 months treatment. Severity of findings was similar at 4 and 12 weeks and the findings were partially reversible. An increase in agonal bleeding of the thymus was also noted in HD males after 3 months. Lympho-histiocytic inflammation (mild) was observed in numerous tissues/organs at a slightly greater incidence/severity in HD animals than in control animals. The NOAEL was identified as 0.2 mg/kg/day roflumilast based on histological findings. The study did not include toxicokinetic data.

In a 6-month study (14/96), rats (20/sex/dose) were given roflumilast at doses of 0, 0.5. 1.5 or 2.5 mg/kg/day. The Control and HD groups also included 8 additional mice per sex to study reversibility of lesions after a recovery period of 4 weeks. Mid- and high-dose females had a decreased number of estrus events versus control animals. HD males had slightly increased testes (18.1%), seminal vesicle (11.6%), and prostate weight (6.6%) following the dosing period, and seminal vesicle weight was still increased following the recovery period [↑ 18%]. MD and HD females had increased ovary weight (23.3% and 31.1%, respectively). Microscopic findings included a non-reversible disruption of the nasal olfactory epithelium and an influx of round cells beneath the basement membrane of olfactory epithelium in mid- and high-dose animals (respective incidence in the C, LD, MD and HD groups was 0/20, 0/20, 16/20, 27/28 in males and 0/20, 0/20, 17/20, 24/28 in females). A non-reversible increase in epididymal spermatoceles and tubular developmental defects of the testes were observed in mid- and high-dose males (incidence: 1/20 and 4/20). Focal gastric erosion, peritonitis, and ulceration were noted in HD animals. One HD female had a mammary gland adenoma. The NOAEL was identified as 0.5 mg/kg/day due to microscopic findings in the nasal cavity which was associated with an AUC of 7.4 μg.h/L.

A second 6-month study (191/2000) was conducted to further define the dose response of roflumilast between 0.5 and 1 mg/kg/day. Rats (20/sex/dose) were given roflumilast at doses of 0 or 0.8 mg/kg/day. The Control and HD groups also included 8 additional mice per sex to study reversibility of lesions after a recovery period of 4 weeks. Leukocyte numbers were elevated in males and females (22-36%) and remained elevated following the recovery period (12%). Absolute testes weight was increased in males (21%) but resolved following the recovery period. In the nasal cavity, atrophy/disorganization of the epithelium associated with inflammatory exudate in the lumen was observed in one treated male. Transitional epithelial hyperplasia and inflammation were also observed. The NOAEL for this study was 0.8 mg/kg/day and was associated with an AUC of 30.9 μg.hr/L.

Hamsters:

A 3-month oral dose-ranging study was performed in hamsters (#252/98) prior to the carcinogenicity study. Hamsters (10/sex/group) were given Roflumilast at doses of 0, 4, 8, and 16 mg/kg/day. Body weight gain was significantly reduced in HD females (-34%) and food consumption was decreased in all male treated groups and in HD females. Gross findings

included adrenal discoloration and thymic involution in HD males and females. Histopathological findings included dysspermia and tubular atrophy of the testes in all treatment groups and seminal vesicle atrophy in the HD group (LD and MD groups were not assessed). Olfactory epithelial disorganization was noted in the MD and HD groups, and olfactory epithelial necrosis was noted in one HD male. Other notable findings included prostatic atrophy, pancreatic granuloma in the HD group, and hyperemia of the adrenal glands in one HD male and one HD female, and findings in the eyes of HD males and females. The NOAEL was 4 mg/kg/day and was associated with a mean AUC of 44.4 μg.h/L.

Dogs:

Oral toxicity studies up to 12 months in treatment showed that roflumilast affected the heart in dogs. These studies included 1, 6, and 12-month studies. The respective roflumilast dose levels were 0, 2, 6, and 18 mg/kg/day in a 1-month study (#68/95); 0, 0.2, 1.0 and 4.0 mg/kg/day in a 6-month study (#94/96); 0, 0.2, 0.6, and 2.0 in the 12-month study (#132/2000). Slight and dose-related increases in the incidence of abnormalities were observed in the heart. The findings included chronic inflammatory changes in the right atrium and right auricle. The change was characterized as mild lymph-histiocytic infiltration with slight subserosal edema or hemorrhage. All changes were reversible. The NOAEL was 2.0, 0.2 and 0.6 mg/kg/day in the 1, 6 and 12 month studies, respectively, with respective AUCs of 827.6, 203.7, and510.1 μg.h/L.

In a 1-month study (68/95), dogs (3/sex/dose) were given Roflumilast at doses of 0, 2, 6, or 18 mg/kg/day. The HD group also included 2 additional dogs per sex to study reversibility of lesions after a recovery period of 4 weeks. One MD male died on Day 21 due to invagination of the colon. Alkaline phosphatase levels were increased (76-90%) in 6 HD animals on Day 23. Gross finding were noted in the heart (bleeding in the right auricle of 1 MD and 2 HD animals, as well as a nodule, scar tissue, and bleeding in the left auricle of 1 HD animal), stomach (swollen mucosa in 1 LD, 2 MD, and 3 HD animals), intestine (red spots at the ileocecal valve and swollen mucosa in HD animals), and liver (dose-related marked lobe pattern and swollen liver). Histopathological findings were noted in the heart of MD and HD animals. Two animals displayed moderate to severe subacute inflammation of the myocardium and nutritive vessels in the right atrium and right auricle. One HD animal showed slight focal neutrophilic infiltration in the right auricle. Heart findings were to be reversible. Tubular degeneration of the testes with dysspermia in the epididymides was reported in one HD male. The NOAEL was identified as 2 mg/kg/day and was associated with a $AUC_{0\text{-}24hr}$ of 827.6 μg.hr/L.

In a 6-month study (94/96), dogs (5/sex/dose) were given roflumilast at doses of 0, 0.5/0.2, 2/1 or 8/4 mg/kg/day. The HD group also included 2 additional dogs per sex to study reversibility of lesions after a recovery period of 4 weeks. The primary microscopic changes included mild to moderate subacute to chronic reactive inflammatory changes in the right atrium (1 HD male) or right auricle (1 HD male; 1-MD female and 2-HD females). One female also demonstrated lympho-histiocytic infiltration following the recovery period. The low-dose of 0.2 mg /kg/day was identified as the NOAEL for this study and was associated with an $AUC_{0\text{-}inf}$ of 203.7 μg/l.h.

In a 12-month study (132/2000), dogs (5/sex/dose) were given Roflumilast at 0, 0.2, 0.6, and 2 mg/kg/day. The HD group also included 2 additional dogs per sex to study reversibility of lesions after a recovery period of 4 weeks. Gross pathological findings included 2-3 mm blood cysts in the heart of HD group (one in each sex). Gross findings correlated with microscopic

findings in the heart, including minimal focal epicardial hemosiderin deposits at the right auricle (incidence: 2/5 –M and 3/5-F) and minimal to mild hemorrhages in HD animals (incidence: 2/5 in each sex). The hemorrhages were reversible. The NOAEL was identified as 0.6 mg/kg/day due to heart and was associated with an $AUC_{0\text{-}24hr}$ of 510.1 μg/l*hr at 52 weeks.

Monkeys:

Oral toxicity studies up to 9-months did not identify any treatment-related effect in monkeys at roflumilast doses up to 0.5 mg/kg/day (Studies #232/2001, 242/2001 and 182/2002). Monkeys were dosed orally with 0, 0.1, 0.25 or 0.5-mg/kg/day roflumilast for 4 weeks or 9 months. Three monkeys per sex per dose were sacrificed after 4 weeks of treatment. Four monkeys per sex per dose were sacrificed at the end of 9-month treatment. Two additional monkeys per sex per dose in the C and HD groups were sacrificed at a 1-month recovery period. No treatment-related effects were observed except for the HD group showed a brief loss (up to 13%) in body weight. The NOAEL was 0.25 mg/kg/day because of the loss in body weight with an associated AUC of 253.1 53.1 μg.h/L.

2.6.6.1.3 Genetic toxicology

Roflumilast was positive for induction of micronucleus formation in an *in vivo* mouse micronucleus test, but was negative in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosome aberration assay in human lymphocytes, *in vitro* HPRT test with V79 cells, an *in vitro* micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and *in vivo* mouse bone marrow chromosome aberration assay. In the *in vivo* mouse micronucleus assay, roflumilast induced micronuclei at oral doses of 300 mg/kg at 48 hours and 900 mg/kg 24 and 48 hours. The Division, in conjunction with the Genetic Toxicology Committee, concluded that the sponsor had adequately assessed concerns regarding the genotoxic potential of roflumilast since both in vitro and in vivo chromosome aberration studies were negative [ref.: Nonclinical Review #1, p96].

2.6.6.1.4 Carcinogenicity:

The evaluation of carcinogenic potential of roflumilast in hamsters was completed in two 2-year studies in Syrian golden hamsters. In the first study, 60 hamsters/sex/dose were treated with 0.5, 1, 4 and 8 mg/kg/day of roflumilast by oral gavage for 103 weeks. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males, but not the females, showed a statistically significant trend for increased mortality ($P < 0.004$). There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The females showed a statistically significant trend for an increased incidence of undifferentiated carcinomas in the nasal cavity. The incidences for the nasal carcinomas were 4/60 in the high dose group and 0/60 for the remaining groups (Table 20). No remarkable neoplastic findings were observed in the males of any roflumilast-treated groups.

The second study was conducted as a supplemental study to the first study to comply with the Center's ECAC's recommendation on roflumilast dose selection. Sixty hamsters per sex were treated with 16 mg/kg/day of roflumilast for 103 weeks. An additional 30 hamsters per sex that did not receive any treatment served as cage controls. Both roflumilast-treated males and

females showed significant increases in mortality. The roflumilast treated groups showed numerical, but statistically non-significant increases in the incidence of undifferentiated carcinomas in the nasal cavity (incidence: 0/30 – cage control and 5/60 – roflumilast in each sex). However, the background incidence of nasal tumors is very low (0.03% or 1/2649). Based on the totality of the data from the two studies, the Executive Carcinogenicity Assessment Committee of the Center concluded on May 10, 2005 that roflumilast was carcinogenic in hamsters at doses of 8 and 16 mg/kg/day. See Section 2.6.6.9.1 – Carcinogenicity discussion about the interpretation of the carcinogenicity study data and its relevance to humans.

Table 20 Tumor Prevalence in the 2-yr Carcinogenicity Study in Hamsters

Tumor	Sex	Study No.	Tumor Prevalence (%) [a]							P-Value (Exact)
			Control		Roflumilast (mg/kg/day)					
			Cage	Veh.	0.25	1	4	8	16	
Carcinoma,	M	7/2002	1.7	0	0	0	0	0	-	NS
undifferentiated		233/2003	0	-	-	-	-	-	8.3	0.0587 [c]
	F	7/2002	0	0	0	0	0	6.7	-	.0006 [b]
		233/2003	0	-	-	-	-	-	8.3	.081 [c]
Adenocarcinoma,	M	233/2003	0	-	-	-	-	-	1.7	NS
Bowman's gland	F	233/2003	0	-	-	-	-	-	1.7	NS
Adenoma,	M	233/2003	0	-	-	-	-	-	1.7	NS
Bowman's gland	F	233/2003	0	-	-	-	-	-	3.3	NS
Total		7/2002	0.8	0	0	0	0	3.3	-	-
		233/2003	0	-	-	-	-	-	12.5	-

a. Extracted from Table 2.6.6 – 24 of the submission. n = 50 and 30/sex/group for studies 7/2002 and 233/2003, respectively.
b. Against vehicle control (asymptotic method)
c. Against cage control (exact method)

In mice, B6C3F1 mice (50/sex/dose) were treated with roflumilast by oral gavage for 103 weeks. The respective roflumilast doses were 0.5, 2.0, 6.0 and 18 mg/kg/day in males and 0.5, 2.0, 6.0 and 12 mg/kg/day in females. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males showed a statistically significant and dose-related trend in increased mortality ($P < 0.0000$). The high dose groups showed a statistically significant decreases in body weight relative to the vehicle (↓ 25.8% and ↓ 14.3% in males and females, respectively, $p < 0.05$) or cage control (↓ 21.7% and ↓ 12.1% in males and females, respectively, $P < 0.05$). Roflumilast-treated mice did not show statistically significant increases in the incidence of any tumors.

2.6.6.1.5 Reproductive and Developmental Toxicity:

Animal toxicity studies were conducted to evaluate the effect of roflumilast on fertility, embryofetal development, and pregnancy and deliver. Effects on fertility were studied in rats and mice. Effects on embryofetal development were studied in rats and rabbits. Effects on pregnancy and delivery were studied in mice. Studies were also conducted to evaluate the effects of roflumilast on post-natal development. The studies revealed that roflumilast decreased fertility in rats, and caused pre- and post-implantation losses in rats when both males and females were treated. Roflumilast treatment resulted in stillborns in pregnant rats and mice. Roflumilast

also disrupted the delivery process and resulted in fetal deaths in rats and mice. Roflumilast was, however, non-teratogenic in rats and rabbits although it may delay bone ossification.

2.6.6.1.5.1 Fertility

Effects of roflumilast on fertility were evaluated in rats and mice (Reports 19/97, 114/2002 and M33/2002). Only Report 19/97 showed that roflumilast affected male fertility rate at 1.8-mg/kg/day (Table 21). The remaining two studies (one each in rats and mice; Reports 114/2002 and M33/200) did not reveal any effect of roflumilast on fertility. The designs of these two studies, however, differed from Report 17/97. Report 114/2002 used only females and Report M33/2002 used only males. The totality of the data showed that roflumilast decreased male fertility in rats.

Table 21 Male Fertility Studies of Roflumilast

Species	Time of Treatment	Roflumilast (mg/kg/day)	Major Findings	Study #
Rat , M + F	M: 70 d prior to and through mating F: 14 d Bef. mating to GD 15	0, 0.2**, 0.6**, 1.8	↓ fertility, ↑ epididymal sperm granuloma, ↑ post implantation loss , ↓ live fetuses	19/97
Rat , F	14 d before & through mating	0, 0.5, 0.8, **1.5**	No effect on female fertility	114/2002
Mouse, M	18 weeks	0, 4, 12, **36**	No effect on male fertility	M33/2002

* Bold face indicates NOAEL.

In Report 19/97, both male and female Wistar rats (28/sex/dose) were treated with 0 (C), 0.2 (LD), 0.6 (MD), or 1.8 (HD)-mg/kg/day before and during the mating. The pre-mating treatment duration was 70 and 14 days in males and females, respectively. The female treatment continued until gestation day 15. Treatment-related effects were observed in the MD and HD group. The effect included decreases in copulation rate, fertility rate in the HD males and increases in pre- and post- implantation losses and the number of live fetus in the MD and HD females. The compromises in fertility index were accompanied by morphological changes in the male reproductive organs: testicular tubular atrophy and epididymal spermiogenic granuloma, hypospermia and dysspermia. The respective male fertility parameters in the C, LD, MD and HD groups were 89.2%, 100%, 92.3% and 64.2% in fertility rate; 28, 28, 27 and 22 in number of males which mated; and 25, 28, 26 and 18 ($P < 0.001$) in the number of fertile males. The respective mean implantation loss in the C, LD, MD and HD groups was 4.7%, 5.5%, 14.8% ($p < 0.05$) and 30.7%* ($p < 0.01$) in post implantation loss and 11.4%, 11.6%, 21.5%* ($p < 0.05$) and 17.5%* ($p < 0.01$). The mean litter size was 11.4, 11.5, 9.9 and 8.2* (↓ 21%, $p < 0.05$) in the C, LD, MD and HD groups, respectively. The NOAEL for fertility effects was the mid-dose of 0.6 mg/kg while the NOAEL for embryo-fetal development was the low-dose of 0.2 mg/kg. AUC was not determined in the study.

The above findings prompted a follow-up study to investigate the fertility effect of roflumilast in females only (Report 114/2202). Female rats were treated with 0, 0.5, 0.8 and 1.5-mg/kg/day roflumilast from 14 days prior to mating to 7 days after mating. The parameters included fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses and live fetus. The study did not show any effect on fertility

parameters; nor did it reveal any apparent toxicity at the HD group. There were, however, significant differences in design of Report 114/2002: 1) males were not treated, 2) the roflumilast dose was lower.

Effects of roflumilast on male fertility in mice were evaluated in a modified 6-month general toxicity study (Report 33/2002). Male mice (20/sex/dose) that had been dosed with 0, 4, 12 or 36-mg/kg/day roflumilast scheduled to dose for 26 weeks. After 18 weeks treatment, they were allowed to mate with untreated females (Ratio = 1 to 1). The pregnant females were sacrificed on gestation day 15 for the evaluation of fertility parameters. No effects on fertility parameters were observed at any doses. The NOAEL for general toxicity findings was 4 mg/kg/day which had a mean AUC of 153.1 μg.h/L.

Nonclinical findings of the effects of roflumilast on male fertility have been addressed clinically. A 3-month clinical trial (Report 98/2002) was completed to study the effects of roflumilast on male fertility in humans. The medical discipline is reviewing the report.

2.6.6.1.5.2 Embryofetal development

Roflumilast was not teratogenic in mice, rats or rabbits; the drug, however, may delay fetal development and cause stillbirths when given to mice during pregnancy. Fetal toxicity of roflumilast may be related to its tocolytic effect. Table 22 presents an overview of the studies evaluating the effect of roflumilast on embryofetal development and pregnancy.

Table 22 Pregnancy and Fetal Developmental Studies Roflumilast

Species	Treatment duration	Roflumilast (mg/kg/day)	Major Findings	Study #
Embryofetal development				
Rat, F	GD 6 – 15	0, **0.2**, 0.6, 1.8	Incomplete bone ossification	8/96
Rabbit, F	GD 6 – 18	0, 0.2, **0.4**, 0.8	No teratogenic effect	191/95
Pre- and post –natal development				
Mouse, F	GD 6 – 18	0, 2, 6, 12	↑ stillborns & ↓ pup viability, delivery problems	125/2002
Mouse, F	GD 6 - 18	0, 12		126/2002
Mouse, F	GD 6 – 15, LD 1 - 21	0, **1.5**, 3, 6		127/2002

a. Bold face indicates NOAEL. GD, gestation days; LD, lactation days.

Embryo-fetal developmental toxicity studies in mice, rats and rabbits were performed. Pregnant females were dosed during the period of gestation days 6 – 18, 6 – 15, 6 – 18 in mice, rats and rabbits, respectively. The respective roflumilast doses ranged 0.2 - 1.8, 0.2 – 0.8 and 1.5 - 12 mg/kg/day in mice, rats and in rabbits, respectively. No significant increases in the incidence of malformations were observed in any species. The statistically significant increases in the incidence of incomplete ossification of skull bones were observed in the MD and HD groups in rats. The respective incidence in the C, LD, MD and HD was (# fetus/litter) was 53/23, 49/18, 59/22 and 89/29 ($P < 0.05$) in inter-parietal bones and 117/11, 20/11, 34/15 ($P < 0.05$) and 46/23 ($P < 0.05$) in parietal bones. The NOAEL for embryo-fetal development was 1.5, 0.2 and 0.2 mg/kg/day in mice, rats and rabbits, respectively. AUCs were not determined.

2.6.6.1.5.3 Pre- and Post-Natal Development

The effect of roflumilast on peri- and post-natal delivery was studied in mice. Three studies showed that roflumilast is tocolytic and can cause significant harm to fetuses if given to pregnant dams (Tables 28 and 31). In Study 125/2002, pregnant mice (20-23/dose, NMRI) receiving orally 2, 6 and 12-mg/kg/day roflumilast during pregnancy (GD 6 – 18). Numbers of live births, stillborns and pup viability and development were evaluated. Results of the study are summarized in Table 27. Briefly, roflumilast treatment caused dose-related increases in the number of stillborns and litter losses and decrease in pup viability and post-natal development. Statistically significant increases in number of stillborns ($P < 0.01$) were observed in all treated groups (Prevalence: 1.7%, 16.4%, 29.9% and 75.0% in C, LD, MD and HD groups, respectively). Other respective parameters in C, LD, MD and HD groups were 0, 2, 6 and 14 ($p < 0.01$) in number of total litter loses; 95.3%, 96.6%, 73.0% and 35.0% in post-natal viability (to Day 4); 97%, 94%, 96% and 57% in forelimb grip reflex and 93%, 92%, 85% and 57% in pinna detachment.

Report 126/2002 investigated the temporal effect of roflumilast on pregnancy and delivery in mice. Pregnant mice (10-12/group) were given vehicle (G1) or 12 mg/kg/day roflumilast orally during lactation (lactation day 1 – 20) and at different times of pregnancy: Gestation Days 6 - 16 (G2), 15 – 18 (G3), or 18 (later time of the day, G4). A control group received the vehicle during lactation and GD 6 – 16. The treated dams showed significant difficulty in delivery. Groups 3 and 4 dams showed increases in the incidence of deaths and early sacrifice due to moribund conditions (incidence: 0/15, 0/15, 2/15 and 3/15 in Groups 1, 2, 3 and 4, respectively). The death and sacrifice occurred around the time of delivery due to delivery complications. The roflumilast treatment groups showed significant increases in the incidence of stillborns. The respective frequencies of stillborns in Groups 1, 2, 3 and 4 was 0% (0/10), 33.3% (4/12), 90% (9/10. $p < 0.01$) and 100% (6/6, $p < 0.01$) in dams; and 0.0% (0/116), 19%% (18/93, $p < 0.01$), 56% (50/89, $p < 0.01$) and 45% (32/71, $p < 0.01$) in pups. The pup viability index on lactation day 4 was 99.1%, 86.7% ($P < 0.01$), 20.5% ($P < 0.01$) and 25.6% ($P < 0.01$) in Groups 1, 2, 3 and 4, respectively.

Report 127/2002 used a finer dose selection to further investigate effects of roflumilast on pre- and post-natal development. Female mice (30/dose, NMRI) were given orally 0, 1.5, 3 or 6-mg/kg/day roflumilast during gestation days 6 – 15 and the lactation period. Pregnancy outcomes were examined at delivery. Behavioral developmental effects were evaluated in pups. Dose-related effects on number of stillborns, litter sizes, pup viability and locomotor activity were observed in the MD and HD groups. The respective frequencies in the C, LD, MD and HD groups were 3.4% (1/29), 3.6% (1/28), 11.1% (3/28) and 22.2% (6/28) in litters with stillborns; and 0.03% (1/366), 0.03% (1/330), 3.1% (10/323, $p < 0.05$) and 8.8% (24/271, $p < 0.01$) in prevalence of stillborn pups. The mean litter size was 12.6, 11.8, 12.0 and 9.9 ($p < 0.05$) pups/litter in the C, LD, MD and HD groups, respectively. The decreases in pup viability were observed both pre and after lactation day 4 (culling). The frequency of litters with live pups at birth, but no pups, on day 4 was 0%, 0%, 0% and 8% in C, LD, MD and HD groups, respectively. The frequency of pup deaths in the post-culling period was 1.1%, 0.3%, 3.2% and 4.5% ($p < 0.05$) in C, LD, MD and HD groups, respectively. The HD dams showed minimal and statistically non-significant decreases in mean body weight (3.9%).

2.6.6.1.6 Local Tolerance

Local tolerance of roflumilast was studied by intramuscular injection in rats; and intravenous, para-venous, and intra-arterial injections in rabbits; and intracutaneous and epitaneous injections in guinea pigs. Slight to moderate petechial hemorrhage in the surrounding areas of the injection sites (0.1 ml of 0.02% roflumilast) were observed in rats. Slight irritation/inflammation at the injection sites were observed in at each route of administration (0.5 – 1.0 ml of 0.02% roflumilast) in rabbits. No irritation or sensitization potential was observed at 2 x 0.1ml of 0.5% roflumilast, intracutaneously in guinea pigs.

2.6.6.1.7 Other Toxicity Studies

Toxicity studies were completed to evaluate the general and genetic toxicity profile of three roflumilast metabolites: roflumilast N-oxide, ADCP and ADCP N-oxide. Studies of roflumilast N-oxide included both general and genetic toxicity studies while only genetic toxicity studies were completed for ADCP and ADCP N-oxide.

2.6.6.1.7.1 Roflumilast N-oxide Toxicity

General toxicity studies of roflumilast N-oxide have been completed in mice, rats and dogs. Table 23 provides an overview of these studies. The treatment duration was 6, 1 and 12 months in mice, rats and dogs, respectively. The toxicity profile of roflumilast N-oxide is generally similar to that of roflumilast. The target organs of roflumilast N-oxide toxicity include the nose, heart, gastrointestinal tract and the male reproductive organs. Roflumilast tested negative in a bacterial mutation assay (Ames test) and a mammalian chromosomal aberration assay in V79 cells.

Table 23 Oral Repeat-Dose Toxicity Studies of Roflumilast N-oxide

Species	Durat'n (month)	Roflumilast (mg/kg/day)	N/sex/ dose	Target Organs of Toxicity	Study#	Rev. #
Mouse	6	0, **4**, 10, 25	20 + (8)	Olfactory, prostate, adrenal	54/2002	6 [a]
Rat	1	0, .4, 1.2, 3.6	20 + (8)	Olfactory, testis, epididymides, prostate, seminal vesicles, GI tract	116/99	1
Dog	1	0, 0.6, **1.2**, 2.4	3 (+ 2)	Heart, testis, epididymides	33/99	1, 3
	12	0, .1, .4, .8, **1.2**	5 (+ 2)	None	162/2001	1

a. Reviews 1, 3 and 6 refer to reviews completed by Dr. Timothy McGovern on 24-JUL-2000, 13-JAN-2003 and the current review, respectively.

Mice

In a 26-week study (54/2002, 52/2002), mice (20/sex/dose) were given roflumilast N-oxide by oral gavage at doses of 0, 4, 10 or 25-mg/kg/day. Control and HD groups also included 8 additional mice to study reversibility of lesions after a recovery period of 4 weeks. In addition, each treatment group included 25 mice for evaluation of plasma roflumilast levels. Finally, after 18 weeks of treatment, each male was paired with an untreated female (age 12 – 13 weeks) to evaluate the fertility rate. Pregnant females were sacrificed on gestations day 15 for fetal

developmental evaluations. Dose-related, unscheduled deaths were observed (4 HD males and 1, 1, and 2 LD, MD, and HD females, respectively). Dose-related decreases in body weight were observed in all treatment groups. Significant, dose-related decreases in prostate weight were noted (down 23.6%, 27.8% and 33.3% compared to control), and HD males showed significant increases in adrenal gland weight. Histopathological findings were noted in the nasal cavity of HD females and in the adrenal cortex in HD males and females. No treatment-related effects on male fertility were observed. Systemic exposure to roflumilast and roflumilast N-oxide increased supra-proportionally to the dose. The NOAEL was 4 mg/kg/day with respective AUCs of 14.4 and 1419.4 µg.h/L for roflumilast and roflumilast N-oxide.

Rats

In a 4-week study (116/99), rats (10/sex/group) were given roflumilast N-oxide at doses of 0, 0.4, 1.2, or 3.6 mg/kg/day. The control and HD groups also included an additional 8 rats per sex to study reversibility of lesions after a recovery period of 4 weeks. In addition, 6 rats per sex per group were used for toxicokinetic analyses. Two HD females were sacrificed in moribund condition on Days 17 and 25, with primary histopathological findings of inflammatory changes in the ileum, jejunum, and pancreas. HD males and females had increased leukocytes (approximately 30%) and segmented neutrophils (approximately 500%). Fewer estrus events were noted in HD females during the dosing period versus controls. Reversible changes in organ weights included increased adrenal weights (38.0 – 59%) and decreased thymus weights (30.0% - 41.1%) in HD males and females, and increased testes (28.0%) weight in HD males. Gross findings in HD animals included small prostate, seminal vesicles and uterus, involuted thymus, and swollen intestine. Histopathological findings included inflammation of the intestine (incidence: 2/10 and 5/10 in males and females, respectively), and pancreas (2/10 and 5/10 in males and females, respectively) in HD animals. HD males had non-reversible spermiogenic effects in the epididymides, as well as tubular dilatation of the testes (8/10) and atrophy of the prostate (2/10) and seminal vesicles (2/10). Thymic atrophy was noted in HD males (3/10) and females (7/10) and uterine atrophy (2/10) was noted in HD females. A NOAEL was not determined since findings in the prostate, seminal vesicles, and uterus in the HD group were not examined in the lower dose groups.

Dogs

In a 1-month study (33/99), dogs (3/sex/dose) were given roflumilast N-oxide at doses of 0, 0.6, 1.2, and 2.4 mg/kg/day. The HD group also included 2 additional females and 3 additional males to study reversibility of lesions after a recovery period of 4 weeks. Two HD males in the recovery group were sacrificed in moribund condition on days 8 and 21. These animals showed massive bleeding at the right auriculum following a gross pathological evaluation. Histopathological findings associated with these animals included a moderate to severe myocarditis of the right atrium and were characterized by moderate to severe lymphohistiocytic and purulent infiltration with hemorrhage. The changes were accompanied by a moderate-to-severe activation of mesenchymal connective tissue, reflected by fibroblastic proliferation and de-novo synthesis of juvenile capillaries. The surviving recovery animal did not display this effect. Reversible findings were also noted in the left ventricle of one HD female (inflammation, degeneration). Reversible prostatic atrophy and fibrosis of low severity was also noted in HD

males. The NOAEL was identified as 1.2 mg/kg/day and was associated with an $AUC_{0\text{-}24hr}$ of 97.2 μg/l.h for B9502-044 and an $AUC_{0\text{-}24hr}$ of 26.2 μg/l.h for metabolite B9302-107.

In a 52-week study (162/2001), dogs (5/sex/dose) were given roflumilast N-oxide at doses of 0, 0.1, 0.4, 0.8, and 1.2 mg/kg/day. The HD group also included 2 additional animals per sex to study reversibility of lesions after a recovery period of 4 weeks. Andrology assessments did not show any definitive drug-related effects. The NOAEL was identified as the HD of 1.2 mg/kg/day, as no definitive target organs of toxicity were noted. The NOAEL was associated with an $AUC_{0\text{-}\infty}$ of 483 μg/l.h.

2.6.6.1.7.2 ADCP

ADCP tested negative in in vivo mouse micronucleus test (Report 106/98) and DNA ^{32}P-postlabeling assay in rat tissues (Report 14E/99).

2.6.6.2 Single-dose toxicity

No new single-dose toxicity data were submitted. Studies assessing the acute toxicity of roflumilast were reviewed previously by the Division. See Pharmacology and Toxicology Review No. 1 (Appendix 1) completed by Dr. Timothy McGovern on July 24, 2000 (p 41 – 52) for detailed review of these studies.

2.6.6.3 Repeat-dose toxicity

The application submitted oral toxicity studies of roflumilast up to 6, 6, 3, 12, and 10 months in treatment duration in mice, rats, hamsters, dogs, and monkeys, respectively. All but the 6-month studies have been reviewed previously. Refer to Pharmacology and Toxicology Reviews 1, 3 and 4 (Appendices 1, 2 and 3) for detailed review of these studies. The following is a review of the 6-month mouse study (Report 33/2002).

Study Title: Roflumilast: 26-week repeated-dose oral toxicity (gavage) study in the B6C3F1 mouse, including testing on the male fertility (Reports #33/2002 and 197/2001)

Key Study Findings:

- Male mice treated with 12 and 36-mg/kg/day roflumilast for 6 months showed changes in the heart (peri-arteritis), nose, lung (peri-vascular inflammation), stomach (epithelial hyperplasia and hyperkeratosis), uterus and vagina (atrophy), and adrenal glands (hypertrophy).
- Male fertility was not affected. The effect on testes was equivocal.
- NOAEL was 4 mg/kg/day with an associated AUC of 153.1 ng*h/mL.

Study number: Report 33/2002, (b) (4)
Location in electronic submission: Section 4.2.3.2

Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 20, 2001
Study termination date:	September 19, 2001
Report date:	June 27, 2003 , 2003
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298596, purity 101.4%, impurities 0.6%
Formulation/vehicle:	Antiform suspension containing Methocel (2-3 drops in 200-mL Antiform)

Methods:

Male mice (20/sex/dose) were given roflumilast by oral gavage 0, 4, 12 or 36-mg/kg/day for 26 weeks. The vehicle and high dose groups also contain 8 additional mice to study reversibility of lesions. In addition, each treatment group included 25 mice for evaluation of plasma roflumilast levels. Finally, after 18 weeks into the treatment, each male was paired with an untreated female (age 12 – 13 weeks) to evaluate the fertility rate. Pregnant females were sacrificed on gestation day 15 for fetal developmental evaluations.

Doses	0, 4, 12 or 36 mg/kg/day
Species/strain:	Mice, B6C3F1
#/sex/group (main study):	20 /sex/dose; also included were 20 untreated females/dose to evaluate male fertility
For reversibility	8/sex in the vehicle and HD groups
For toxicokinetics:	25 /sex/dose
Age:	11 - 12 weeks at commencement of treatment
Weight:	M; 17.0 – 27.5 g; F: 15.8 – 21.1 g
Route, volume:	Oral gavage, 10 mL/kg
Treatment duration:	Once daily from 2 weeks before mating until 7 days post mating. The mating period was up to 3 weeks.

Observations and times:

Mortality:	Daily
Clinical signs:	Daily
Body Weight:	Weekly
Food consumption:	Weekly
Paring:	Male to female ratio of 1:1
Toxicokinetics:	Weeks 1 (day 1), 3 (day 15), 13 and 26
Clinical pathology:	Weeks 13, 26 and 30
Necropsy and histology:	A complete panel of organs at end of the treatment and recovery periods in C and HD groups; adrenals, heart, nasal cavity, prostate, testes, epididymides, ovaries, uterus, vagina, and tissues with gross lesions in LD and MD groups
Maternal examination:	Fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses

Results:

Mortality: No treatment related deaths were observed.

Clinical Signs: No treatment-related effect was observed.

Body Weight: All treated males and HD females showed decreases in body weight. At the end of the study, the mean body weight reduction was 8.8%, 13.6%, 18.5% in LD, MD and HD males and 13.4% in HD females, respectively.

Hematology: The MD and HD groups and occasionally the LD group, showed slight, but statistically increases in segmented neutrophils (relative 0 – 25%), decreases in lymphocyte counts (up to 60%), eosinophil counts (up to 67%) and white blood cell counts. All findings were reversible.

Clinical chemistry: The MD and HD groups, and occasionally the LD group, showed slight but statistically significant increases in bilirubin (up to 100%), total protein (up to 5%), phosphorus (up to 25%), chloride (up to 2%) and cholinesterase (up to 50%) and decreases in triglycerides (up to 55%). All findings were reversible.

Urinalysis: No treatment-related effects were observed.

Organ weight: Reduced weights were noted for ovaries (44.4%) and pituitary gland (33.3%) in HD females, for spleen (21 – 34%) in HD males and females, for thymus in MD (26%) and HD (41%). Increased adrenal gland weights were noted in MD and HD males (21% in both groups). All findings were reversible.

Histopathology: Organs with most noticeable findings were the adrenal glands, lung, and stomach in both sexes and the nasal cavity and the reproductive organs in the females. The adrenal glands showed increases in the incidences of cortex hypertrophy in all treatment groups (Table 24). The lung showed increases in the incidence of perivascular information in the HD group. The stomach showed increases in the incidence of hyperkeratosis in the HD group. The nasal cavity showed increases in the incidence of nasal lesions in the MD and HD female groups. The nasal lesions included olfactory degeneration, nerve fiber atrophy and hypertrophy of the Bowman's gland. The findings in the female reproductive organs included the atrophy of uterus, cervix and vagina in all treatment groups.

Table 24 Histopathological Findings in 6-Month Study in Mice (Report 33/2002)

Sex	Male				Female			
Roflumilast (mg/kg/day)	0	4	12	36	0	4	12	36
N/group	20	20	20	20	20	20	20	20
Heart/ Peri-arteritis	0	0	1 (3.0)	2 (3.0)	0	0	1 (2.0)	0
Nasal cavity/ olfactory degen.	0	0	0	0	0	0	2 (1.5)	17 (2.1)
Nerve fiber atrophy	0	0	0	0	0	0	0	6 (2.0)
Bowman's hyperplasia	0	0	0	0	0	0	0	18 (2.0)
Lung/ Perivascular inflamm.	0	-	0	2 (2.0)	0	0	0	3 (2.0)
Stomach/ hyperkeratosis	0	-	0	3 (3.0)	0	1 (2.0)	0	3 (2.0)
Epithelial hyperplasia	0	-	0	3 (3.0)	0	-	0	2 (2.0)
Uterus/ atrophy					0	1 (2.0)	4 (2.3)	8 (2.4)
Cervix/ atrophy					0	1 (2.0)	4 (2.8)	8 (2.5)
Vagina/ atrophy					0	2 (2.0)	3 (2.3)	2 (2.0)
Adrenal cortex/ hypertrophy	0	-	5 (2.0)	9 (1.9)	0	5 (2.0)	9 (1.9)	11 (2.0)
Testes/ tubular degeneration	0	2	6	0				
Sperm stasis	0	0	0	2				

Male fertility: No treatment-related effects were observed.

Plasma drug levels: Plasma levels of roflumilast and its metabolite levels increased generally in supra-proportionally to dose (Table 25). The mean plasma roflumilast AUCs were 153, 690 and 6,122 µg.h/L in the 4, 12 and 36 mg/kg/day group on day 184, respectively. At lower doses (i.e., 4 and 12 mg mg/kg/day), roflumilast N-oxide AUCs were approximately three times roflumilast AUCs while roflumilast and ADCP N-oxide levels were similar. ADCP levels were the lowest.

Table 25 Plasma Roflumilast and Roflumilast N-oxide Levels in 6-mo Roflumilast N-oxide toxicity Study Mice (Study 33/2002)

	Plasma $AUC_{0-\infty}$ (µg.h/L) [a]		
Roflumilast (mg/kg/day, PO)	4 mg	12 mg	36 mg
Roflumilast [b]	153.1	689.7	6122.5
Roflumilast N-oxide [b]	512.5	2196.7	15,929
ADCP [c]	NA	NA	163.7
ADCP N-oxide [b]	196.2	503.5	3,816.4

a. 26-week values: the mean of males and females. (Source: Report 197/2002).
b. AUC 0-24 hr value.
c. AUC 0-8 hr value.

Study Title: Comparison of the spermatological data in beagle dogs found in the repeated dose toxicity studies roflumilast (parent compound) and roflumilast N-oxide (metabolite) Report 34/2002

This is an expert report by (b) (4) evaluating the spermatogenic data of roflumilast-treated dogs. (b) (4) was a Professor at the Institute für Reproductions medizin, Tierärztliche Hoscschcile Hannover, Denmark. The report evaluated Reports 94/94 (6-month roflumilast) and 13001/2000 (12-month roflumilast N-oxide) in comparison to the historic data (Report 35/2002). The report concluded that neither roflumilast nor roflumilast N-oxide showed any significant effect on the spermatogenic parameters in dogs. Dr. Timothy McGovern conducted a comprehensive evaluation of male reproductive toxicity of roflumilast previously in a P/T review completed on January 30, 2003. The review concluded that roflumilast had no effect on male reproductive organs in dogs (p48) in the 6- and 12-month studies but did have some effect in a 4-week study. The effect included tubular degeneration and dysspermia in the HD group (1/3 each); both changes were reversible. Roflumilast doses in the 4-week study (Report 68/95), the potential effect of roflumilast on male reproductive organs in the 4-week study in dogs cannot be excluded.

The respective roflumilast doses in the LD, MD and HD groups were 2, 6, and 18 mg/kg/day in the 4-week study; 0.2, 1.0 and 4.0 mg/kg/day in the 6-month study; and 0.2, 0.6 and 2.0 mg/kg/day in the 12-month study. The respective plasma AUCs in the LD, MD and HD groups at the last week of the treatment were 991.9, 2304.6 and 3547.6 µg.h/L in the 4-week study; 224.5, 519.5 and 1860.7 µg.H/L in the 6-month study; and 180.1, 462.3 and 1015.3 µg.h/L in the 12-month study.

Study Title: Combined 4-week/42-Week Oral (Gavage 39) Administration Toxicity Study in Adult Cynomolgus Monkey with an 8-Week Treatment-Free Period

Key Study Findings:

- Roflumilast disrupted menstrual cycles in female monkeys. The high dose (0.5 mg/kg/day) group showed statistically significant prolongation of the menstrual cycles associated with the lack of the cyclic nature of female hormones.
- The NOAEL was 0.1 mg/kg/day.

Study number:	Report 232/2001; (b) (4)
Location in electronic submission:	Section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 2, 2001
Report date:	November 20, 2002
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298596, purity 100.4%, impurities 0.6%
Formulation/vehicle:	Antiform suspension containing Methocel (2-3 drops in 200-mL Antiform)

Methods:

Cynomolgus monkeys (4/sex/dose) were given by oral gavage 0, 0.1, 0.25 or 0.5-mg/kg/day of roflumilast for 43 weeks. The vehicle and high groups contained 2 additional monkeys/sex for evaluations of the reversibility of any lesions. Monkeys were sacrificed at the end of the treatment or the recovery period.

Doses	0, 0.1, 0.25 or 0.5-mg/kg/day
Species/strain:	Cynomolgus monkeys
#/sex/group (main study):	4 /sex/dose
For reversibility	2/sex in the vehicle and HD groups
For toxicokinetics:	No additional
Age:	4 – 9 years of age
Weight:	M; 3.9 – 8.2 kg; F: 2.4 – 5.8 kg
Route, volume:	Oral gavage, 10 mL/kg
Treatment duration:	Once daily from 2 weeks before mating until 7 days post mating. The mating period was up to 3 weeks.

Observations and times:

Mortality:	Daily in weekdays
Clinical signs:	Daily
Body Weight:	Weekly
Food consumption:	Weekly
Toxicokinetics:	Days 1 and weeks 4 and 43 at hr 0, 1, 2, 3, 4, 6, 8, 12 and 24

Clinical pathology:	Pre-treatment and weeks 4, 13, 26 and 42
Hormonal analysis:	Pre-dose and weeks 1, 2, 3, 4, 13, 17, 21, 26, 30 and 42 in males for analysis of testosterone T and inhibin B in males; Pre-dose; weeks 4, 8, 13, 17, 21, 26, 30, 35, 39 and 42; and every 3 days during the periods of days 239 – 294 and days 305 – 350 for analysis of estradiol and progesterone in females;
Ophthalmology:	Pre-treatment and weeks 4, 13, 26 and 43
EKG:	Pre-treatment and weeks 4, 13, 26 and 42
Vaginal smear:	Daily beginning at least 4 weeks prior the treatment for menstrual cycles
Testicular volume:	Pre-treatment and weeks 4, 13, 26 and 42
Spermatogenesis stage:	Pre-treatment and weeks 4, 13, 26 and 42 using flow cytometry
Necropsy and histology:	A complete panel of organs at end of the treatment and recovery periods in C and HD groups; adrenals, heart, nasal cavity, prostate, testes, epididymides, ovaries, uterus, vagina, and tissues with gross lesions in LD and MD groups

Results:

Mortality: Two HD males died (#405 on day 171) or were sacrificed (#404 on day 22). Monkey 405 showed slight inflammation in various organs that included colon, heart, lung, mandibular lymph node. The mesenteric lymph node displayed a severe inflammation. There was, however, no evidence of vasculitis. The report states that "[t]he cause of death was not clear and there was no finding of sufficient severity to explain the moribund condition of the animal." Monkey 404 showed cortical hypertrophy of adrenal glands, single cell necrosis in the liver accompanied with severely increased liver enzymes, lymphoid depletion of the lymphoid tissues. The most severe findings were multiple necrotic skin lesions and acute inflammation with bacterial growth in the left eye, hemorrhage and severe abscesses on the auxillary skin. The report state that "[t]he above findings were considered incidental and not related to the test article toxicity because similar lesions were not present in the other high dose animals."

One control male (#105) was sacrificed on day 171 due to poor physical condition associated with pneumonia.

Clinical Signs: The HD group (1 M and 3 F) showed abnormal appearance and posture not observed in other groups. Male monkey (#406) showed prostrate/lying position, reduced food intake and lower body temperature and swollen eyelids. Female monkeys (454, 455 and 456) showed hunched position and thin appearance on single occasions.

Body Weight: Statistically significant losses in body weight were observed during the first two weeks of treatment (Figure 3). The mean loss was approximately 4.5% and 9.5% in males and females, respectively. The low body weight remained throughout the study. The respective mean body weight in the C, LD, MD and HD groups was 5.8, 5.7, 5.9 and 5.9 kg on day 0 and 6.2, 5.9, 6.5 and 5.1 kg on day 294 in males; 3.2, 3.0, 2.9 and 3.2 kg on day 0 and 3.6, 3.4, 3.3 and 3.3 kg on day 294. The body weight rebounded during the recovery period.

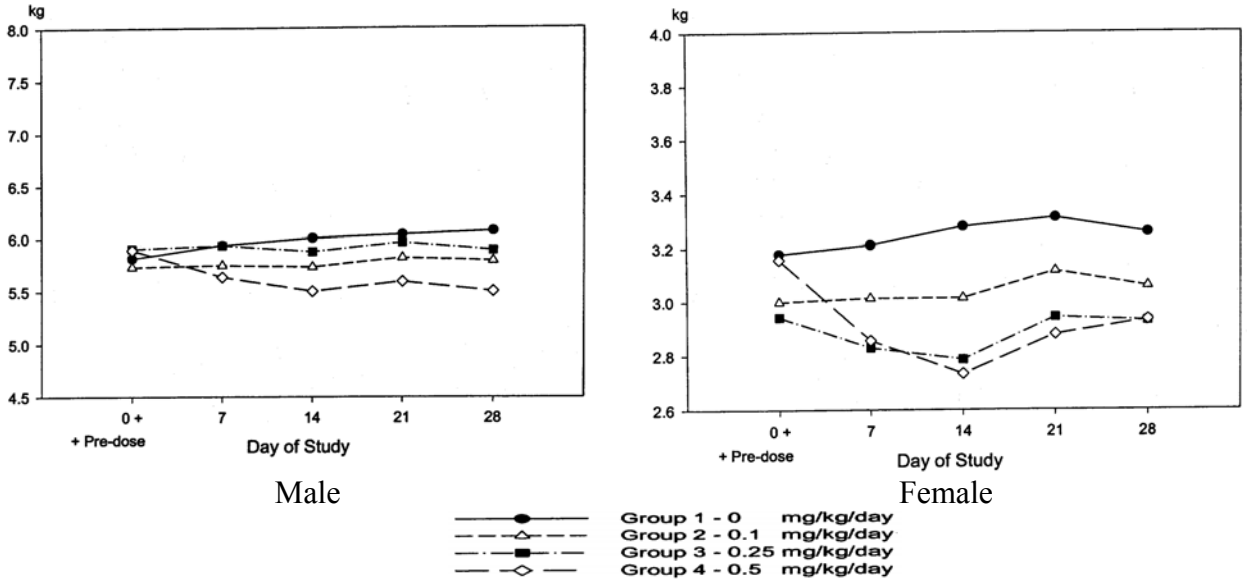


Figure 4 Mean body-weight time course of the 42 week monkey study.

Food Consumption: Statistically significant decreases in food consumption were observed during the first 3 weeks of treatment. The mean loss was approximately 7-18% and 11.6% in males and females, respectively.

Ophthalmic examinations: No treatment-related effect was observed.

Blood pressure: No treatment-related effect was observed.

EKG: No treatment-related effect was observed.

Menstrual cycles: Dose-dependent prolongation of the menstrual cycles was observed (Table 26). Only the HD group, however, reached statistically significant levels during the first third of the study.

Hormonal analysis: Timings of hormone peaks (estradiol and progesterone) could not be determined in the HD female group due to changes in menstrual cycle length. No treatment-related effects were observed on testosterone T and inhibin B levels in males.

Hematology: No treatment-related effect was observed.

Clinical chemistry: No treatment-related effect was observed.

Urinalysis: No treatment-related effects were observed.

Table 26 Effect of Roflumilast on Menstrual Cycles in Monkeys

	Menstrual	Cycle Length (days, mean ± SD) [a]			
	cycle	0	0.1 mg	0.25 mg	0.5 mg
Pre-treatment	Cycle 1	40 ± 13	30 ± 5	32 ± 5	30 ± 3
	Cycle 4	32 ± 4	54	35 ± 11	24
Treatment	Cycle 1	33 ± 9	34 ± 13	81 ± 83	101 ± 81 *
	Cycle 4	34 ± 9	37 ± 4	57	61 ± 20 *
	Cycle 7	31 ± 4	36 ± 8	-	61
	Cycle 8	33 ± 5	35	-	-

a. Numbers lack of SD reflected small sample sizes (n = ≤ 2).

Organ weight: No treatment-related effect was observed.

Histopathology: Not remarkable changes were observed except Monkey 405 which died on day 171 showed slight inflammation in various organs that included colon, heart, lung, mandibular lymph node. The mesenteric lymph node displayed a severe inflammation. There was, however, no evidence of vasculitis.

Spermatogenesis staging: No treatment-related effects were observed.

Plasma drug levels*:* Plasma levels of roflumilast and its metabolites levels increased generally in supra-proportionally to dose (Table 27). The mean plasma roflumilast AUCs were 153, 690 and 6,122 µg.h/L in the 4, 12 and 36 mg/kg/day group on day 184, respectively. At lower doses (i.e., 4 and 12 mg mg/kg/day), roflumilast N-oxide AUCs were approximately three times roflumilast AUCs while roflumilast and ADCP N-oxide levels were similar. ADCP levels were the lowest.

Table 27 Plasma Roflumilast and Metabolite Levels in Monkeys (Study 108/2002)

	Plasma $AUC_{0-\infty}$ (µg.h/L) [a]		
Roflumilast (mg/kg/day, PO)	0.1 mg	0.25 mg	0.5 mg
Roflumilast	80.3	205.2	556.3
Roflumilast N-oxide	340.0	835.0	1,684.5
ADCP	-	39.3	35.5

a. Weeks 42/43 value.

2.6.6.4 Genetic toxicology

Reports of genotoxicity testing except for Report 143/2002 have been reviewed previously by Dr. Timothy McGovern Nonclinical Review #1 completed on July 24, 2000. Results of the reports have been summarized in Section 2.6.6.1 – Overall Toxicology Summary. The following is the review and evaluation of the Report 143/2002.

Study title: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay for azo-dyes with B9302-107 (Roflumilast)

Key findings:

- All criteria for a valid assay were met.
- B9302-107 was negative for mutagenic potential in bacterial reverse mutation assays under the conditions tested.

Study no.: 12/2005

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location (b) (4)

Date of study initiation: October 7, 2004

GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: B9302-107, Batch No. 298569, 100.15% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* (*S. typhimurium*): TA1537, TA98, TA1535, and TA100; *Escherichia coli* (*E. coli*): WP2 uvrA

Doses used in definitive study: Experiment I – 3, 10, 33, 100, 333, 1000, 2500, and 5000 µg/plate with and without metabolic activation (non-induced hamster liver S9, 30% v/v) for all strains; Experiment II – 33, 100, 333, 1000, 2500, and 5000 µg/plate, with and without metabolic activation (non-induced hamster liver S9, 5% v/v) for all strains.

Basis of dose selection: Toxicity of B9302-107 was evaluated in all strains in a pre-experiment at the same doses and experimental conditions as in Experiment I (3-5000 µg/plate). The pre-experiment was reported as Experiment I because there were evaluable plates (> 0 colonies) at 5 or more concentrations. Plates incubated with B9302-107 showed normal background growth up to 5000 µg/plate, with and without metabolic activation. In addition, no toxic effects were noted in B9302-107 treated groups, as evidenced by a reduction in the number of revertants.

Negative controls: Solvent (DMSO) and untreated (medium) controls

Positive controls:

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)
TA1535, TA100	-	sodium azide	10
TA1537	-	4-nitro-o-phenylene-diamine	50
TA98	-	4-nitro-o-phenylene-diamine	10
WP2*uvr*A	-	methyl methane sulfonate	4 µl/plate
TA1535, TA100, TA1537	+	2-aminoanthracene	2.5
TA98	+	congo red	500
WP2*uvr*A	+	2-aminoanthracene	10

Incubation and sampling times: Treated plates were incubated at least 48 hours at 37°C prior to colony counting.

Results

Study validity: The definitive studies (Experiments I and II) utilized the pre-incubation method to examine the mutagenicity induction potential of B9302-107 on four *S. typhimurium* strains and one *E. coli* strain. The sponsor examined all necessary strains for the completion of the bacterial reverse mutation battery. Each concentration of B9302-107, as well as positive and negative controls in the presence and absence of metabolic activation was tested in triplicate. The positive controls induced marked increases of reverse mutations. For tester strains TA98, TA100, and WP2uvrA, the test substance was considered a mutagen when the number of revertants was >2-fold vehicle control values. For tester strains TA1535 and TA1537, the test substance was considered a mutagen when the number of revertants was > 3-fold vehicle control values. In addition, a dose-related increase in revertants was considered biologically relevant if the threshold was exceeded at more than one concentration. An increase in revertants at one

concentration only was considered biologically relevant if the result was reproduced in a second independent experiment. All criteria for a valid assay were met.

Study outcome: B9302-107 did not increase mutant frequency in any tester strain at any dose level, with or without metabolic activation. Positive controls increased revertant frequency in a strain-dependent manner. Roflumilast was negative for mutagenic potential in bacterial reverse mutation assays for azo-dyes under the conditions tested.

Study title: ^{32}P-Postlabeling assay for detection of adduct formation by Roflumilast (B9302-107) in hamster nasal mucosa and liver DNA

Key findings: No formation of DNA adducts from Roflumilast or its metabolites, specifically ADCP, was detected under the conditions of the assay.

Study no.: 143/2002

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location:
(b) (4)

Date of study initiation: February 19, 2002
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: B9302-107, Lot No. 200 589 000, 99.3% pure

Methods

Strains/species/cell line: Female Syrian Gold hamsters, n=4 per treatment group, received Roflumilast or positive or negative controls via intragastric installation (gavage) daily for 7 days.

Doses used in definitive study: 1 and 8 mg/kg/day of Roflumilast for 7 days

Basis of dose selection: Doses were selected based on the top dose used in the hamster Roflumilast carcinogenicity study.

Negative controls: 4% aqueous methylcellulose (Roflumilast vehicle, 1 mL/kg/day) and olive oil (2,6-Xylidine vehicle, 2.5 mL/kg/day) for 7 days

Positive controls: 310 mg/kg/day of 2, 6-Xylidine for 7 days

Incubation and sampling times: Animals received a daily dose of Roflumilast or positive or negative control via gavage daily for 7 days. Animals were sacrificed 24 hrs after the final dose and liver and nasal mucosa were removed for determination of DNA adduct formation in liver and nasal mucosa.

Results

Study validity: The study was valid. The purpose of this study was to assess the potential of Roflumilast to form DNA adducts, specifically, 4-amino-3, 5-dichloropyridine (ADCP) adducts formed from the monocyclic aromatic amine metabolite of Roflumilast. ^{32}-P-labeled DNA adducts were resolved using 2D-thin-layer chromatography (TLC). Chromatograms from Roflumilast-treated animals were compared against chromatograms from positive and negative controls. N-acetoxy-ADCP- and N-hydroxy-2,6-Xylidine-modified DNA samples were used as standards. A positive result is identified when chromatograms from Roflumilast-treated animals exhibited a reproducible pattern of additional radioactive spots not present in the controls.

Study outcome: There were no unscheduled deaths or signs of morbidity. No formation of DNA adducts from roflumilast or its metabolites, specifically ADCP, was detected under the conditions of the assay. DNA adducts were detected for the positive controls.

Study title: Action of B9302-107 on mutations affecting the hypoxanthine-guanine phosphoribosyl transferase locus in V79 cells (HPRT test)

Key findings:

- This study did not adequately assess the potential of B9302-107 to induce mutations in the HPRT locus in Chinese hamster V79 cells under the conditions tested.

Study no.: 67/97

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location: Byk Gulden, Institute of Pathology and Toxicology, FT4, Friedrich-Ebert-Damm 101, 22047 Hamburg, Germany
Date of study initiation: June 11, 1996
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: B9302-107, Batch No. AM50/039, 98.4% pure

Methods

Strains/species/cell line: Chinese hamster V79 cell line

Doses used in definitive study: Main experiment 1 (HD0426) – 50, 75, 100, 125, and 150 µmol/l (equivalent to 0.02, 0.03, 0.04, 0.05, and 0.06 mg/mL), without metabolic activation; Main experiment 2 (HE0426 and HF0426) – 25, 50, 75, 100, 125, and 150 µmol/l (equivalent to 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg/mL), with and without metabolic activation. Due to the formation of dose-dependent precipitation (study report did not note dose at which precipitation was first observed), treatment media from Main experiment 1 (HE0426) were analyzed and determined to be test compound B9302-107 (internal communication dated July 3, 1996). Experiments were repeated at the following lower concentrations of B9302-107 - 1.25, 2.5, 5, 10 and 20 µmol/l (equivalent to 0.0005, 0.001, 0.002, 0.004, and 0.008 mg/mL), with (HI0426, HN0426, HP0426) and without (HG0426, HJ0426, HQ0426, HS0426) metabolic activation.

Basis of dose selection: Toxicity of B9302-107, with and without metabolic activation, was evaluated in a pre-experiment at 50, 75, 100, 125, and 150 μmol/l (equivalent to 0.02, 0.03, 0.04, 0.05, and 0.06 mg/mL). Approximately 100% plating efficiency was observed at all concentrations; therefore, B9302-107 was not toxic to V79 cells in the pre-experiment. A dose-dependent precipitation was also observed (study report did not note dose at which precipitation was first observed).

Negative controls: Solvent (DMSO) and untreated (medium) controls

Positive controls: Without S9 mix: ethyl methanesulfonate (8000 μmol/l); With S9 mix: dimethylbenzanthracene (60 μmol/l).

Incubation and sampling times: Cells were treated for 4 hrs at 37°C.

Results

Study validity: In Main experiment I, one experiment was performed without metabolic activation (HD0426). In Main experiment II, two experiments were performed with and without metabolic activation (HE0426, with S9; HF0426, without S9). In the repeat, lower-dose experiments, three independent experiments were performed without metabolic activation (HI0426, HN0426, HP0426) and four independent experiments were performed with metabolic activation (HG0426, HJ0426, HQ0426, HS0426). For all experiments, a single flask of one million cells was treated for each treatment condition (test article, positive and negative controls). First plating efficiency was set up with 2 culture flasks per group (100 cells each with the exception of HS0426 where 200 cells were used). For selection of mutants, 8 culture flasks were used for untreated controls and 4 flasks were used in B9302-107 groups, and solvent and positive controls. Triplicate flasks were used for the survival test in the selection phase (100 cells each with the exception of HS0426 where 200 cells were used). Criteria for determining a positive result were a 1) reproducible concentration-related increase in mutant frequency, and a 2) reproducible positive response for at least one the test substance concentrations. Mutant colonies were hand-counted.

Study outcome: *Experiments I and II*: In experiment HD046, the dose-independent range of cell toxicity was 3 to 27% and the dose-independent range of mutants was 3.3 to 4.0 per million living cells versus 2.0 and 1.0 mutants per million cells for solvent and untreated controls. In HE0426, no cell toxicity was observed and the dose-independent range of mutants was 10.8 to 19.4 per million living cells versus 18.3 and 6.3 per million cells for solvent and untreated controls. In HF0426, no cell toxicity was observed and the dose-independent range of mutants was 5.6 to 24.3 per million living cells versus 10.7 and 16.2 per million cells for solvent and untreated controls. *Lower-dose experiments without S9*: In HI0426, the dose-dependent range of cell toxicity was 11 to 28% and the range of mutants (dose-dependent from 2.5 to 20 μmol/l) was 17.2 to 27.6 per million living cells versus 29.1 and 33.4 per million cells for solvent and untreated controls. In HN0426, the first relative plating efficiencies were high (>146%) and the second relative plating efficiencies were low (58.3 and 62.7% in the controls). The dose-independent range of mutants was 51.7 to 82.1 per million living cells versus 62.1 and 89.4 per million cells for solvent and untreated controls. The experiment was repeated with new cells

from (b) (4) due to the high spontaneous mutation rates in solvent and untreated controls. In HP0426, the dose-independent range of cell toxicity was up to 23% and the range of mutants (dose-dependent from 2.5 to 20 μmol/l) was 2.7 to 10.1 per million living cells versus 7.1 and 9.1 per million cells for solvent and untreated controls. *Lower-dose experiments with S9*: In HG0426, the dose-independent range of cell toxicity was up to 25% and the dose-independent range of mutants was 7.9 to 36.8 per million living cells versus 27.9 and 19.1 per million cells for solvent and untreated controls. As above, new V79 cells from (b) (4) were used for the next experiment due to high spontaneous mutation rates in solvent and untreated controls. In HJ0426, no cell toxicity was observed and the dose-independent range of mutants was 21.8 to 68.8 per million living cells versus 54.1 and 48.3 per million cells for solvent and untreated controls. As above, the next experiment was repeated with new cells from (b) (4) due to high spontaneous mutation rates in solvent and untreated controls. In HQ0426, cell toxicity at the first plating was 17% at the highest dose of 20 μmol/l and the dose-independent range of second plating cell toxicity was high, 38 to 61%. Therefore, the dose-independent range of mutants per million living cells was high, ranging from 7.9 to 50.4 per million cells versus 19.5 and 17.2 per million cells for solvent and untreated controls. In HS0426, the dose-independent range of cell toxicity was up to 15% and the dose-independent range of mutants was 9.9 to 14.8 per million living cells versus 11.9 and 9.9 for solvent and untreated controls. In all main and lower-dose experiments with and without S9, the positive controls clearly increased mutation rates.

B9302-107 did not appear to induce mutations in the HPRT locus in Chinese hamster V79 cells under the conditions tested. However, data interpretation for this assay was difficult. B9302-107 was poorly soluble in DMSO at concentrations well below 5 mg/mL. The study report notes that a dose-dependent precipitate was observed in the pre-experiment and in Main experiment I; however, the report does not note the dose at which the precipitate was first observed, it is unclear whether the cells in the lower-dose experiments were dosed up to the lowest precipitating concentration of test article. In addition, V79 cells from three different sources were used for repeat experiments throughout the study (see above) due to concerns about high spontaneous mutation rates in solvent and untreated controls. Therefore, this reviewer concludes that this study did not adequately assess the potential of B9302-107 to induce mutations in the HPRT locus in Chinese hamster V79 cells under the conditions tested.

2.6.6.5 Carcinogenicity

Three carcinogenicity studies were completed to evaluate the carcinogenicity potential of roflumilast in mice and hamsters. The Center's Executive Carcinogenicity Assessment Committee (Exec. CAC) reviewed the studies previously on October 10, 2005. The Committee concluded that 1) roflumilast treatment at daily doses of 8 and 16 mg/kg/day cause nasal tumors in hamsters and 2) no evidence of tumorigenicity was seen in mice. See the minutes of February 10, 2005 and January 19, 2010 ECEC meetings, Pharmacology and Toxicology Review No. 5 (Appendix 4) completed by Dr. Luqi Pei on June 27, 2007 under IND 57,883 and the memorandum to NDA 22-522 file completed by Dr. Luqi Pei on January 25, 2010 for additional information. Also see Section 2.6.6.9.2 – Discussion about interpretation of relevance of the animal carcinogenicity studies to humans.

2.6.6.6 Reproductive and developmental toxicology

A number of studies evaluating the reproductive and developmental potential of roflumilast were completed and submitted. The studies evaluated the effect of roflumilast on fertility, embryofetal development and pregnancy in rats, rabbits, and mice. Roflumilast decreased male fertility in rats, disrupted the pregnancy in mice and rats, and decreased pup viability in rats. Some studies were previously reviewed [Reports 19/97, 191/95 and 8/92]. See Appendices 2 and 3 for detailed review of the studies. The current review evaluates the studies that have not been previously reviewed. These studies included Reports 114/2002, 125/2002, 126/2002, and 127/2002. These studies primarily evaluate the effect of roflumilast on pregnancy and delivery.

Study Title: Toxicity of roflumilast for reproduction. Study for effects on female fertility and early embryonic development to implantation in the rat, p.o. (Report 114/2002)

Key Study Findings:

- Female Wister rats treated with roflumilast up to 1.5 mg/kg/day from 14 days prior to mating to 7 days after mating did not show any effect on any fertility parameters. The study, however, did not achieve MTD. The high dose was below the NOAEL value for general toxicity. The parameters included fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses and live fetus.

Study number:	Report 114/2002, Study RP0815
Location in electronic submission:	Section 4.2.3.5
Conducting laboratory and location:	Altana Pharma AG, RPD/T3, Friedrich-Ebert-Damm 101, 22047 Hamburg, Germany
Date of study initiation:	March 22, 2002
Study termination date:	April 24, 2002
Report date:	January 8, 2003
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298569, purity 101.4%, impurities 0.6%
Formulation/vehicle:	Antiform suspension containing Methocel (2-3 drops in 200-mL Antiform)

Methods:

Adult rats (28/dose) were given by oral gavage (gastric tube) 0, 0.5, 0.8 and 1.5 mg/kg/day of roflumilast from 2 weeks before mating to 7 days post coitus. (The mating period may last up to 3 weeks in some rats.) Rats were sacrificed on Gestation Day 15 (i.e., 15 days post coitus) for fertility parameter examinations. Coitus was determined by vaginal smears. The parameters included fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses and live fetus.

Doses	0, 0.5, 0.8, 1.5 mg/kg/day

Species/strain:	Female Rats, Wistar [(Crl:WI)WU]
#/sex/group (main study):	28 /sex/dose
For toxicokinetics:	None
Age:	14 weeks at commencement of treatment
Weight:	199 - 274 g
Route, volume:	Oral gavage, 10 mL/kg
Treatment duration:	Once daily from 2 weeks before mating until 7 days post mating. The mating period was up to 3 weeks.

Observations and times:

Mortality:	Daily
Clinical signs:	Daily
Body Weight:	Day 1, Twice per week prior to mating, daily during and after mating
Food consumption:	Day 1, Twice per week prior to mating, daily during and after mating
Paring:	Male to female ratio of 1:1
Vaginal smear:	Daily during mating period
Toxicokinetics:	Not determined
Maternal examination:	Fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses

Results:

Mortality: No deaths occurred in any groups.

Clinical Signs: No treatment-related effect was observed.

Body Weight: No treatment-related effects were observed. The respective mean body weight in the control, LD, MD and HD groups was 197, 198, 198 and 195 g on day 1 and 207, 208, 208 and 206 g prior to mating (day 14).

Food Consumption: No treatment-related effect was observed.

Reproduction Data: No treatment-related effect was observed on any of the following parameters: fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses, and live fetuses.

Evaluation: The study is inadequate in characterizing the effect of roflumilast on fertility in female rats. The primary reason for the conclusion was the lack of any signs of general toxicity of the drug in the pregnant rats. No treatment-related effect was observed in any of the parameters: body weight, body weight gain, food consumption, as well as fertility parameters. The study attempted to further explore the effect of the roflumilast identified previously in Report 19/97, as Segment I fertility study that showed roflumilast at 0.6 and 1.8 mg/kg/day decreased fertility rate and increased pre- and post-implantation losses. The finding of this study is insufficient to dismiss the findings in previous study due to the lack of toxicokinetic parameters and treatment in males as well as the shorter treatment of the current study. See Section 2.6.6.9 for additional discussion.

Study Title: Study for Effects of Pre-and Post-natal Development with Roflumilast in NMRI Mice (Report 125/2002)

Study number:	Report 125/2002, Study 12G01016
Location in electronic submission:	Section 4.2.3.5.3
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 10, 2001
Study completion date:	November 20, 2001
Report date:	February 25, 2003
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298569, purity 101.4%, impurities 0.6%
Formulation/vehicle:	Methocel suspension containing Antiform (10-15-drops Antiform in 1,000-mL Methocel)

Methods:

Pregnant mice (20-23/dose) were treated orally (via a stomach tube) with 0, (vehicle), 2, 6 or 12-mg/kg/day roflumilast from gestation day 6 to the end of lactation, except for the day of delivery. Pregnancy parameters such as dam weight, length of pregnancy duration, liter size, pup weight, the number of live and stillborns were assessed. Pup viability and developmental parameters were also assessed. The developmental parameters included grip reflex of the forelimb, surface righting, pinna detachment, slope test, and eye opening. The study was terminated on the day of weaning because of the small number of pups available for evaluation.

Doses	0, 2, 6, or 12 mg/kg/day
Species/strain:	Mice, NMRI(Crl:NMRI BR)
#/sex/group (main study):	20 - 23 pregnant females/group
For toxicokinetics:	24 pregnant roflumilast-treated females
Age:	Approximately 11 weeks at mating time
Weight:	26 – 34 g
Route, volume:	Oral (stomach tube), 5 mL/kg
Treatment duration:	Gestation days 6 to weaning except for the delivery day

Observations and times:

Mortality:	Daily
Clinical signs:	Daily
Body Weight:	Daily prenatally and gestation days 0, 3, 6 – 19, and lactation days 0, 4, 7, 14 and 21
Food consumption:	Gestation days 3, 6, 9, 12, 15 and 18 and lactation days 0, 4, 7, 14 and 21
Paring:	Male to female ratio of 1:2
Vaginal smear:	Daily during mating period
Maternal examination:	Delivery parameters, liter size, pup weight, dam weight
F1 examination:	Viability and developmental parameters: grip reflex of the

forelimb (day 2), surface righting (day 2), pinna detachment (day 4), slope test (day 10) and eye opening day 19)

Necropsy: At weaning, premature deaths, or moribund sacrifices

Results:

Mortality: No treatment-related effects were observed.

Body weight: Decreases in body weight and/or weight gains were seen in the MD and HD group dams during gestation days 16 – 18. The HD group dams showed statistically significant decreases in both mean body weight (up to ↓ 15%, $p < 0.01$) and weight gain (up to ↓ 34%). The MD dams showed most decreases in mean body weight gains (up to ↓28%).

Food consumption: No treatment-related effects were observed.

Table 28 Summary findings of Pre- and Post natal developmental study in mice (125/2002)

Roflumilast (mg/kg/day)	**0**	**2**	**6**	**12**
F0 Females				
Pregnant #	20	20	21	23
No. died or sacrificed moribund	1	0	0	1
Clinical observations (#dams), Piloerection	0	2	2	8
Decreased activity	0	1	2	2
Necropsy observations (n dams), Stomach white area	0	1	6*	13**
Stomach thickened	0	0	2	6*
Gestation body weight (GD 18)	57.6 g	↓ 1.6%	↓ 8.0%	↓ 15.3%
Lactation body weight (LD 14)	45.1 g	↑ 1.8%	↓ 1.1%	↓ 10.0%
Lactation body weight (LD 21)	38.7g	↑ 2.8%	↑ 2.3%	↓ 6.5%
Gestation food consumption (GD 15 - 18)	7 g	0%	↑ 14.3%	↑ 28.6%
Lactation food consumption (LD 14 -21)	29 g	↓ 3.4%	↓ 6.9%	↓ 37.9%
Mean duration of pregnancy (day)	19.1	19.1	19.2	19.5**
Abnormal parturition - Delivery problems (#dams)	0	1	4	9
F1 litters (pre-weaning)				
No. litters evaluated	19	17	15	7
Mean no. of implantations	12.9	12.4	11.5	11.7
Mean no. pups/litter Day 0	12.4	11.3	8.3**	6.3**
Mean no. live born pups/litter	12.2	10.5	8.1**	2.9**
Stillborn: total #litters	4	7	12	14**
Percentage	1.7%	16.4%**	29.9%**	75.0%**
Postnatal survival to day 4 (%)	95.3	96.6	73.0	35.0
Weight gain(g, from birth to weaning)	11.8	12.5	13.9	15.6
Postnatal survival to weaning (%)	99.5	100.0	92.1	100.0
No. of total litter losses	0	2	6	14**
Slope test (% of pups reaching criteria)	72	76	73	14
Grip reflex forelimb (% reaching criteria)	97	94	96	57
Pinna detachment (% reaching criteria)	93	92	85	57

**, $p < 0.01$.

F_0 Pregnancy and delivery Outcome: Treatment-related effects were observed in MD and HD groups, with severe effects in HD group. The effects included non-statistically significant increases in pregnancy period, and statistically significant increases in the incidence in stillborn pups and viability in live born pups (Table 28, previous page)). Some dams delivered no live

pups. Statistically significant decreases in litter sizes and pup numbers were observed in the MD (↓ 32.0%) and HD (↓ 48.3%) groups.

Study Title: Study to investigate the effects of roflumilast on delivery in NMRI mice including toxicokinetics in dams and fetuses (Report 126/2002)

Key Study Findings:

- Roflumilast is a tocolytic agent that significantly interferes with the delivery process in pregnant mice.
- Roflumilast at 12 mg/kg/day given pregnant mice during GD 16 – 18 prolonged labor and resulted in deaths of dams and stillborns. The viability index of pups was also decreased significantly.
- Roflumilast at 12 mg/kg/day given pregnant mice between GD 12 - 16 resulted in increases in stillborns and decreases in pup viability index.

Study number:	Report 126/2002, Study 12G01031
Location in electronic submission:	Section 4.2.3.5.3
Conducting laboratory and location:	Altana Pharma AG, RPD/T3, Friedrich-Ebert-Damm 101, 22047 Hamburg, Germany
Date of study initiation:	July 2, 2001
Study completion date:	October 12, 2001
Report date:	February 25, 2003
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298569, purity 101.4%, impurities 0.6%
Formulation/vehicle:	Methocel suspension containing Antiform (10-15-drops Antiform in 1,000-mL Methocel)

Methods:

Pregnant mice (15/dose) were treated with vehicle or 12-mg/kg/day roflumilast during a portion of the pregnancy period and the whole lactation period (Table 29). The treatment duration in dams was divided into three sections according to gestation days: GD 6 – 16 (Group 2), GD 16 – 18 (Group 3) and (GD 18 only, Group 4). Treatment in the lactation period was same for G2 – G4: post partum days (PPD) 1 - 20. Both dams were sacrificed on PPD 21 for maternal and fetal examinations.

Table 29 Design of Pre- and Post-natal Study in Mice (Report 126/2002)

Group No.	Treatment Category	Treatment Period (days) [a]		#Mice/group
		Gestation	Post Partum	
1	Vehicle	6 - 16	1 - 20	15
2	Pre-birth	6 - 16	1 - 20	15
3	Birth A	16 - 18	1 - 20	15
4	Birth B	18 [b]	1-20	15
5 - 7	Kinetics	14 - 18	-	24 [c]

a. Dams were treated during gestation and lactation periods.

b. This dose was given at later afternoon. The dosing in other groups was given in the morning.

c. 8 dams/time point at 0.25, 2 and 4 hr after the last treatment (Day 18).

Vehicle was 4% hydroxypropylmethylcellulose (containing 10- 15 drops of Med Antiform per 1000-ml). The treatment schedules of Groups 1 and 2 were identical. The route of administration was oral gavage (5 ml/kg in volume). Plasma drug levels were determined on satellite mice (8/time point) on Day 18 post coitus. Coitus was determined by vaginal smears.

Doses	0, 12 mg/kg/day
Species/strain:	Mice, NMRI(Crl:NMRI BR)
#/sex/group (main study):	15 pregnant females/group
For toxicokinetics:	24 pregnant roflumilast-treated females
Age:	Approximately 11 weeks at mating time
Weight:	26 – 34 g
Route, volume:	Oral gavage, 5-mL/kg
Treatment duration:	See Table (above)

Observations and times:

Mortality:	Daily
Clinical signs:	Daily
Body Weight:	Daily prenatally and Days 0, 4, 7 14 and 21 post partum
Food consumption:	Not measured
Paring:	Male to female ratio of 1:2
Vaginal smear:	Daily during mating period
Toxicokinetics:	0.25, 2 and 4 hr post dosing on day 18
Maternal examination:	Delivery parameters, liter size, pup weight, dam weight

Results:

Mortality: Deaths and/or early sacrifices happened in pregnant mice receiving roflumilast during gestation days 16 - 18 (Table 30). One G4 dam (#403) was found dead on GD 20; four dams [two each in G3 (#304 and #314) and G4 (#404 and #406)] were sacrificed around the delivery time due to moribund conditions.[2]

Table 30 Clinical Observations in Pre- and Post-natal Study in Mice

	Control Vehicle	Pre-birth GD 6 - 16	Birth A GD 16 - 18	Birth B GD 18
#Dam sacrificed (moribund)	0/0	0/0	2	2
#Dam found dead	0/0	0/0	0/0	1
#Dam with delivery problem	0/0	0/0	0/0	7

Clinical Signs: A significant number of G4 (7/11) dams showed delivery problems. There were no detailed descriptions of the problems; however, they were most likely related to the tocolytic effect of roflumilast.

[2] There was also a treatment-unrelated death in G4: Dam #401 was sacrificed before receiving any treatment on the morning of GD 18; this dam is not considered treatment-related. Post natal data did not include this dam either.

Dam Body Weight: No treatment-related effects were observed.

Food Consumption: No treatment-related effect was observed.

Pregnancy and delivery Outcome: Increases in the incidence of stillborns were observed in all treatment groups, but higher incidences were observed in Group 3 and 4 (Table 31). The whole litter was lost in some dams.

Table 31 Pregnancy and Offspring Outcome (Report 126/2002)

	C	G2	G3	G4
Dam data				
Dams with live born #	10	10	6*	6
Dams completing delivery #	10	12	10	6
Dams with stillborn pups [#, (%)]	0 (0)	4 (33.3)	9 (90)**	6 (100)**
Litters with live born but no pus on day 4	0 (0)	1 (10)	4 (66.7)*	1 (16.7)
Litter data				
Pup livered (total #)	116	93	89	71
Live born	116	75**	39**	39**
Still born [#, (%)]	0 (0)	18 (19)**	50 (56)**	32 (45)**
Died in lactation day 1 - 4	1 (0.9)	10 (13)**	31 (80)**	29 (74)**
Viability index (%, day 4)	99.1	86.7**	20.5**	25.6**

*, $p < 0.05$; **, $p < 0.01$.

Fetal Outcome: In addition to increases in incidence of stillborns, the viability of the offspring was reduced in all treatment groups (Table). The most severe reduction occurred in dams treated during the Gestation days 16 – 18.

Plasma AUCs $_{(0\text{-}4h)}$ were 667.6 and 949.4 μg.h/L for roflumilast and Roflumilast N-oxide, respectively.

Study Title: Study for effects on pre- and postnatal development with 89302-1 07 in NMRl mice (Report 127/2002)

Key Study Findings:

- Roflumilast treatment at 3 and 6 mg/kg/day during pregnancy and lactation period resulted in increases in the incidence of stillborns and decreases in pup viability and locomotor activity.
- The NOAEL was 1.5 mg/kg/day.

Study number:	Report 127/2002, Study 12G02001
Location in electronic submission:	Section 4.2.3.5.3
Conducting laboratory and location:	Altana Pharma AG, RPD/T3, Friedrich-Ebert-Damm 101, 22047 Hamburg, Germany
Date of study initiation:	January 28, 2002
Study completion date:	June 13, 2002
Report date:	February 25, 2003
GLP compliance:	Yes, with a signed statement

QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch 298569, purity 101.4%, impurities 0.6%
Formulation/vehicle:	Methocel suspension containing Antiform (10-15-drops Antiform in 1,000-mL Methocel)

Methods:

Pregnant mice (30/dose) were treated with 0, 1.5, 3, or 6-mg/kg/day roflumilast during the pregnancy (GD 6 – 15) and lactation (PPD 1 – 20) periods. Pregnancy outcomes were assessed at the time of delivery. Pups (F1) were culled to 4 pups/sex/litter on PPD4 and were allowed to mature for developmental and behavior evaluations. Some of the pups (1/sex/liter, total of 22 – 27/sex/group) were allowed to mate with the opposite sex of other litters at age of 14 weeks. Pregnant F1 females were sacrificed on gestation day 14 on fertility parameter examinations while F1 males were sacrificed after mating.

Doses	0, 1.5, 3 and 6 mg/kg/day
Species/strain:	Mice, NMRI(Crl:NMRI BR)
#/sex/group (main study):	30 pregnant females/group
For toxicokinetics:	pregnant roflumilast-treated females
Age:	Approximately 11 weeks at mating time
Weight:	26 – 34 g
Route, volume:	Oral gavage, 5-mL/kg
Treatment duration:	See Table (above)

Observations and times:

Maternal (F0):	
Mortality:	Daily
Clinical signs:	Daily
Body Weight:	GD 0, 3, 6-19; PPD 0, 4, 7, 14 and 21
Food consumption:	GD 3, 6, 9, 12, 15, 18; PPD 0, 4, 7, 14 and 21
Paring:	Male to female ratio of 1:2
Vaginal smear:	Daily during mating period
Toxicokinetics:	0.25, 2 and 4 hr post dosing on day 18
Maternal examination:	Delivery parameters, liter size, pup weight, dam weight
F1 Offspring:	
Mortality/ Clinical signs:	Daily
Culling:	To 4 pups/sex/litter on lactation day 4
Body Weight:	Days (age) 0, 4, 7, 14 and 21; weekly thereafter till mating; Gestation days 0, 7 and 14
Pup ontogeny:	Development of grip reflex of the forelimb (age of 2 days), surface righting (2 d), pinna detachment (4 d), slope test (10 d) and eye opening (16 d), sexual maturation, spontaneous locomotor activity (50 – 60 d), learning test (water maze test, 60 – 80 d)
Mating:	Week 14 until pregnancy occur or maximum mating period of 3 weeks; Paring of female ratio of 1:1.
Sacrifice:	F: Gestation day 14; M: after mating

Fertility parameters: uterine weight, the number of Corpora lutea, implantation sites, early and late resorption, life and dead embryos

Results:

Mortality: No treatment-related effects were observed. Four F0 females were found dead or sacrificed due to moribund conditions. These deaths were not considered treatment-related because of the lack of dose-response relationship and apparent dosing errors. Among them, three F0 mice (one in C, LD and MD group each) were found dead during the lactation period on PPD 11, 16 and 13, respectively. Necropsy failed to show any significant abnormality. One HD mouse (#422) was sacrificed due to moribund condition on GD 16. The poor condition was a result of a dosing error - necropsy revealed dark red lungs and a liquid filled pleural cavity.

Clinical Signs: No treatment-related effects were observed.

Dam Body Weight: The HD dose dam showed statistically significant increases in mean body weight during the lactation period (↑ 7% on PPD 21, $p < 0.05$). The nonstatistically significant decreases in body weight, however, were observed during the gestation period (↓ 3.9% on GD 15, $P > 0.05$).

Food Consumption: No remarkable effects were observed.

F_0 Pregnancy and delivery Outcome: Treatment-related effects were observed in MD and HD groups, with severe effects in HD group. The effects included non-statistically significant increases in pregnancy period, and statistically significant increases in the incidence in stillborn pups and viability in live born pups (Table 32). Some dams delivered no live pups. Statistically significant decreases in litter sizes and pups were noted in the MD and HD groups. Dose-related decreases in pup viability during lactation were noted, but only the HD group reached statistical significance.

Table 32 Pregnancy and Offspring Outcome (Report 127/2002)

Roflumilast (mg/kg/day)	0	1.5	3	6
F0 Dam data				
No. of Pregnant mice (dams)	29	28	28	28
Duration of gestation (days)	19.0	19.1	19.1	19.4
Dams with stillborn pups [#, (%)]	1 (3.4)	1 (3.6)	3 (11.1)	6 (22.2)
Dams with all stillborn pups [#, (%)]	0 (0)	0 (0)	1 (3.7)	2 (7.4)
Litter data (F1)				
Pup livered (total #)	366	330	323	271
Live born (total#, %)	365 (99.7)	329 (99.7)	313 (96.9)*	247 (91.1)**
Stillborn (total#, %)	1 (0.3)	1 (0.3)	10 (3.1)*	24 (8.8)**
Litters with live born but no pups on day 4	0 (0)	0 (0)	0 (0)	2 (8)
Mean litter size at birth (#pup/litter)	12.6	11.8	12.0	9.9**
Mean litter size on day 4 (pre-culling)	11.0	11	11	8.2 **
Culled pups (total) on day 4	109	92	80	32
Died in lactation day 5 - 21	4 (1.1)	1 (0.3)*	10 (3.2%)	11 (4.5)*
Pups (total) surviving 21 days	201	208	189	161
Viability index (%, day 4)	87.7	93.9	91.7	82.6

*, $p < 0.05$; **, $p < 0.01$.

The HD F1 offspring showed decreases in rearing parameters (Table 33). The decreases in both the number and time of rearing were statistically significant ($P < 0.05$).

Table 33 F1 Postnatal Development Outcomes (Report 127/2002)

	C	G2	G3	G4
Mean rearing time (sec.), at 90 minutes	128.2	122.2	115.6	92.8*
Rearing No. at 90 minutes	188.3	179.9	166.3	131.2*
Mean rearing time (sec.), total	792.1	763.3	704.0	555.1*
No Rearing. total	1109.0	1086.2	962.2	774.4*

*, $p < 0.05$; **, $p < 0.01$.

F1 Fertility parameters: No treatment-related effects were observed.

2.6.6.7 Local tolerance

Local tolerance of roflumilast was studied by intramuscular injection in rats; and intravenous, para-venous, and intra-arterial injections in rabbits; and intracutaneous and epitaneous injections in guinea pigs. Slight to moderate petechial hemorrhage in the surrounding areas of the injection sites (0.1 ml of 0.02% roflumilast) were observed in rats (Study# 122/96). Slight irritation/inflammation at the injection sites were observed at each route of administration (0.5 – 1.0 ml of 0.02% roflumilast) in rabbits (Study#123/96). No irritation or sensitization potential was observed at 2 x 0.1 ml of 0.5% roflumilast, intracutaneously in guinea pigs (Study #131/95). See Pharmacology and Toxicology Review completed by Dr. Timothy McGovern on July 24, 2004 (Appendix 3).

Local tolerance of roflumilast N-oxide was studied using the guinea pig maximization test. For the first and second inductions, guinea pigs were treated with 25% roflumilast N-oxide intracutaneously and topically. Roflumilast N-oxide 25% did not show sensitizing properties following a third topical challenge (Study 202/99).

2.6.6.8 Special toxicology studies

2.6.6.8.1 Mechanistic study

Study 19/2203: **Mechanistic studies on the cytochrome P450-dependent metabolism and toxicity of roflumilast and ADCP in the rat olfactory epithelium**

This report showed that P450 enzymes played an important role in the nasal toxicity of roflumilast. Male Wistar rats (n=3/group) were pre-treated introperitoneally with vehicle (10 ml/kg), phorone (250 mg/kg), or metyrapone (50 mg/kg) 30 minutes prior to the oral administration of roflumilast. Rats were sacrificed (time unspecified) for miscroscopic examinations of the nasal cavity. Phorone that depletes the intracellular glutathione levels significantly enhanced nasal toxicity of 5-mg/kg roflumilast. Metyrapone (50 mg/kg, i.p.) that inhibits P450 enzymes attenuated the nasal lesions of 100-mg/kg roflumilast (p.o.).

2.6.6.8.2 Roflumilast N-Oxide Toxicity Studies

Study Title: B95002-044: 26-Week repeated-dose oral toxicity (gavage) study in the B6C3F1 mouse, including testing on the male fertility (Reports #54/2002 and 52/2002)

Key Study Findings:

- Mice treated with 25-mg/kg/day showed olfactory epithelial hyaline inclusions in the nasal cavity and adrenal cortex hypertrophy.
- There was no effect on male fertility at up to 25 mg/kg/day.
- The NOAEL was 4 mg/kg/day of roflumilast N-oxide and was associated with an AUC of 981.5 µg.h/L.

Study number:	Report 54/2002, (b) (4)
Location in electronic submission:	Section 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 20, 2001
Study termination date:	September 25, 2001
Report date:	June 27, 2003 , 2003
GLP compliance:	Yes, with a signed statement
QA reports:	Yes, with a signed statement
Drug lot # & purity:	Batch ZI28/103/001
Formulation/vehicle:	Antiform suspension containing Methocel (2-3 drops in 200-mL Antiform)

Methods:

Male mice (20/sex/dose) were given by oral gavage 0, 4, 10 or 25-mg/kg/day of roflumilast-N-oxide for 26 weeks. The vehicle and high groups also contain 8 additional mice to study reversibility of lesions after a recovery period of 4 weeks. In addition, each treatment group included 25 mice for evaluation of plasma roflumilast levels. Finally, after 18 weeks into the treatment, each male was pared with an untreated female (age 12 – 13 weeks) to evaluate the fertility rate. Pregnant females were sacrificed on gestations day 15 for fetal developmental evaluations.

Doses	0, 4, 10 or 25 mg/kg/day
Species/strain:	Mice, B6C3F1
#/sex/group (main study):	20 /sex/dose; also included was 20 untreated females/dose to evaluate male fertility
For reversibility	8/sex in the vehicle and HD groups
For toxicokinetics:	25 /sex/dose
Age:	11 - 12 weeks at commencement of treatment
Weight:	M; 22-8 – 31.8 g; F: 17.3 – 22.6 g
Route, volume:	Oral gavage, 10 mL/kg

Treatment duration: 6 months

Observations and times:

Mortality:	Daily
Clinical signs:	Daily
Body Weight:	Weekly
Food consumption:	Weekly
Paring:	Male to female ratio of 1:1
Toxicokinetics:	Weeks 1 (day 1), 3 (day 15), 13 and 26 (pre-dose, and 0.5, 1, 2, 3, 4, 6, 8 and 24 hr post dosing (n = 5/time point). Blood (0.2 ml) was drawn from the retro-orbital plexus.
Clinical pathology:	Weeks 13, 26 and 30
Necropsy and histology:	A complete panel of organs at end of the treatment and recovery periods in C and HD groups; adrenals, heart, nasal cavity, prostate, testes, epididymides, ovaries, uterus, vagina, and tissues with gross lesions in LD and MD groups
Maternal examination:	Fertility rate, implantation rate, numbers and locations of corpora lutea and implantations, pre- and post-implantation losses

Results:

Mortality: Dose-related increases in the number of deaths were observed. The respective death number in the C, LD, MD and HD group was 0, 0, 0, 4 in males and 0, 1, 1 and 2 in the females. The cause of death was not identified.

Clinical Signs: No treatment-related effect was observed.

Body Weight: All treatment groups showed decreases in mean body weight. The respective decrease in the LD, MD and HD groups was at week 26 was 8.1% ($P < 0.01$), 13.0% ($P < 0.01$), 15.6% ($P < 0.01$) in males and 3.8%, 5.2.0% ($P < 0.05$), 15.1% ($P < 0.01$) in females.

Food consumption: The HD females showed statistically significant decreases in food consumption (↓ 11.8%, $p < 0.01$).

Hematology: There were slight, dose-related and occasional statistically significant decreases in platelet counts (⩽ 12%), lymphocyte counts (⩽ 50%), and hemoglobin concentration (≤ 5%) and increases in segmented neutrophils (⩽ 100%).

Clinical chemistry: There were slight, dose-related and occasional statistically significant increases in concentrations of hemoglobin (≤ 300%), total protein (< 10%), calcium (< 10%), ß-globulin (≤ 20%), albumin (≤ 11%) alkaline phosphatase (< 15%) and cholinesterase (≤100%).

Urinalysis: No treatment-related effects were observed.

Organ weight: The LD, MD and HD males showed significant decreases in prostate weight: down 23.6*, 27.8* and 33.3%, respectively, compared to control. The HD males showed significant increases in adrenal gland weight (↑42.9%).

Histopathology: The HD group showed changes in the nasal cavity and adrenal cortex (Table 34). Olfactory epithelial cell hyaline inclusions were observed in the nasal cavity. Adrenal cortex hypertrophy (diffused) was observed in the adrenal glands.

Table 34 Histopathological Findings in 6-Month Study in Mice (Report 54/2002)

Sex	Male				Female			
Roflumilast (mg/kg/day)	0	4	12	36	0	4	12	36
N/group	20	20	20	20	20	20	20	20
Nasal cavity/ olfac. Epithel. Hyaline inclusion	0	0	0	0	0	2	1	7
Lacrimal gland/ lymp. Infilt.	3	0/1	0	10	3	0/1	0/1	7
Adrenal cortex/ hypertrophy	0	0	0	14	0	1	0	2

Male fertility: No treatment-related effects were observed. There were no differences in pregnancy rate and any other parameters evaluated between the control and Roflumilast N-oxide treatment groups.

Plasma drug levels: Plasma roflumilast N-oxide levels rose supra-proportionally to doses (Table 35). The AUCs were approximately 1,400, 4,800 and 23,400 at oral doses of 4, 10 and 15 mg/kg/day at week 26 of the treatment (Table 39).

Table 35 Plasma Roflumilast and Roflumilast N-oxide Levels in 6-mo Roflumilast N-oxide toxicity Study Mice (Study 54/2002)

	Plasma AUC $_{0\text{-}24\ hr}$ (µg.h/L) [a]			Cmax (µg/L)		
Oral roflumilast dose	4 mg	10 mg	15 mg	4 mg	10 mg	15 mg
Plasma Roflumilast	14.4 (*)	95.4	348.8	2.57	8.73	33.9
Plasma roflumilast N-oxide	1419.4	4847.9	23457.8	366.8	731.6	4052.7

a. 26-week values: the mean of males and females. (Source: Report 52/2002).

Roflumilast N-oxide tested negative in a bacterial gene mutation assay (Ames test, Report 225E/99) and in vitro mammalian cell chromosomal damage test using V79 cells (Report 135/99).

Study title: Micronucleus test with V79 cells in vitro with BYK22890

Key findings:

- All criteria for a valid assay were met.
- BYK22890 did not induce micronuclei in Chinese hamster V79 cells in vitro under the conditions tested.

Study no.: 135/99

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location:
Byk Gulden
Institute of Pathology and Toxicology, FPT
Friedrich-Ebert-Damm 101
22047 Hamburg, Germany
Date of study initiation: April 12, 1999
GLP compliance: Yes

QA reports: yes (X) no ( )
Drug, lot #, and % purity: BYK22890, Batch No. AM54/049/002, 98.4% purity

Methods

Strains/species/cell line: Chinese hamster V79 cell line

Doses used in definitive study: Mitotic shake off (MSO) method – 80, 100, 120, 160, and 200 μg/ml, without metabolic activation; MSO method - 20, 40, 80 μg/ml, with metabolic activation; Mixed population (MIP) method - 80, 100, 120, 160, and 200 μg/ml, without metabolic activation. For each dose group, one culture flask (MSO method) or 1 Quadriperm dish (MIP method) was tested.

Basis of dose selection: BYK22890 was tested up to toxic concentrations. In the MSO experiment without metabolic activation, treatment with 200 μg/ml BYK22890 reduced cell densities by ~50%. The study report also states that in the MIP experiment without metabolic activation, treatment with 300 and 500 μg/ml BYK22890 detached all cells.

Negative controls: Solvent control (1% DMSO)

Positive controls: Without S9 mix: mitomycin C (0.0316 and 0.1 μg/ml); With S9 mix: cyclophosphamide (3.16 μg/ml)

Incubation and sampling times: Cells were incubated at 37°C and 5% CO_2 for the following times: without S9 mix – 24 hrs without recovery; with S9 mix – 3 hrs with 21 hr recovery.

Results

Study validity: For each dose group, one culture flask (MSO method) or 1 Quadriperm dish (MIP method) was tested. Criteria for determining a positive result were 1) incidence of micronuclei at 3-fold increase than controls, 2) a clear dose-effect relationship, and 3) reproducibility of effects. Evaluation of micronuclei was performed using a research microscope, and 1000 cells were counted per experimental group. For determination of the proliferation index (PI) in the MIP experiment, 2000 cells were counted per experimental group. All criteria for a valid assay were met.

Study outcome: In the MSO experiment without metabolic activation, treatment with 200 μg/ml BYK22890 reduced cell densities by ~50%. The number of micronucleated cells in the BYK22890-treated groups was between 11 and 19, versus 13 in the solvent control group. In the MSO experiment with metabolic activation, treatment up to 80 μg/ml did not affect cell densities, thus indicating no toxicity. The number of micronucleated cells in the BYK22890-treated groups was between 5 and 7, versus 8 in the solvent control group. In the MIP experiment without metabolic activation, the PI for BYK22890-treated groups decreased in a dose-related manner compared to the solvent control. The PI was decreased ~50% at 200 μg/ml compared to the solvent control. The number of micronucleated cells in the BYK22890-treated groups was between 9 and 13, versus 7 in the solvent control group. Positive controls clearly increased mutation rates, and negative controls did not affect mutation rates. BYK22890 did not induce micronuclei in Chinese hamster V79 cells in vitro under the conditions tested.

Study title: Spontaneous incidence of polychromatic erythrocytes with micronuclei in NMRI mice

Key findings: The incidence of micronucleated PCEs ranged from 0 to 8. The group means ranged from 1.2 to 4.3, and the group medians ranged from 1 to 4 for preparations with 10 animals (male or female) per group.

Study no.: 133/99

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location: Byk Gulden Institute of Pathology and Toxicology, Byk Gulden Lomberg Chemische Fabrik GmbH, D-D78467 Konstanz.
Date of study initiation: April 27, 1998
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: N/A

Methods

Strains/species/cell line: Male and female mice (NMRI; (b) (4)
120 males and 120 females, distributed in groups of 10 males and 10 females per preparation date.

Doses used in definitive study: No test substance was administered. This study was conducted to determine the spontaneous incidence rates of polychromatic erythrocytes with micronuclei in untreated NMRI mice.

Basis of dose selection: N/A

Negative controls: N/A

Positive controls: N/A

Incubation and sampling times: N/A

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Micronucleus frequency was determined by analyzing the number of micronucleated polychromatic erythrocytes (PCEs) from at least 2000 PCEs per animal (n=10/sex from each untreated group for a total of 12 groups). PCE:NCE ratio was determined by scoring the number of PCEs and NCEs observed while scoring at least 1000 erythrocytes per animal.

Study outcome: The incidence of micronucleated PCEs ranged from 0 to 8. The group means ranged from 1.2 to 4.3, and the group medians ranged from 1 to 4 for preparations with 10 animals (male or female) per group.

Study title: Reverse mutation assay using bacteria (*Salmonella typhimurium*) with BYK22890 (B9502-044)

Key findings:

- All criteria for a valid assay were met.
- B9302-107 was negative for mutagenic potential in bacterial reverse mutation assays under the conditions tested.

Study no.: 225E/99

Volume #, and page #: Electronic submission, location 4.2.3.3
Conducting laboratory and location:
(b) (4)

Date of study initiation: March 23, 1999
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: BYK22890 (B9502-044), Batch No. AM54/049/002, 102.4% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* (*S. typhimurium*): TA1537, TA100, TA102, TA1537 and TA98

Doses used in definitive study: Experiment I and Experiment II – 31.6, 100.0, 316.2, 1000.0, 2500.0, and 5000.0 µg/plate, with and without metabolic activation. Experiment I and Experiment II utilized the plate incorporation and pre-incubation methods, respectively, to examine the mutagenicity induction potential of BYK22890 on five *S. typhimurium* strains.

Basis of dose selection: Toxicity of BYK22890 was evaluated in strains TA98 and TA100 in a pre-experiment at doses of 3.16, 10.0, 31.6, 100.0, 316.2, 1000.0, 2500.0, and 5000.0 µg/plate and with the same experimental conditions as in Experiment I (plate incorporation test). The pre-experiment with strains TA98 and TA100 was reported as part of the main Experiment I. Strains TA98 and TA100 showed toxicity as evidenced by reduced background growth and a decreased number of spontaneous mutants at doses ≥ 2500 µg/plate, with and without metabolic activation.

Negative controls: Solvent (DMSO) and untreated (medium) controls

Positive controls:

Tester Strain(s)	S9	Positive Control	Dose (µg/plate)
TA1535, TA100	-	sodium azide	10
TA1537	-	4-nitro-o-phenylene-diamine	10
TA98	-	4-nitro-o-phenylene-diamine	40
TA102	-	methyl methane sulfonate	1
TA1535, TA1537, TA98, TA100	+	2-aminoanthracene	2.5
TA102	+	2-aminoanthracene	10

Incubation and sampling times: In Experiment II, cells were pre-incubated with test-article or controls for 60 min prior to plating. For Experiments I and II, plates were incubated at least 48 hours at 37°C prior to colony counting.

Results

Study validity: The definitive studies (Experiments I and II) utilized the plate incorporation (Experiment I) and pre-incubation methods (Experiment II) to examine the mutagenicity induction potential of BYK22890 on five *S. typhimurium* strains. The sponsor examined all necessary strains for the completion of the bacterial reverse mutation battery. Each concentration of BYK22890, as well as positive and negative controls in the presence and absence of metabolic activation was tested in triplicate. The positive controls induced significant increases of reverse mutations. For tester strains TA100 and TA102, the test substance was considered a mutagen when the number of revertants was >2-fold vehicle control values. For tester strains TA1535, TA1537, and TA98, the test substance was considered a mutagen when the number of revertants was > 3-fold vehicle control values. In addition, a dose-related increase in revertants was considered biologically relevant if the threshold was exceeded at more than one concentration. An increase in revertants at one concentration only was considered biologically relevant if the result was reproduced in a second independent experiment. All criteria for a valid assay were met.

Study outcome: BYK22890 did not increase mutant frequency in any tester strain at any dose level, with or without metabolic activation. Positive controls increased revertant frequency in a strain-dependent manner. Negative controls did not increase revertant frequency in any tester strain. BYK22890 was negative for mutagenic potential in bacterial reverse mutation assays under the conditions tested.

2.6.6.8.3 ADCP Studies

ADCP tested negative in in vivo mouse micronucleus test (Report 106/98) and DNA ^{32}P-postlabeling assay in rat tissues (Report 14E/99).

2.6.6.9 Discussions and Conclusion

The toxicity profiles of roflumilast have been discussed previously in 5 nonclinical reviews. These reviews were completed by Dr. Timothy McGovern on February 2, 1999, July 24, 2000 (Original Review), May 29, 2001 (Review #2) and January 30, 2003 (Review #3); and by Dr. Luqi Pei on June 27, 2007 (Review #5). The 02-FEB-1999 review primarily evaluated proposed carcinogenicity study protocols in hamsters and mice. The original review (also named Review #1) was a comprehensive review that evaluated also relevant issues of the IND in its early stage. Review #2 evaluated the male reproductive toxicity of roflumilast in animals and its relevance to humans. Review #3 evaluated the general toxicity of roflumilast in dogs and further analyzed the male reproductive toxicity of roflumilast. Review #5 evaluated the carcinogenic study results of roflumilast. The current discussions provide updates to the toxicity profile of the drug. It also

discusses interpretations of findings of carcinogenicity and reproductive toxicology studies of roflumilast.

2.6.6.9.1 Target Organs of Toxicities

The target organs of toxicity included the nasal cavity (mice, rats and hamster), the male reproductive system (mice, rats, hamsters and dogs), the gastrointestinal tract (rats and monkeys), and the heart (dogs, Table 36). The organs in the reproductive system in rats included the testes and epididymides. The morphological lesions the reproductive system in rats are accompanied by functional compromises such as decreases in male fertility.

Table 36 Oral Repeat-Dose General Toxicity Studies of Roflumilast

Species	Durat'n	Roflumilast (mg/kg/day)	N/sex/ dose	Target Organs of Toxicity	Study#	Rev. # a
Mouse	3	0, 6, 12, 18	10	Olfactory epithelium, adrenals	216/98	1
	6	0, 4, 12, 36	20 (+8) [b]	Olfactory epithelium, adrenals, uterus/vagina, testis, heart (M)	33/2002	6
Rat	1	0, 0.5, 2, 8	10 (+8)	male reproductive organs, stomach, intestine, peritoneum	81/95	1
	4	0, 0.02, **0.2**, 2	8 (+8)	Olfactory epithelium, testes, epididymides	38/98	1
	6	0, **0.5**, 1.5, 2.5	20 (+8)	Olfactory, epididymides	14/96	1
	6	0, **0.8**	20 (+8)	-	191/2000	1
Juven.	3	0, 0.2, 0.5, **0.8**	10 (+8)	-	62/99	2
Hamster	3	0, **4**, 8, 16	10	Olfactory, prostate, epidid.	252/98	1, 3
Dog	1	0, **2**, 6, 18	5 (+2)	Heart, testis	68/95	1
	6	0, **0.2**, 1, 4	5 (+2)	Heart , testis	94/96	1
	12	0, 0.2, **0.6**, 2.0	5 (+2)	Heart, prostate	132/2000	1
Monkey	1	0.5	3	Stomach,	95/2001	1, 3
	9	0, 0.1, 0.25, **0.5**	4 (+2)	Female reproductive physiology	232/2001	6

a. Review Nos.1, 2, 3, 5 and 6 indicate nonclinical reviews competed by Dr. Timothy McGovern on 24-JUL-2000, 29-MAY-2001, 31-JAN-2003, by Dr. Luqi Pei on 27-JUL-2007, and the current review, respectively.

b. Number in the parenthesis indicates the number of animals used in the recovery group (control and high dose only).

Nose: The lesions in the nasal cavity appear to be limited to rodents. The nose is the most sensitive target organ of roflumilast toxicity in rodents. The severity of nasal lesions per rodent species in descending order was rats, hamsters and mice. Rats appear to be the most sensitive species to this toxicity. This toxicity is attributed to the special ability of rat nasal epithelium to take up ADCP and then to convert it into ADCP N-oxide, a precursor of a reactive intermediate. The neoplastic nasal lesions included epithelial disorganization, degeneration, necrosis and nerve fiber atrophy of olfactory area. Concerns about the nasal toxicity in rats resulted in the selection of the hamsters as a species of choice for the carcinogenicity studies. The hamsters showed significant increases in nasal tumors. See Section 2.6.6.9.2 for discussion about interpretation of the carcinogenicity findings. The respective NOAELs in mice, rats, hamsters, dogs and monkeys were 4, 0.8, 4, 0.6 and 0.25 mg/kg/day in nominal doses associated with 153.1, 30.9, 44.4, 510.1 and 251.3 μg.h/L as plasma AUCs.

Reproductive System: Roflumilast affects the reproductive system in both males and female animals. Morphological changes were observed in both males and females in mice and in males

only in rats, hamsters and dogs. Physiological disturbances were observed in females in rats and monkeys. Male rats showed decreased fertility. See Sections 2.6.6.9.3 and 2.6.6.9.3 for discussions of the effects of roflumilast on male and female reproductive systems.

The morphological change was accompanied by functional changes in male rats. Rats treated with 1.8 mg/kg/day of roflumilast exhibited a 25%-reduction in fertility rate (i.e., 89.2% and 64.2% in 0 and 1.8 mg/kg/day groups, respectively) when both males and females were treated in a Segment I fertility study (Report 19/97). See Section 2.6.6.1.5.1 – Fertility for additional information about the effect of roflumilast on male fertility. The respective NOAELs for male reproductive toxicity (morphologic) in mice, rats and dogs were 12, 0.8, and 0.6 mg/kg/day in nominal doses and 689.7, 30.7, and 510.1 μg.h/L in the 3-month study. [3]

Cardiovascular Toxicity: Roflumilast affected the cardiovascular system in dogs, mice and monkeys (Table 37). Dogs treated with >0.6-mg/kg/day roflumilast for 12 months showed cardiac lesions of focal hemorrhages, hemosiderin deposits and lympho-histiocytic cell infiltration in the right atria/auricles Male mice treated with ⩽12-mg/kg/day roflumilast for 6-months showed moderate peri-arteritis in the heart. Monkeys treated with 0.5-mg/kg/day roflumilast for a month showed myocarditis. The respective NOAELs for cardiac lesions in mice, dogs and monkeys were 4, 0.2, and 0.25 mg/kg/day on nominal doses and 153.1, 203.7 and 251.3 μg.h/L in plasma AUCs.

Table 37 Cardiac Findings in Roflumilast Toxicity Studies

Species	Durat'n (month)	Roflumilast (mg/kg/day)	Finding	Sex	C	LD	MD	HD	Study#
Mouse	3	0, 6, 12, 18	None						216/98
	6	0, 4, 12, 36	Peri/arteritis	M	0/20	0/20	1/20	2/20	33/2002
				F	0/20	0/20	1/20	0/20	
Dog	1	0, **2**, 6, 18	Myocarditis	M	0/3	0/3	0/3	2/3	68/95
			Myofiber degeneration	M	-	-	-	2/2	
	6	0, 0.2, **1**, 4	Myocardial Hemosid.	M	0/5	0/5	0/5	1/5	94/96
	12	0, 0.2, **0.6**,	Hemorrhage	M	1/5	0/5	2/5	4/7	
		2.0		F	0/5	1/5	0/5	2/5	132/2000
			Hemosid.	M	0/5	1/5	0/5	5/7	
				F	0/5	0/5	0/5	4/7	
Monkey	1	0, 0.1, **0.25**,			0/3	0/3	0	1/3	
	9	0.5	Myocarditis	M	0/4	0/4	0/4	0/4	232/2001

hemosid. = Hemosiderin deposits. (Report 132/2000 at right atrium).

Gastrointestinal Tract: Roflumilast treatment-related effects on the gastrointestinal (GI) tract were observed in rats, dogs and monkeys; but not in mice and hamsters. Rats and monkeys showed morphological changes while dogs showed only functional changes. In a 4-week oral study (Report 81/95), Wistar rats treated with 8.0-mg/kg/day roflumilast for 4 weeks showed serositis/inflammation in jejunum (64%, or 23/36 in incidence) and peritonitis (33%, or 12/36 in incidence), and stomach erosion (31% or, 5/16, males only). No GI findings were observed at roflumilast doses of 2.0-mg/kg/day in the study, or up to 2.5 mg/kg/day in a 6-month study (Reports 14/96).

[3] The NOAEL in rats were from Report 191/2000. This NOAEL were determined based on the totality of the data. The study has the longest treatment duration and highest NOAEL value among 5 rat studies.

In monkeys, minimal acute inflammation or inflammation foci were noted in the pyloric region of the stomach. Report 232/2001 was a 42-week oral toxicity study with a 4-week interim analysis. Cynomolgus monkeys were dosed orally with 0.1, 0.25 or 0.5-mg/kg/day roflumilast for 4 or 42 weeks. Five of 6-HD monkeys treated for 4 weeks showed pyloric inflammation at the stomach, although there was no apparent treatment-related effect at 42 weeks. The results suggest that monkeys could have adapted to the roflumilast treatment after prolonged use. The NOAEL in monkeys is 0.25 mg/kg/day associated with an AUC of 251.3 μg.h/L.

Dogs treated with roflumilast showed emesis and hypersalivation. Both vomiting and hypersalivation occurred in a dose-dependent manner at ⩾2.0 mg/kg/day in a 4-week study (Reports 68/95) and 0.2 mg/kg/day in a 6-month study (Report 94/96) and 0.6 mg/kg/day in a 12-month study (Report 132/2000). No morphological lesions of the GI tract were seen in dogs.

The respective NOAELs for GI effects of roflumilast in mice, dogs and monkeys were 2.5, 0.2, and 0.25 mg/kg/day on nominal doses associated with respective AUCs of 78.7, 203.7 and 251.3 μg.h/L in plasma AUCs. These AUC values provided safety margins of at least 5, a value greater than the usual requirement of 2.

2.6.6.9.2 Carcinogenicity

Roflumilast at daily doses of 8 and 16 mg/kg/day for 2 years caused statistically significant increases in the incidence of nasal tumors in hamsters, but not at doses up to 18 mg/kg/day in mice, or at ≤ 4-mg/kg/day in hamsters (Reports 7/2002, 233/2002 and #97/2001). The tumor incidence was ≤0.08%, 0%, 3.3% and 12.5 in control, 1 – 4, 8 and 16 mg/kg/day roflumilast groups, respectively. Relevance of the hamster tumor findings to humans is unknown due to differences in tissue ADCP N-oxide levels between rodents and humans. In rodent nasal cavity Cytochrome P450 enzyme CYP2G1 converts ADCP to ADCP N-oxide and then to ADCP N-oxide epoxide intermediate, which will deplete intracellular glutathione and result in subsequent protein crosslink. Human nasal tissues apparently lack active enzymes to convert ADCP to ADCP N-oxide, but ADCP N-oxide is found in human urine (approximately 10.5% of roflumilast dose) and plasma. Tissues and enzymes involved in the production and metabolism of ADCP N-oxide in humans are unknown. The lack of understanding of human metabolism of roflumilast to ADCP N-oxide suggests that the hamster tumor data could be relevant to humans.

The above conclusion disagrees with the applicant who has been arguing that the hamster tumor findings are irrelevant to humans. The applicant has submitted four documents evaluating the carcinogenic potential of roflumilast in rodents and the relevance of rodent tumors to humans. The documents are:

- Assessment of the tumorigenic potential of roflumilast (B9302-107, Report #259/2008)
- Assessment of the tumorigenic potential of roflumilast (B9302-107, Gerd Bode et al.)
- Roflumilast: Causal Relationship between Olfactory Metabolism and Olfactory Toxicity in Rodents (Report # 255/2008)
- Roflumilast: Causal Relationship between Olfactory Metabolism and Olfactory Toxicity in Rodents (Report # 159/2002)

These reports argued that the hamster nasal tumors are irrelevant to humans based on the differences in ADCP N-oxide metabolism in nasal, lung and liver tissues between rodents and humans. The arguments were based on the premise that 1) humans lacked counterparts of rodent nasal Cytochrome P450 enzyme CYP2G1 that converts ADCP to ADCP N-oxide and then to

ADCP N-oxide epoxide intermediate, which will deplete intracellular glutathione and result in subsequent protein crosslinks, and 2) humans showed no detectable amount of ADCP N-oxide in plasma.

The second assertion of the sponsor (above) is inaccurate based on the following findings 1) ADCP N-oxide has been detected in human plasma (≤ 19.1 μg.h/L), 2) the compound accounts for 15% of the total drugs in the human urine, and 3) urine is the primary out of excretion of orally administered roflumilast (about 70%), according to the clinical pharmacology discipline, and 4) tissues and enzymes involved in the production and metabolism of ADCP N-oxide in humans are unknown.

Overall, relevance of the hamster tumor findings to humans is unknown due to the lack of understanding of human metabolism of roflumilast to generate ADCP N-oxide suggests that the hamster tumor data could be relevant to humans. The conclusion was relayed to the sponsor via teleconference on January 29, 2010.

See the following documents for detailed discussions about the interpretation of the hamster carcinogenicity data: the Minutes of the January 19, 2010, October 5, 2005, and February 9, 1999 ECAC meetings; Nonclinical Reviews 1 and 5 completed by Drs. Timothy McGovern and Luqi Pei on July 30, 2000 (p165–166) and June 27, 2007, respectively; and Memorandum by Dr. Luqi Pei dated of January 25, 2010 which documents the most recent Agency discussions.

2.6.6.9.3 Male Reproductive Toxicity

Roflumilast decreases male fertility in rats and affects morphology in male reproductive organs in rats, mice and dogs. Morphological changes were present in one or more of these organs: prostate, testes, epididymides, seminal vesicles, pending animal species. Atrophy was observed in the prostate. Testes showed one or more of the following: tubular atrophy, dilation, degeneration and developmental defects as well as spermiogenic disturbance. Findings in the seminal vesicle included necrosis and atrophy. Findings in the epididymides included oligospermia, spermiogenic granuloma, and spermatocele. Functional changes were observed in rats but not in mice, dogs or humans and are unknown in hamsters and monkeys. Morphological changes were observed in rats, hamsters, dogs; but not in monkeys. See Section 2.6.6.1.2 General Toxicity/Target Organs of Toxicity/Male Reproductive organs detail summaries of the findings. The following section discusses the interpretation of the findings.

Summary of Male Reproductive Toxicity Data

Table 38 summarizes the effects of roflumilast on the male reproductive system. Functionally, roflumilast decreases male fertility in rats, but not in mice. The drug did not affect sperm parameters in dogs and humans. Morphologically, changes in the male reproductive organs were most pronounced in rats; less obvious in mice, hamsters and dogs, and not present in monkeys.

Table 38 Summary Findings of Roflumilast in Male Reproductive System

Species	Durat'n (mo.)	Roflumilast (mg/kg/day)	Finding	C	LD	MD	HD	Study#
Mouse	3	0, 6, 12, 18	None					216/98
	6	0, 4, 12, 36	Testis: Tub. Degeneratn	0/20	2/20	6/20	0/20	33/2002
			Sperm stasis	0/20	0/20	0/20	2/20	
Rat	1	0, 0.5, 2, 8	Testis: Spermatoge. Δ	0/10	0/10	1/10	4/10	81/95
			Epid: oligo/aspermia	0/10	1/10	0/10	4/10	
			Spermiog. granuloma	0/10	0/10	1/10	3/10	
			Seminal Ves.: atrophy	0/10	0/10	0/10	3/10	
			Prostate: atrophy	0/10	0/10	0/10	3/10	
	4	0, 0.02, **0.2**, 2 [a]	Testis: atrophy	3/8	0/8	1/8	6/8	38/98
			Giant cells/dyspermia	4/8	1/8	3/8	8/8	
			Epid: oligo/aspermia	2/8	0/8	2/8	5/8	
	6	0, **0.5**, 1.5, 2.5	Epid: Sperm granuloma	0/20	0/20	1/20	4/20	14/96
	6	0, **0.8**	None					191/2000
	2 ½	0, 0.2, **0.6**, 1.8	Fertility	89%	100%	92%	64%	19/97
Juven.	3	0, 0.2, 0.5, **0.8**	None					62/99
Hamster	3	0, 4, 8, 16	Prostate: atrophy	0/10	N/A	N/A	3/10	252/98
			Epid: dysspermia	6/10 (1.3)	10/10 (3.3)	8/10 (2.7)	8/10 3.0)	
			Semi: atrophy	0/10 (0)	N/A	NA	4/10 (0.8)	
			Testis: tubular atrophy	8/10 (1)	10/10 (3.4)	9/10 (3.4)	9/10 (3.5)	
Dog	1	0, **2**, 6, 18	Testis: Tubular degen.	0/3	0/3	0/3	1/3	68/95
			Epid.: dysspermia	0/3	0/3	0/3	1/3	
	6	0, **0.2**, 1, 4	Testis: Tubular degen.	0/5	1/5	0/5	2/5	94/96
	12	0, 0.2, **0.6**, 2.0	Prostate: atrophy	1/5	0/5	0/5	2/5	132/2000

a. Bold face indicates NOAEL of the study.

- Briefly, rats treated with1.8 mg/kg/day roflumilast exhibited a 25%-reduction in fertility rate (i.e., 89.2% and 64.2% in 0 and 1.8 mg/kg/day groups, respectively) when both males and females were treated in a Segment I fertility study (Report 19/97). Rats treated with ≥ 1.5-mg/kg/day roflumilast showed dose-dependent increases in the incidence and severity of changes in the male reproductive system: atrophy in the prostate; tubular atrophy, dilation, degeneration and developmental defects as well as spermiogenic disturbance in testes; necrosis and atrophy in the seminal vesicle; oligospermia, spermiogenic granuloma, and spermatocele in epididymides. The NOAEL on the male reproductive organs were 0.8 mg/kg/kg in rats, based on the totality of data.

- Hamsters treated with 4 to 16-mg/kg/day roflumilast for 3 months showed increases in the incidence and severity of atrophy in the prostate, seminal vesicles, testis and epididymides (Report 252/98).

- Dogs treated with 18-mg/kg/day roflumilast for 4 weeks showed changes in epididymides (dysspermia, 1/3) and testes (tubular degeneration, 1/3) (Report 68/95). Dogs treated with 4-mg/kg/day roflumilast for 6 months showed tubular degeneration in the testis (Report 94/96). Dogs treated with 2-mg/kg/day roflumilast for 12 months showed prostate atrophy (Report 132/2000). No abnormalities in the male reproductive organs were observed at roflumilast doses up to 1 and 0.6 mg/kg/day in 6- and 12-month studies. No effect on sperm parameters was observed in the 6-month study at doses up to 4 mg/kg/day in dogs. The

respective NOAEL in the 1, 6 and 12 month studies was 6, 1 and 2 mg/kg/day in nominal doses and 2304.6, 519.5 and 462.3 μg.h/L in plasma AUCs.

- Mice at doses ⩾ 12 mg/kg/day roflumilast showed increases in incidence of tubular degeneration in the testis.
- Monkeys did not show any treatment-related effects on the male reproductive organs at roflumilast doses up to 0.5 mg/kg/day for 9 months.
- Humans, at the intended clinical dose of 500-mg/kg/day roflumilast, did not show any treatment-related effect (CP-052). The clinical discipline reviewed these data.

Applicant's Evaluations of Roflumilast on Male Reproductive Organs

The applicant has submitted a number of documents evaluating the male reproductive toxicity of roflumilast in animals and the relevance of animal data to humans. These documents are:

- Roflumilast: Safety Assessment for Male Reproductive Parameters (Report #256/2008)
- Expert Report on Roflumilast: Effect on Male Reproductive Organs (Report #225/2002), (b) (4) .
- Comparison of the Spermatological Data in Beagle Dogs Found in the Repeated Dose Toxicity Studies Roflumilast (Report 34/2002), (b) (4) (2002). [4]
- Historic data on the Semen Quality of Beagle Dog Bred for Experimental Studies (35/2002), (b) (4) (2002).
- Summary of the Results Concerning Spermiograms of Dogs Treated with the Test Compound Roflumilast or Roflumilast N-oxide (Report 22/2001), (b) (4) 2001).
- Peer Review of Histological Findings in the Testes and Epididymides of Rats, Dogs, mice and hamsters treated orally with B9302-107 and B9502-044" (Report #20/2001), Drs. (b) (4) and K Tuck (Byk Gulden, the former name of the applicant).
- Expert Statement on Testicular Toxicity and its Relevance to Man" (Report #25/2001), (b) (4) (2001).

Among the above documents, Report #256/2008 is the applicant's most recent evaluation of the male reproductive toxicity of roflumilast. It contained not only information in Reports 20/2201 and 25/2001 that were previously submitted under IND 57,883 but also the applicants most up-dated interpretation of the all available data. [5] Report 256/2008 concludes that the male reproductive toxicity of roflumilast is limited to rats only. The report cites conclusions of Report 225/2002 as its justifications. Report 225/2002 concludes the following:

1. "The treatment-related lesions in the male reproductive organs were confined to rats given high doses of roflumilast (≥ 1.5 mg/kg/day) or roflumilast N-oxide (3.6 mg/kg/day). Lesions were characterized by epididymal sperm granuloma and associated changes in testes of rats. No treatment-related sperm granuloma or testicular effects were seen in mouse, hamster, dog and monkey. Primary lesion is likely to be in the efferent

[4] (b) (4)

[5] The NDA submission did not contain Reports 20/2201 and 25/2001 but the critical information has been captured in Review 3. So resubmissions of these reports are not necessary.

duct of the epididymides or proximal section of the caput. The caudal granulomas are likely to be lesions present in the efferent ductules, a rat-specific epithelial response. Testicular tubular lesions are secondary to epididymal and efferent ductule lesions. Seminiferous tubular lesions seen in roflumilast and roflumilast N-oxide treated rats are not hormonally mediated… it could be concluded that the nature of epididymal and associated testicular lesions in rats are less likely to appear in other species (p9)".

2. "It was unclear that the treatment related changes in the male reproductive organs after administration of Roflumilast or Roflumilast N-oxide were confined to rats only (p5)."
3. The male reproductive toxicity of roflumilast in rats is species-specific due to differences in anatomic structures of epididymides among rodents and other species accounted (Figure 4). The funnel-like efferent ductules make rats more susceptible to chemical induced fluid accumulation in the testis and back pressure atrophy.

Figure 5 Efferent ductile formations in animals and humans.

Division's Evaluations of Roflumilast on Male Reproductive Organs

The Division evaluated the effect of roflumilast on the male reproductive system previously. The evaluation was documented in Nonclinical Reviews #2 (p16-17) and #3 (p49-53) completed by Dr. Timothy McGovern completed on May 29, 2001 and on January 30, 2003, respectively. Review 2 found that roflumilast impaired male fertility rate by 25% in rats (p16). Concerns about the male reproductive toxicity of roflumilast were communicated to the IND sponsor at least as early as December 6, 2001 (Ref.: minutes of the 06-DEC-01 End-of-Phase 2 meeting).

Review 3 was a comprehensive evaluation of male reproductive toxicity of roflumilast. It considered all, but two repeat-dose toxicity studies: 6-month mouse study (Report 33/2002) and 9-month monkey study (Report 232/2001). The review also considered sponsor's evaluation of the male reproductive toxicity in Reports 20/2001 and 25/2001. The review tentatively concluded that roflumilast had no effect on male reproductive organs in dogs in any of 1, 6 and 12-month studies although one (of 3) HD dog in the 1-month study (Report 68/95) showed unilateral tubular degeneration in testis and dysspermia in epididymides (p48). Also observed in the HD group of the 1-month study were increased testis weight (24-28%), and smaller sizes of prostate (2/3) and seminal vesicles (2/3). The review noted that the applicant has not submitted information requested in a December 6, 2001 meeting. The request asked the applicant to submit individual animal evaluations and rationales for differences between Reports 68/95 and 20/2001. The review stated that the requested information would enable the Division to make a final determination on the male reproductive toxicity of roflumilast.

Reports 34/2002, 35/2002 and 22/2001 are expert reviews of the dog spermatological data. Reports 34/2002 and 22/2001 are almost identical. They analyze spermatological data of 6-month study (Report 64/96) and a 12-month roflumilast N-oxide study (Report 13001/2000) in dogs. Report 35/2002 provides historic background data of spermatological parameters from 45

untreated beagle dogs. Report 35/2002 concluded that neither roflumilast nor roflumilast N-oxide showed any significant effect on the spermatogenic parameters in dogs. The report also contains individual animal data requested in Review 3. The report concluded that neither roflumilast nor roflumilast N-oxide showed any significant effect on the spermatogenic parameters in dogs.

Report 20/2001 was a peer review of histological findings in testes and epididymides of rats (≤ 6 mo.), mice (≤ 3 mo.), hamsters (≤ 3 mo.) and dogs (≤ 6 mo.). Report 25/2001 was an expert statement on testicular toxicity of roflumilast and its relevance to man. Overall, these reports concluded that the male reproductive toxicity of roflumilast is limited to rats and were irrelevant man.

Data of roflumilast on the male reproductive organs presented in Table 38 and summarized previously showed more effects than the applicant has indicated. Briefly, the finding was not observed in rats only. It was also observed in hamsters, dogs, and mice. However, the applicant has completed a 3-month clinical trial evaluating the male reproductive parameters (Report 98/2002). The sponsor concluded that "[T]he totality of clinical and non-clinical data indicates no significant risk of roflumilast administration on male reproductive system in humans and supports the safe administration of 500 μg/day roflumilast in patients (p14, Report 256/2008)." The medical discipline is evaluating the clinical data.

2.6.6.9.4 Female Reproductive Toxicity

Effects of roflumilast on the female reproductive organs were less pronounced than on male reproductive organs. Table 39 summarizes the effects of roflumilast on the female reproductive system. Mice, rats and monkeys showed changes in the female reproductive organs while dogs and hamsters did not. Briefly,

- Mice treated with ≥ 12-mg/kg/day roflumilast showed increases in the incidence of uterus and cervix atrophy (Report 33/2002). The respective incidence in the control, 4, 12 and 36-mg/kg/day groups was 0/20, 1/20 (2.0, mean severity), 4/20 (2.3) and 8/20 (2.4) for uterus atrophy and 0/20, 1/20 (2.0), 4/20 (2.8) and 8/20 (2.5) for cervix atrophy.
- Rats treated with 2.0 and 2.5-mg/kg/day roflumilast for 3 or 6 months, respectively, showed decreases in the number of estrus events and estradiol levels (Reports 33/98 and 14/96). Report 19/97 showed decreases in fertility index when both males and females were treated with roflumilast although Report 114/2002 showed no significant effect on female fertility when only females were treated from 2 weeks prior to and through mating.
- Monkeys treated with ≥ 0.25-mg/kg/day roflumilast showed disruption of menstrual cycles (Report 232/2001). The mean length of the first menstrual cycle during treatment was 33 ± 9, 34 ± 13, 81 ± 83 and 101 ± 81 days for the control, 0.1, 0.25 and 0.5-mg/kg/day groups, respectively. Only the HD group, however, reached statistically significant levels during the first third of the study. Associated with the prolonged menstrual cycling was the lack of cyclic changes in the plasma female hormones.

Table 39 Summary Findings of Roflumilast in Female Reproductive System

Species	Durat'n (mo.)	Roflumilast (mg/kg/day)	Finding	C	LD	MD	HD	Study#
Mouse	3	0, 6, 12, 18	None					216/98
	6	0, 4, 12, 36	OV.: ↓ Copora lutea#	0/20	0/20	1/20	12/20	33/2002
			UT.: Inactivation	0/20	1/20	4/20	8/20	
			VA.: amestic mucosa	1/20	2/20	2/20	4/20	
			Inflammation	0/20	3/20	3/20	3/20	
			Inactivation	0/20	3/20	2/20	7/20	
Rat	1	0, 0.5, 2, 8	None					81/95
	3	0, .02, **0.2**, 2	None				A [a]	38/98
	6	0, **0.5**, 1.5, 2.5	None				A	14/96
	6	0, **0.8**	None					191/2000
	2 ½	0, 0.2, **0.6**, 1.8	Fertility	89%	100%	92%	64%	19/97
Juven.	3	0, 0.2, 0.5, **0.8**	None					62/99
Dog	1	0, **2**, 6, 18	None					68/95
	6	0, **0.2**, 1, 4	None					94/96
	12	0, 0.2, **0.6**, 2.0						132/2000

a. A, fewer estrus events. The reports did not provide numerical descriptions.

The above data suggest that roflumilast may adversely affect the female reproductive system in animals. The mechanism of the female reproductive toxicity of roflumilast is unknown at present. The applicant argued that the effect of roflumilast in female be "due to stress and/or irritation and not directly indicative of a direct drug effect" (p 49, Report 260/2008, Section 4.2.3.7.7 of the submission). It is noted that roflumilast severely and adversely affect pregnancy when given to pregnant animals (ref. Reproductive and Developmental toxicity).

2.6.6.9.5 Embryofetal development

Roflumilast was not teratogenic in rats or rabbits. The drug, however, delayed fetal bone ossification and caused stillbirths when given to rats and mice at high doses during pregnancy. See Section 2.6.6.1 – Overall Toxicology Summary for bone ossification results. See the Pregnancy and Delivery section (below) for discussions on stillbirths. Both effects of bone ossification and pregnancy should be described in the product labeling.

2.6.6.9.5.1 Pregnancy and Delivery

Three studies in mice consistently showed that roflumilast given to pregnant dams can harm the fetus and affect labor and delivery (Table 32). In Study 125/2002, pregnant mice (20-23/dose, NMRI) receiving orally 2, 6 and 12-mg/kg/day roflumilast during pregnancy (GD 6 – 18) showed significant delivery problems resulting in deaths and stillborns. In Report 126/2002, pregnancy mice (10-12/group) were given 12 mg/kg/day roflumilast orally during lactation (lactation day 1 – 20) and different time of pregnancy: Gestation Days 6 - 16 (G2), 15 – 18 (G3), or 18 (later time of the day, G4). A control group received the vehicle during lactation and GD 6 – 16. The treated dams showed significant difficulty in delivery. Groups 3 and 4 dams showed increases in the incidence of deaths and early sacrifice due to moribund conditions (incidence: 0/15, 0/15, 2/15 and 3/15 in Groups 1, 2, 3 and 4, respectively). The death and sacrifice occurred around the time of delivery due to delivery complications. Additional 7 G4 dams showed delivery problems. The roflumilast treatment groups showed significant increase in the incidence

of stillborns. The respective frequencies of stillborns in Groups 1, 2, 3 and 4 were 0% (0/10), 33.3% (4/12), 90% (9/10. $p < 0.01$) and 100% (6/6, $p < 0.01$) in dams; and 0.0% (0/116), 19% (18/93, $p < 0.01$), 56% (50/89, $p < 0.01$) and 45% (32/71, $p < 0.01$) in pups.

Study 127/2002 showed that treatment with 3 and 6-mg/kg/day roflumilast during pregnancy and lactation periods resulted in stillborns and decreases in pup viability and locomotor activity. Female mice (30/dose, NMRI) were given orally 0, 1.5, 3 or 6-mg/kg/day roflumilast during gestation days 6 – 15 and the lactation period. Pregnancy outcomes were examined at delivery. The MD and HD groups showed treatment-related effects, with severer effects seen in HD group. The effects included increases in the number of stillborns and decreases in litter sizes, pup viability and locomotor activity. The respective frequencies of stillborns in the C, LD, MD and HD groups was 3.4% (1/29), 3.6% (1/28), 11.1% (3/28) and 22.2% (6/28) in dams; and 0.03% (1/366), 0.03% (1/330), 3.1% (10/323, $p < 0.05$) and 8.8% (24/271, $p < 0.01$) in pups. The mean litter size was 12.6, 11.8, 12.0 and 9.9 ($p < 0.05$) pups/litter in the C, LD, MD and HD groups, respectively.

2.6.6.9.5.2 Post –natal Development

Reports 125/2002 and 127/2002 also showed that treatment with ⩾3-mg/kg/day roflumilast during pregnancy and lactation periods resulted in decreases in pup viability and development of locomotor activity. Pup viability was determined in two periods: pre and post culling that was carried out on post natal day 4. Report 127/2007 showed effects on both pre and post culling periods. The frequency of litters in C, LD, MD and HD groups with live pups at birth, but no pups, at the time of culling was 0%, 0%, 0% and 8%, respectively. The frequency of pup deaths in the post-culling period was 1.1%, 0.3%, 3.2% and 4.5% ($p < 0.05$) in C, LD, MD and HD groups,, respectively.

Report 125/2002 showed that 97%, 94%, 96% and 57% in forelimb grip reflex and 93%, 92%, 85% and 57% in pinna detachment. In Report 127/2007, female mice (30/dose, NMRI) were given orally 0, 1.5, 3 or 6-mg/kg/day roflumilast during gestation days 6 – 15 and the lactation period. Pregnancy outcomes were examined at delivery. Behavioral developmental effects were evaluated in pups. The MD and HD groups showed treatment-related effects, with severe effects seen in HD group. The effects included decreases in pup viability and locomotor activity. The decreases in pup viability were observed both pre and after culling (lactation day 4). There were dose-related but statistically non-significant decreases in body weight in the dams during the pregnancy period (decreases of 1.5%, 2.7% and 3.9% in the LD, MD and HD groups, respectively).

Report 126/2002 also showed that the pup viability index on lactation day 4 was 99.1%, 86.7% ($P < 0.01$), 20.5% ($P < 0.01$) and 25.6% ($P < 0.01$) in Groups 1, 2, 3 and 4, respectively.

2.6.6.9.6 Metabolites

Roflumilast, after oral administration, produces three metabolites in animals and humans. They are Roflumilast N-oxide, ADCP and ADCP N-oxide. Roflumilast N-oxide is the most abundant across species; its concentrations are significantly higher than roflumilast. General toxicity studies of roflumilast N-oxide up to 6, 1 and 12 months in the treatment duration have been completed in mice, rats and dogs, respectively. These studies showed that roflumilast and roflumilast N-oxide possess similar toxicity profiles. The target organs of toxicity of the 2

compounds were also similar. Roflumilast tested negative in a bacterial mutation assay (Ames test) and a mammalian chromosomal aberration assay in V79 cells. ADCP tested negative in mouse micronucleus test and DNA ^{32}P-postlabeling assay in rat tissues. There is no toxicity study of ADCP N-oxide which accounts for approximately 10% of the administered dose in humans, based on the urine drug data (Ref.: Dr. Yun Xu via email dated March 16, 2010). No additional toxicity study for either compound is needed as there were sufficient exposure ratios for all 3 metabolites in animal toxicity studies of roflumilast and roflumilast N-oxide.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:
The applicant has submitted all required nonclinical studies to characterize the nonclinical safety profile of the drug. Major findings of the studies include: 1) nasal tumors in hamsters, 2) stillbirths and maternal healthy in pregnant mice, 3) delay in post-natal development in mice, 4) decreases in fertility in male rats, 5) cardiovascular system effects and 6) the gastrointestinal effects. Nonclinical concerns about the above findings can be addressed through clinical evaluations and labeling. From the nonclinical perspective, approval of the application is recommended if clinical safety evaluation or overall risk-benefit analysis warrants the approval of the application.

A number of nonclinical studies have been completed to characterize pharmacological and toxicological profiles of the drug. The studies evaluated primary and secondary activities, safety pharmacology, and pharmacokinetics of roflumilast in animals. They also evaluated the general toxicity, genetic toxicity, carcinogenicity, and reproductive and developmental toxicity of the drug. They identified the following organs/systems as the target organs of roflumilast toxicity: the nasal cavity, cardiovascular system, and gastrointestinal tract and reproductive system. Roflumilast is a non-genotoxic carcinogen in hamsters, but not in mice. The results have been summarized and discussed in great detail in previous sections. See Section 2.6.6.1. – Overall Toxicology Summary and Section 2.6.6.9. – Discussion and Conclusion. The following is a brief summary of nonclinical findings and their interpretations.

Roflumilast is a selective phosphodiesterase type-4 (PDE4) inhibitor to be marketed for treatment of chronic obstructive pulmonary disease (COPD), an inflammatory disease. Pharmacological activity of roflumilast was evaluated in vivo and in vitro. Roflumilast possesses bronchodilatory and anti-inflammatory properties. Both properties are attributed to its ability to increase the intracellular cAMP, an intracellular messenger that is present in many cells and is hydrolyzed by phosphodiesterases. Increases in cAMP levels in tracheobronchial smooth muscles result in muscle relaxation and bronchial dilation. The increases of cAMP levels in some inflammatory cells results in functional suppression of the cells.

Pharmacokinetics of roflumilast was studied in mice, rats, hamsters, dogs and monkeys. Roflumilast is readily absorbed after oral administration. Maximum plasma concentrations after

oral administration were reached within an hour in mice, rats and rabbits, approximately 4 hours in hamsters and dogs, and 8 hours in humans, respectively. The oral bioavailability of roflumilast was 25%, 11%, 7%, 9%, 19%, 48% and 64% in mice, rats, hamsters, rabbits, dogs, monkeys, and humans, respectively. Roflumilast in the plasma is present predominantly in bound form. The fraction of the free drug was 3.7%, 2.0%, 2.9%, 4.8%, 2.2%, 1.6%, 2.1%, 1.1% and 1.1% in mice, rats, hamsters, guinea pigs, rabbits, dogs, monkeys, mini pigs, and humans, respectively. After oral administration, fecal excretion is predominant in mice and dogs while urine excretion is predominant in humans. Other species were somewhere in between.

The toxicology program of the application included studies of general toxicity, genetic toxicity, carcinogenicity, reproductive and embryofetal developmental toxicity and other toxicities. General toxicity of oral administered roflumilast was evaluated in several animal species. The treatment duration was up to 6, 6, 3, 12, and 9 months in treatment duration in mice, rats, hamsters, dogs, and monkeys, respectively. The studies identified the following organs as the target organs of toxicity: the nasal cavity, the male reproductive system, the gastrointestinal tract, and the cardiovascular system.

Nasal effects: The lesions in the nasal cavity appear to be limited to rodents. The lesions included non-neoplastic and neoplastic changes. Lesions occurred in rats, hamsters, and mice, in descending order of severity. Rats appear to be the most sensitive species. This appears to be attributed to the special ability of rat nasal epithelium to take up ADCP and then to convert it into ADCP N-oxide, a precursor of a reactive intermediate. The non-neoplastic nasal lesions included epithelial disorganization, degeneration, necrosis and nerve fiber atrophy of the olfactory area. The NOAEL was 4, 0.8, 4, 0.6 and 0.25 mg/kg/day in mice, rats, hamsters, dogs and monkeys, respectively. The respective safety margins in mice, rats, hamsters, dogs and monkeys were 4.7, 0.9, 1.3, 15.5, and 7.6 for roflumilast and 10.3, 16.2 and 15.3, unavailable and unavailable for ADCP N-oxide. See carcinogenicity section (below) for neoplastic findings.

Reproductive Organs: Roflumilast affected the reproductive system in both males and females. Morphological changes in the male reproductive organs were most pronounced in rats; less obvious in mice, hamsters and dogs, and absent in monkeys. Rats treated up to 1.8 mg/kg/day showed morphological changes in the following organs: the prostate (atrophy), testes (tubular atrophy degeneration and atrophy, spermatogenic disturbances), epididymides (oligospermia and granuloma), and seminal vesicles (atrophy). Dogs at ⩾1.8-mg/kg/day roflumilast showed testis degeneration and atrophy. Mice treated with 36-mg/kg/day roflumilast showed a slight increase in the incidence of sperm stasis in the testes. These morphological changes were associated with a decrease in fertility in rats. See the Fertility section (below) for the effect of roflumilast on fertility. The NOAELs were 12, 0.8, 16, 1 and 0.5 mg/kg/day in mice, rats hamsters, dogs and monkeys, respectively. The safety margin was 21, 0.9, 3.2, 17.9 and 24.9 in mice, rats, hamsters, dogs and monkeys, respectively.

The effect of roflumilast on female reproductive organs was less pronounced. Mice treated with ≥ 12-mg/kg/day roflumilast showed increases in the incidence of uterine and cervical atrophy. Rats treated with 2.0 and 2.5-mg/kg/day roflumilast for 3 or 6 months, respectively, showed decreases in the number of estrus events and estradiol levels. Rats also showed decreases in fertility index when both males and females were treated with roflumilast although no significant effect was observed when only one sex was treated. Monkeys treated with ≥ 0.25-mg/kg/day roflumilast showed disruption of menstrual cycles. The NOAEL on female reproductive organs

was 4, 0.8, and 0.25 mg/kg/day. The safety margin was 21, 0.9, and 24.9 in mice, rats, and monkeys, respectively.

Cardiovascular effects: Roflumilast affected the cardiovascular system in dogs, mice and monkeys. Dogs treated with > 0.6-mg/kg/day roflumilast for 12 months showed cardiac lesions such as focal hemorrhages, hemosiderin deposits and lympho-histiocytic cell infiltration in the right atria/auricles Male mice treated with ⩽12-mg/kg/day roflumilast for 6-months showed moderate peri-arteritis in the heart. Monkeys treated with 0.5-mg/kg/day roflumilast for a month showed myocarditis. The respective NOAELs for cardiac lesions in mice, dogs and monkeys were 4, 0.2, and 0.25 mg/kg/day on nominal doses and 153.1, 203.7 and 251.3 μg.h/L in plasma AUCs. These AUC values provided safety margins of at least 5.

Effects of Gastrointestinal tract: Roflumilast treatment-related effects on the gastrointestinal (GI) tract were observed in rats, dogs and monkeys; but not in mice and hamsters. Wistar rats treated with 8.0-mg/kg/day roflumilast for 4 weeks showed serositis/inflammation in jejunum, peritonitis, and stomach erosion. No GI findings were observed at roflumilast doses up to 2.5 mg/kg/day in a 6-month rat study. In monkeys, minimal acute inflammation or inflammation foci were noted in the pyloric region of the stomach after roflumilast treatment up to 0.5 mg/kg/day for up to 42 weeks. The respective NOAELs for GI effects of roflumilast in rats, and monkeys were 2.5 and 0.25 mg/kg/day on nominal doses and 78.7 and 251.3 μg.h/L in plasma AUCs.

Genotoxicity: Genetic toxicity of roflumilast was evaluated in vitro and in vivo. Roflumilast tested positively in an in vivo mouse micronucleus test, but negatively in the following assays: Ames test for bacterial gene mutation, in vitro chromosome aberration assay in human lymphocytes, in vitro HPRT test with V79 cells, an in vitro micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and in vivo mouse bone marrow chromosome aberration assay.

Carcinogenicity: Roflumilast was carcinogenic in hamsters, but not in mice. Carcinogenic potential of roflumilast was evaluated in 2-year oral studies in hamsters and mice. Roflumilast at daily doses of 8 and 16 mg/kg/day for 2 years caused statistically significant increases in the incidence of nasal tumors in hamsters. Hamsters were treated with 0 (cage), 0 (vehicle), 0.5, 1, 4, 8 or 16 mg/kg/day of roflumilast by oral gavage for 103 weeks. Dose-related increases in the incidence of nasal tumors were observed. The tumor prevalence was ≤ 0.8%, 0%, 0%, 0%, 0%, 3.3% and 12.5% in the cage control, vehicle control, 0.5, 1.0, 4.0, 8.0 and 16-mg/kg/day groups, respectively. Mice treated with up to 18-mg/kg/day roflumilast for 2 years did not show any increases in the incidence of any tumors.

The carcinogenicity of roflumilast appears attributed to a metabolite, ADCP N-oxide that is further converted to a reactive intermediate, ADCP N-oxide epoxide in the nasal tissues. Both steps are catalyzed by Cytochrome enzyme P450 CYP 2G1 in rodents. The epoxide would result in protein crosslinking and tumor formation. Human nasal tissues apparently lack active enzymes to convert ADCP to ADCP N-oxide, but ADCP N-oxide is found in human plasma and urine. Relevance of the tumor finding to humans is unknown since the tissues and enzymes involved in the production of ADCP N-oxide and its potential down stream metabolite are unknown in humans. The hamster tumor finding should be described in the product labeling.

The respective exposure (AUC) ratios at 4, 8 and 16 mg/kg/day between rats and humans was 1.1, 1.8 and 12.5 for roflumilast and 34.7, 78.1 and 96 for ADCP N-oxide. See Section 2.6.6.1.4 – Overall Toxicology Summary /Carcinogenicity for additional information on study results. See

Section 2.6.6.9.1 – Discussion and Conclusion/Carcinogenicity for interpretation and relevance of the hamster tumor findings to humans.

Effects on Fertility, Pregnancy and Embryofetal Development:

Fertility: A fertility study in rats showed that roflumilast at 1.8 mg/kg/day decreased fertility rate by 25% ($P < 0.05$), but not at $\leqslant$ 0.6 mg/kg/day when both males and females where treated. Such an effect, however, was not observed when only one sex was treated at similar doses. Male mice treated with up to 36-mg/kg/day roflumilast did not show any effect on fertility either. The safety margin for the male fertility effect in rats was 0.9, based on plasma roflumilast AUC. There are, however, clinical data available to address the nonclinical fertility finding. A 3-month clinical trial was completed to study the effects of roflumilast on male fertility in humans. It appears that roflumilast at 500 μg/patient/day had no effects on sperm and fertility parameters evaluated. The fertility effect of roflumilast should be described in the product labeling.

Pregnancy and Embryofetal Development: Effects of roflumilast on the reproductive system and embryofetal development were studied in mice, rats, and rabbits. Roflumilast treatment at 2, 6 and 12 mg/kg/day during pregnancy resulted in dose-related increases in stillborns, maternal deaths, and decreases pup viability in mice. The effect is more pronounced when the drug is given in late pregnancy, especially prior to delivery. This effect is attributed to the tocolytic effect of roflumilast. No treatment-related effect was seen at 1.5 mg/kg/day. Roflumilast is not teratogenic in rats and rabbits, although rats showed statistically significant increases in the incidence of incomplete bone ossification (a minor variation) at 0.6 and 1.8 mg/kg/day. The NOAEL for embryofetal developmental effects was 1.5 and 0.2 mg/kg/day in mice, rats and rabbits, respectively. The embryofetal developmental effects of roflumilast should be described in the labeling.

Post-Natal Development: Roflumilast caused statistically significant decreases in viability and grip reflex and delay in pinna attachment in pups in mice when dams were dosed with 12-mg/kg/day of the drug during pregnancy and lactation. No such effects were observed at $\geqslant$ 6 mg/kg/day.

The nonclinical characterization of roflumilast has been completed. Data submitted in the application showed that roflumilast is a carcinogen in hamsters, but not in mice. Roflumilast is non-teratogenic in rats and rabbits but can cause significant harm to fetuses when given to pregnant mice. Roflumilast decreases male fertility in rats, but does not appear to affect semen quality in humans. Risks identified with roflumilast treatment in animals have been conveyed to the clinical discipline to facilitate the overall risk/benefit evaluation. There is no outstanding nonclinical issue regarding the nonclinical safety evaluation of roflumilast. From the nonclinical perspective, the approval of the application is recommended if the clinical safety evaluation concludes that an approval may be granted.

Unresolved toxicology issues (if any): None.

Recommendations:

1. From the nonclinical perspective, approval of the application is recommended if clinical safety evaluation or overall risk-benefit analysis warrants the approval of the application.

2. Findings of carcinogenicity, male reproductive toxicity and effects of roflumilast on pregnancy should be described in the labeling.

Luqi Pei, Ph.D.
Senior Pharmacologist

Appendix:
1. Pharmacology review No. 1
2. Pharmacology review No. 2
3. Pharmacology review No. 3
4. Pharmacology review No. 5
5. Memorandum by Dr. Luqi Pei dated January 29, 2010

Appendix 1

Pharmacology and Toxicology Review No. 1
by Dr. Timothy McGovern Completed on July 24, 2000
In IND 57,883

HFD-570 : DIVISION OF PULMONARY AND ALLERGY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Review

IND No.	57,883	**Serial No.**	000	**Submission Date**:	16 FEB 1999
			001		03 MAR 1999
			002		09 MAR 1999
			000		19 APR 1999
			007		29 JUN 1999

Reviewer: Timothy J. McGovern, Ph.D. **Review Completed:** 24 JUL 2000

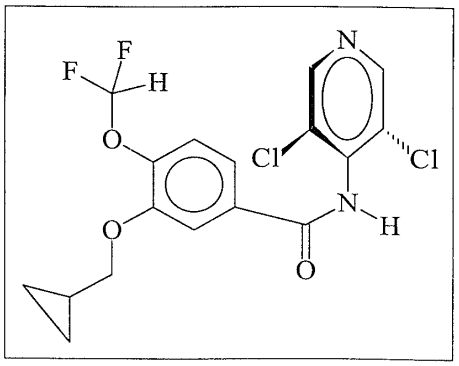
Information to be Conveyed to Sponsor: Yes (4), No ( )

Sponsor: Byk Gulden Pharmaceuticals through Altana, Inc. (local agent)

Drug Names: Roflumilast (BY-217) *Code Name:* B9302-107

Chemical Name: 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloro-pyrid-4-yl)-benzamide

Structure:



Molecular Weight: 403.2 g/mol

Formula: $C_{17}H_{14}Cl_2F_2N_2O_3$

Related INDs/NDAs/DMFs: None

Class: Phosphodiesterase (PDE IV) inhibitor

Indication: Asthma and COPD

Route of Administration: Oral (tablet)

Clinical Formulation B:	**Components**	**Amount/tablet type (mg)**
	Micronized BY 217	0.25 or 0.5
	Lactose monohydrate	(b) (4)
	Maize starch	
	Polyvidone (b) (4)	
	Magnesium stearate	
	Total:	

The sponsor states that 3 drug formulations will be used throughout the drug development program. Formulation A was used in Phase I trials, Formulation B is proposed for Phase IIa and IIb trials in Europe and the US, and Formulation C for Phase IIb trials in the US and Phase III trials. The 6-month toxicity study in dogs utilized the Formulation A tablet.

(b) (4)

The sponsor expects to initiate a Phase II b trial (12 weeks administration plus 9 months maintenance) comparing 0.25 and 0.5 mg of roflumilast vs placebo in the year 2001.

Previous Clinical Experience: Phase Ia studies in Europe showed that 0.5 mg for up to 28 days is safe and well-tolerated. Phase IIa studies in Europe and South Africa have shown that 0.5 mg over 6 weeks is safe and efficacious.

Previous Review: A Pre-IND meeting was held with the sponsor on 5/29/98 to discuss issues relating to histological findings in the olfactory epithelium of rats and the use of hamsters rather than rats for carcinogenicity studies. See the Meeting Minutes for a review of this discussion. In addition, the sponsor submitted a carcinogenicity package for Carcinogenicity Assessment Committee concurrence on the use of the hamster in lieu of the rat and dose selection in hamster and mouse carcinogenicity studies (see minutes of Carcinogenicity Assessment Committee, February 9, 1999). The review of studies done in preparation for this meeting is attached to this Original IND Review. The studies reviewed in the attachment are not reviewed again in the current review but are discussed in proper context in the Overall Summary and Evaluation. Submissions 001 and 002 are responses to a Division inquiry concerning drug formulations used in particular studies.

In a teleconference held 18 MAR 1999 (see minutes), the sponsor was informed that the Division intended to place the proposed clinical trial on hold due to the positive findings in the in vivo micronucleus mouse assay and due to structural and toxicological similarities with another compound in the Division which was positive in 2-year rat and mouse carcinogenicity assays. The sponsor commented that they had recently completed DNA adduct studies which showed

that there was no binding of test compound with DNA and that the positive findings were due to a different, non-genotoxic mechanism from the other drug. The sponsor asked that the Division inactivate the IND and they would submit the reports of the since completed studies. The Division recommended that the sponsor notify other national agencies who hosted clinical studies of our concern with this compound. Submissions 000 (19 APR 1999) and 007 include studies and references the sponsor submitted in order to reactivate the IND.

The following table summarizes the studies submitted and reviewed in the original IND:

Preclinical Studies Submitted and Reviewed in this IND:

Study	Res. Report # Study #	Vol.
Pharmacodynamics:		
Effect of IP administered B9302-107 on cell accumulation and protein concentration in BAL and on allergic dyspnea	98/94	1.4
Selective inhibition of PDE IV by B9302-107	124/94	1.4
Inhibition of ROS formation and leukotriene synthesis in human PMNL by B9302-107	131/94	1.4
Potency of B9302-107 to relax guinea pig isolated trachea	182/94	1.4
Inhibition of antigen induced trachea contraction in vitro by B9302-107	195/94	1.4
Interaction of B9302-107 with M_3 and H_1-receptors in guinea pig isolated ileum	197/94	1.4
Antagonism of B9302-107 at α2-adrenoreceptors and adenosine A_1-receptors in the field stimulated guinea pig ileum	209/94	1.4
Inhibition of SRS-A-mediated bronchoconstriction and lethality in anaesthetized guinea pig by IV administered B9302-107	215/94 K	1.4
Influence of B9302-107 IV on lung function and cardio-vascular parameters in anesthetized spontaneously breathing guinea pig	3/95	1.4
Effect of B9302-107 po on the SRS-A mediated broncho-constriction in guinea pig pretreated 1, 4 or 12 h before antigen challenge	28/95	1.4
Effect of orally administered B9302-107 on cell accumulation and protein concentration in BAL and on allergic dyspnea in guinea pig	35/95	1.4
Inhibition of SRS-A mediated bronchoconstriction in anesthetized mechanically ventilated guinea pig by iv B9302-107	59/95	1.4
Possible interaction of B9302-107 with adenosine A_2-receptors in guinea pig thoracic aorta	76/95	1.4
Effect of iv B9302-107 on cell accumulation and protein concen-tration in BAL of ovalbumin-sensitized Brown-Norway rats	104/95	1.4
Effect of orally administered B9302-107 on cell accumulation and protein concentration in BAL of ovalbumin-sensitized Brown-Norway rats	105/95	1.4
Inhibition of SRS-A mediated bronchoconstriction in anesthetized mechanically ventilated guinea pig by po B9302-107	42/96	1.4
Inhibition of SRS-A mediated bronchoconstriction in anesthetized mechanically ventilated guinea pig by B9302-107 insufflated into the lung as dry powder formulation	101/96K1	1.4
Effect of iv B9302-107 on the serotonin-induced broncho-constriction and on baseline lung mechanics and CV parameters in anesthetized, mechanically ventilated Brown-Norway rats	165/96	1.4
Effect of iv B9302-107 on histamine induced bronch0-constriction and on baseline lung mechanics and cardiovascular parameters in	209/96	1.4

Study	Res. Report #	Study #	Vol.
anesthetized, mechanically ventilated guinea pig			
Effect of roflumilast metabolites (B9502-054, B9502-044, B9302-077 and B9202-045) on phosphodiesterase 3 and 4 activities	160/98		1.4
Inhibition of lipopolysaccharide-induced tumor necrosis factor alpha release in human whole blood Roflumilast	253/98		1.4
Interaction of B9302-107 with β_1-adrenoceptors in guinea pig right atrium	206/94		1.4
Interaction of B9302-107 with alpha1β-adrenoceptors in guinea pig spleen strip	207/94		1.4
Inhibition of the lipopolysaccharide-induced tumor necrosis factor-alpha release in human monocytes and monocyte-derived dendritic cells and macrophages by Roflumilast	37/99		5.8
Evaluation of B9302-107 as an inhibitor of human P450 enzymes	12E/99	XT053098	5.8
Safety Pharmacology:			
Investigation of B9302-107 in the guinea pig Lagendorff heart	118/94		1.4
Irwin screen, female rats, oral administration	134/94		1.4
Irwin screen, female rats, iv administration	135/94		1.4
Influence of repeated administration of B9302-107 on behavior and body weight of female rats; preliminary study	138/94		1.4
Irwin screen, female mice, oral administration	149/94		1.4
Irwin screen, female mice, iv administration	150/94		1.4
Influence of B9302-107 po on body temperature of female mice	200/94		1.4
Influence of B9302-107 po on electrically induced seizures in mice	211/94		1.4
Influence of B9302-107 po on neuromuscular function of female mice	212/94		1.4
Influence of B9302-107 po on pentetrazole-induced seizures in mice	213/94		1.4
Influence of B9302-107 on action potential and force of contraction in guinea pig isolated papillary muscle	222/94		1.4
Influence of repeated po administered B9302-107 on behavior and body weight of female mice: preliminary study	231/94		1.4
Investigation on possible interaction of B9302-107 with alpha1A adrenoceptors	233/94		1.4
Influence of B9302-107 po on gastrointestinal motility of female mice	237/94		1.4
Influence of B9302-107 po on locomotor activity of mice in light beam cages	29/95		1.4
Effect of single iv and po doses of B9302-107on blood pressure and heart rate in conscious rats	39/95		1.4
Influence of B9302-107 po on pupil diameter of female mice	43/95		1.4
Effects on renal function and serum glucose after single po administration of B9302-107 to rats	98/95		1.4
Effect of iv B9302-107 on basal acid secretion in the lumen perfused stomach of the anesthetized rat (Ghosh-Schild rat)	108/95		1.5
Effect of B9302-107 iv on blood pressure, heart rate, LVP and dP/dt in pithed rats	111/95		1.5
Hemodynamic and respiratory effects of B9302-107, iv infusion in anesthetized cats	116/95		1.5
Influence of B9302-107 iv on GI motility of female mice	124/96		1.5
Influence of B9302-107 iv on body temperature of female mice	127/96		1.5
Influence of B9302-107 emulsion iv on behavior of female mice	140/96		1.5
Relaxation of the intact and endothelium-denuded rat aorta by	219/98		1.5

Study	Res. Report #	Study #	Vol.
roflumilast and its N-oxide metabolite BYK22890: A comparison with the PDE III/IV inhibitor zardaverine	221/98		1.5
Interaction of roflumilast metabolite, BYK22890 with alpha1D-adrenoceptors in rat isolated thoracic aorta	231/98K		1.5
Interaction of roflumilast with adenosine A2a-receptors in the guinea pig Langendorff heart	180/94		1.5
Investigation of the vasodilatory effect of B9302-107 in the isolated perfused rat kidney	224/94		1.5
Influence of B9302-107 po on hexobarbitol-induced loss of righting reflex in female mice	228/94		1.5
Influence of B9302-107 po on ethanol-induced loss of righting reflex in female mice			
Pharmacokinetics:	152/95	H17/FKM/201	1.5
PK of [14C]-B9302-107 in dog, single oral and iv administration	162/95	RH17/FKM/204/206/209	1.5
PK of [14C]-B9302-107 in the rat following single IV and oral (id) dose	199/95K1	9535(IDR); H17/FKM/102(FKM)	1.5
Absolute bioavailability of a tablet formulation of B9302-107 in dogs	175/96	9615(IDR)	1.5
Absolute bioavailability of tablet formulation in dogs	32/97	9635(IDR);H07/FKM/102	1.6
Relative bioavailability of new tablet formulation of B9302-107 in beagle dogs	155/97	K07/FKM/101; LK0392	1.6
PK of B9302-107 in female rabbit, single oral and iv dose	21/98	R45/FKM/201/202/207/209	1.6
Absorption and disposition of [^{14}C]-B9202-045 (DCAP) in the rat	22/98	C07/FKM/203/204	1.7
Absorption and disposition of [^{14}C]-roflumilast in the hamsters	159/98	H07/FKM/110	1.7
Retrospective determination of B9502-044 in dog plasma following a single oral dose of B9302-107	226/98	R07/FKM/109; PK98002	1.7
Comparative PK of B9302-107 and B9502-044 in the rat	157/95	H17/FKM/101; BD0334	1.7
PK of B9302-107 during the 4-week toxicity study in dogs	44/96	R07/FKM/209	1.7
PK of [^{14}C]- B9302-107 following multiple oral doses to the rat	97/96	R07/FKM/101;	1.7
PK of B9302-107 in a 6-month toxicity study in rats	159/97	LR0365 AD0430	1.7
TK of B9302-107 and its metabolites B9202-045 and B9502-054 in mice after a single oral high-dose	170/97	PK97001/B;N07/FKM/101	1.8
TK of B9302-107 and its metabolites B9202-045 and B9502-054 in Syrian hamster after a single oral high-dose	188/97	PK97001/A;C07/FKM/102	1.8
TK of B9202-045 in Syrian hamster after a single oral dose	262/97	PK97002;C07/FKM/103	1.8
TK of B9302-107 in mice after a single oral doses	14/98	LM0502;N07/FKM/102	1.9
TK of B9202-045 in mice following single oral doses	176/96	LM0502; N07/FKM/102	1.9
Comparative TK of B9302-107 and B9202-045 in rats	48/97	R07/FKM/103; WR0380	1.9
Comparative TK of B9302-107, B9202-045 and B9502-054 in rats	54/97	07/FKM/106; WR0435	1.9
TK of B9202-045 in rats following repeated oral administration	145/97	R07/FKM/107; WR0436	1.9
TK of B9302-107 during 14-day iv toxicity study in dogs	147/97	H07/FKM/105; ED0425	1.10
6-month TK of B9302-107 in dogs following oral administration			

Study	Res. Report #	Study #	Vol.
	12/98	H07/FKM/104; CD0367	1.10
TK of B9302-107 and metabolites B9202-045, B9502-054, and B9502-044 in dogs following repeated oral administration of 18 mg/kg B9302-107	133/97	H07/FKM/109; BD0334	1.10
Tissue distribution of [^{14}C]-B9302-107 in the mouse following single oral or iv dose	174/98	N07/FKM/204-205	1.10
Whole-body autoradiography of [^{14}C]-roflumilast in rats following single oral or iv dose	175/98	R17/FKM/201	1.10
Whole-body autoradiography of [^{14}C]-roflumilast in rats following single oral or iv dose	142/97	R45/FKM/203/204	1.10
Formulation of N-oxide metabolites of B9302-107 and B9202-045 by microsomal fractions of nasal mucosa in various species in vitro	150/97	R07/FKM/216	1.10
Metabolism of [^{14}C]-roflumilast in the dog	224/97		1.10
Metabolism of [^{14}C]-roflumilast in the mouse	42E/99	H07/FKM/201	5.2
Autoradiographic studies of ^{14}C- and ^{3}H-labeled B9302-107 and ^{14}C-labeled B9202-045 in nasal passages of rats	110/99	N07/FKM/206	5.2
Orientative PK of B9302-107 and its metabolite B9502-044 and determination of cAMP and erythropoietin following a single oral dose of 900 mg/kg B9302-107 to mice.	131/99	1998-06-16 N217/FKM/103	5.2
Orientative study to investigate the effects of metyrapone on tissue distribution and metabolism of ADCP in the rat	BYG 057/992968	R217/FKM/229	5.3
Comparative in vitro metabolism using microsomes prepared from nasal olfactory and respiratory epithelium and liver of rat, mouse, hamster, dog, monkey and human.	12E/99	XT053098	5.8
Evaluation of B9302-107 as an inhibitor of human P450 enzymes			
Toxicology:			
Acute Toxicology:	91/95	OR0347	1.11
Rats, oral administration of B9302-107	114/96	OR0417	1.12
Rats, IV administration of B9302-107	92/95	OM0346	1.11
Mice, oral administration of B9302-107	115/96	OM0418	1.11
Mice, IV administration of B9302-107	NA, pg 166		5.2
Histology Interim report	118/99	WM0627	5.2
Interim report on hematology	34E/99	NA	5.3
Effects of the PDE IV inhibitor B9302-107 on the nasal cavity in hamster, mice and rats			
Multiple Dose Toxicology:	81/95	BR0335	1.12
4-week toxicity, Wistar rats, oral administration	38/98	BR0499	1.14
4-week/3-month toxicity, rats, oral administration	113/96	ER0416	1.19
2-week toxicity, Wistar rats, IV administration	68/95	BD0334	1.15
4-week toxicity, dogs, oral (suspension) administration	159/96	ED0425	1.20
2-week toxicity, dogs, IV administration	94/96	CD0367	1.23
6-month toxicity, dogs, oral (tablet) administration	216/98	GM0550	5.6/5.7
Toxicity of B9302-107 in mice (po) for 3 months (screening)	252/98	GH0562	5.8
Toxicity of B9302-107 in hamsters (po) for 3 months (screening)			
Genetic Toxicology:	14E/99	R-1867	5.2
^{32}P-postlabeling assay for detection of adduct formation by 2,6 xylidine, 4,4'methylenebis(2-chloroaniline), 4-amino-3,5 dichloropyridine and 2-acetylaminofluorene in rat nasal mucosa, liver and testes DNA: a pilot study	71E/99	R-1866	5.2
^{32}P-postlabeling assay for detection of adduct formation by Roflumilast (B9302-107) in rat nasal mucosa, liver and testes			

Study	Res. Report #	Study #	Vol.
DNA	92/99	0583	5.3
Testing of roflumilast for mutagenic activity by means of the bone marrow chromosome aberration test in mouse after oral administration.			
Specialty Toxicology:	122/96	0427	1.27
Local toxicity of B9302-107 after single im injection in Wistar rats	123/96	0428	1.27
Local toxicity of B9302-107 after single iv, paravenous, or intra-arterial injection in rabbits	131/95	VG0354	1.27
Guinea pig maximization test using B9302-107 (topical)			

Studies Not Reviewed in this IND: (b) (4)

(b) (4)

Studies Previously Reviewed (see Attachment): A number of studies, listed below, were reviewed in preparation for a Carcinogenicity Assessment Committee meeting.

Study	Res. Report #		Vol.
Pharmacokinetics:			
Tissue distribution of [^{14}C]- B9302-107 in rat, single oral and iv administration	133/95	R17/FKM/207/208/212	1.5
Tissue distribution in mouse, single oral and iv administration	133/97*		
Preliminary biotransformation in hamsters, oral and iv	197/96*		
Pharmacokinetics in rats following single oral doses	199/96		1.6
PK of B9302-107 and metabolites in hamsters, single oral doses	200/96	R07/FKM/104 PK96003;	1.6
PK of B9202-045 (metabolite), rats, single oral doses	35/97	C07/FKM/101	1.6
Preliminary biotransformation in Syrian hamsters following administration of single iv or oral [^{14}C]-B9302-107 dose	197/96	R07/FKM/105 PK96002	1.10
Metabolism of [^{14}C]-roflumilast in the rat	141/97	R07/FKM/222	1.10
Multiple Dose Toxicology:			
6-month toxicity, Wistar rats, oral administration	14/96	CR0364	1.21
Comparative oral toxicity of single/repeated doses of B9302-107 or B9202-045 in Wistar rats	194/95	WR0380	1.27
Comparative oral toxicity of single/repeated doses of B9302-107, B9202-045 or B9502-054 in Wistar rats (screening study)	128/96	WR0435	1.27
Comparative oral toxicity of single/repeated doses of B9202-045 in Wistar rats (screening study)	129/96	WR0436	1.27
Histopathological assessment of nasal and testicular toxicity in hamsters and mice following single/multiple administration of B9302-107, B9202-045 and B9502-054	4D/98		1.27
Carcinogenicity:			
Rationale for Species selection for carcinogenicity studies	313P/98		1.24
Genetic Toxicology:			
Reverse mutation assay (Ames) using B9302-107	127E/95	951059	1.26
In vitro chromosome aberration using human lymphocytes with B9302-107	129/95	0333	1.26
HPRT test in V79 cells using B9302-107	67/97	0426	1.26
In vitro micronucleus test with V79 cells using B9302-107	113/97	0480	1.26
In vivo mouse micronucleus test using B9302-107 (oral)	106/96	MM0415	1.26
In vivo mouse micronucleus test using B9202-045 (oral)	106/98	MM0535	1.26

* Studies submitted in Pre-IND package but not in IND submission.

Note: Portions of this review were excerpted directly from the sponsor's submission.

PHARMACOLOGY:

Mechanism of Action: Studies demonstrating the mechanism of action of Roflumilast are summarized in Table 1. B9302-107 showed primary inhibition activity with PDE IV receptors with minimal inhibition of PDE I-III and V receptors. The metabolite B9502-044 was the only metabolite tested that showed significant inhibition of PDE IV activity (3-fold weaker than the parent) while none of the metabolites showed any PDE III activity. No reactivity with M_3 or H_1, $alpha_2$-adrenoceptors, adenosine A_1 and A_2-receptors, adenosine A_{2A}-receptors, $alpha_{1D}$-adrenoceptor, β_1-adrenoceptors, $alpha_{1B}$-adrenoceptors was observed. B9302-107 did, however, exhibit 50 to 220-fold greater potency than SB207499 in inhibiting lipopolysaccharide-induced TNF-α release in human monocytes, macrophages and dendritic cells. In addition, a weak vasodilator effect in rat renal vasculature (12-20% vasodilation of cirazoline-induced vasoconstriction) at 10^{-9} to 10^{-7} M was noted. In an in vitro assessment of intact and endothelium denuded male rat aorta relaxation, endothelium-dependent relaxation was noted confirming PDE IV selectivity. The metabolite B9502-044 was 4- to 5-fold weaker than the parent in this assay.

Table 1: Pharmacology studies demonstrating mechanism of action of B9302-107.

Parameter/Model	**Study #**	**Activity**
Phosphodiesterase receptor inhibition PDE 1 (bovine brain) PDE II (rat heart) PDE III and V (human platelets) PDE IV (human neutrophils)	124/94	Inhibition of PDE IV activity: IC50 = 0.74 nmol/l < 15% inhibition of PDE I, II, III and V at 1 μmol/l
Phosphodiesterase receptor inhibition of B9502-054, B9502-044, B9302-077 and B9202-045 PDE III (human platelets) PDE IV (human neutrophils)	160/98	None of the tested compounds inhibited PDE III activity at concentrations up to 1 μmol/l. Inhibition of PDE IV activity: B9502-044: IC50 = 2 nmol/l B9302-077: IC50 = 186 nmol/l B9202-045: no activity up to 1 μmol/l B9502-054: no activity up to 1 μmol/l
Interaction with M_3 and H_1-receptors in guinea pig isolated ileum	197/94	No effect on acetylcholine- or histamine-induced ileal contraction at Roflumilast concentrations of 10^{-6} to 3×10^{-5} M and 10^{-7} to 3×10^{-6} M, respectively.
Interaction with α_2-adrenoceptors and adenosine A_1-receptors in field stimulated guinea pig ileum	209/94	No antagonism of α_2-adrenoceptors and adenosine A_1-receptors by Roflumilast (up to 10^{-5} M)
Interaction with adenosine A_2-receptors of guinea pig thoracic aorta in vitro	76/95	No interaction with adenosine A_2-receptors by Roflumilast (up to 10^{-4} M).
In vitro assessment of intact and endothelium denuded male rat aorta relaxation	219/98	B9302-107: -log(EC50)M = 6.67 and 5.18 for intact and endothelium denuded aorta rings, respectively B9502-044 was 4-5-fold weaker than parent: -log(EC50)M = 5.96 and 4.58, respectively Endothelium-dependent relaxation confirming PDE4 selectivity Zardaverine (PDE 3/4 inhibitor): -log(EC50)M = 6.66 and 6.09 for intact and endothelium denuded aorta rings
Interaction of B9502-044 with	221/98	No $alpha_{1D}$-adrenoceptor antagonism observed at 10^{-7} to

Parameter/Model	Study #	Activity
$alpha_{1D}$-adrenoceptors in male rat isolated thoracic aorta		3×10^{-5} M. Slight depression of noradrenaline-induced contraction likely due to PDE inhibition
Interaction of B9302-107 with adenosine A_{2A}-receptors in guinea pig Langendorff heart	231 /98K	B9302-107 ineffective up to 3 x 10-7 M in inhibiting 5'-N-ethylcarboxamidoadensoine-evoked vasodilation. Reference antagonists (8-SPT and ZM 241385) antagonized vasodilation (-log(EC50)M = 7.41 and 10.82, respectively)
Vasodilatory effect in isolated perfused male rat kidney	180/94	Weak vasodilator effect in rat renal vasculature (12-20% vasodilation of cirazoline-induced vasoconstriction) at 10-9 to 10-7 M.
Interaction of B9302-107 with B_1-adrenoceptors in isolated guinea pig right atrium	206/94	Blockade and synergism of receptor-mediated cAMP accumulation excluded as equilibration with 10-7 to 3×10^{-5} M did not modify concentration-response-curve of isoprenaline to enhance beating rate.
Interaction of B9302-107 with alpha1B-adrenoceptors in isolated guinea pig spleen strip	207/94	Significant antagonism excluded as equilibration with 10^{-7} to 3×10^{-5} M did not modify concentration-response-curve of noradrenaline for contracting tissue, although contraction was 46 to 53% in the presence of B9302-107 compared to control tissue.
Interaction of B9302-107 with $alpha_{1A}$-adrenoceptors in isolated prostatic segments of rat vas deferens	233/94	Gradual depression of noradrenaline-induced concentration response curves by test substance (10^{-7} to 10^{-6} M). Effect also observed with vehicle alone (DMSO). Results exclude any significant competitive interaction with smooth muscle alpha1A-adrenoceptors
Inhibition of lipopolysaccharide-induced TNF-α release in human monocytes and monocyte-derived dendritic cells and macrophages by Roflumilast	37/99	B9302-107 exhibited 50 to 220-fold greater potency than SB207499 in inhibiting TNF-α release in human monocytes, macrophages and dendritic cells. Monocytes: IC40 = 0.02 μM Macrophages with PGE2 + motapizone: IC30 = 0.005 μM Dendritic cells: Without motapizone: IC20 = 0.004 With motapizone: IC30 = 0.005

Drug Activity Related to Proposed Indication: The results from in vitro/in vivo models used to support the potential usefulness of B9302-107 in the therapy of Asthma/COPD are summarized in Table 2. B9302-107 demonstrated activity in inhibiting inflammatory cell accumulation, reactive oxygen species release, LTB4 synthesis, methacholine or OA-induced tracheal contraction, decreases in airway conductance and dynamic compliance induced by SRS-A, histamine-induced decreased expiratory flow and tidal volume, serotonin-induced bronchoconstriction, and TNF-α synthesis. In addition, normalization of BAL protein concentration was noted following OA challenge. These findings were noted in guinea pigs and Brown Norway rats, as well as in human lymphocytes (TNF-α synthesis). No influence on the action potential and contraction force of isolated male guinea pig papillary muscle was noted.

Table 2: Activity of B9302-107 related to proposed indication.

Parameter/Model	**Report #**	**Activity**
OA challenged male guinea pigs: Inflammatory cell and protein accumulation	98/94	ID50 for inflammatory cell accumulation Macrophage: 2 x 2.2 μmol/kg ip Neutrophil: 2 x 1.3 μmol/kg, ip Eosinophil: 2 x 4.8 μmol/kg. ip ID50 for normalization of protein concentration in BAL: 2 x 2.2 μmol/kg Bronchodilatory/antiallergic properties (1-10 μmol/kg, ip)
Human polymorphonuclear leukocytes (PMNL)	131/94	Inhibition of fMLP-stimulated reactive oxygen species release: IC50 = 3.2 nmol/l Inhibition of fMLP/thimerasol-stimulated LTB4 synthesis: IC50 = 1.2 nmol/l No effect (< 20%) on unspecifically (A23187-stimulated) LT synthesis
Methacholine-induced contraction of guinea pig trachea in vitro	182/94	-log EC50 (M): 8.80 RP 73401: 8.82 comparable Ro 20-1724: 7.54 ~20-fold weaker (±)-rolipram: 7.50 ~20-fold weaker
OA-induced in vitro contraction of sensitized guinea pig trachea	195/94	~ 50% inhibition of submaximal contractions at 10-8 g/ml OA by 10-7 M Roflumilast.
SRS-A-mediated broncho-constriction in anaesthetized guinea pigs	215/94K	Inhibition of SRS-A-mediated decrease in dynamic compliance and conductance. ED50 = 0.11 and 0.95 μmol/kg iv, respectively at 15 minutes before OA challenge and 0.022 and 0.096 μmol/kg 60 minutes prior to challenge.
SRS-A-mediated broncho-constriction in male anaesthetized guinea pigs	28/95	Significant inhibition of SRS-A-mediated decrease of dynamic compliance of the lung (84-85%) and airway conductance (45-57%) by 1 μmol /kg po Roflumilast administered 1 and 4 hours prior to OA challenge. No activity at 12 hours pretreatment.
Anaesthetized, spontaneously breathing male guinea pigs	3/95	Weak inhibition of histamine-induced decreased expiratory flow and TV (ED30 at 60 minutes = 9.5 and 19 μmol/kg, iv; interval 0-60 minutes ED30 = 21 and 66 μmol/kg, respectively). ED50 for dug induced increased respiratory rate = 5.2 and 1.3 μmol/kg at 60 minutes and 0-60 minute interval, respectively. Negligible influence on Vmax, TV, systolic and diastolic arterial BP, and HR
Sensitized, OA-challenged guinea pigs	35/95	Dose-dependent inhibition of BAL macrophage, neutrophil and lymphocyte accumulation at 6 and 20 μmol/kg, po; ID50 = 5, 3, and 5 μmol/kg, respectively. Eosiniphil ID50 = 7 μmol/kg. Exerted bronchodilatory or antiallergic properties. Protected from anaphylactic dyspnea at all doses (2-20 μmol/kg) within 10 min after start of OA challenge
SRS-A-mediated broncho-constriction in anaesthetized, mechanically ventilated male guinea pigs	59/95	Dose-dependent decrease of dynamic compliance of lung and airway conductance. ED50 of inhibition of compliance or conductance decrease = 0.3 and 0.11 μmol/kg, iv, respectively, when injected 60 minutes prior to OA challenge.
OA-sensitized/challenged male Brown Norway rats	104/95	Dose-dependent inhibition of BAL protein, macrophage, neutrophil, eosinophil and lymphocyte accumulation at 0.1 to 10 μmol/kg, iv; ID50 = 0.5, < 0.1, 0.5 μmol/kg for protein, neutrophil and eosinophil, respectively.
OA-sensitized/challenged male Brown Norway rats	105/95	Inhibition of BAL protein, macrophage, neutrophil, eosinophil and lymphocyte accumulation at 1 and 3 μmol/kg, po

Parameter/Model	Report #	Activity
		ID50 = 1.04, 0.51, 0.22 μmol/kg for protein, neutrophil and eosinophil, respectively.
SRS-A-mediated broncho-constriction in anaesthetized, mechanically ventilated male guinea pigs	42/96	ED50 for inhibition of decrease of lung dynamic compliance and airway conductance = 1.4 and 1.53 μmol/kg, po following dosing 60 minutes prior to OA challenge. Dose of 1.5 μmol/kg had no effect administered orally 4 hours prior to OA challenge.
SRS-A-mediated broncho-constriction in anaesthetized, mechanically ventilated male guinea pigs	101/ 96K1	ED50 for inhibition of decrease of lung dynamic compliance and airway conductance = 0.111 and 0.065 μmol/kg, insufflated as dry powder in the lung, following dosing 60 minutes prior to OA challenge. Dose of 0.1 μmol/kg significantly reduced lung dynamic compliance by 52% and airway conductance by 56% when insufflated 4 hr prior to OA challenge
Serotonin-induced broncho-constriction, baseline lung mechanics, cardiovascular parameters in anesthetized, mechanically ventilated male Brown-Norway rats	165/96	Inhibition of serotonin-induced decrease of conductance (ED50=0.11 μmol/kg) 2 min after iv administration. ED50 = 4.4 μmol/kg at 60 min and 11.7 μmol/kg at 120 min Pulmonary mechanics not affected. Heart rate increased at 0.1 to 10 μmol/kg; blood pressure decreased at 1 to 10 μmol/kg.
Histamine-induced broncho-constriction, baseline lung mechanics, cardiovascular parameters in anesthetized, mechanically ventilated male guinea pigs	209/96	Inhibition of histamine-induced decrease of conductance (ED50=0.9 μmol/kg) 2 min after iv administration. ED50 = 7.1 μmol/kg at 60 min and > 10 μmol/kg at 120 min Pulmonary mechanics not affected. Heart rate/blood pressure show trend towards being increased at doses of 0.1 to 10 μmol/kg
Human whole blood – inhibition of LPS-induced TNF-α release	253/98	Concentration-dependent inhibition of TNF-α synthesis (IC30 = 0.05 μM vs 5 μM for SB207499, a PDE IV selective inhibitor)
Influence on action potential and contraction force of isolated male guinea pig papillary muscle	222/94	No significant effect on resting membrane potential, amplitude of action potential, duration, upstroke velocity of action potential or force of contraction (0.1 to 10 μM).

Summary of Pharmacology: B9302-107 showed primary inhibition activity with PDE IV receptors with minimal inhibition of PDE I-III and V receptors. In addition, no reactivity with M_3 or H_1, alpha$_2$-adrenoceptors, adenosine A_1 and A_2-receptors, adenosine A_{2A}-receptors, alpha$_{1D}$-adrenoceptor, β_1-adrenoceptors, alpha$_{1B}$-adrenoceptors was observed. The metabolite B9502-044 was the only metabolite tested that showed significant inhibition of PDE IV activity. B9302-107 did, however, exhibit 50 to 220-fold greater potency than SB207499 in inhibiting lipopolysaccharide-induced TNF-α release in human monocytes, macrophages and dendritic cells. In addition, a weak vasodilator effect in rat renal vasculature (12-20% vasodilation of cirazoline-induced vasoconstriction) at 10^{-9} to 10^{-7} M was noted. An in vitro assessment of intact and endothelium denuded male rat aorta relaxation demonstrated B9302-107-induced endothelium-dependent relaxation, confirming PDE IV selectivity; the metabolite B9502-044 was 4- to 5-fold weaker than the parent. Results from in vitro/in vivo models used to support the potential usefulness of B9302-107 in the therapy of Asthma/COPD demonstrated activity in

inhibiting inflammatory cell accumulation, reactive oxygen species release, LTB_4 synthesis, methacholine or OA-induced tracheal contraction, decreases in airway conductance and dynamic compliance induced by SRS-A, histamine-induced decreased expiratory flow and tidal volume, serotonin-induced bronchoconstriction, and TNF-α synthesis. In addition, normalization of BAL protein concentration was noted following OA challenge. These findings were noted in guinea pigs and Brown Norway rats, as well as in human lymphocytes (TNF-α synthesis). No influence on the action potential and contraction force of isolated male guinea pig papillary muscle was noted.

SAFETY PHARMACOLOGY:

The results of various safety pharmacology studies are summarized in Table 3. Behavioral effects following single or repeated oral dosing in female NMRI mice included reduced activity and vigilance, stalking gait, tremor, hypernoe, segregation, and limb splay lying. Two of five mice died after 11 days dosing at 30 mg/kg. Other findings included a prolongation of hexobarbitol-induced loss of righting reflex (76 and 590%, respectively at 10 or 30 mg/kg) and a dose-dependent increase in ethanol-induced sleeping time (89-368% at 3-30 mg/kg). IV dosing in mice induced similar signs as well as touch escape with vocalization and decreased palpebral fissure. Similar findings were observed in female Sprague Dawley rats following single or repeated oral (gavage) dosing: reduced activity (> 3 mg/kg), head twitches, forepaw shaking, increased grooming, and brown/bloody colored noses and eyes with decreased palpebral fissure (>10 mg/kg). Following repeated dosing, rats were sacrificed after 3-10 days due to poor condition. A single IV dose (0.3-30 mg/kg) in rats demonstrated reduced activity at all doses, while other signs were noted at all but the lowest dose. Cardiovascular effects included a significant increase in HR (62%, oral; 5-12%, IV) and a decrease in blood pressure in male normotensive rats (0.12 to 1.2 mg/kg) following oral (10 mg/kg) or IV dosing (12 to 26 mm Hg). A cumulative IV injection in pithed anesthetized male rats, (0.3-20 μmol/kg) increased systolic arterial pressure (16%) and dP/dtmax (9%) at the high dose and decreased diastolic pressure (24-29%, three highest doses), while a dose-dependent, sustained increase in blood pressure (46-63%), heart rate (up to 18%), and cardiac contractility (3-fold) were noted in cats (0.1-7 mg/kg). A rise in the ST-phase (two highest doses) and increased breathing rate and respiratory minute volume (33 and 32%) were also observed in cats. Bolus injections (10^{-11} to 10^{-7} M) increased coronary flow (21-47% at 10^{-8} to 10^{-7} M) in male guinea pig Langendorff hearts. Results also suggest that B9302-107 can depress CNS function and reduce the threshold for penetrazole-induced seizures as a dose- and time-dependent reduction in coordination capacity (30-93%) was observed in female NMRI mice (3-30 mg/kg, po); the no effect dose was 1 mg/kg. Animals also exhibited increased tonic convulsions and lethality in a potentiation test at 30 mg/kg and shortened time to tonic convulsions and death at lower doses. Anticonvulsive properties in an inhibition test and protection against maximal electroshock after dosing (1-30 mg/kg, po) were not noted although the high dose increased lethality. Other findings included minor decreases in intestinal motility and weight, increased stomach basal acid secretion, reduced locomotion and rectal body temperature, and reduced urine excretion of correlated with increased urine osmolality, and urine Na+ and K+.

Table 3: Results of safety pharmacology studies with B9302-107.

Parameter	Activity
CNS/Behaviour (Irwin test) Female NMRI mice	**Locomotor activity**: Dose- and time-dependent reduction of locomotion at the mid- and high-doses (0.3, 3 or 30 mg/kg B9302-107, po). Duration of effect was ~ 30 minutes at 3 mg/kg, 9 hours at 30 mg/kg with a rapid onset. **Pupil diameter:** No miotic or mydriatic activity following oral administration of 0.3-30 mg/kg **Oral (gavage):** single dose of 1, 3, 10, 30, 100, 300 or 1000 mg/kg): ↓ activity and vigilance, stalking gait, tremor, hypernoe, segregation, and limb splay lying at 3 mg/kg or higher. No lethality was noted. Repeated dosing (1, 3, 10, 30 or 100 mg/kg up to 11 days): stalking gait, pending abdomen, and deteriorating condition at 2 highest doses; 2/5 died on day 11 at 30 mg/kg. Dosing (1, 3, 10, 30 mg/kg) 60 minutes prior to tail injection of hexobarbitol prolonged hexobarbitol-induced loss of righting reflex (76 and 590%, respectively) at 10 and 30 mg/kg. Ethanol-induced sleeping time dose-dependently ↑ at the three highest doses (89, 147 and 368%, respectively); latency time not affected. Data suggest a potential kinetic or dynamic interaction of B9302-107 with hexobarbitol and ethanol. **IV dosing**: single dose of 0.3, 1, 3, 10 or 30 mg/kg (10 ml/kg, 1 minute): ↓ activity at all doses, and hypernoe, touch escape with vocalization, tremor on touch, segregation and ↓ palpebral fissure at doses greater than 0.3 mg/kg. Repeated administration at doses of 0.1-8 mg/kg (10 ml/kg within 1 minute): signs included those above and prostration at the low dose. No lethality or body weight effects noted. Female NMRI mice (3-30 mg/kg, po): ↓ coordination capacity (30-93% for 30->240 minutes), no effect on grip strength; no effect dose of 1 mg/kg. In a potentiation test (80 mg/kg penetrazole injected sc 60 minutes after dosing, 1-30 mg/kg, po), ↑ tonic convulsions and lethality (9 of 10 vs 1 of 10 in controls) at high dose; ↓ time to occurrence of tonic convulsions (8.6 to 10.6 seconds vs 27.6 seconds in controls) and death (1-3 of 10) at lower doses. In inhibition test (120 mg/kg penetrazole injected sc 60 minutes after dosing), no anticonvulsive properties nor protection against maximal electroshock (60 minutes after administration of 30 mg/kg (po) but ↑ lethality. B9302-107 may depress CNS function, reduce threshold for seizures and aggravate seizures and/or sequelae but does not impair peripheral function. **Oral (gavage):** single dose of 1, 10, 30, 100 or 300 mg/kg: ↓ activity at 10 mg/kg, and head twitches, forepaw shaking, ↑ grooming, brown/bloody colored noses and eyes with ↓ palpebral fissure at 30 to 300 mg/kg. Repeated dosing (3, 10, or 30 mg/kg): low-dose animals were sacrificed after 10 days, mid-dose at day 4 and high-dose at day 3 due to poor condition. Observations at 10 and 30 mg/kg included those above and ungroomed fur. No accumulation of CNS-specific symptoms noted. No signs noted at low dose. **IV dosing:** single dose of 0.3, 1, 3, 10, or 30 mg/kg: ↓ activity (all doses), ↓ vigilance, ↑ grooming, head twitches, forepaw shaking, brown/ bloody colored noses and eyes with ↓ palpebral fissure was noted at 1 to 30 mg/kg.
Female Sprague Dawley rats	
Cardiovascular/Hemodynamic/	Pithed anesthetized male Sprague-Dawley rats (0.3, +0.7, +2, +7, +20

Parameter	Activity
Respiratory Effects:	μmol/kg; iv, 1 ml/kg; 0.2 ml/min): non-statistically significant ↑ systolic arterial pressure (16% at the high dose) and dP/dtmax (9%, high dose), and ↓ diastolic pressure (24-29% at the three highest doses). Heart rate and left ventricular pressure not significantly affected. Male normotensive rats (0.12, 0.4 and 1.2 mg/kg, IV): dose-dependent ↓ mean arterial pressure (12 to 26 mm Hg); maximal effect at 30-60 minutes. ↑ HR noted in first 60 minutes; maximal effect of 12, 6 and 5%, respectively. ↓ changes in AUC time interval (0-360 minutes) of 11, 5 and 12 beats per minute, respectively. Oral administration (10 mg/kg): significant ↑ HR (47 bpm (29%) vs 29 bpm in controls) and ↓ blood pressure (13 mm Hg vs ↑ of 8 mm Hg in controls) in AUC interval (0-60 minutes). ↓ BP maximal at 40 minutes (16%) persisting to 360 minutes while HR gradually declined. Male guinea pig Langendorff hearts (bolus injections of 10^{-11} to 10^{-7} M): no effects on heart rate, left ventricular pressure or rate of maximal pressure rise. Coronary flow ↑ 21-47% at 10^{-8} to 10^{-7} M. Cats (cumulative infusion of 0.1, 0.2, 0.7, 2 and 7 mg/kg; 15 minute infusions administered 45 minutes apart): dose-dependent ↑ blood pressure (left ventricular pressure, systolic and diastolic arterial pressure, up to 46-63%), heart rate (up to 18%), and cardiac contractility (3-fold). Blood pressure and contractility reacted biphasically at the 3 higher doses; slight changes at low doses. ↑ ST-phase in the ECG of 3/5 animals at 2 mg/kg and 2 of 5 at 7 mg/kg. ↑ breathing rate and respiratory minute volume (33 and 32%)
Renal function:	Male Wistar rats (1, 3, 10 mg/kg, oral gavage): ↓ urine excretion (33-65%) correlated with ↑ urine osmolality (105 to 190%) within 2 hours. Dose-dependent ↑ urine Na (51 and 170%) and K excretion (110 and 120%) at 3 and 10 mg/kg, respectively, correlated with ↓ mean serum K (13%; 10 mg/kg) within 6 hours. ↑ mean serum glucose (30% at 10 mg/kg) within 2 hours. ↑ piloerection (1 of 5) and reduced activity (5/5 vs 0/5 in other groups) at 10 mg/kg.
Body temperature:	Female NMRI mice (1, 3, 10 or 30 mg/kg, oral gavage): dose- and time-dependent ↓ rectal body temperature (0.8-1.5 degrees for 90 min at 3 mg/kg to 1.9-3.5 degrees for 240 minutes at 30 mg/kg). The no effect dose was 1 mg/kg. Similarly, an emulsion (0.3, 1 or 3 mg/kg; 10 ml/kg, 1 minute, IV), induced ↓ rectal body temperature: 0.5-1.7 degrees, up to 1 hour at low dose; 1-2.6 degrees for up to 1.5 hours at mid-dose. Effect at 3 mg/kg IV (↓ 2-2.6 degree, 2 hours) similar to that at 10 mg/kg oral (↓ 0.9-1.9 degrees for 90 min).
Stomach basal acid secretion:	Female anesthetized Sprague Dawley rats (starved 24 hours prior to treatment); 0.1 to 10 μmol/kg, IV (1 ml/kg): in situ ↑ basal acid secretion in lumen perfused stomach (Ghosh-Schild rat); 17-100% at 0.1 and 0.3 μmol/kg over 45-60 minute period and up to 480% at 1 μmol/kg for > 3.5 hours). 3 and 10 μmol/kg induced no further increase.
GI motility:	Female NMRI mice (0.3, 1, 3, 10, or 30 mg/kg, oral or 0.3, 1 or 3 mg/kg; 10 ml/kg in 1 minute, IV emulsion): small, non-dose-related reduction in length (6-22%) and weight (7-12%) of charcoal labeled small intestine and dose-related ↑ stomach weight (19 to 126%) indicating inhibition of stomach emptying. No effect dose of 0.3 mg/kg.

Summary of Safety Pharmacology: The effects of B9302-107 on behavior, cardiovascular function, hemodynamics/respiration, neuromuscular function, stomach acid secretion, GI motility, body temperature, locomotor activity, pupil diameter and renal function were assessed. Behavioral effects in female mice and rats following single and repeated oral and IV dosing included reduced activity and vigilance, stalking gait, tremor, hypernoe, segregation, and limb splay lying, touch escape with vocalization, decreased palpebral fissure, head twitches, forepaw shaking, increased grooming, brown/bloody colored noses and eyes and decreased palpebral fissure. Two of five mice died after 11 days dosing at 30 mg/kg, po, and rats were sacrificed after 3-10 days of dosing (3-30 mg/kg, po) due to poor condition. Other findings included a prolongation of hexobarbitol-induced loss of righting reflex (76 and 590%, respectively at 10 or 30 mg/kg) and a dose-dependent increase in ethanol-induced sleeping time (89-368% at 3-30 mg/kg). Cardiovascular effects included a significant increase in HR (62%) and a decrease in blood pressure in male normotensive rats after oral administration (10 mg/kg). Similarly findings were noted following IV injection (0.12 to 1.2 mg/kg): reduced mean arterial pressure (12 to 26 mm Hg) and increased HR (5-12%). A cumulative IV injection in pithed anesthetized male rats, (0.3-20 μmol/kg) increased systolic arterial pressure (16%) and dP/dtmax (9%) at the high dose and decreased diastolic pressure (24-29%, three highest doses), while a sustained increase in blood pressure (46-63%), heart rate (up to 18%), and cardiac contractility (3-fold) were noted in cats (0.1, 0.2, 0.7, 2 and 7 mg/kg). Increased ST-phase (two highest doses), breathing rate and respiratory minute volume (33 and 32%) were also observed in cats. Bolus injections (10^{-11} to 10^{-7} M) increased coronary flow (21-47% at 10^{-8} to 10^{-7} M) in male guinea pig Langendorff hearts. B9302-107 also depressed CNS function in female NMRI mice following oral dosing (3-30 mg/kg), reducing the threshold for penetrazole-induced seizures and coordination capacity (30-93%); the no effect dose was 1 mg/kg. Animals also exhibited increased tonic convulsions and lethality (9/10 vs 1/10 in controls) in a potentiation test at 30 mg/kg and shortened time to tonic convulsions (~17 seconds) and death (1-3 of 10) at lower doses; the high dose increased lethality. Other findings included increased basal acid secretion (17-100%) in anesthetized female rats (0.1 to 0.3 μmol/kg, IV) which plateaued at doses of 1-10 μmol/kg (up to 480%), reduced locomotion in female NMRI mice (3 or 30 mg/kg, po), reduced intestinal motility (6-22% reduction in length and 7-12% weight reduction of small intestine) and increased stomach weight (19 to 126%) following oral (0.3 to 30 mg/kg) or IV administration (0.3 to 3 mg/kg) in female NMRI mice (no effect dose was 0.3 mg/kg), decreased rectal body temperature (0.5 to 2.6 degrees) in female NMRI mice following oral (gavage; 1 to 30 mg/kg) or intravenous dosing (0.3 to 3 mg/kg) for up to four hours after dosing, and altered renal function (decreased urine excretion by 33-65% correlated with increased urine osmolality by 105 to 190% in male rats following oral dosing of 1-10 mg/kg; increased urine Na+ by 51 and 170% and K+ excretion by 110 and 120% at mid- and high-doses, respectively, which correlated with decreased serum K+ by 13% at 10 mg/kg; increased serum glucose by 30% at the high dose.

PHARMACOKINETICS & TOXICOKINETICS:

Single dose PK parameters: Kinetic studies were performed in rats, dogs, mice and hamsters. Some of these studies assessed the parent compound as well as the metabolites B9502-044 (N-oxide of the parent compound), B9502-045 and B9502-054 (N-oxide of B9502-045).

Rats:
Table 4 summarizes PK findings following administration of a single dose of 10 mg/kg po with 1 mg/kg id, or 1 mg/kg IV [^{14}C]-B9302-107 in male Sprague Dawley rats. The data indicate that the majority of administered drug was metabolized as very little unchanged drug was detected. Elimination following IV and oral administration was biphasic and monophasic, respectively.

Table 4. Pharmacokinetics of B9302-107 in male Sprague Dawley rats following single dosing.

	[^{14}C]-radioactivity		**Unchanged B9302-107**	
Route of admin.	iv	po / id	iv	po / id
Dose (mg/kg)	1	10 / 1	1	10 / 1
AUC (µg hr/ml)	11.42	37.61	0.67	0.31
Cl (l/hr/kg)	0.088	-	1.6	-
Vd area (l/kg)	5.83	-	22.98	-
T1/2 (hr)	46	34	11	5
Cmax (µg/ml)	-	0.86	-	0.034
Tmax (hr)	-	8-10	-	1.5-4

Systemic exposure (AUC) to B9302-107 in male Sprague Dawley rats was ~ 16-fold lower than exposure to its N-oxide following a single oral administration of 20 mg/kg B9302-107 (Table 5). Following IV administration of 1 mg/kg B9302-107, the N-oxide was observed at a 4-fold greater level than the parent. Some conversion to the parent compound (< 1%) was also observed when B9502-044 was administered directly.

Table 5. PK assessment following a single dose of B9302-107 in male Sprague Dawley rats.

Parameter	**B9302-107**	**B9502-044**	**B9302-107**	**B9502-044**	**B9302-107**	**B9502-044**
Dose	20 mg B9302-107/kg, po		20 mg B9502-044/kg, po		1 mg B9302-107/kg, iv	
AUC (0-24 h) (µg.h/ml)	0.643	10.375	0.076	15.937	0.260	1.089
Cmax (µg/ml)	0.094	0.731	0.021	2.081	0.355	0.432
Tmax (h)	0.5	7	1.42	1.42		
t1/2 (h)	6.4	15.33	2.27	9.42	0.91	0.77
Cl/kg (l/hr)					3.85	-
Vdarea/kg (l)					5.06	-

Table 6 summarizes kinetic data from male Sprague Dawley rats administered single doses of either 5 mg/kg, po, or 1 mg/kg, iv, of B9202-045. Time to maximum concentration was within 1.5 hour and elimination half-life for the administered drug was 3.1 hours. Elimination of total radioactivity was significantly longer (26-33 hours).

Table 6. PK in Sprague Dawley rats following single po or iv dose of B9202-045

Parameter	**Dose group (mg/kg)**		
	^{14}C		B9202-045
	1 iv	5, po	5, po
$AUC_{(0\text{-}inf)}$ (μg h/ml)	4.79	29.74	12.82
Cmax (μg/ml)	-	3.261	2.112
t1/2 (h)	33.4	26.1	3.1
tmax (h)	-	1.5	1
Cl (l/hr/kg)	0.209	-	-
Vd(area) (l/kg)	10.062	-	-

Dogs:
In dogs, systemic exposures to B9302-107 following a single dose with B9302-107 (0.05 to 2 mg/kg, po, Formulation A tablet, or 0.03 mg/kg, IV) increased proportionally with dose up to the highest which increased supra-proportionally and elimination half-lives increased 2- to 3-fold at the two highest oral doses tested (Table 7). Detected levels of B9202-045 were 3-14% of parent drug levels and the levels of the N-oxide of the parent drug were 2% of parent drug levels.

Table 7. PK results following single dose administration of B9302-107 in dogs.

Parameter	Dose (B9302-107)					
	0.03 mg/kg IV	0.05 mg/kg PO (T)	0.25 mg/kg PO (T)	0.75 mg/kg PO (T)	0.03 mg/kg IV	2.0 mg/kg PO (T)
Analyte: B9302-107						
T1/2 (hr)	1.49	1.90	1.83	4.3	2.0	7.1
Tmax (hr)		1.19	1.50	1.88		2.58
Cmax (μg/l)		8.904	27.718	70.669		145.21
AUC (μg/l.h)	22.52	18.61	86.77	326.70	42.52	1851.9
Cl/kg (l/hr)	1.397				0.726	
Vdarea/kg (l)	2.977				2.009	
Analyte: B9202-045						
T1/2 (hr)	1.73	2.2	3.2	4.0		
Tmax (hr)	0.53	2.0	2.19	2.63		
Cmax (μg/l)	0.262	0.418	2.517	5.162		
AUC (μg/l.h)	0.69	1.86	11.98	31.44		
% AUC of AUC of parent cmpd	3.06	9.99	13.81	9.62		
Analyte: B9502-044						
T1/2 (hr)				2.5		
Tmax (hr)				1.8		
Cmax (μg/l)				6.656		
AUC (μg/l.h)				6.42		
% AUC of AUC of parent cmpd				1.97		

Due to a change in drug formulation, the sponsor assessed the comparable pharmacokinetics of Formulations A and B in male dogs (Table 8). Although equivalence was not formally demonstrated since the upper confidence interval for absolute bioavailability was out of range

(140%), systemic exposure for the new tablet formulation (B) measured by AUC was comparable to that observed with Formulation A following oral administration of 0.75 mg/kg. It should also be noted that the systemic exposure reported in this study for Formulation A is significantly greater than that reported in the previous table at a similar dose. The reason for the difference in exposure is unclear.

Table 8. Single dose PK comparisons of Tablet Formulations A and B in dogs.

	Dose (0.75 mg/kg)	
	Tablet Formulation A	Tablet Formulation B
T1/2 (hr)	3.6	3.4
Tmax (hr)	2.0	1.33
Cmax (μg/l)	120.80	115.60
AUC(0-inf) (μg/l h)	701.7	703.97

Systemic exposure to B9302-107 following administration of a suspension formulation was only 56% of that observed at a comparable dose in tablet form in 7 year old dogs (Table 9). Unchanged drug accounted for 41% of total radioactivity indicating significant drug metabolism. The Tmax was achieved within 8 hours after oral dosing and elimination half-life ranged from 4-11 hours.

Table 9. Single dose PK comparisons of suspension formulation in dogs.

Parameter	[^{14}C]-radioactivity		Unchanged B9302-107	
	0.03 mg/kg IV	2 mg/kg PO (S)	0.03 mg/kg IV	2 mg/kg PO (S)
T1/2 (hr)	8	11	4	7
Tmax (hr)		1.5-8		1-8
Cmax (μg/l)		152		106
AUC (μg/l.h)	236	2543	80	1054
Cl/kg (l/hr)	0.129		0.382	
Vdarea/kg (l)	1.46		2.099	

Hamsters:
Following single oral administration of 300 mg/kg B9302-107, 240 mg/kg B9202-045 or B9502-054 in 4% methocel in male Syrian hamsters, exposure to B9502-044 was 6.3-fold greater than the parent compound following administration of B9302-107 (Table 10). Hamsters were similar to the rat in this regard. Exposure to B9202-045 was 56% of parent while levels of B9502-054 were similar to that of B9302-107. Tmax was significantly greater than in the mouse at 14 hours. Exposure to the N-oxide of B9202-045 (B9502-054) was an order of magnitude lower than B9202-045 regardless of which of the two compounds was administered. The exposure levels of the two metabolites were orders of magnitude greater than that observed following administration of B9302-107.

Table 10. PK assessment of single dose administration in male hamsters.

Parameter	**Analyte**			
	B9302-107	B9202-045	B9502-044	B9502-054
Administration of 300 mg/kg B9302-107				
$AUC_{(0\text{-}24h)}$ (mg/l h)	9.18	5.12	57.94	9.97
Cmax (mg/l)	0.9	0.5	4.3	0.67
tmax (h)	14.2	24	4	21.2
Administration of 240 mg/kg B9202-045				
$AUC_{(0\text{-}24h)}$ (mg/l h)	NA	1394.9	NA	78.73
Cmax (mg/l)		80.36		22.72
tmax (h)		48		67.2
Administration of 240 mg/kg B9502-054				
$AUC_{(0\text{-}24h)}$ (mg/l h)	NA	1149.1	NA	140.16
Cmax (mg/l)		60.09		19.24
tmax (h)		24.8		15.2

NA: not assessed.

When male Syrian hamsters were administered single, oral doses of 0.05-1.5 mg/kg B9202-045 in 4% methocel, exposure to B9202-045 increased proportionally with increasing dose (Table 11). Exposure to its N-oxide was ~ 2.3-fold greater than exposure to B9202-045 at a dose of 1.5 mg/kg, indicating significant metabolism, in contrast to higher doses (Table 10). The Tmax was achieved within 1.5 to 2 hours.

Table 11. PK assessment of single B9202-045 dose administration in male hamsters.

Parameter	**Analyte**			
	B9202-045			B9502-054
Dose (mg B9202-045/kg)	0.05	0.5	1.5	1.5
$AUC_{(0\text{-}8h)}$ (µg/l h)	39.22	495.84	1589	3694.3
Cmax (µg/l)	9.87	125.06	402.34	750
tmax (h)	2	1.6	1.6	2.2

Mice:

A single oral dose of B9302-107 (300 mg/kg) demonstrated that B9502-044 (its N-oxide) was the primary analyte in male B6/C3 F1 mice and was detected at 2.65-fold of the parent compound. Formation of B9202-045 and its N-oxide (B9502-054) was minor in this strain of mice (Table 12). Following exposure to B9202-045 or its N-oxide, there was conversion from one form to the other resulting in similar exposure levels, which were orders of magnitude greater than those following administration of B9302-107. Time to maximum concentrations were greater following administration of B9302-107 (4.5 to 10.4 hours) than the two metabolites (1-6 hours).

Table 12. PK assessment of single dose administration in male mice.

Parameter	Analyte			
	B9302-107	B9202-045	B9502-044	B9502-054
Administration of 300 mg/kg B9302-107				
$AUC_{(0\text{-}24h)}$ (µg/l h)	20696	667.5	54906	963.5
Cmax (µg/l)	2352.7	63.7	5320.7	274.7
tmax (h)	5.2	8.8	10.4	4.5
Administration of 240 mg/kg B9202-045				
$AUC_{(0\text{-}24h)}$ (mg/l h)	NA	508.6	NA	333.7
Cmax (mg/l)		47.18		18.69
tmax (h)		3.8		5.8
Administration of 240 mg/kg B9502-054				
$AUC_{(0\text{-}24h)}$ (mg/l h)	NA	403.29	NA	399.6
Cmax (mg/l)		43.9		47.12
tmax (h)		2.4		1

NA: not assessed.

At lower single oral doses (0.5-9 mg/kg of B9302-107 in 4% methocel), systemic exposure to B9302-107 increased proportionally from the low to mid doses and sub-proportionally from the mid to high doses (Table 13). At the high dose, the main metabolite was the B9202-044, observed at slightly greater levels than the parent compound. Low levels of B9202-045 were observed but its N-oxide was detected at levels slightly below that of the parent compound. The Tmax ranged from 1 to 2 hours in all cases. When administered single oral doses of 0.05-1.5 mg/kg of B9202-045, B9202-045 exposure increased proportionally from the mid to high doses; tmax was ~ 1 hour. Systemic exposure to its N-oxide was ~ 19-fold greater than to B9202-045 itself in contrast to observations at higher doses, indicating extensive metabolism in the mouse.

Table 13. PK assessment of single dose administration in male mice.

Parameter	Analyte									
	B9302-107			B9502-044	B9202-045	B9502-054	B9202-045	B9202-045	B9202-045	B9502-054
Dose (mg B9302-107/kg)	0.5	1.5	9	9	9	9				
$AUC_{(0\text{-}8h)}$ (µg/l h)	25.02	70.36	279.95	308.58	19.73	240.08				
Cmax (µg/l)	10.11	20.54	105.45	105.56	6.69	58.2				
tmax (h)	1.4	1.2	1	1.6	1.4	2				
Dose (mg B9202-045/kg)							0.05	0.5	1.5	1.5
$AUC_{(0\text{-}8h)}$ (µg/l h)							na	76.33	179.74	3401.9
Cmax (µg/l)							na	27.42	82.49	1096.6
tmax (h)							na	1	1	1.2

The kinetic data for B9302-107 and its N-oxide were similar in male NMRI mice administered a single oral (gavage) dose of 900 mg/kg B9302-107 (Table 14). Plasma cAMP was significantly increased up to 14-fold at 8 hours following dosing, and urine cAMP and creatinine levels increased by 17.3 and 2.2-fold. Plasma erythropoietin levels showed no change although it is questionable as to why this substance was detected at pre-dose but not at 24 hours after dosing.

Table 14. Single dosing with B9302-107 (900 mg/kg) in male NMRI mice.

Parameter	Control	B9302-107	B9502-044
AUC (0-24 h; µg/l h)		96670	104770
Cmax (µg/l)		7072	6259
Tmax (h)		11.2	11.2
Plasma cAMP (pmol/ml)			
Pre-dose	101.2	83.9	
1	140.2	1240.3	
3	108.4	1523.4	
8	122.9	816.4	
24 hr	61.8	616.9	
Plasma erythropoietin (mU/ml)			
Pre-dose	0.99	0.83	
1	1.51	1.86	
3	1.13	0.81	
8	0.43	1.27	
24 hr	0	0	
Urine cAMP (nmol/ml)	17.9	310	
Urine Creatinine (µmol/ml)	1.03	2.25	
Urine cAMP/Creatinine	17.4	137.8	

Rabbits:

Pharmacokinetic parameters for non-pregnant female rabbits following a single oral or iv administration of B9302-107 are summarized in Table 15. Serum concentrations in pregnant rabbits (0.5 hours following administration) were ~ 6-fold greater on Day 29 than on Day 18 post coitum. In addition, drug was able to cross the placenta as serum concentrations on Day 29 post coitum were ~ 1.3-fold greater in the fetus than in the parent.

Table 15. PK in rabbits (non-pregnant females, pregnant rabbits and fetuses) following single po or iv dose of B9302-107.

Parameter	**Dose group (mg/kg)**				
	Non pregnant female		Pregnant		Fetus
	0.02 iv	0.8 po	0.8, po, Day 18 PC	0.8, po, Day 29 PC	0.8, po, Day 29 PC
$AUC_{(0\text{-}inf)}$ (µg/l h)	15.57 (12.09-20.04)	16.32 (13.2-20.19)			
Cmax (µg/l)	22.2 (15.7-31.4)	3.1 (2.1-4.7)			
Mean serum conc. (µg/l) ± SD			3.4 ± 1.89	18.37 ± 14.1	24.44 ± 9.57
t1/2 (h)	1.3 (0.8-2.2)	-			
tmax (h)	0.08	2.67			
Cl/kg (l/hr)	1.28 (0.998-1.65)	-			
Vd(area)/kg (l)	2.42 (1.65-3.56)	-			

Repeat dose PK parameters:

Rats:
Following repeated oral dosing (single/7-day dosing with radioactive B9302-107 in 4% methocel) in male Sprague Dawley rats, plasma concentrations taken 8 hours after dosing attained steady state on Day 4, at levels approximately 2.25 times greater than day 1 levels (Table 16). The AUC levels remained 2.4-fold greater after the seventh consecutive dose compared to the first indicating the potential for drug accumulation.

Table 16. PK in Sprague Dawley rats following single/multiple oral dose of B9302-107

Parameter	2 mg/kg B9302-107	
Day of assessment	1	7
$AUC_{(0\text{-}24)}$ (μg equiv.hr/ml)	3.6	8.77
Cmax (μg/ml)	0.216	0.487
tmax (h)	8	4-10

Repeated oral dosing (7-day) with B9302-107 (8 mg/kg in 4% methocel) or B9202-045 (6.4 mg/kg in HCl) in male Wistar rats showed enterohepatic circulation and resulted in a systemic exposure to the respective compounds that was greater for B92020-045 by ~ 27-fold than that of B9302-107 (Table 17), taking into consideration that the compounds were administered in a molar ratio of 1:2 (B9302-107:B9202-045). In a follow-up 7-day oral dosing study in which B9302-107 (8 mg/kg in 4% methocel), B9202-045 (6.4 mg/kg in HCl) or B9502-054 (6.4 mg/kg) were assessed, systemic exposure to B9202-045 was greater by ~ 13-fold that of B9302-107 (n = 1) when considering compound administration was in a molar ratio of 1:2 (B9302-107:B9202-045). Following administration of B9302-107, B9202-045 exposure was 3.2-fold greater than the parent compound. Following administration of B9502-054, B9202-045 exposure was 1.4-fold greater than its N-oxide, indicating a significant conversion to the non-oxidized form. Tmax for all compounds ranged from 1-6 hours and clearance half-life was ~ 3-5 hours (measured only for B9202-045). This assessment was performed as part of toxicity studies reviewed previously (see Attachment, studies 194/95 and 128/96).

Table 17. PK in Wistar rats following 7-day oral dosing of B9302-107 and metabolites

Parameter	**Analyte**		
	B9302-107	B202-045	B9202-054
	8 mg/kg B9302-107		
$AUC_{(0-8)}$ (µg/ml.hr)	0.246		
Cmax (µg/ml)	0.054		
tmax (h)	3.4		
T1/2	NA		
	6.4 mg/kg B9202-045		
$AUC_{(0-8)}$ (µg/ml.hr)		13.636	
Cmax (µg/ml)		2.555	
tmax (h)		2.2	
T1/2		3.2	
	8 mg/kg B9302-107		
$AUC_{(0-24)}$ (µg/ml.hr)	0.973 (n=1)	3.194	
Cmax (µg/ml)	0.063	0.228	
tmax (h)	7.4	6	
T1/2	NA	NA	
	6.4 mg/kg B9202-045		
$AUC_{(0-24)}$ (µg/ml.hr)		24.917	
Cmax (µg/ml)		2.696	
tmax (h)		1.2	
T1/2		4.3	
	6.4 mg/kg B9502-054		
$AUC_{(0-24)}$ (µg/ml.hr)		13.820 (n=1)	9.574
Cmax (µg/ml)		1.509	1.998
tmax (h)		3.6	1
T1/2		4.9	4

NA: not assessed.

Systemic exposure increased at a slightly greater than proportional rate with increasing dose after 7-day oral dosing of male Wistar rats with B9202-045 (0.05-6 mg/kg in HCl; Table 18). Tmax ranged from 1-2 hours and elimination half-life ranged from 3.4 to 4.4 hours. These values are slightly greater than those observed after single dose administration. This assessment was performed as part of a toxicity study reviewed previously (see Attachment, study 129/96).

Table 18. Systemic exposure in rats to B9202-045 following 7-day administration of B9202-045.

Parameter	**Dose group (mg/kg)**				
	0.05	0.5	1.5	3	6
$AUC_{(0-24)}$ (µg/ml.h)	0.105	1.398	4.396	10.053	23.602
Cmax (µg/ml)	0.018	0.168	0.555	1.216	2.383
Tmax (h)	1	1.2	1.4	1.2	1.8
T1/2 (h)	4.4	4.4	3.6	3.4	3.5

During a 6 month oral administration study in Wistar rats (14/96; reviewed previously, see Attachment), systemic exposure to B9302-107 increased proportionally with increasing dose (Table 19). The $AUC_{(0\text{-inf})}$ was not ascertainable due to enterohepatic circulation, demonstrated by increasing serum concentration at various timepoints between 4 and 24 hours; elimination half-lives were also not determined and plasma concentrations were below LLOQ at 24 hours after dosing in most animals. Systemic exposures increased at the mid and high-dose from day 91 to day 184 by 41 and 81%, respectively, indicating potential drug accumulation. Tmax was similar among all treatment groups (~1-3 hours). The NOAEL for this study was the low dose of 0.5 mg/kg.

Table 19. Kinetics of 6 month oral administration of B9302-107 in rats.

Parameter	**Day**	**Dose group (mg/kg)**		
		0.5	1.5	2.5
$AUC_{(0\text{-}8)}$ (µg/l.h)	1	7.4	31.52	46.27
	91	16.87	29.28	43.50
	184	9.74	41.28	78.73
Cmax (µg/l)	1	2.66	9.12	9.66
	91	5.13	8.7	10.15
	184	2.60	12.86	19.13
Tmax (h)	1	1	1	2.83
	91	0.92	0.82	1.08
	184	1.58	2.17	2.08

Dogs:
Four week and 6 month toxicokinetic assessment following oral dosing was performed as part of toxicity studies and the data are reviewed in detail with those studies. Table 20 summarizes the data. Four week administration (suspension in hard gelatin capsule) resulted in a systemic exposure which increased proportionally from a dose of 2 to 6 mg/kg, although the increase was sub-proportional at the high dose indicating saturation of drug absorption. Drug accumulation was apparent at the two higher doses. A follow up 4-week oral study in dogs assessed the systemic exposure of B9302-107 and B9202-045 following daily administration of B9302-107 and produced similar results. In contrast to the findings in the rat, parent compound levels were approximately 4-fold greater than B9202-045 levels. Also, a longer elimination half-life was noted for the parent compound (4.5-10 hr) than for B9202-045 (~3 h). Levels of B9502-044 and B9502-054 were below detection limits. In the 6 month study, the pharmacokinetic parameters remained constant throughout the study and systemic exposures to B9302-107 (tablet) increased sub-proportionally. Elimination half-life ranged from 4.5 to 13 hours in the 4 week study, increasing at the high dose, and 2-4 hours in the 6 month study. The tmax ranged from ~ 1.4 to 5.5 hours.

Table 20. Pharmacokinetics following 4 week and 6-month oral administration in dogs.

Parameter	Day	Analyte							
		B9302-107							B9202-045
mg B9302-107/kg		0.2	1	2	4	6	18	18	18
AUC (μg/l hr)	1			991.9		2304.6	3547.6	3384.3	127.86
	3	224.5	519.5		1860.7				
	25			827.6		3061.1	4182.8	3458.9	151.94
	86	183.3	496.8		1597.3				
	178	203.7	588.5		1982.0				
AUC ratio (%): B9202-045/ B9302-107	1							Reference	3.77
	25								4.39

Clearance, volume of distribution and AUC data were not ascertainable in a 14-day IV study with B9302-107 (0.01-0.06 mg/kg) since drug levels in terminal phases of elimination were below LOQ. The Cmax, however, increased proportionally with increasing dose (6.55-54.61 μg/l) and remained constant throughout the study; tmax ranged from 0.25 to 0.38 hours.

Table 21 summarizes the absorption and bioavailability of orally administered B9302-107. Absorption was moderate (32%) in male Sprague Dawley rats while bioavailability was low (4%) indicating high first-pass metabolism. Bioavailability in 7 year old dogs following a single oral administration of a B9302-107 suspension (2 mg/kg) was 15-19% but increased significantly (48-61%) following administration of a tablet (Formulation A, 0.05 to 2 mg/kg) which may be explained by rate limited dissolution prior to absorption. Formulation B was shown to have comparable bioavailability in male dogs (106% of reference Formulation A). Bioavailability in male hamsters and mice (34-48%) was similar to that in dogs administered tablet while non-pregnant female rabbits were similar to rats (2%), possibly due to a pronounced first pass effect or low absorption.

Table 21. Bioavailability B9302-107 following single dosing.

Species	Dose (mg/kg)	Bioavailability (%)	Absorption (%)
Rat	10 (po)/1 (id)	4	32
Dog	2, suspension	15-19	
Dog	0.05-2, tablet (A)	48-61	
Mouse	1	34	
Hamster, male	1	48	
Rabbit, female	0.8	2	

Absorption of B9202-045 was rapid and extensive (99%) following a single oral administration of 5 mg/kg B9202-045 in male Sprague Dawley rats.

Distribution: Assessment of tissue distribution of [^{14}C] or [^{12}C]-B9302-107 by whole body autoradiography (no quantitative data) was performed in male Sprague Dawley rats. A single 1 mg/kg, iv (with PEG), administration resulted in highest concentrations detected in lungs, liver, adrenals, kidneys after 1 hour. Highest levels after 4 and 8 hours were in the large intestine and nasal mucosa followed by the liver and stomach. Radioactivity persisted in the nasal mucosa at 168 hours after dosing. An oral dose of 10 mg/kg (in 4% methocel) resulted in slow absorption,

with peak levels detected at 1-4 hours in the stomach contents, intestine and liver. Pronounced uptake and retention in nasal mucosa were again noted from 4 to 168 hours. The distribution of [^{14}C] or [^{12}C]-B9202-045 following a single 1 mg/kg iv dose administration (in aqueous solution with HCl) resulted in highest radiation concentrations in stomach, intestinal contents, preputial gland in first hour following iv administration. After 8 hours, highest concentrations were detected in the nasal mucosa followed by the intestines and liver; radioactivity persisted in nasal mucosa and preputial gland until 96 hours (last time point investigated). Following oral dosing (5 mg/kg), B9202-045 was rapidly absorbed, with highest concentrations in the stomach, nasal mucosa, preputial gland, liver and kidneys.

In a study to assess the uptake and binding of radioactivity in the nasal passages, male Wistar rats were administered a single peroral dose of ^{14}C labeled B9302-107 (10 mg/kg) and B9202-045 (1 mg/kg) or (benzene ring ^{3}H) B9302-107 (10 mg/kg). A selective uptake of radioactivity in the sustentacular cells and Bowman's glands of the dorsomedial part of the olfactory region was observed in rats administered ^{14}C labeled B9302-107 and B9202-045 with levels markedly higher in B9202-045 treated rats. Binding of B9202-045 was not noted in the olfactory neurons. Also, no tissue bound radioactivity was observed in the olfactory mucosa of rats administered ^{3}H B9302-107, indicating that the reactive metabolite (B9202-045) does not contain the benzene ring structure and that B9302-107 is metabolized into a reactive product which becomes bound to the tissue structure of the olfactory mucosa in Wistar rats.

Distribution following 7 day oral dosing with radioactive B9302-107 (2 mg/kg in 4% methocel) po, in male Sprague Dawley rats, was consistent with single dose studies as highest concentrations were noted in the nasal mucosa, followed by the liver and kidneys at 8 hours after dosing (Table 22). Nasal mucosa levels were ~ 8 times greater than those in the plasma. Peak tissue radioactivity levels following a single dose of B9202-045 (5 mg/kg, po) occurred at 0.5-1 hours followed by a secondary peak at 2-4 hours; peak concentrations in the nasal mucosa occurred 4 hours after dosing. Terminal half-lives ranged from 6-41 hours with some radioactivity detected in the nose after 96 hours. The metabolite B9502-054 was present in the nose and lungs, but not the liver and kidneys, suggesting formation took place extra-hepatically.

Table 22. Tissue distribution in male rats after oral dosing with B9302-107 or B9202-045

Parameter	**B9302-107** (mg-equiv/l)	**B9202-045** (mg-equiv/l)
	Day 7	Day 1
Nose	3.63 (8 hrs)	15.89 (4 hrs)
Liver	1.79 (8 hrs)	5.99 (0.5 hrs)
Kidneys	1.14 (82 hrs)	6.7 (2 hrs)
Testes	0.21 (8 hrs)	-
Lungs	0.45 (8 hrs)	-
Plasma	0.44	-
Fat	-	10.37 (0.5 hrs)
Heart	-	4.17 (4 hrs)

The effects of metyrapone (50 mg/kg, ip), an unspecific p450 inhibitor, on tissue distribution of pyridine ring labeled ^{14}C-B9202-045 (single oral dose of 1 mg/kg) in plasma, liver and nose of

male Wistar rats are summarized in Table 23. Levels in plasma and liver were decreased 2 to 3-fold in the presence of metyrapone when compared to animals administered B9202-045 alone. In the nose, concentrations were similar at 30 minutes but decreased by 2-fold in the presence of metyrapone at 2 hours. Three major bands were detected in the nose and plasma (B9202-045, B9502-054, unidentified polar compound), while in the liver most of the radioactivity represented B9202-045; no B9502-054 was detected. In the presence of metyrapone, levels of B9502-054 and B9202-045 were increased by 6-27% and a new metabolite, M4, was formed. The amount of polar components in the control experiment observed in the nose (40-60%) was reduced, amounting to 9-12% of the total in the nose. In plasma, the increase in B9202-045 was more pronounced than in the nose, while minor changes were noted in the liver; no B9502-054 was produced. The data indicate that cytochrome P450 is involved in the metabolic activation of the B9502-054, the major metabolite observed in the rat nose.

Table 23: Effects of metyrapone on distribution of drug-related materials.

					Radioactive bands (% of spotted material)			
	Organ/tissue	Time (h)	% extracted with MeOH	Tiss. Conc (µg eq/g)	B9202-045	B9502-054	M4	Polar components
Without metyrapone	Plasma	0.5 2	100 100	0.769 0.747	29 37	47 52	- -	24 11
	Liver	0.5 2	97 95	1.597 1.439	91 89	- -	- -	9 11
	Nose	0.5 2	87 78	2.939 2.28	38 23	20 14	- -	40 60
With Metyrapone (50 mg/kg)	Plasma	0.5 2	100 100	0.41 0.239	52 50	37 40	- -	10 8
	Liver	0.5 2	98 97	0.90 0.877	85 83	- -	- -	13 15
	Nose	0.5 2	98 97	2.752 1.171	44 40	39 41	8 7	9 12

Tissue distribution of [^{14}C]-B9302-107 (1 mg/kg, po or iv) in male NMRI mice differed depending upon route of administration (Table 24). Following oral dosing, peak levels were noted at 1-4 hours in the bone marrow, adrenals, and liver; levels were 7 and 4-fold greater in the liver and adrenals than in plasma. The terminal half-lives were 42 and 88 hours in the liver and adrenals, respectively. With IV dosing, highest concentrations were detected in the lungs, liver, adrenals, kidneys and plasma, with the lung showing a 5-fold increase compared to plasma; terminal half-lives ranged from 32 hours in fat to 87 hours in liver. Radioactivity levels were less than 1% of peak tissue values after 96 hours. Significant levels were not detected in the nose in this study, although it was observed in a metabolism study (see next section). Male hamsters displayed similarities to the mouse in that highest radioactivity concentrations were detected in the liver, adrenals, and kidneys. However, radioactivity was detected in the nasal epithelium and thyroid at 4 hours while none was detected in the lung or bone marrow. Highest concentrations were seen in the liver and nose after four days. Following IV dosing, highest levels were noted at 72 hours. Tissue elimination half-lives ranged from 28.6 to 48 hours. The parent compound (not observed in plasma), N-oxides and polar compounds were detected in the liver, kidneys, lungs and nose.

Table 24. Tissue distribution of B9302-017 in mice and hamsters.

Parameter	Dose group (mg/kg)			
	Mouse		Syrian hamster	
	1 iv	1 po	1 iv	1 po
Peak Drug Disposition (µg equiv./l)				
Lungs	5.282	0.072	-	-
Liver	5.167	0.375	0.229 (72 hrs)	0.536 (4 hrs)
Adrenals	2.742	0.67	0.103 (72 hrs)	0.235 (4 hrs)
Kidneys	1.217	0.09	0.052 (72 hrs)	0.179 (4 hrs)
Bone marrow	0.807	1.989	-	-
Nose	-	-	0.057 (72 hrs)	0.047 (4 hrs)
Thyroid	-	-	0.062 (72 hrs)	0.119 (4 hrs)
Plasma	1.153	0.10	-	-
AUC(0-96h) (µg equiv.hr/g)				
Lungs	12.54	0.98		
Liver	14.69	5.59		
Adrenals	3.21	5.18		
Kidneys	3.77	1.44		
Plasma	4.63	1.59		

Metabolism: The metabolism of B9302-107 following a single dose in dogs is summarized in Table 25. In serum, unchanged drug was the major radioactive [^{14}C] component and gradually disappeared with the formation of M9 (unknown) following IV dosing (0.03 mg/kg). Results following oral dosing (2 mg/kg, suspension) were similar to IV dosing although three other minor metabolites were detected. B9202-045 was detected only in trace amounts. In urine, the major metabolite was M4 (unknown; 63-76%) regardless of dosing route and two other metabolites were identified as B9302-007 (dealkylation at 3-position), B9302-102 (cleavage of amide bond). Parent drug and B9202-045 were not detected. In the feces, much higher levels of parent drug were detected after oral dosing (91-98%) than IV (3-11%). The major metabolite after IV dosing was M5 (27-35%) and M9 (7-25)% of dose.

Table 25. Metabolism of B9302-107 in dogs.

	Dose/route	Time after Dose (h)	% of spotted material						
			Parent	B9202-045	M4	M5	M9	M10	B9302-077
Serum	0.03 mg/kg, IV	4	32-65		4-5		14-47	0-3	
	2.0 mg/kg PO	4	30-51	0-4	7-8	3-5	9-37	0-9	
Urine	0.03 mg/kg, IV	0-24	0		69-76	3-5	0		0
	2.0 mg/kg PO	0-24			63-75				6-13
Feces	0.03 mg/kg, IV	0-24	3-11		4-12	27-35	7-25		6-15
	2.0 mg/kg PO	0-24	91-98						

Metabolism of [^{14}C]-B9302-107 in male NMRI mouse following single dose administration of 1 mg/kg po (4% methocel) or IV (in PEG) is summarized in Table 26. In plasma, a similar metabolic profile was observed with either route of administration; highest levels of B9402-044, followed by B9502-054, M3, M5 and M11. Unchanged drug accounted for most of the radioactivity in the liver, kidneys, lung, and nose. Detected metabolites included B9502-044,

M3, M12, and B9302-077. B9502-054 was detected only in the nose and plasma. Traces of B9202-045 (1-8%) was detected in all tissues and organs investigated. Following IV administration, only traces of parent drug appeared in feces and none in the urine. With oral administration, unchanged parent was the primary compound detected in feces (85%) but was undetected in urine. Major metabolites in urine and feces were B9502-054 (feces only, 14-24%), B9302-077, M3 and M4.

Table 26. Metabolism of B9302-107 in mice.

Component	**Dose/ route**	**Time after Dose (h)**	**Radioactive bands (% of spotted material)**										
			Parent	**B9502-044**	**B9202-045**	**B9502-054**	**M3**	**M4**	**M5**	**M11**	**M12**	**B9302-077**	**Origin**
Plasma	1 mg/kg, IV	0.5 4	28 6	20 7		9 19	11 6		2 5	3 5			24 46
	1 mg/kg PO	0.5 4	11 2	26 9		5 10	11 9		0 7	9 8			24 38
Liver	1 mg/kg, IV	0.5 4	55 7		3 3		5 6				6 13	10 42	16 22
	1 mg/kg PO	0.5 4	62 20		2 3		3 7				4 10	13 27	11 21
Kidneys	1 mg/kg, IV	0.5 4	65 7	2 2	5 7		6 5				2 4	7 46	8 22
	1 mg/kg PO	0.5 4	36 22	13 11	3 8		5 6				3 5	19 21	8 14
Lungs	1 mg/kg, IV	0.5 4	60 40	4 3	2 8		6 6					4 5	14 24
	1 mg/kg PO	0.5 4	48 27	9 10	3 4		2 6					3 4	14 20
Nose	1 mg/kg, IV	0.5 4	65 20	3 6	1 2	- -	5 8					4 13	13 31
	1 mg/kg PO	0.5 4	37 18	14 11	- -	7 12	5 10					- -	27 34
Urine	1 mg/kg, IV	0-24	0			24	3	0				10	57
	1 mg/kg PO	0-24	0			14	6	5				11	56
Feces	1 mg/kg, IV	0-24	1			0	21	8				13	44
	1 mg/kg PO	0-24	85			0	1	2				2	6

Metabolism in Syrian hamsters following single oral dosing is summarized in Table 27. The N-oxide of the parent was noted at highest levels after 1 hour in plasma followed by B9502-054; no parent drug was detected. By 24 hours, M3 had increased as the N-oxide decreased. Relatively high levels of parent drug were detected in the liver, kidneys, lungs and nose after 1 hour with increasing levels of M3 by 24 hours. Lesser amounts of B9502-044, B9202-045, B9502-054, and B9302-107 were noted in these tissues. Parent drug was primarily detected in the feces (83%) while B9302-077 and polar compounds were the major metabolites present in the urine.

Table 27. Metabolite profiles after oral dosing in hamsters

Sample	Time after Dose (h)	Radioactive band (% of spotted material)						
		Parent	**B9502-044**	**B9202-045**	**B9502-054**	**M3**	**B9302-077**	**Origin**
Plasma	1 24		24 2		13 8	9 29		45 39
Liver	1 24	24 3	1 3	4 1	7 4	10 17	4 3	42 43
Kidneys	1 24	37 6	9 6	3 5	3 3	3 6	- -	37 58
Lungs	1 24	44 4	3 0	4 4	7 0	4 18	7 0	22 60
Nose	1 24	37 0	4 0	4 0	4 3	6 37	5 2	31 55
Urine	0-24	2		0	3	4	12	69
Feces	0-24	83		1	1	2	2	7

The in vitro metabolism of ^{14}C-labeled B9302-107 and B9202-045 was evaluated using hepatic and respiratory microsomes from rat, mouse, hamster, cynomolgus monkey and human donors (Table 28). The compounds were incubated for 2-4 hours with microsomes at concentrations of 100 and/or 1000 ng/ml. Recovery was approximately 40% of the radioactivity associated with B9302-107 and B9202-045. In general, microsomes incubated with B9302-107 were not able to form B9202-045 or its N-oxide (M2, B9502-054) with some exceptions. Metabolites M7 and M9 appeared to be the major metabolites with lower levels of M1, M3, and M4. Interestingly, rat nasal microsomes did not produce B9502-054, thought to be responsible for nasal toxicity in this species.

Table 28: Metabolite formation following microsome incubation with Roflumilast.

			Metabolite (% region of interest)														
Tissue microsome	Conc. ng/ml	Incubation time	Species	B9202-045	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M14
Liver	100	2	Rat	1.23	1.09		2.22	3.34			5.25		70.3				
			Mouse		5.54	8.16		3.37		1.59	24.76		17.34				0.91
			Hamster		7.7		12.3	4.46			11.55		25.39				
			Dog					2.38	3.81	3.68	6.86		8.43				
			Monkey				15.78				12.41		25.85				
			Human			0.41		0.65			5.06		11.08				
		4	Rat	1.53	1.66		2.83	3.57			4.96		69.97				
			Mouse		7.42	8.08		4.25		1.91	24.45		16.9				1.06
			Hamster		9.2		17.98	7.18			8.66		15.5				
			Dog					4.67	3.79	6.94	8.79		12.41				
			Monkey				24.6				8.11		27.53				
			Human			0.67		0.99			8.34		16.26				
	1000	2	Rat	1.92	1.38		1.96				6.51		61				
			Mouse		4.0	7.8		4.46		1.23	15.4		13.0				
			Hamster				<	<	*		16.3		36.8				
			Dog		0.76	0.4		2.85	1.69		3.89		4.10				
			Monkey			2.09	2.51				7.88		14.77				
			Human					0.17			1.72		3.30				
Olfactory	100	2	Rat		4.02		9.79				12.5	3.04	12.24	0.96	0.66	0.59	
			Hamster		8.06		13.37	2.77	3.84	4.96	7.29					2.15	
			Dog		6.49	3.51		5.19	2.8		16.52	4.68	4.65	1.51			
			Human														
	1000		Rat				6.1				3.72	4.59	6.15				
			Mouse							3.94	1.89						
			Hamster				3.4	5.9			2.55					2.77	
			Dog					3.06	1.41		7.76		2.72				
			Monkey								0.56		0.78				
			Human														
Respiratory	100	2	Mouse			0.89				1.62	0.78						
			Hamster				1.27	7.01		1.34	1.78		0.77			0.62	
			Dog								0.23	1.37	0.18				
			Monkey								0.44		0.53				
			Human														
	1000		Rat				0.66				0.36	0.4					
			Mouse														
			Hamster				0.78	1.5			0.77		0.3			0.79	
			Dog									0.27					
			Monkey														
			Human														

Microsome incubation studies were also performed with B9202-045 to primarily assess the ability of nasal microsomes to convert B9202-045 to B9502-054, its N-oxide believed responsible for nasal lesions found in rodents; results are summarized in Table 29. Olfactory and respiratory microsomes from rats, mice, hamsters and dogs produced large amounts of B9502-054. In contrast, human microsomes produced no B9502-054 while monkey olfactory microsomes produced trace amounts. Small amounts of M3 were also produced. Similar results were observed with liver microsomes from mice and hamsters with much lower amounts produced by rat, dog, monkey and human microsomes. The results indicate that nasal lesions induced by B9502-054 are not of concern in humans since human nasal microsomes do not convert B9202-045 to B9502-054.

Table 29: Metabolite formation following microsome incubation with B9202-045.

			Metabolite (% / pmoles/hr/mg protein)								
Tissue	Conc. ng/ml	Incubation time	Species	B9502-054	M1	M2	M3	M4	M10	M11	M14
Liver	100	2	Rat Mouse Hamster Dog Monkey Human	3.78 / 22.65 88.42 / 530.52 93.3 / 559.8 1.13 / 6.75 12.38 / 74.25 1.07 / 6.41			0.60 0.32 0.38				0.36
		4	Rat Mouse Hamster Dog Monkey Human	4.27 / 12.81 90.5 / 271.5 94.83 / 284.48 1.96 / 5.87 15.01 / 45.0 1.28 / 3.84			0.77 0.39 0.33				0.43
	1000	2	Rat Mouse Hamster Dog Monkey Human	0.36 / 21.3 24.29 / 1457.1 52.67 / 3159.9 0.19 / 11.1 2.23 / 133.5 0.15 / 9.0			 0.22 0.25			 0.26	
Olfactory	100	2	Rat Hamster Dog Monkey Human	91.98 / 551.88 90.18 / 541.05 89.22 / 535.3 1.27 / 7.59	 0.91 0.40		0.66 1.0 0.71				1.13
	1000		Rat Mouse Hamster Dog Human	89.84 / 5390.1 77.69 / 4661.4 61.55 / 3692.7 88.38 / 5303		0.2	0.18 0.52 0.41	 2.84 1.95			
Respiratory	100	2	Mouse Hamster Dog Human	88.15 / 528.9 77.03 / 462.18 94.92 / 569.52				3.24			
	1000		Rat Hamster Dog Monkey Human	72.05 / 4323 29.12 / 1747.2 37.91 / 2364.6			0.31	1.39	 0.69		

In order to assess xenobiotic-metabolizing capability, cytochrome P450 concentrations were measured in microsomes (primarily liver; Table 30). A single determination in rat olfactory microsomes was about half of that found in the liver, while dog olfactory microsomes had slightly higher concentrations than those measured in liver microsomes. The results suggest a greater level of activity in the olfactory epithelium than in the liver or respiratory epithelium of the dog while liver activity was greater in humans and rats. Cytochrome P450 activity was not detected in the olfactory microsomes from the human nor the respiratory microsomes from the dog or human.

Table 30: Cyt P450 (nmol/mg protein) levels in microsome samples.

Species	Tissue Sample		
	Liver	**Olfactory**	**Respiratory**
Rat	0.458	0.268	Not tested
Mouse	0.517	Not tested	Not tested
Hamster	0.63	Not tested	Not tested
Dog	0.311	0.37	Not detected
Monkey	0.62	Not tested	Not tested
Human	0.307	Not detected	Not detected

The catalytic role of the CYP 2A subfamily of cytochrome P450, believed to be involved in the metabolic activation of nasal toxicants, in 7-hydroxylation of coumarin and steroid hydroxylation reactions was assessed. Coumarin 7-hydroxylase activity was not detected in liver microsomes from the rat and mouse, with low levels from hamster and dog and much higher activities in pooled samples from human and monkeys (Table 31). Significantly greater activity was determined in olfactory microsomes from all species tested except humans, with a 2.5- to 4-fold increase in hamsters compared to rats and dogs. Rat, dog and hamster olfactory epithelia appear to have a higher capacity (35 to 120-fold) than the liver for metabolism of coumarin. In contrast, olfactory and respiratory enzyme activity was not detected in humans.

Table 31: Coumarin 7-hydroxylase (pmol/min/mg protein) levels in microsome samples.

Species	Tissue Sample		
	Liver	**Olfactory**	**Respiratory**
Rat	Not detected	122.08	Not tested
Mouse	Not detected	Not tested	Not tested
Hamster	14.22	492.69	Not tested
Dog	5.23	197.39	5.3
Monkey	234.31	Not tested	Not tested
Human	191.62	Not detected	Not detected

The metabolism of testosterone by microsomes incubated with B9302-107 was assessed to reflect the relative quantities of the enzymes of cytochrome P450. 15α- and 16β-hydroxytestosterones were generated using rat and dog olfactory microsomes only (Table 32), while 7α-hydroxytestosterones were produced by hamster liver and dog olfactory microsomes, suggesting that these tissues posses CYP2A-mediated activity. Although generation of 6β-HTT metabolite (liver microsomes from all species, olfactory microsomes from dog), 6α metabolite (olfactory microsomes from dog), 16α-hydroxytestosterone (liver microsomes from rat, mouse and dog; olfactory microsomes from dog), 2α/2β-hydroxytestosterone combination (rat and hamster liver microsomes and olfactory microsomes from the rat and dog), Metabolite X (rat olfactory and hamster liver microsomes) and androstenedione (all liver microsomes except monkey and human) were observed, the cytochrome P450 isoforms responsible for their production are unclear. No testosterone metabolizing activity was noted in human olfactory or respiratory microsomes indicating the absence or very low presence of cytochrome P450-mediated activity.

Table 32. Steroid production in microsomes incubated with B9302-107.

Species	Tissue	15α-HTT	7α-HTT	6β-HTT	6α-HTT	16α-HTT	16β-HTT	2α/2β-HTT	ASD	Met X
Rat	Liver	ND	ND	1.12	ND	2.022	ND	1.694	1.393	ND
	Olfactory	2.9	ND	ND	ND	ND	1.18	1.949	ND	1.821
Mouse	Liver	ND	ND	0.964	ND	0.235	ND	ND	0.855	ND
Hamster	Liver	ND	1.644	2.728	ND	ND	ND	1.08	0.666	0.496
Dog	Liver	ND	ND	0.414	ND	0.287	ND	ND	0.308	ND
	Olfactory	0.828	0.175	0.381	0.295	0.272	0.107	0.446	1.858	ND
	Respiratory	ND	ND	ND	ND	ND	ND	ND	ND	ND
Monkey	Liver	ND	ND	4.465	ND	ND	ND	ND	ND	ND
Human	Liver	ND	ND	1.84	ND	ND	ND	ND	ND	ND
	Olfactory	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Respiratory	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: not detected

The in vitro biotransformation of B9302-107 and B9202-045 to their respective N-oxides was investigated in microsomal fractions incubated with radioactive test substance (1 μg/ml of liver and nasal mucosa of rat (Sprague Dawley and Wistar), mouse (NMRI), hamster (Syrian) and dog (Beagle) and microsomal fractions of human respiratory tissue) for 2 hours. No significant species differences were noted in rodent nasal tissue as B9502-054 was the main metabolite of B9202-045 (Table 33). Differences in metabolism of B9302-107 to B9502-044 were noted with little observed in the rat, 30% metabolized in hamster to form polar metabolites and the mouse in between. Liver microsome metabolism of B9202-045 in rats was slower than in the nose. Increasing amounts of B9502-054 were formed in rat (trace), mouse (~10%) and hamster (~35%). Similarly, B9502-044 formation from B9302-107 was most pronounced in hamsters. In dogs, a short in vitro half-life for B9202-045 was observed in the nasal and respiratory fraction. More metabolites were found in olfactory tissue with B9302-107. In humans, monooxygenase activities were not noted in respratory microsomes as levels of B9302-107 were stable and only traces of B9202-045 polar metabolites were observed. The oxidative pathway in nasal microsomes of all animal species was inhibited in vitro by PMSF and metyrapone.

Table 33. In vitro conversion of B9302-107 or B9202-045 to their respective N-oxides.

Species	**Tissue**	**Formation of N-oxide**	
	Microsomal fraction	**B9302-107 to B9502-044**	**B9202-045 to B9502-054**
Rat	Olfactory/respiratory mix	-	++
Mouse	Olfactory/respiratory mix	+	++
Hamster	Olfactory/respiratory mix	+	++
Dog	olfactory	++	+++
Dog	respiratory	++	+++
Human	respiratory	-	-
Rat	liver	+	-
Mouse	liver	+	+
Hamster	liver	+++	++

Activity classified from none (-) to strong (+++)

In an evaluation of B9302-107 as a direct acting (metabolism independent) reversible inhibitor of P450 activity or as a metabolism dependent reversible or irreversible inhibitor, pooled human liver microsomes were incubated with marker substrates at a concentration equal to Km/2, Km

and 4Km in the presence or absence of B9302-107 at concentrations ranging from 0.1 to 100 μM (metabolism independent) or pre-incubated with B9302-107 and NADPH for 15 minutes to allow for the generation of metabolites that could inhibit cytochrome P450 (metabolism dependent). Study results showed B9302-107 has little or no capacity to inhibit CYP2A6, CYP2B6, CYP2E1 or CYP4A9/11 and that B9302-107 is a competitive inhibitor of CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 with Ki values of 13, 2.4, 2, 5.3, and 0.77 μM, respectively. These results suggest that B9302-107 is not expected to cause drug interactions with drugs that are metabolized by P450 enzymes evaluated in this study. B9302-107 also demonstrated little capacity as a reversible or irreversible metabolism-dependent inhibitor of any of the P450 enzymes evaluated except for CYP2B6, which was weakly inhibited in an irreversible metabolism dependent manner.

Excretion: The excretion of drug-related radioactivity in various species is summarized in Table 34. Excretion in male Sprague Dawley rats was primarily urinary following IV administration and fecal after oral administration following administration of a single dose of 10 mg/kg po, 1 mg/kg id, or 1 mg/kg IV [^{14}C]-B9302-107. Biliary excretion accounted for ~ 20% of the administered dose following intraduodenal administration. Comparable results were observed after single and 7-day oral dosing with radioactive B9302-107 (2 mg/kg in 4% methocel), as fecal excretion was again the primary route of elimination at both time points, although urinary excretion increased with dosing duration. Excretion in dogs, mice and hamsters was similar as excretion was primarily fecal following oral dosing with increased urinary excretion after IV dosing.

Table 34. Excretion of [^{14}C]-radioactivity related to B9302-107.

Species	Route of administration	Dose (mg/kg)	Day of sample	Urine	Feces	Bile	Total recovery
Rat	Iv	1	1	69	31	29	100
	Po/id	10/1	1	36	70	20	106
	Po	2	1	18.5	68.6	-	87.2
	Po	2	7	50.7	62.6	-	113.3
Dog	IV	0.03	1	33	58	-	91
	PO	2	1	7	88	-	95
Mouse	IV	1	1	30.3	60.7	-	91
	PO	1	1	7.8	59.8	-	67.6
Hamster	IV	1	1	50.23	40.02	-	90.25
	PO	1	1	24.36	63.27	-	87.63

In contrast, excretion of drug-related radioactivity was primarily via the kidneys in male Sprague Dawley rats administered single doses of either 5 mg/kg, po, or 1 mg/kg, iv, of the metabolite B9202-045 (Table 35). Approximately 87% of dose was excreted within 3 days and the major components in urine consisted of parent drug (15%), its N-oxide (23%) and M2 (16.7%) with the remainder was associated with polar compounds.

Table 35. Excretion in Sprague Dawley rats following single dosing with B9202-045.

Parameter	**Dose group (mg/kg)**	
	[^{14}C]-radioactivity	
	1, iv	5, po
Urine (% of dose)	87.01	86.63
Feces (% of dose)	1.79	2.03
Recovery (% of dose)	88.8	88.64

Summary of Pharmacokinetics and Toxicokinetics: Single dose PK studies were performed in rats, dogs, mice, hamsters and rabbits and a summary of systemic exposure in each species is presented in Table 36. Systemic exposure to B9302-107 increased proportionally with increasing dose in rats and sub-proportionally in dogs and mice. Elimination half-lives ranged from 1 to 7 hours in rats and dogs. Rats were previously shown to produce a 5-fold increase in levels of B9202-045 compared to levels of the parent compound and 25-fold increase in B9502-044. Hamsters were similar in terms of metabolite production although the ratios were 7 and 137, respectively. Mice and dogs produced very low levels of B9202-045; levels of B9502-044 were similar to parent in the mouse and only 2% in the dog. Humans also produced relatively low levels of B9202-045 (12% of parent) and B9502-044 (73%). Clearance in rats was 4 l/hr and volume of distribution was 5 l following IV administration and similar values were observed in dogs (1.4 l/hr and 3 l, respectively) and females rabbits (1.3 l/hr and 2.4 l, respectively).

Table 36. Pharmacokinetics of B9302-107 and metabolites after single dose administration.

Species	Dose (mg/kg)	Admin route	Parameter	B9302-107	B9202-045	B9502-044	B9502-054
Rat	0.5 1.5 2.5 10/1 20	PO Po/id PO	AUC (0-8)(μg.h/ml) AUC (μg.h/ml) AUC (0-24)(μg.h/ml)	7.4 31.52 46.27 0.31 0.643			
Dog	0.75 2 2 2 6 18	PO tablet PO, suspension PO, suspension in capsule	AUC (0-∞) (μg/l h) AUC ratio AUC (0-∞) (μg/l h) AUC (0-∞) (μg/l h) AUC (0-24) (μg/l hr) AUC (0-24) (μg/l hr) AUC (0-24) (μg/l hr) AUC ratio	326.70 1851.9 1054 991.9 2304.6 3547.6	31.44 0.10 127.86 0.04	6.42 0.02	
Hamster	1.5 300	 PO	AUC (0-8) (μg/l.h) AUC ratio AUC (0-24)(μg/l.hr) AUC ratio	2.09 9180	15.34 7.34 5120 0.56	286.59 137.12 57940 6.3	39.94* 9970 1.09
Mouse	0.5 1.5 9 900	PO	AUC (0-8) (μg/l.h) AUC (0-8) (μg/l.h) AUC (0-8) (μg/l.h) AUC ratio AUC (0-24)(μg.h/l) AUC ratio	25.02 70.36 279.95 96670	 19.73 0.07	 308.58 1.1 104770 1.08	 240.08 0.86
Rabbits	5	PO	AUC (0-inf)(μg/l.hr)	12820			

Systemic exposure following multiple dose administration in rats and dogs are summarized in Table 37. Exposure continued to increase proportionally with dose in rats and a significantly greater exposure to the metabolite B202-045 was observed, consistent with observations following single dose administration. At an oral dose of 2.5 mg/kg, systemic exposure to B9302-107 was ~70% greater after 184 days compared to Day 1, indicating a potential for drug accumulation. In dogs, systemic exposure increased sub-proportionally with increasing dose and parameters were similar to those following single dose administration. Elimination half-life ranged from 3-4 hours and time to maximum concentration was 1-3 hours in rats; values in dogs were 2-13 hours and 1.5-4 hours with oral dosing.

Table 37. Pharmacokinetics of B9302-107 and metabolites after multiple administration.

Species	Dose (mg/kg)	Duration (days)	Admin route	Parameter	B9302-107	B9202-045
Rat	8	7	PO	AUC (0-8)(μg/hr.l) AUC ratio	246-973	13636 13-55
	0.5 1.5 2.5	184	PO	AUC (μg h/ml) AUC (0-24)(μg h/ml)	9.74 41.28 78.73	
Dog	2 6 18	25	PO, suspension in capsule	AUC (0-∞) (μg/l.h) AUC ratio	827.6 3061 3460-4183	 151.94 0.04
	0.2 1 4	178	PO, tablet	AUC (0-∞) (μg/l.h)	204 589 1982	

Drug absorption was 32% in rats following oral/id dosing while absolute bioavailability was low in rats and female rabbits (2-4%) but greater in dogs, hamsters and mice (34-61%). The greatest levels of drug-related radioactivity were observed in the nose, kidney and liver of the rat. Administration of metyrapone, a P450 inhibitor, was demonstrated to be involved in the activation of B9502-054, the major metabolite in the rat nose which is believed responsible for B9302-107-related nasal lesions. The lungs, liver, adrenals and bone marrow were the primary sites of drug distribution in mice and hamsters while only low to undetected levels were observed in the nose. The dominant biotransformation route in all species was N-oxidation of the parent drug (B9502-044), cleavage of the parent to yield B9202-045 which in turn was oxidized to B9502-054, or O-dealkylation of the parent compound. Other unidentified metabolites included M3, M4, M5, M6, M7, M8 and M11. In vitro studies with hepatic and respiratory microsomes demonstrated that B9302-107 was converted to B9202-045 or B9502-054 only by rat liver microsomes in this assay. Incubation with B9202-045 resulted in formation of B9502-054 by rat, hamster, mouse and dog olfactory and respiratory microsomes while none was produced with human microsomes and only very little with monkey microsomes. Excretion of B9302-107 related radioactivity was primarily fecal in rats, dogs, mice and hamsters following single or repeated oral administration (60-88%). Urinary excretion increased following intravenous administration and was the predominant route in rats and hamsters (50-69%; 30-33% in dogs and mice).

TOXICOLOGY

ACUTE TOXICITY:

Single dose toxicity after oral administration of B9302-107 to mice
Study No.: OM0346 *Report No.*: 92/95 *Volume*: 1.11

Study Dates: Starting date 4/6/1995; report issued 7/26/1995
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM46/251; purity = 98.61 to 98.82%) suspended in 4% methocel
Concentration: 60-240 mg/ml
Dose Volume: 10 ml/kg
GLP: Yes.
QA report: Yes.

Methods: NMRI mice (6 weeks old; 16 to 28 g) were assigned to the following treatment groups:

Dose (mg B9302-107/kg):	0	600	1100	1600	2000	2400
No./sex	2	5	5	5	5	5

Doses were based upon a dose-range finding study in which deaths occurred at doses ranging from 1200 to 2000 mg/kg (1 of 4 at the high dose). In the current study, each mouse received a single oral (gavage) dose of test drug or vehicle control. Animals were then observed for 14 days after which all animals were autopsied. The following observations were made:

Clinical observation . . . up to 3x daily
Body weight . . . . . daily
Food consumption . . . . daily
Water consumption . . . daily
Gross pathology . . . . . at sacrifice; organs examined include gastro-intestinal mucosa, thyroid and adrenals
Histopathology . . . . . at sacrifice; organs/tissues examined include abnormalities, heart, lungs, liver, kidneys, spleen, and intestine, stomach of animals killed terminally
Toxicokinetics . . . . . not assessed

Results:

Mortality: Two of 10 animals in the 1100 mg/kg group and 6-7 animals in each of the higher three dose groups died primarily within 24-48 hours of dosing while one was noted on day 4 (Table 38). The approximated LD_{50} value was between 1100 and 1600 mg/kg.

Clinical observations: Animals in all treatment groups exhibited piloerection, hunched position, ptosis, and reduced activity within 30-60 minutes of dosing (Table 38). A slight dose-

dependence in incidence and duration of action was noted. Animals dying spontaneously also exhibited limpness or ataxia.

Table 38. Observations following a single oral dose of B9302-107.

Dose (mg/kg)	0		600		1100		1600		2000		2400	
	M	F	M	F	M	F	M	F	M	F	M	F
Mortality	0	0	0	0	1	1	4	3	3	3	3	4
Clin obs. (Day 1)												
Hunched position	0	0	2	3	3	2	1	1	2	2	0	0
Piloerection	0	0	5	5	5	5	5	3	3	3	3	2
Ptosis	0	0	5	5	5	5	5	3	2	3	3	2
Red activity	0	0	5	5	5	5	5	4	4	5	5	5
Dead in cage	0	0	0	0	0	0	1	0	0	0	0	0

Body weight: Animals which died lost ~ 1-3 grams. Surviving animals in all treatment groups exhibited stagnated body weight gain for ~ 1 week followed by a recovery.

Food consumption: No noticeable food consumption was noted in animals which eventually died spontaneously. Food consumption in surviving animals was non-existent (day 1) or reduced (first 3 days) followed by a normalization to control values.

Water consumption: Markedly reduced water consumption was noted in animals dying spontaneously. Slight to moderate reduction was noted in surviving animals in the first week followed by normalization to control values.

Gross observations: Mucosal ulcers and bleedings in the glandular stomach were noted in most animals dying spontaneously in addition to bloody contents in the intestine and thickening of the jejunum and ileum. In most surviving animals, the inner surface of the forestomach was partly thickened and a spotty white discoloration was noted. Surviving animals in the high dose group (n=2) exhibited red liver spots, and ulcer, petechial hemorhage and thickening of the glandular stomach. At the dose of 2000, (n=2) ulcer and multiple thickening. 1600 (n =3; multiple ulcers forestomach.

Histopathology: In most surviving animals, findings included hyperkeratosis, epithelial hyperplasia, mixed cell infiltration and bacterial overgrowth of the stomach. Some animals exhibited intravasal or extravasal deposits of foamy material and panarteritis, although these findings were not dose-related.

The minimum lethal dose of B9302-107 was 1100 mg/kg. The maximum non-lethal dose was 600 mg/kg. Of the organs assessed, toxicity was noted in the stomachs of animals from all dose groups.

Single dose toxicity after intravenous administration of B9302-107 to mice
Study No.: OM0418 *Report No.*: 115/96 *Volume*: 1.11

Study Dates: Starting date 5/20/1996; report issued 11/11/1996
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch BY217-11-1-1; purity = 96.62%) emulsion
Concentration: 0.8 mg/ml
Dose Volume: 15-25 ml/kg
GLP: Yes.
QA report: Yes.

Methods: NMRI mice (6 weeks old; 23 to 33 g) were assigned to the following treatment groups:

Dose (mg B9302-107/kg):	0	12	16	20
No./sex	2	5	5	5

Each mouse received an intravenous injection of test drug or vehicle. Animals were then observed for 14 days after which all surviving animals were autopsied. The following observations were made:

Clinical observation . . . up to 3x daily
Body weight . . . . . daily
Food consumption . . . . daily
Water consumption . . . daily
Gross pathology . . . . . at sacrifice; organs examined include gastro-intestinal mucosa, thyroid and adrenals
Histopathology . . . . . at sacrifice; organs/tissues examined include abnormalities, heart, lungs, liver, kidneys, spleen, brain, nasal/paranasal cavities, testes, epididymides, ovaries, uterus, intestine, and injection site of the control and high dose groups only

Results:

Mortality: None observed.

Clinical observations: Increased respiration rate, reduced activity, piloerection, ptosis were noted on day 1 only up to 8 hours after administration. In single animals, abdominal or hunched position and limpness were observed (Table 39). Animals were considered normal on day 2.

Table 39. Clinical observations following single dose administration.

Dose (mg/kg)	0		12		16		20	
	M	F	M	F	M	F	M	F
Clinical observations								
Increased respiration rate	0	0	0	1	3	2	4	2
Limpness	0	0	0	0	1	1	1	2
Piloerection	0	0	2	0	1	0	1	1
Ptosis	0	0	5	5	5	5	4	5
Reduced activity	1	0	5	5	5	4	5	5

Body weight: Stagnation or slight loss in body weight was noted in all groups on Days 2 or 3. Afterwards, all groups gained weight comparably.

Food consumption: Drug-related effects on food consumption were not observed.

Water consumption: Drug-related effects on water consumption were not observed.

Gross observations: No treatment-related findings were noted.

Histopathology: No definitive treatment-related findings were noted although one of five high dose females exhibited tubular cell regeneration of the kidney.

Thus, the study results show that B9302-107 was not lethal at intravenous doses up to 20 mg/kg.

Interim report on influence of B9302-107 on hematological parameters after single oral dosing to mice

Study No.: WM0627 *Report No.*: 118/99 *Volume*: 5.2

Study Dates: Starting date 4/19/99; report issued 5/12/99
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM50/126)
Concentration: Not reported.
Dose Volume: 10 ml/kg
GLP: Sponsor states report was audited but report was not signed for GLP.
QA report: No.

The goal of this study was to prove the influence of B9302-107 on hematological parameters, especially on red blood cell mass, after single, high oral doses. It is assumed by this reviewer, though not stated by the sponsor, that this study was performed to support the sponsor's claim that positive findings in an in vitro mouse chromosome aberration study is due to an erythropoietic effect of B9302-107.

Methods: Male NMRI mice (6 weeks old) were assigned to the following treatment groups:

Dose (mg B9302-107/kg):	0	100	300	900
No.	24	24	24	24

Each mouse received a single dose of test drug by oral administration. Blood samples were taken from half of the animals at 24 hours after dosing and on day 8, and from the other half at 48 hours after dosing and on day 9. A control group was also included in this study although it was not stated which testing condition the control group represented. Similarly, it was not stated what substance was used to dissolve the test compound. Hematological parameters were evaluated following collection of blood samples.

Results: At 24 and 48 hours following dosing, a dose-related increase in erythrocyte counts (3-17%) was observed (Table 40). Hemoglobin (24 hours only) and hematocrit (2-15%) were also increased in mid- and high-dose animals. Leukocyte counts were decreased in all treatment groups, though the effect was not dose-related. Differential blood counts revealed increased neutrophil (up to 533%) and decreased lymphocyte numbers (up to 73%) in a dose-related manner. By days 8 and 9, erythrocyte count, hematocrit and hemoglobin were reduced in B9302-107 treated groups. MCV index was slightly decreased in high-dose animals while MCHC index was slightly increased. Differential blood counts continued to indicate increased neutrophil and decreased lymphocyte numbers.

In conclusion, very slight increases in erythrocyte counts and hematocrit and hemoglobin levels were noted following single, oral dose administration in mice. Greater changes were noted in terms of neutrophil (increased) and leukocyte (decreased) counts. It should be noted that a 3-month mouse study (216/98) at doses up to 18 mg/kg gave no indication of an erythropoietic effect.

Table 40. Hematological changes in mice following a single oral dose administration.

Dose (mg B9302-107/kg)	100	300	900
Erythrocytes			
% Δ from control – day 2	3	5	8
% Δ from control – day 3	5	8	17
% Δ from control – day 8	-7	-14	-6
% Δ from control – day 9	-1	-11	-10
Hematocrit			
% Δ from control – day 2	2	7	7
% Δ from control – day 3	-2	2	7
% Δ from control – day 8	-2	-12	-10
% Δ from control – day 9	-2	-9	-14
Hemoglobin			
% Δ from control – day 2	3	5	7
% Δ from control – day 3	2	10	15
% Δ from control – day 8	-4	-13	-6
% Δ from control – day 9	-2	-10	-10
MCV			
% Δ from control – day 2	--	--	--
% Δ from control – day 3	-4	-2	-2
% Δ from control – day 8	4	2	-6
% Δ from control – day 9	--	4	-4
MCHC			
% Δ from control – day 2	-1	--	-1
% Δ from control – day 3	--	3	1
% Δ from control – day 8	-1	-1	7
% Δ from control – day 9	-1	-2	4
Segmented neutrophils			
% Δ from control – day 2	42	292	533
% Δ from control – day 3	-3	76	86
% Δ from control – day 8	--	267	478
% Δ from control – day 9	-23	56	228
Lymphocytes			
% Δ from control – day 2	-7	-41	-73
% Δ from control – day 3	--	-30	-36
% Δ from control – day 8	-1	-39	-48
% Δ from control – day 9	2	-6	-27

Single dose toxicity after oral administration of B9302-107 to Wistar rats

Study No.: OR0347 *Report No.*: 91/95 *Volume*: 1.11

Study Dates: Starting date 4/6/1995; report issued 7/28/1995
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM46/251; purity = 98.61 to 98.82%) suspended in 4% methocel
Concentration: 10-70 mg/ml
Dose Volume: 10 ml/kg
GLP: Yes.
QA report: Yes.

Methods: Wistar rats (6 weeks old; 117-187 g) were assigned to the following treatment groups:

Dose (mg B9302-107/kg):	0	100	400	700
No./sex	2	5	5	5

Dose selection in this study was based upon a dose-range finding study in which deaths occurred at doses ranging from 500 to 1500 mg/kg. In the current study, each rat received a single oral (gavage) dose of test drug or vehicle. Animals were then observed for 14 days after which all animals were autopsied. The following observations were made:

Clinical observation . . . up to 3x daily
Body weight . . . . . daily
Food consumption . . . . daily
Water consumption . . . daily
Gross pathology . . . . . at sacrifice; organs examined include gastro-intestinal mucosa, thyroid and adrenals
Histopathology . . . . . at sacrifice; organs/tissues examined include abnormalities, heart, lungs, liver, kidneys, spleen, and intestine, stomach of animals killed terminally

Results:

Mortality: Three of five high-dose males and 2 of five mid- and high-dose females died between days 3 and 7 (Table 41).

Clinical observations: Animals in all drug treatment groups exhibited piloerection, hunched position, ptosis, increased respiration rate and reduced activity within 30 minutes of dosing (Table 44). A slight dose-dependence in incidence and duration of action (up to one week) was noted. Animals dying spontaneously also exhibited limpness, hyperthermia, gasping, or general tremor.

Table 41. Clinical observations following single dose administration.

Dose (mg/kg)	0		100		400		700	
	M	F	M	F	M	F	M	F
Mortality	0	0	0	0	0	2	3	2
Clin obs. (Day 1)								
Choking, gasping	0	0	1	2	3	2	1	1
Hunched position	0	0	5	5	5	5	5	5
Increased respiration	0	0	3	2	5	5	5	4
Piloerection	0	0	5	4	5	5	5	5
Ptosis	0	0	5	3	5	5	5	5
Reduced activity	0	0	5	5	5	5	5	5
Tremor	0	1	0	0	0	0	2	1

Body weight: Animals which died lost ~ 20-40 grams. Surviving animals in all treatment groups exhibited stagnated body weight gain for ~ 1 week followed by a recovery. However, body weight gain over the course of the study was reduced in males (40, 78, and 38% in the low, mid

and high-dose groups, respectively) and females (38, 25, and 62% in the low, mid and high-dose groups, respectively).

Food consumption: No noticeable food consumption was noted in animals which eventually died spontaneously. Food consumption in surviving animals was non-existent (day 1) or reduced (up to 75% in first 3 days) followed by a normalization to control values.

Water consumption: Markedly reduced water consumption was noted in animals dying spontaneously. Slight to moderate reduction (up to 55%) was noted in surviving animals in the first week followed by normalization to control values.

Gross observations: Mucosal red spots and hemorrhages in the glandular stomach were noted in most animals dying spontaneously and dark red or black contents were noted in the intestine (Table 42). In most surviving animals treated with B9302-107, the mucosal surface of the glandular stomach was partly covered with white layers. Also testes were reduced and appeared soft in two low- and mid-dose males.

Table 42. Gross findings following single dose administration.

Dose (mg/kg)	0		100		400		700	
	M	F	M	F	M	F	M	F
# of animals examined	2	2	5	5	5	5	5	5
Stomach								
White mucosal layer	1	0	3	1	2	2	4	1
Testes								
Reduced	0		1		2		0	
Large white soft area	0		2		2		0	
Spleen								
Pale grey layers	0	0	0	1	1	0	0	0
White layers	0	0	0	2	0	0	0	0
Lung								
Pale	0	0	0	0	0	1	0	0
Pale red/red	0	0	0	0	0	2	2	1

Histopathology: A thickening of the vessel wall of the mesenterium and intestinal serosa with intimal blebs and vascular inflammation were observed in single cases (Table 43). Two low- and mid-dose males exhibited atrophy or spermiogenic disturbance with calcification or necrosis of the testes. It should be noted that the nasal epithelium was not examined in this study.

Table 43. Histological findings in rats following single oral dose of B9302-107.

Dose (mg/kg)	0		100		400		700	
	M	F	M	F	M	F	M	F
# of animals examined	2	2	5	5	5	3	2	3
Stomach								
Hyperkeratosis	0	0	1	0	0	0	0	0
Mesenterial mixed cell infiltration	0	0	1	0	0	0	0	0
Chief cell atrophy	0	0	2	0	2	0	1	0
Vascular dilatation	0	0	2	1	1	0	0	0
Vessel wall thickening	0	0	0	1	2	0	1	0
Intimal blebs	0	0	0	1	1	0	1	0
Serositis	0	0	0	0	1	0	0	0
Testes								
Atrophy	0		2		1		0	
Calcification	0		1		1		0	
Necrosis	0		1		1		0	
Perivasc. infiltration	0		0		1		0	
Spermiogenic disturb.	0		0		1		0	
Jejunum								
Paneth cell hyperplas	0	0	2	2	1	0	0	0
Gland. Ectasia	0	0	1	0	0	0	0	1
Actv Peyer's patches	0	0	2	2	1	0	1	0
Vasc intimal blebs	0	0	3	1	1	1	1	1
Mes/serosal inflamm	0	0	1	0	0	0	0	0
Periarteritis	0	0	1	0	0	0	0	0
Serositis	0	0	1	1	1	0	0	0
Vasculitis	0	0	0	1	0	0	0	0
Mes granul inflamm	0	0	0	1	1	0	0	0
Vessel wall thickening	0	0	0	1	2	0	1	0
Lymphocytic stasis	0	0	0	0	1	0	0	0
Villous shortening	0	0	0	0	0	1	0	0

B9302-107 was lethal at doses of 400 mg/kg in females and 700 mg/kg in males. Non-lethal doses of 400 mg/kg and 100 mg/kg were noted in males and females, respectively. Of the organs assessed histopathologically, findings were noted in the testes, stomach and jejunum although they were not necessarily dose-related.

Single dose toxicity after intravenous administration of B9302-107 to Wistar rats
Study No.: OR0417 *Report No.*: 114/96 *Volume*: 1.12

Study Dates: Starting date 5/20/1996; report issued 10/18/1996
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch BY217-11-1-1; purity = 96.62%) emulsion
Concentration: 0.8 mg/ml
Dose Volume: 15-25 ml/kg
GLP: Yes.
QA report: Yes.

Methods: Wistar (6 weeks old; 131 to 200 g) were assigned to the following treatment groups:

Dose (mg B9302-107/kg):	0	12	16	20
No./sex	2	5	5	5

Each rat received an intravenous injection of test drug or vehicle. Animals were then observed for 14 days after which all surviving animals were autopsied. The following observations were made:

Clinical observation . . . up to 3x daily
Body weight . . . . . daily
Food consumption . . . . daily
Water consumption . . . daily
Gross pathology . . . . . at sacrifice; organs examined include gastro-intestinal mucosa, thyroid and adrenals
Histopathology . . . . . at sacrifice; organs/tissues examined include abnormalities, heart, lungs, liver, kidneys, spleen, brain, nasal/paranasal cavities, testes, epididymides, ovaries, uterus, injection site and intestine
Toxicokinetics . . . . . not assessed

Results:

Mortality: An increased incidence of mortality compared to control animals was noted in the mid- and high-dose groups (Table 44). Most deaths occurred immediately or within 2-5 minutes of injection. The latest death occurred 30 minutes after injection.

Clinical observations: On day 1, primary findings included increased respiration rate, dyspnea, ptosis, abdominal or hunched position, reduced activity, and limpness (Table 44). No differences between treatment groups were noted and findings were observed for up to 5 hours after dosing. Piloerection and/or reduced activity were noted in animals from the low- and high-dose groups up to day 3.

Table 44. Observations in rats following single iv dose of B9302-107.

Dose (mg/kg)	0		12		16		20	
	M	F	M	F	M	F	M	F
Mortality	1	2	2	1	4	3	5	3
Clin. observations								
Dyspnea	1	0	3	3	3	2	0	0
Lying flat	1	0	4	4	3	3	2	3
Choking	1	0	2	1	2	3	3	2
Hunched position	1	0	3	4	1	2	0	0
Increased respiration	0	0	4	4	1	2	0	3
Limp	1	0	4	2	2	1	2	3
Piloerection	1	0	3	4	1	2	0	1
Ptosis	0	0	3	4	1	2	0	2
Reduced activity	1	0	5	5	4	4	2	4

Body weight: In surviving animals, body weight stagnated (controls) or decreased (B9302-107 treated groups) during the first 3 days after administration. From then on, animals gained weight comparably but were below historical values.

Food consumption: Food consumption was reduced in surviving animals treated with B9302-107 up to day 3. Afterwards, consumption ranged from slightly reduced to normal.

Water consumption: Moderately reduced water consumption was noted in surviving drug-treated animals on days 1-2. Afterwards, consumption was normal. Slight to moderate reduction was noted in surviving animals in the first week followed by normalization to control values.

Gross observations: No dose-dependent findings were noted although findings included emphysema in the lung (1 of 4 and 2 of 5 mid and high-dose males) or lobular markings and dark discoloration of the livers.

Histopathology: A slight disorganization and necrotic inflammation of the olfactory epithelium with slight hyperplasia of the basal cell layer was noted in the two high dose females which survived to study termination.

Lethality related to the administration of B9302-107 was noted at a dose of 16 mg/kg or greater. Of the organs assessed histologically, toxicity was noted in the olfactory epithelium at the high dose.

Interim Histology Report to Byk Gulden Study WR0536: Modulation of the toxicity of B9302-107 and B9202-045 by phorone (GSH-depletion) and metyrapone (P450-inhibition)

Study No.: WR0536 *Report No.*: Not provided *Volume:* 5.2

Male Wistar rats were administered a single dose of 5 mg/kg B9302-107 or 0.5 mg/kg B9202-045 (assumed to be oral) with or without ip pretreatment with phorone (250 mg/kg). Animals pretreated with metyrapone have not been evaluated to date. Nasal/paranasal cavities, testes, epididydimes, prostate and seminal vesicles were evaluated histologically. The sponsor states that pretreatment with phorone resulted in a depletion of GSH-enzymes in the olfactory epithelium, although no data to this effect was provided. A pronounced increase in morphologic alterations of the olfactory epithelium was observed following pretreatment with phorone compared to animals that were not pretreated (Table 45). This type of effcet was not noted in the testes/epididymides or in prostate/seminal vesicles. The data suggest that depletion of glutathione S transferase may play a role in B9302-107- or B9202-045-induced nasal toxicity.

Table 45: Histological findings in male Wistar rats following single dose administration.

Group (n=3/group)	Dose mg/kg	Nasal/paranasal	Testes, epididymides	Prostate/seminal vesicles
Control	0	NAD	Slight epididymal histio-cytic infiltration in 1 of 3	NAD
B9302-107 without phorone	5	NAD	Slight epididymal histio-cytic infiltration in 1 of 3; Testes: moderate tubular degeneration and slight dysspermia in 1 of 3	NAD
B9302-107 with phorone	5	Moderate to severe necrosis of the olfactory epithelium; 3 of 3	Testes: moderate tubular degeneration , giant cells, dys- /oligospermia in 1 of 3	NAD
B9202-045 without phorone	0.5	NAD	Slight epididymal histio-cytic infiltration in 1 of 3	NAD
B9202-045 with phorone	0.5	Severe necrosis of the olfactory epithelium covered by reparative processes in 3 of 3	Severe eosinophilic infiltration of the peritoneal serosa in 1 of 3	NAD

Effects of the B9302-107 on the nasal cavity in hamsters, mice and rats

Study No.: NA *Report No.*: 34E/99 *Volume:* 5.3

In order to obtain further information on the localized toxicity in the nasal cavity of rats, hamsters and mice after treatment with B9302-107, male Wistar rats, Syrian Golden hamsters and B6C3F1 mice were orally administered (10 ml/kg) a single dose of amino-dichloropyridine moiety labeled [^{14}C]-B9302-107 (1.5 or 8 mg/kg) or phenyl moiety labeled [^{3}H]-B9302-107 (0.5, 1.5 or 8 mg/kg) in 4% methocel. Animals were sacrificed at 8 and 48 hours after dosing and skulls with nasal cavities were processed for light microscopic autoradiography as summarized in Table 46.

Table 46. Schedule for animal sacrifice.

	[^{14}C]-B9302-107		[^{3}H]-B9302-107		
Dose (mg/kg):	1.5	8	0.5	1.5	8
No. rats – 8 hours	3	3	3	3	3
No. rats – 48 hours	3	3	3	3	3
No. hamsters – 8 hours	3	3	3	3	3
No. hamsters – 48 hours	3	3	3	3	3
No. mice – 8 hours	0	3	0	0	3
No. mice – 48 hours	0	3	0	0	3

Results: None of the animals administered [^{3}H]-B9302-107 demonstrated bound radioactivity in their nasal cavities, indicating that the parent compound B9302-107 is not responsible for the reported nasal toxicity. Labeling of the amino-dichloropyridine moiety of B9302-107 was necessary to achieve binding. A metabolite activation appears to be a pre-requisite for binding since the parent compound does not bind to the nasal mucosa. Among animals treated with [^{14}C]-B9302-107, no labeling was detected in mice, hamsters showed barely detectable labeling and rats of all dose groups showed heavy labeling.

Quantitative data in rats and hamsters are summarized in Table 47. Bound radioactivity in rats treated with the [^{14}C]-B9302-107 was maximally concentrated in cells of the olfactory submucous glands located in endoturbinals at the caudal part of the olfactory turbinates and immediately adjacent to the cribiform plate. A gradual decrease in bound radioactivity in submucous glands towards the apical portion of the olfactory region was noted. Densitometry of bound radioactivity in submucous glands of rats was comparable between the two doses but tended to increase from 8 hours to 48 hours after dosing. Evaluation of the olfactory epithelium revealed increased radioactivity binding in the high dose group than in the low and increased binding at 48 hours than at 8 hours. In addition, binding was lower in the olfactory epithelium than in the submucous glands. Evaluation of binding by cell type was not possible due to the high levels of radioactivity.

Table 47: Densitometric values and number of developed silver grains per cell in tissue of animals given [^{14}C]-B9302-107.

Dose (mg/kg)	Time of sacrifice (hr)	Submucous glands	Olfactory epithelium	Sustentacular cells
Rat (Densitometric values)				
1.5	8 48	83,136 88,739	46,697 72,783	
8	8 48	79,152 88,799	60,034 76,214	
Hamster (Developed silver grains/cell)				
1.5	8 48	4.92 8.37		1.33 3.69
8	8 48	7.52 8.91		3.73 4.92

Since bound radioactivity levels in hamsters was too low to be measured (indicating that levels in hamsters were at least ten times less than those in rats), quantitation of autoradiograms was performed manually by counting developed silver grains per cell. Only cells of the submucousal glands of the olfactory region and sustentacular cells of the olfactory epithelium were labeled with counts increasing with increasing dose and time.

MULTIPLE-DOSE TOXICITY:

Rat, 14-day Intravenous Toxicity

Report No.: 113/96 *Protocol No.*: ER0416 *Volume*: 1.19

Study Dates: Starting date 5/20/96; report issued 12/9/96
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch# BY217-11-1-1; purity = 96.6%)
Concentration: 0.8 mg B9302-107/ml
Dose Volume: 5 ml/kg/day
GLP: Yes.
QA report: Yes.

Methods: Wistar rats (6 weeks old; 127-227 g) were assigned to the following treatment groups:

Dose (mg/kg/day)	0	0.5	1	2
No./sex toxicity study	10	10	10	10

Each rat received a daily dose of vehicle or test drug by intravenous injection in the lateral tail vein for 14 days. The following observations were made:

Clinical observation . . . 5 times daily
Body weight . . . . . daily
Food consumption . . . . daily
Water consumption . . . daily
Ophthalmoscopy . . . . . not assessed
Hematology . . . . . Day 14
Clinical chemistry . . . . Day 14
Urinalysis . . . . . Day 13/14
Enzyme induction . . . . not performed
Organ weights . . . . . at sacrifice; For specific organs weighed see Appendix (page 91-92).
Gross pathology . . . . . at sacrifice; microscopic evaluation included gastro-intestinal mucosa, thyroid, adrenals.
Histopathology . . . . . at sacrifice; examination of all organs for control and high-dose groups, animals dying early, and nasal and paranasal cavities, testes, and epididimydes from animals in the low and mid-dose groups. For specific organs examined see Appendix (page 91-92).
Toxicokinetics . . . . . not assessed

Results:

Mortality: No deaths were reported.

Clinical Observations: A dose-dependent, increased incidence of increased respiration, piloerection, reduced activity, hunched position, ptosis, hypersalivation and chromodacryorrhea was noted during the study period (Table 48).

Body Weight: Body weight gain was reduced in all treatment groups (12-41%) although statistical significance was achieved only in mid-dose males (Table 48).

Food Intake: Mean food consumption was reduced in all treatment groups (8-19%) from the second or third day of treatment (Table 48).

Water consumption: Reduced consumption in all male drug treatment groups (14-25%) although statistical significance was not achieved (Table 48).

Table 48. Clinical changes in rats following 14 day intravenous dosing with B9302-107.

Dose (mg/kg)	0		0.5		1		2	
	M	F	M	F	M	F	M	F
Clinical observations								
Chromodacryorrhea, nose	0	0	4	1	6	3	8	8
Hunched position	0	0	4	6	6	7	8	9
Increased respiration	0	0	2	3	8	7	7	10
Piloerection	1	1	4	4	4	5	5	5
Reduced activity	1	2	2	4	7	6	7	10
Ptosis	1	1	2	2	2	1	1	5
Hypersalivation	0	0	0	0	1	0	1	3
Body weight gain			Day 15	Day 8	Day 15	Day 8	Day 15	Day 8
% change from control			↓31	↓25	↓41	↓12	↓33	↓12
Food consumption								
Days 1-7			↓10	↓10	↓15	↓8	↓19	↓17
Days 8-14			↓13	↓13	↓16	↓8	↓15	↓11
Water consumption								
Days 1-7			↓14	↑7	↓22	↓5	↓21	↓2
Days 8-14			↓20	↓1	↓25	↓8	↓18	↑4

Hematology: No drug-related findings were noted.

Clinical Chemistry: A dose-dependent increase in ALAT was observed (7-85%; significant at all doses in females and high dose in males; Table 49). In addition, serum glucose levels were slightly increased (24%) in high dose males.

Urinalysis: Dose-dependent increases in urine osmolality (up to 40%), calcium (up to 377 and 916% in males and females, respectively) and chloride (up to 108 and 205% in males and females, respectively) were observed after 14 days of drug administration (Table 49). pH values were slightly decreased at all doses in males and at the mid and high doses in females.

Table 49. Clinical chemistry and urinalysis findings in rats dosed intravenously for 14 days.

	Males				Females			
Dose (mg /kg/d)	Control	0.5	1	2	Control	0.5	1	2
Clinical Chemistry								
Serum glucose								
%Δ vs control group		↑13	↑11	↑24		↑3	↑5	↑4
ALAT								
%Δ vs control group		↑7	↑20	↑60		↑15	↑38	↑85
Urinalysis								
pH	7	6.4	6.2	6.2	6.4	6.4	6.2	5.8
Osmolality								
%Δ vs control group		no Δ	↑24	↑25		↑20	↑25	↑40
Calcium								
%Δ vs control group		↑106	↑290	↑377		↑232	↑402	↑916
Chlorine								
%Δ vs control group		↑51	↑91	↑108		↑94	↑101	↑205

Shaded areas indicate a statistically significant difference from control group values.

Organ Weights: A slight decrease in absolute spleen (10-17%) and thymus weight (13-20%) was noted in B9302-107 treated animals.

Gross Pathology: Although the report summary reported an increased incidence of paleness and reduced size in the thyroid, no gross pathology data was submitted. No significant histological findings were observed in the thyroid.

Histopathology: The primary histological findings were noted in the olfactory epithelium and nasal cavity of all treatment groups (Table 50) and included olfactory single cell necrosis, degeneration of olfactory epithelium, disorganization of olfactory epithelium (rearrangement response), and hyperplasia of basal cell of olfactory epithelium (reparative response). Severity tended to be greater females. Findings in other organs were generally of low severity.

Table 50. Histopathological changes following 14-day B9302-107 IV administration in rats.

	Males				Females			
Dose (mg/kg/d)	0	0.5	1	2	0	0.5	1	2
Adrenals n=	**10**	**0**	**0**	**10**	**10**	**0**	**0**	**10**
-cortical hyperplasia	1(0.03)			2(0.15)	0			0
Liver	**10**	**0**	**0**	**10**	**10**	**0**	**0**	**10**
-lympho-histiocytic infilt	4(0.43)			7(0.65)	6(0.33)			5(0.38)
Liver, iron stain	**10**	**0**	**0**	**10**	**10**	**0**	**0**	**10**
-Kupfer cell storage	0			2(0.4)	0			0
Lung	**10**	**0**	**0**	**10**	**10**	**0**	**0**	**10**
-foreign body granuloma	0			3(0.28)	1(0.13)			2(0.15)
-interstit lymphocyt infil	5(0.63)			7(0.6)	2(0.18)			8(0.48)
Nasal cavities	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
-olfactory epith degen	0	2(0.25)	1(0.05)	6(1.35)	0	0	4(0.63)	10(2.5)
-disorg. of olfact epith	0	2(0.4)	2(0.2)	10(2.3)	0	3(0.55)	4(0.65)	10(3.0)
-olfact single cell necros	0	10(1.1)	10(1.1)	10(0.8)	0	10(1.3)	10(1.35)	10(1.2)
-olfact basal cell hyperpl	0	4(0.23)	6(0.33)	10(2.4)	0	8(0.7)	5(0.75)	10(2.8)

(severity): 1 – minimal; 2 – mild; 3 – moderate; 4- marked

A NOAEL could not be selected for this study due to adverse findings at the lowest dose in the nasal cavities, reduced body weight gain and clinical observations. The primary target organ of toxicity was identified as the nasal cavity.

Rat, 4-week Oral (gavage) Toxicity

Report No.: 81/95 *Study No.*: BR0335 *Volume*: 1.12

Study Dates: Starting date 1/26/95; report issued 8/7/95
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM46/251; purity = 98.61 - 98.82%) in 4% methocel E15
Concentration: 0.05 to 0.8 mg/ml
Dose Volume: 10 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

Methods: Wistar rats (6 weeks old; 127-211 g) were assigned to the following treatment groups:

Dose (mg/kg/day):	0	0.5	2	8
No./sex 4-week study	10	10	10	10
No./sex 4-week recovery	8	0	0	8

Each rat received a daily oral (gavage) dose of vehicle or test drug for 4-weeks. Autopsies were performed following both dosing periods and 4-weeks following the dosing period. The following observations were made:

Clinical observation . . . 4 times daily
Body weight . . . . . days –1, 1; 2x/week, day 30, 57/58
Food consumption . . . . day 1; 2x/week, day 30, 57/58
Water consumption . . . day 1; 2x/week, day 30, 57/58
Ophthalmoscopy . . . . . day –6, 28, 55
ECG . . . . . day 21, 49
Hematology . . . . . day -5; day 29, 56/57
Clinical chemistry . . . . day -5; day 29, 56/57
Urinalysis . . . . . day 28/29, 55/56/57
Enzyme induction . . . . not performed
Vaginal smears . . . . . Days 1-30, 57/58
Organ weights . . . . . at sacrifice; (for specific organs see Addendum, (page 91-92).
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice; all organs/tissues from vehicle control and high-dose B9302-107, rats dying prior to scheduled necropsy and all gross lesions (for specific tissues/organs see Addendum, page 91-92). In low- and mid-dose groups, intestine, stomach, testes, epididymides, spleen, thymus and prostate were examined.
Toxicokinetics . . . . . not assessed.

Results:

Mortality: Eight high dose males and eight high-dose females died or were killed in extremis mainly during weeks 3 and 4 (Table 51).

Clinical Observations: High-dose animals exhibited piloerection, chromodac, emaciation, hunched position, increased respiration rate, limpness, reduced activity, ptosis, swollen abdomen, penis prolaps, and reduced defecation after one week of treatment (Table 51). During recovery, most animals appeared normal within one week.

Body Weight: Reduced body weight gain was noted in high-dose animals (75-84%; Table 51). This effect was reversible in surviving recovery animals.

Food Intake: Food consumption was significantly reduced in high dose animals (25-40%; Table 51). Recovery was almost immediate once dosing regimen ended.

Table 51. Clinical observations following administration of B9302-107.

Dose (mg/kg)	0		0.5		2		8	
	M	F	M	F	M	F	M	F
Mortality	0	0	0	0	0	0	8/18	8/18
Clinical observations								
Chromodacryorrhea, nose	0	0	0	0	0	0	9	14
Diarrhea	0	0	0	0	0	0	4	6
Emaciation	0	0	0	0	0	0	14	13
Hunched position	1	2	1	2	2	4	18	18
Penis prolaps	0		0		0		4	
Increased respiration	0	0	0	0	0	0	4	6
Tonus reduced - limp	0	0	0	0	0	0	5	9
Pale skin, mucous membr	0	0	0	0	0	0	6	9
Piloerection	1	2	1	2	2	4	18	18
Reduced activity	0	0	0	2	2	3	18	18
Ptosis	0	0	0	2	2	3	17	18
Swollen abdomen, soft	0	0	0	0	0	0	12	14
Body weight gain % change from control			↓6	↑2	↓7	↑6	↓84	↓75
Food consumption Days 21-27			↑1	↑1	no Δ	↑3	↓40	↓25

Water consumption: No toxicologically significant treatment-related effects.

Ophthalmoscopy: The cornea of one or both eyes in 3 males of the high dose group were slightly opaque. It was unclear whether this effect was related to drug treatment or blood sampling.

Hematology: Increased leukocyte (61% in females) and thrombocyte counts (22% in males), increased PMN (450%), and decreased lymphocyte counts (40-45%) were observed in the high-dose groups (Table 52). These effects were reversible following the recovery period.

Clinical Chemistry: In the high dose groups, decreased levels of glucose (30%) and creatinin (10-15%), and increased ALAT (21-29%), creatinine kinase (18-57%) and serum urea (96-113%) were noted (Table 52). Recovery was noted following cessation of dosing except for glucose and creatinine kinase levels which were decreased 19% and increased 37%, respectively, in female recovery animals.

Urinalysis: Reversible increases in urine osmolality (52% in males), sodium (205-245%), chlorine (134-226%), calcium (up to 458%) and urine volume (32-56%) were observed in high dose animals (Table 52). Calcium levels were also increased in low and mid-dose females and mid-dose males. Hemoglobin levels were still increased following the recovery period.

Table 52. Clinical chemistry findings in rats following 4 week dosing with B9302-107.

	Males			Females		
Dose (mg /kg/d)	0.5	2	8	0.5	2	8
Hematology						
Seg. neutrophils						
%Δ vs control group	↓37	↓12	↑450	↓33	↓22	↑444
Lymphocytes						
%Δ vs control group	↑4	↑1	↓40	↑3	↑1	↓45
Leukocytes						
%Δ vs control group	↑1	↑6	↑5	↑13	↑19	↑61
Thrombocytes						
%Δ vs control group	↑3	↑5	↑22	↓3	↑1	↑10
Clinical Chemistry						
ALAT						
%Δ vs control group	↑14	↑14	↑21	no Δ	↑29	↑29
Glucose						
%Δ vs control group	↓1	↓8	↓30	↑3	↑6	↓31
Urea						
%Δ vs control group	↑10	↑7	↑113	↑7	↑5	↑96
Creatinin						
%Δ vs control group	↑6	↑2	↓15	↑6	no Δ	↓10
Creatin Kinase						
%Δ vs control group	↓23	↓23	↑18	↑6	↑19	↑57
Urinalysis						
Protein	- to ++	(+) to ++	- to ++	- to +++	- to +	- to (+)	- to ++	- to +++
Hemoglobin	-	-	-	- to +++	-	- to +	- to (+)	- to ++
Osmolality						
%Δ vs control group	↑9	↑13	↑52	↓5	↑13	↑7
Volume						
%Δ vs control group	↓9	↓5	↑32	↑5	↓7	↑56
Sodium						
%Δ vs control group	↓18	↑19	↑205	↓1	↑19	↑245
Calcium						
%Δ vs control group	↑22	↑130	↑426	↑83	↑131	↑458
Chlorine						
%Δ vs control group	↑1	↑37	↑134	↑12	↑28	↑226

Shaded areas indicate significant difference from control groups.

Vaginal Smears: A low number of estrus events and a prolongation of phase of diestrus during treatment was noted in high dose females; six of ten females demonstrated 0-2 estrus events while all other groups had at least 3 and as many as 7. This effect was reversible.

Electrocardiogram: Significant increases in QRS time (males and females: 19 msec versus 18 msec in control) and QαT-time (37 msec versus 25 msec in control; females: 39 msec versus 32 msec in control) was observed in high dose animals. Higher QT time values were also noted in the high-dose groups (males: 87 msec versus 77 msec in control; females: 85 msec versus 76 msec in control). These values were still increased in the one male recovery animal, while in females the effects were reversible, although the QT value was reduced in high dose females (67 msec versus 83 msec in control).

Organ Weights: Absolute organ weight decreases were noted in high dose males in the heart (23%), spleen (39%), seminal vesicles (57%), and prostate (47%) (Table 53). Thymus weight was decreased in both high-dose males and females (60-71%), while adrenal weight was slightly increased (26%) in high dose males. Decreases were not noted in relative (to body weight) organ weights and weights were increased in the liver, lungs, and brain (27-70%, 23-30%, and 21-35%, respectively).

Table 53. Absolute organ weight changes in rats administered B9302-107.

	Males			Females		
Dose (mg /kg/d)	0.5	2	8	0.5	2	8
Heart						
%Δ vs control group	↑2	↓1	↓23	↑3	↑3	↓11
Spleen						
%Δ vs control group	↓9	↓7	↓39	↓5	↓5	↓23
Adrenals						
%Δ vs control group	↑3	↑8	↑26	↑4	↑9	↑23
Thymus						
%Δ vs control group	↓4	↓10	↓60	↓5	↓7	↓71
Seminal vesicles						
%Δ vs control group	↑14	↑10	↓57			
Prostate						
%Δ vs control group	↑6	↑3	↓47			

Gross Pathology: According to the summary report, findings were primarily at the high dose. In animals dying early, emaciation, peritonitis, and enlargement of small intestine were the main findings. In surviving high-dose animals, hemorrhage and enlargement of the stomach and small intestine, peritonitis, atrophy of the thymus and accessory organs, and atrophy of testes were noted. However, no detailed data tables were submitted to confirm the sponsor's report.

Histopathology: Nasal cavities were not examined in this study. Dying high-dose animals demonstrated gastric erosions, mild degenerative testicular spermiogenic disturbance associated with epididymidal oligospermia and spermiogenic granuloma, splenic follicular atrophy, a slight increase in splenic hemosiderin content, and peritonitis (perisplenitis, ovarian peritonitis; Table 54). Liver and lung congestion were reported in all males which died early. Surviving high-dose

animals displayed gastric erosions (males), thymic atrophy, splenic folicular atrophy, an increase in splenic hemosiderin content (females), degenerative testicular spermiogenic disturbance associated with epididymidal oligospermia and spermiogenic granuloma, prostatic and seminal vesicle atrophy, peritonitis (perisplenitis, ovarian peritonitis). Males showed less replacement of cellular bone marrow fat, and marked hypermyelopoiesis in at least 2 animals. In mid-dose animals, serositis, submucosal inflammation, goblet cell hyperplasia, Paneth cell hyperplasia, vascular dilatation, villous necrosis were all noted in the jejunum and ileum. Serositis spread from the mesenterium to the intestinal serosa. Mid-dose males also displayed gastric erosions (4), and spermiogenic granuloma formation (1).

In recovery animals, oligospermia, peritonitis, (hepatic serositis, ovarian, perispenitis, purulent lymphadenitis), and jejunal inflammation associated with serositis were noted.

Table 54. Histopathological changes following 4-week oral B9302-107 administration in rats.

	Males				Females			
Dose (mg/kg/d)	0	0.5	2	8	0	0.5	2	8
Adrenals n=	**10**	**0**	**0**	**17**	**10**	**0**	**0**	**12**
-cortical fatty vacuoles	0			5	0			1
-hemmorhage	0			0	0			1
Epididymides	**10**	**10**	**10**	**17**				
-Oligospermia	0	1	0	8				
-Aspermia	0	0	0	1				
-Spermiogen granuloma	0	0	0	6				
Gastric fundus	**10**	**10**	**10**	**14**	**10**	**10**	**10**	**14**
-Erosion	0	0	4	9	0	0	0	0
Parathyroid	**8**	**0**	**0**	**16**	**9**	**0**	**0**	**2**
-Fibrosis	0			4	2			1
Spleen	**10**	**10**	**10**	**17**	**10**	**10**	**10**	**12**
-follicular atrophy	0	0	0	11	0	0	0	5
-perisplenitis	0	0	0	3	0	0	0	5
Testes	**10**	**10**	**10**	**17**				
-spermiogenic disturb	0	0	1	8				
Bone marrow, tibia	**10**	**0**	**0**	**9**	**10**	**0**	**0**	**4**
-fat replacement								
little/no	0			5	1			2
mild	5			3	5			2
moderate	5			1	4			0
-myelopoiesis								
usual	5			0	4			0
moderate	3			4	5			3
marked	2			3	1			1
very marked	0			2	0			0
Prostate	**10**	**8**	**10**	**9**				
-atrophy	0	0	0	3				
Seminal vesicle	**10**	**0**	**0**	**9**				
-atrophy	0			3				
Thymus	**10**	**10**	**10**	**7**	**10**	**10**	**10**	**3**
-atrophy	0	0	0	2	0	0	0	2
Thyroid, follicle	**10**	**0**	**0**	**9**	**10**	**0**	**0**	**4**
Edema	0			2	0			0
Urinary bladder	**10**	**0**	**0**	**7**	**10**	**0**	**0**	**4**
Edema	2			5	2			0
Pancreas	**10**	**0**	**0**	**9**	**10**	**0**	**0**	**4**
edema	0			1	0			2

Since the nasal cavities, a primary target organ of toxicity in the rat, were not assessed in this study, a NOAEL could not be determined. In addition, findings were noted in high dose males in seminal vesicles, parathyroids and adrenals but those organs were not assessed at the low- and mid-doses. Target organs of toxicity include the heart, adrenals, epididymides, stomach (males), parathyroid, testes, prostate, and seminal vesicles in males and the thymus and spleen in males and females. The estrus cycle of females also appears to be affected.

Rat, 4-week/3-month Oral (gavage) Toxicity with 8 Week Recovery

Report No.: 38/98 *Study No.*: BR0499 *Volume*: 1.14

Study Dates: Starting date 8/26/97; report issued 8/25/98
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM50/126; purity = 99.8%) in 4% methocel E15
Concentration: 0.02-2 mg/ml
Dose Volume: 1 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

Methods: Wistar rats (6 weeks old; males: 122-191 g, females: 97-149 g) were assigned to the following treatment groups:

Dose (mg/kg/day):	0	0.02	0.2	2
No./sex 4-week study	8	8	8	8
No./sex 3-month study	8	8	8	8
No./sex 8-week recovery after 3 month dosing	8	0	0	8

Each rat received a daily oral (gavage) dose of vehicle or test drug for 4-weeks or 3-months. Autopsies were performed following both dosing periods and 8-weeks following the 3-month dosing period. The following observations were made:

Clinical observation . . . up to 4 times daily
Body weight . . . . . days –1, 1; 2x/week, day 30, 87, 143
Food consumption . . . . day 1; 2x/week, day 30, 87, 143
Water consumption . . . day 1; 2x/week, day 30, 87, 143
Ophthalmoscopy . . . . . day –6, 28, 85, 141
EKG . . . . . not performed
Hematology . . . . . day -5; day 29, 86, 142
Clinical chemistry . . . . day -5; day 29, 86, 142
Urinalysis . . . . . Days 28/29, 85/86, 141/142
Enzyme induction . . . . not performed
Vaginal smears . . . . . Days 1-30, 90-117
Organ weights . . . . . at sacrifice; (for specific organs see Addendum, pages 91-92)
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice; examination of all organs in all animals treated for three months and recovery animals; in control and high-dose animals treated for 4 weeks only nasal/paranasal cavities, testes, epididymides, heart, lung, liver, adrenals, spleen, kidneys, thyroids, ovaries, oviducts, uterus and vagina; in low- and mid-dose animals treated for 4 weeks only nasal/paranasal cavities, testes, and epididymides (for specific tissues/organs see Addendum, page 91-92).
Toxicokinetics . . . . . not assessed.

Results:
Mortality: One female control rat was killed in moribund condition on Day 111.

Clinical Observations: A reversible polyuria was noted in 2 high dose females in the first week and 4 high-dose males at the end of the 3-month dosing period (Table 55).

Body Weight: No significant treatment-related effects.

Food Intake: No toxicologically significant treatment-related effects.

Water Consumption: A slight increase in water consumption (9-11% in males; 10-23% in females) was noted in high dose animals 2-3 weeks after start of dosing. Consumption was comparable following recovery period.

Ophthalmoscopy: No toxicologically significant treatment-related effects.

Hematology: A slight and reversible increase in leukocyte counts were noted after 4-week and 3-months of treatment at the high dose (Table 55).

Table 55. Clinical observations and hematology findings in rats.

	Males					Females				
Dose (mg /kg/d)	0	0.02	0.2	2	2-R	0	0.02	0.2	2	2-R
Clinical observations										
Polyuria	0	0	0	4	0	0	0	0	2	0
Hematology – 4 wks										
Leukocytes										
%Δ vs control group		↑10	↑6	↑12	na		↑10	↑4	↑18	na
Hematology – 3 mos										
Leukocytes										
%Δ vs control group		↑18	↑29	↑38	↓7		↑1	↑1	↑25	↑16

Clinical Chemistry: No toxicologically significant treatment-related effects were noted. There were some slight but significant changes in certain parameters (glucose reduced 11-15% in high dose females; reduced triglycerides 13-29% in high-dose animals; reduced serum cholinesterase 17-18% in high dose females). Triglyceride levels were still reduced by 25% in females after the recovery period.

Urinalysis: After 4 weeks and 3 months, an increase in calcium excretion (53-326% in males; 32-76% in females) and a decrease in urine osmolality (12-33%) were noted at the high dose. These findings were reversible.

Vaginal smears: A lower number of estrus events at the high dose was noted during weeks 1-4 as 14 of 24 high-dose females underwent estrus events less than or equal to 4 times, while in the other groups 17 of 24 controls, 13 of 16 low-dose and 10 of 16 mid-dose animals exhibited 5 or greater estrus events. No differences were observed during recovery period.

Organ Weights: Slight increases in absolute testes (24% after 3 months) and liver weights (females only; 19% after 4 weeks and 3 months) were noted at the high dose (Table 56). Organ weights were comparable to control values following the recovery period. No significant changes in relative organ weights were noted.

Gross Pathology: Gross findings were noted at the high dose and included the occurrence of head nodules of the epididymides, discoloration of glandular stomach (males and females), enlarged testes and discoloration of thyroids (males) and diminished thyroids in females (Table 56). The findings in the thyroids were not fully reversible.

Table 56. Organ weight and gross changes following 3-month B9302-107 dosing in rats.

	Males						Females					
Dose (mg/kg/d)	0	0.02	0.2	2	0-R	2-R	0	0.02	0.2	2	0-R	2-R
Absolute Organ Weight												
Liver												
%Δ vs control group		↓1	↑10	↓5		↓1		↑8	↑13	↑19		↑1
Testes												
%Δ vs control group		↑2	↑5	↑24		↑12						
Gross Pathology n =	**7**	**8**	**8**	**8**	**7**	**8**	**8**	**8**	**8**	**8**	**8**	**7**
Stomach, glandular												
-discoloration	0	0	0	1	0	0	1	1	0	2	0	0
Thyroids												
-discoloration	1	1	0	3	0	1	2	0	1	2	2	0
-smaller/diminished	1	0	0	0	2	0	0	2	4	4	0	2
Epididymides												
-head: nodule	0	0	0	2	0	0						
Testes												
-enlarged	0	0	0	1	0	0						

Histopathology: After both 4 and 12-weeks, a non-reversible disorganization of the olfactory epithelium including basal cell hyperplasia and a loss of PAS staining of Bowman's glands was noted in all high-dose animals (Table 57). Additionally, a focal atrophy of the germinative testicular epithelium (3 males), formation of giant cells (6 males), and epididymal oligo- and aspermia (4 males) and formation of spermatoceles (2 males was observed at the high dose after 3 months treatment. Severity of findings appears to be similar at 4 and 12 weeks and the findings were partially reversible. An increase in agonal bleeding of the thymus was also noted in high-dose males after 3 months. Lympho-histiocytic inflammation (mild) was observed in numerous tissues/organs at a slightly greater incidence/severity in high dose animals than in control animals.

Table 57. Histopathological changes through 3-months B9302-107 dosing in rats.

	Males				Females			
Dose (mg/kg/d)	0	0.02	0.2	2	0	0.02	0.2	2
4 weeks n=	**8**	**8**	**8**	**8**	**8**	**8**	**8**	**8**
Nasal cavities								
-hyperplasia	0	0	1(1)	8(1-3)	0	1(1)	0	8(2-3)
-epithelial disorganiz	6(1)	3(1-2)	7(1-2)	8(2-4)	4(1-2)	6(1)	5(1-2)	8(3)
-olfactory cell apoptosis	7(1-2)	6(1-2)	8(1-2)	8(1-3)	6(1-2)	8(1-2)	6(1-2)	8(1-2)
-Loss of PAS staining	0	1(1)	2(1)	8(1-3)	1(1)	2(1)	3(1)	8(2-3)
Testes								
-atrophy	0	0	1(4)	1(3)				
-giant cells, dyspermia	0	1(2)	1(4)	2(1-2)				
Epididymides								
-oligo/aspermia	0	0	1(4)	1(3)				
-inflammation	8(1)	7(1-2)	5(1-2)	3(1-2)				
-spermatocele	0	0	0	0				
Adrenals	**8**	**0**	**0**	**8**	**8**	**0**	**0**	**8**
-cortical fat vacuoles	0			1(0.13)	0			0
12 weeks	**8**	**8**	**8**	**8**	**7**	**8**	**8**	**8**
Nasal cavities								
-hyperplasia	0	1(1)	0	8(2-3)	1(1)	0	1(1)	8(2-4)
-epithelial disorganiz	4(1-2)	3(1-2)	4(1)	8(3)	3(1-2)	1(1)	3(1-2)	8(3-4)
-olfactory cell apoptosis	7(1-2)	7(1-2)	6(1-2)	7(1-2)	5(1-2)	4(1-2)	7(1-2)	5(1-2)
-Loss of PAS staining	0	2(1)	0	8(2-4)	2(1)	1(1)	3(1)	8(2-3)
Testes								
-atrophy	1(1)	0	1(3)	3(1-3)				
-giant cells, dyspermia	2(1)	1(1)	3(1)	6(1-2)				
Epididymides								
-oligo/aspermia	1(2)	0	2(1-2)	4(1-2)				
-inflammation	5(1-2)	3(1)	7(1-2)	7(1-4)				
-spermatocele	0	0	0	2(3-4)				
Thymus	**8**	**0**	**0**	**8**	**8**	**0**	**0**	**8**
-agonal bleeding	2(0.3)			6(0.7)	3(0.5)			3(0.2)
8-week Recovery	**8**			**8**	**7**			**8**
Nasal cavities								
-hyperplasia	0			8(1-3)	0			8(3-4)
-epithelial disorganiz	0			8(1-3)	0			8(3-4)
-olfactory cell apoptosis	4(1-2)			7(1-2)	6(1)			8(1)
-Loss of PAS staining	0			0	0			0
-PAS-positive glands	0			5(1-2)	0			8(2-4)
Testes								
-atrophy	2(2-4)			3(1-4)				
-giant cells, dyspermia	2(1)			2(1)				
Epididymides								
-oligo/aspermia	1(4)			1(4)				
-inflammation	8(1-2)			7(1-3)				
-spermatocele	0			1(4)				

* Incidence(severity). Severity based upon 0-4 scale in which 0, 1, 2, 3, 4 indicate none, minimal, mild, moderate or severe, respectively.

The mid-dose of 0.2 mg B9302-107/kg/day was selected as the NOAEL for this study. The target organs of toxicity included the nasal cavities, testes, epididymides and the thymus which

were identified (except for nasal cavities) in the 4-week study reviewed previously. However, some target organs (heart, spleen, adrenals, GI tract, prostate, parathyroid, seminal vesicles) which were identified following 4 week dosing with 8 mg/kg were not identified in the current study utilizing a high dose of only 2 mg/kg.

Dog, 14-day Intravenous Toxicity

Report No.: 159/96 *Protocol No.*: ED0425 *Volume*: 1.20

Study Dates: Starting date 7/19/96; report issued 1/10/97
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch# BY217-10-1-1; purity = 96.62%)
Concentration: 0.8 mg B9302-107/ml
Dose Volume: 1 ml/kg/day
GLP: Yes.
QA report: Yes.

Methods: Beagle dogs (12 months old; 11-13.2 kg) were assigned to the following treatment groups:

Dose (mg/kg/day)	0	0.01	0.03	0.06
No./sex toxicity study	2	2	2	2
No./sex recovery group	0	0	0	2

Each dog received a daily dose of vehicle or test drug by intravenous infusion once a day for 14 days. The following observations were made:

Clinical observation . . . 1 time daily
Body weight . . . . . Days –18, -4, 1, 4, 8, 11, 15, 25, 32, 39
Food consumption . . . . daily
Water consumption . . . not assessed
Physical exam . . . . . weeks –1, 2, and 6
Ophthalmoscopy . . . . . weeks –1, 2, and 7
ECG . . . . . weeks –1, 2, and 6
Hematology . . . . . weeks –2, -1; days 3 and 15, week 6
Clinical chemistry . . . . weeks –2, -1; days 3 and 15
Urinalysis . . . . . Weeks –1 and 6
Enzyme induction . . . . not performed
Organ weights . . . . . at sacrifice; for specific organs weighed see Appendix (page 91-92).
Gross pathology . . . . . at sacrifice; macroscopic evaluation included gastro-intestinal mucosa, thyroid, adrenals.
Histopathology . . . . . at sacrifice; examination of all organs for control and high-dose groups, animals dying early, and nasal and paranasal cavities, testes, and epididimydes from animals in the low and mid-dose groups. For specific organs examined see Appendix (pages 91-92).
Toxicokinetics . . . . . assessed on days 1 and 10; results reported in Study 145/97 and are reviewed within the context of the toxicity study.

Results: The small number of animals per dose group made it difficult to make any conclusive decisions regarding drug-related effects.

Mortality: No deaths were reported.

Clinical Observations: Increased incidence of tremor was observed all dose groups (Table 58).

Table 58: Clinical observations following drug administration.

Dose (mg/kg)	0	0.01	0.03	0.06	0.06-R
Clinical observations Tremor	1	2	3	2	1

Body Weight: No drug-related findings were observed.

Food Intake: No drug-related findings were observed.

Physical Exam: No drug-related findings were noted.

Ophthalmoscopy: No drug-related findings were noted by the sponsor although no data was found in the study report.

Electrocardiogram: No drug-related findings were noted.

Hematology: No drug-related findings were noted.

Clinical Chemistry: No drug-related findings were noted other than a reduction in LDH at the mid and high doses (Table 59).

Table 59. Clinical findings in dogs dosed for 14 days.

	Males			Females		
Dose (mg /kg/d)	0.01	0.03	0.06	0.01	0.03	0.06
LDH %Δ vs control group	↑96	-24	-77	-27	-47	-76

Urinalysis: No drug-related findings were noted.

Organ Weights: No drug-related findings were noted.

Gross Pathology: No drug-related findings were noted.

Histopathology: Mild to minimal edema or hypertrophy of the media of the nutritive vessels in the right auricle were reported in 2 males (low dose and high dose) and one mid dose female. A lymphohistiocytic infiltration in one mid dose female and a lymphocytic infiltration of the

myocardium were found in a high dose female (Table 60). One recovery female demonstrated minimal perivasculitis of nutritive vessels in the right auricle. Other findings occurred in only one high-dose animal and are of unclear significance.

Table 60. Histopathological changes following 14-day B9302-107 IV administration in dogs.

	Males					Females				
Dose (mg/kg/d)	0	0.01	0.03	0.06	0.06-R	0	0.01	0.03	0.06	0.06-R
Duodenum n=	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
atrophy of Brunner's gland	0	0	0	1	1	0	0	1	0	0
Kidney 1	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Vasculitis/perivasculitis	0	0	0	1	0	0	0	0	0	0
Jejunum	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Lymphoid tissue activation	0	0	0	1	0	0	0	0	0	0
Liver, fat-stain	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Kupfer cells	0	0	0	2	1	0	1	1	0	2
Testes 2	**2**	**2**	**2**	**2**	**2**					
Tubulus cell degeneration	0	0	0	1	1					
Heart, auricle, right	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Vascular media edema	0	1	0	1	0	0	0	0	0	0
Vasc media hypertrophy	0	0	0	0	1	0	0	1	0	0
Myocard lymphocyt infilt	0	0	0	0	0	0	0	0	1	0
Perivasc lymphocyt infilt	0	0	0	0	0	0	0	1	0	0
Vasculitis/perivasculitis	0	0	0	0	0	0	0	0	0	1
Mammary gland	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Edema	0	0	0	0	0	0	0	0	1	0
Hyperemia/congestion	0	0	0	0	0	0	0	0	1	0
Ovary 1						**2**	**2**	**2**	**2**	**2**
Corpus luteum cyst						0	0	0	1	0
Follicular cyst						0	0	0	0	1
Ovary 2						**2**	**2**	**2**	**2**	**2**
Corpus luteum cyst						0	0	0	1	0
Parathyroid 1	**2**	**0**	**2**	**2**	**2**	**1**	**1**	**1**	**2**	**2**
Cyst	0	0	0	0	0	0	0	0	1	0
Uterus, uterine cervix						**2**	**2**	**2**	**2**	**2**
Cerv squam hyperplasia						0	0	0	1	0

Toxicokinetics: Systemic exposure (AUC) data were not ascertainable since levels in terminal phases of elimination were below the LOQ. The Cmax, however, increased proportionally with increasing dose and remained constant throughout Day 10 (Table 61).

Table 61. Pharmacokinetics following 2 week IV administration in dogs.

Parameter	**Day**	**Dose group (mg/kg)**		
		0.01	0.03	0.06
Cmax (μg/l)	1	6.55	23.68	54.28
	10	7.50	19.37	54.61
Tmax (h)	1	0.25	0.38	0.38
	10	0.5	0.38	0.25

A NOAEL of 0.03 mg/kg was selected for this study due to various findings at the high dose in the ovaries, and mammary glands, testes, heart, kidneys and duodenum. This dose achieved a Cmax of 19-24 μg/l.

Dog, 4-week Oral (gavage) Toxicity with 4-week Recovery
Protocol No.: BD0334 *Report No.*: 68/95 *Volume*: 1.15

Study Dates: Starting date 2/6/95; report issued 8/3/95
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM46/251; purity = 98.61 - 98.82%) in 4% methocel E15
Concentration: 4-36 mg/ml
Dose Volume: 0.5 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

Methods: Beagle dogs (9.8 months old; 10.5-12.7 kg) were assigned to the following treatment groups:

Dose (mg/kg/day):	0	2	6	18
No./sex 4-week study	3	3	3	3
No./sex 4-week recovery	0	0	0	2

Each dog received a daily oral (gavage) dose of vehicle or test drug for 4-weeks. Autopsies were performed following both dosing periods and 4-weeks following the dosing period. The following observations were made:

Clinical observation . . . daily
Body weight . . . . . days -32, -18, -4 and 2 times weekly
Food consumption . . . . daily
Physical Exam . . . . . weeks -2, 3 and 8
Ophthalmoscopy . . . . . weeks -1, 4 and 8
ECG . . . . . .Weeks -2, -1, days 4, 22/24 and week 8
Blood pressure . . . . . day -12, -7, -4, 5, 10, 23/24, week 8
Hematology . . . . . Week -2, -1, days 3, 23, week 8
Clinical chemistry . . . . Week -2, -1, days 3, 23, week 8
Urinalysis . . . . . .Week -1, 3, 8
Enzyme induction . . . . not performed
Organ weights . . . . . at sacrifice; (for specific organs see Addendum, pages 91-92).
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice; all organs/tissues from all animals (for specific tissues/organs see Addendum, pages 91-92).
Toxicokinetics . . . . . assessed on days 1 and 25; results submitted separately as Study 157/95 and are reviewed within the context of the toxicity study.

Results:

Mortality: One mid-dose male died on Day 21 due to invagination of the colon.

Clinical Observations: All high dose animals exhibited tremor primarily of the hind legs, starting 30 minutes after dosing and lasting at least 2 hours and up to 7 hours after dosing in some (Table 65). Many of these animals also exhibited lethargy. Vomiting was exhibited in animals of all dose groups (30 minutes to 2 hours after dosing). These findings were reversible in recovery animals.

Body Weight: Body weight gain was reduced in high-dose animals from Day 8 onward in males and Day 29 in females as body weight was reduced 0.7-0.9 kg in males and females, respectively (Day 29; Table 62).

Table 62: Clinical observations following 4 week dosing with B9302-107 in dogs.

Dose (mg/kg)	0		2		6		18	
	M	F	M	F	M	F	M	F
Mortality	0	0	0	0	1	0	0	0
Clinical observations								
Tremors	0	0	0	0	0	1	5	5
Lethargy	0	0	0	0	1	0	3	4
Body wt gain Day 29 (kg)	↑0.1	↑0.2	↑0.2	↑0.1	↓0.3	↑0.1	↓0.9	↓0.7

Shaded areas indicated statistically significant change from control values.

Food Intake: Food consumption was significantly reduced in high dose animals (1-35% in males beginning on day 13; 4-40% beginning on day 5 in females). This effect was reversible in recovery animals.

Phyical Exam: No toxicologically significant treatment-related effects.

Blood Pressure: No toxicologically significant treatment-related effects.

Electrocardiogram: No toxicologically significant treatment-related effects.

Ophthalmoscopy: The sponsor reported no toxicologically significant treatment-related effects. However, no data was submitted with the study report.

Hematology: No significant drug-induced changes were noted in mean values. However, at days 3 and 23, leukocyte counts were increased in two high-dose males (two to three-fold) in connection with inflammation of processuss vaginalis and cystititis and one high dose female (three-fold higher on day 23). These effects were reversible following the recovery period. Increased erythrocyte sedimentation rate (21 to 27-fold) compared to mean control values was severe in 2 HD animals after 1 hour on day 23; a smaller increase was noted after 2 hours (13 to 20-fold).

Clinical Chemistry: An increase in alkaline phosphatase and globulin levels were observed at the high dose on Day 23 (Table 63). Alkaline phosphatase levels were increased in one low-dose female, one mid-dose male, and six high-dose animals on day 23.

Table 63. Clinical chemistry findings in dogs.

	Males			Females		
Dose (mg /kg/d)	2	6	18	2	6	18
Clinical Chemistry						
AP						
%Δ vs control group	↓6	↑22	↑76	↑46	↑23	↑90
Globulin						
%Δ vs control group	↑8	↑7	↑23	↑2	↑1	↑22

Shaded areas indicated statistically significant change from control values.

Urinalysis: Increased levels of erythrocytes, phosphate and kidney epithelial cells were noted in high-dose males and females and increased leukocytes numbers were observed in high-dose females, and increased kidney epithelial cells in males and females.

Organ Weights: No toxicologically significant treatment-related effects.

Gross Pathology: Gross findings were observed primarily in the heart, stomach, intestine and liver (Table 64). In the heart, bleeding was observed in the right auricle of 1 mid dose and 2 high dose animals and a nodule and scar tissue as well as bleeding was noted in the left auricle of one high dose animal. Both high dose animals had increased leukocyte counts which were associated with processuss vaginalis and cystititis. In the stomach, a swollen mucosa was observed in one low dose, 2 mid dose, and 3 high dose animals. In one additional 1 high dose animal, swelling of the submucosa was suspected edema. Findings in the intestine consisted of red spots at the ileocecal valve, and swollen mucosa in a few high dose animals. Invagination of the colon was observed in the mid-dose animal which died. A marked lobe pattern was observed in 1 control animal, 2 low dose, 2 mid dose and 5 high dose animals. In addition, a swollen liver was noted in 2 high dose animals.

Table 64. Gross changes following 4-week oral B9302-107 administration in dogs.

	Pooled				
Dose (mg/kg/d)	0	2	6	18	18-R
Heart					
Arterio-ventricular valve					
-bleeding	0	0	1	0	0
Right auricle					
-bleeding	0	0	0	2	0
-nodule, scar	0	0	0	1	0
left auricle					
-bleeding	0	0	0	1	0
Liver					
Marked lobe pattern	1	2	2	3	2
Swollen	0	0	1	2	0
Gall bladder					
Fibrinous content	0	0	0	2	0
White epithelial layers	1	3	2	3	2
Dark discoloration	0	0	0	0	2
Stomach					
Swollen mucosa	0	1	2	2	0
Submucous edema	0	0	0	1	0
Intestine					
Swelling/yellow epithelial layers	0	0	0	1	0
Duodenum					
Dark red	0	0	0	1	0
Jejunum					
Swollen, activation of Peyer's patches	0	0	0	0	1
Ileocecal valve					
Dark red spot, solitary	0	0	0	0	2
Dark red discoloration	0	0	0	0	1
Scrotum / processuss vaginalis					
Pururlent swelling	0	0	0	1	0
Urinary bladder					
Urolthiasis	0	0	0	1	1

Histopathology: Primary findings were noted in the heart at the mid- and high-doses. Two animals exhibited moderate to severe subacute inflammation of myocardium and nutritive vessels in right atrium and right auricle (Table 65). One high dose animal showed slight focal neutrophilic infiltration in the right auricle. Most of the findings appeared to be reversible. An increased incidence of thymic atrophy was noted at the mid- and high-doses and tubular degeneration of the testes with dysspermia in the epididymides were reported in one high dose animal. Various other findings were noted with a slight increase in incidence at the high dose.

Table 65. Histopathological changes following 4-week oral B9302-107 administration in dogs.

	Males					Females				
Dose (mg/kg/d)	0	2	6	18	18-R	0	2	6	18	18-R
n=	**3**	**3**	**3**	**3**	**2**	**3**	**3**	**3**	**3**	**2**
Heart										
Left ventricle										
-bleeding	0	0	0	0	1	0	0	0	0	0
Right atrium										
-capillarisation	0	0	1	1	0	0	0	0	0	0
-bleeding	0	0	1	1	0	0	0	1	0	0
-peri/vasculitis	0	0	1	2	0	0	0	0	0	0
Right auricle										
-neutrophilic infiltr	0	0	0	1	0	0	0	0	0	0
-capillarisation	0	0	0	1	0	0	0	0	0	0
-bleeding	0	0	0	1	0	0	0	0	0	0
-peri/vasculitis	0	0	0	1	0	0	0	0	0	0
-endo/myocarditis	0	0	0	1	0	0	0	0	0	0
Lung										
-broncholisation	0	0	0	1	0	0	0	0	0	0
-emphysema	0	0	1	1	0	0	0	1	0	1
-atelectasis	0	0	0	1	0	0	0	0	0	0
Gastric fundus										
-submucous edema	0	0	0	1	0	0	0	0	0	0
-lympho-histiocytic infiltr	1	0	1	2	1	0	1	0	1	1
-calcification	0	0	0	1	0	0	0	0	0	0
Gastric antrum										
-activ lymph follicle	0	0	0	1	0	0	0	0	0	0
Gastric cardia										
calcification	0	0	0	0	0	0	0	0	2	0
Duodenum										
-hyperemia/congestion	0	0	0	1	0	0	0	0	0	0
Adrenals										
-Z. fasciculata hypertrop	0	0	0	1	0	0	0	0	0	0
Kidney 1										
-pelvic epith hyperplasia	0	0	0	0	1	0	0	0	0	0
Kidney 2										
-interstial nephritis	0	0	0	0	0	0	0	0	1	0
Thymus										
-cyst	0	0	0	1	0	0	0	0	0	0
-atrophy	1	1	2	2	1	0	1	3	3	1
Testes 2										
-tubular degeneration	0	0	0	1	0					
Epididymis 2										
-dyspermia	0	0	0	1	0					
Eye 1										
-conjunctivitis	0	0	0	1	0	0	0	0	0	0
-keratitis	0	0	0	1	0	0	0	0	0	0
Eye 2										
-conjunctivitis	0	0	0	1	0	0	0	0	0	0
-keratitis	0	0	0	1	0	0	0	0	0	0
Thyroid C-cell 1										
-diffuse hyperplasia	0	1	2	1	0	1	1	1	3	0
Thyroid C-cell 2										

	Males					Females				
Dose (mg/kg/d)	0	2	6	18	18-R	0	2	6	18	18-R
-diffuse hyperplasia	0	1	2	1	0	2	1	1	3	0
Thyroid follicle										
-branchiogenic cyst	1	0	0	0	0	0	0	0	1	1
Pancreas										
Chronic pancreatitis	0	0	0	0	0	0	0	0	1	1
Liver										
-pigment deposits	0	0	0	0	0	0	0	0	1	0

Toxicokinetics: Systemic exposure to B9302-107 increased proportionally from a dose of 2 to 6 mg/kg, especially on Day 25 (Table 66) and sub-proportionally at the high dose, indicating saturation of drug absorption. Drug accumulation was apparent at the two higher doses. The elimination half-life increased to 12.8 hours at the high dose and may be due to eneterohepatic recirculation. The t_{max} ranged from ~ 1.5 to 5.5 hours with no indication of dose-dependency.

Table 66. Pharmacokinetics following 4 week oral administration in dogs.

Parameter	**Day**	**Analyte**		
		B9302-107		
mg B9302-107/kg		2	6	18
AUC_{0-24} (µg.h/l)	1	991.9	2304.6	3547.6
	25	827.6	3061.1	4182.8
Cmax (µg/l)	1	199.8	321.5	559.7
	25	159.1	464.6	545.2
t1/2 (h)	1	7.4	9.9	4.7
	25	6.2	4.6	12.8
tmax (h)	1	1.58	5.67	1.75
	25	2.33	2.4	2.0

A NOAEL of 2 mg/kg was identified in this study and was associated with a systemic exposure to B9302-107 of ~ 830-990 . Target organs of toxicity include the heart, thymus, lung, gastrointestinal tract, testes, epididymides and pancrease (females).

Mouse, 3-Month Oral Dose-Ranging Study (screening)
Study No.: GM0550 *Report No.*: R 216/98 *Vol.*: 5.6

Study Dates: Starting date 4/23/1998; report issued 4/30/1999
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch: AM50/126; purity: not reported)
Concentration: Not reported; estimated to be 0.6 to 1.8 mg/ml.
Volume: 10 ml/kg.
GLP: The study was unaudited.
QA report: No.

This study was performed primarily for dose-ranging purposes for a carcinogenicity study. A summary of this report was submitted in the Pre-IND package and was reviewed as a portion of the Pre-IND Carcinogenicity Protocol Review (dated February 2, 1999; see attachment). There are no significant differences between the submissions other than the presence of a more detailed methodology section and the inclusion of food and water consumption data in the current submission.

Methods: Mice (B6C3F1; 6 weeks old; 19-28 g) were assigned to the following treatment groups:

Est. total inhaled dose (mg/kg)	Veh. control	6	12	18
No. of animals/sex	10	10	10	10

Mice were exposed daily (oral gavage) to vehicle control (4% methocel E15 with Med Antifoam C) or test drug for 3 months and the following observations were made:

Clinical observation . . . three times daily
Body weight . . . . . assessed one to two times per week
Food consumption . . . . assessed one to two times per week
Water consumption . . . assessed two times per week
Health exam . . . . . not assessed
Ophthalmoscopy . . . . . not assessed
ECG . . . . . not assessed
Hematology . . . . . Days 90/91/92
Clinical chemistry . . . . not assessed
Urinalysis . . . . . not assessed
Enzyme induction . . . . not assessed
Organ weights . . . . . not assessed
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice (for specific tissues/organs see Addendum, pages 91-92).
Toxicokinetics . . . . . not assessed

Results:

Mortality: One control female and one mid-dose male were found dead in their cages on Days 12 and 55, respectively. The sponsor stated that the cause of death was unclear although the

female displayed lung discoloration with hyperemia/congestion and the male exhibited adrenal discoloration and spots on the liver and lungs (associated with intra-alveolar blood).

Clinical Observations: No drug-related observations were noted.

Body Weight: Only slight differences in initial and final body weights were observed in treated mice (Table 67) However, a statistically significant reduction in body weight gain was reported for exposed males, although a dose-related response was not observed and the reduction was similar to the non-significant reduction observed in females. This reduction in body weight gain is not considered to be biologically significant.

Table 67. Effect of B9302-107 on body weight gain in mice.

	Males				Females			
Dose group	VC	6	12	18	VC	6	12	18
Initial body weight (g)	26	26	25	25	21	20	21	20
Final body weight (g)	32	31	30	30	27	27	27	27
Net BW gain (g)	6	5	5	5	6	7	6	7
%Δ BW gain		↓17	↓17	↓17		↑17	--	↑17

Shaded areas indicate statistically significant difference from control group.

Food consumption: Consumption was slightly increased in males (5-9%) and females (10%) in all drug-treatment groups.

Water consumption: No significant drug-related effects were observed.

Hematology: Reduced numbers of leukocytes in treated females and increased numbers of segmented neutrophils in treated females and mid- and high-dose males were observed (Table 68).

Table 68. Effect of B9302-107 on body weight gain in mice.

	Males			Females		
Dose group (mg/kg)	6	12	18	6	12	18
Leukocytes						
%Δ vs control animals	↓6	↓8	↓19	↓19	↓33	↓44
Seg. neutrophils						
%Δ vs control animals	↑20	↑40	↑35	↑47	↑35	↑47

Shaded areas indicate statistically significant difference from control group.

Gross pathology: No drug-related findings were observed.

Histopathology: A systemic lympho-hystiocytic infiltration was noted in all dose groups including control animals (Table 69). Findings of note included cortical atrophy and hyperemia of the adrenal glands in high-dose males and all treated females, olfactory epithelial degeneration and necrosis were also noted in mid- and high-dose animals, and a slight increase in the incidence and severity of follicular hyperplasia of the spleen in high-dose females. In general, the severity of the observed lesions was minimal in nature.

Table 69. Histopathological changes in mice following 3-month B9302-107 administration.

	Males				Females			
Dose (mg/kg/d)	VC	6	12	18	VC	6	12	18
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Atrophy, cortical x-zone	0	0	0	5(.7)	0	5(.4)	8(.9)	8(1)
Hyperemia of inner cortex	0	0	4(.4)	1(.2)	0	4(.3)	6(.7)	9(1.3)
Harderian glands								
Lympho-histiocytic infiltration	0	NA	NA	1(.1)	1(.2)	NA	NA	1(.1)
Chromodacryorrhea	0	NA	NA	0	0	NA	NA	1(.1)
Eyes								
Uveitis	0	NA	NA	1(.3)	0	NA	NA	0
Kidneys								
Pyelonephritis	0	0	0	1(.3)	0	0	0	0
Lympho-histiocytic infiltration	4(.4)	3(.1)	4(.1)	2(.1)	5(.2)	9(.3)	7(.7)	9(.9)
Lacrimal glands								
Lympho-histiocytic infiltration	1(.03)	NA	NA	7(.4)	0	NA	NA	1(.2)
Nasal cavity								
Olfactory epithelial degeneration	0	0	0	3(.2)	0	0	4(.2)	5(.4)
Olfactory cell necrosis	0	0	1(.1)	2(.4)	0	0	1(.03)	1(.05)
Prostate								
Inflammation	0	NA	NA	1(.1)				
Thymus								
Involution	0	NA	NA	1(.2)	0	NA	NA	1(.2)
Urinary bladder								
Epithelial hyperplasia	0	0	0	1(.1)	0	0	0	0
Acute cystitis	0	0	0	1(.3)	0	0	0	0
Lympho-histiocytic infiltration	5(.1)	3(.1)	4(.1)	5(.1)	3(.1)	6(.2)	3(.1)	5(.2)
Liver								
Lympho-histiocytic infiltration	3(.1)	1(.03)	0	1(.2)	4(.2)	7(.3)	7(.6)	6(.7)
Lungs								
Lympho-histiocytic infiltration	0	1(.2)	1(.2)	0	0	1(.1)	1(.03)	2(.3)
Pancreas								
Serosal lympho-histiocyt infiltr	2(.2)	NA	NA	2(.1)	1(.3)	NA	NA	6(.4)
Lympho-histiocytic infiltration	0	NA	NA	0	0	NA	NA	1(.03)
Spleen								
Serosal Lympho-histiocyt infiltr	0	1(.03)	1(.03)	0	0	0	1(.03)	1(.1)
Follicular hyperplasia	0	0	0	0	1(.2)	0	0	2(.4)
Stomach/forestomach								
Submucosal inflammation	0	NA	NA	0	0	NA	NA	1(.1)
Submandibular glands								
Lympho-histiocytic infiltration	0	NA	NA	0	0	NA	NA	3(.1)

Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe
NA: no histological assessment for this group.

This study was performed primarily for dose-ranging purposes for a carcinogenicity study. Maximum tolerated doses of 18 mg/kg and 12 mg/kg were identified in male and female mice, respectively. The primary target organs of toxicity were the nasal cavity and adrenal glands. These conclusions are identical to those proposed in the review of the report summary submitted previously.

Hamster, 3-Month Oral Dose-Ranging Study (screening)

Study No.: GH0562 *Report No.*: 252/98 *Vol.*: 5.8

Study Dates: Starting date 6/24/1998; report issued 3/16/1999
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch: AM50/126; purity: not reported) in 4% methocel and Med Antifoam C
Concentration: Not reported.
Volume: 10 ml/kg.
GLP: The study was unaudited.
QA report: No.

This study was performed primarily for dose-ranging purposes for a carcinogenicity study. A summary of this report was submitted in the Pre-IND package and was reviewed as a portion of the Pre-IND Carcinogenicity Protocol Review (dated February 2, 1999; see attachment). There are no significant differences between the submissions other than the presence of a more detailed methodology section and the inclusion of food and water consumption data in the current submission.

Methods: Syrian Golden hamsters were assigned to the following treatment groups:

Dose (mg/kg)	Vehicle control	4	8	16
No. of animals/sex	10	10	10	10

Hamsters were exposed daily to B9302-107 for 3-months. The following observations were made:

Clinical observation . . . twice daily
Body weight . . . . . assessed up to 2 days per week
Food consumption . . . . assessed up to 2 days per week
Water consumption . . . assessed up to 2 days per week
Health exam . . . . . not assessed
Ophthalmoscopy . . . . . not assessed
ECG . . . . . not assessed
Hematology . . . . . assessed Day 74/75
Clinical chemistry . . . . not assessed
Urinalysis . . . . . not assessed
Enzyme induction . . . . not assessed
Organ weights . . . . . not assessed
Gross pathology . . . . . at sacrifice (days 95-100)
Histopathology . . . . . at sacrifice (for specific tissues/organs see Addendum, pages 91-92).
Toxicokinetics . . . . . not assessed

Results:

Mortality: Two animals died (one mid-dose and one high-dose male on Days 10 and 22, respectively) due to gavage errors. The mid-dose animal exhibited discoloration and congestion

in the lungs and a watery pale or yellow exudate in the thoracic cavity. Similar findings were reported in the high-dose animal in addition to intra-alveolar blood and discoloration and congestion in the adrenals.

Clinical Observations: No drug-related findings observed.

Body Weight: Body weight gain was dose-dependently reduced in both males and females at Day 85 (Table 70). Although the reduction was greater than or equal to 10% in all dose groups in females and in mid- and high-dose males, only the effect in high-dose females was statistically significant (P value < 0.05, Kruskal-Wallis, two-sided Wilcoxon tests).

Food Consumption: After the first study week, food consumption was significantly reduced in all males groups administered the test substance (14-17%) and high dose females (16%) (Table 70). Consumption returned to control values in males but was still reduced in high dose females from Days 29-91 (13%).

Table 70. Effect of B9302-107 on body weight gain in hamsters.

	Males				Females			
Dose group	VC	4	8	16	VC	4	8	16
Body weight gain	77	76	77	79	73	73	74	73
Initial body weight (g)	118	118	114	112	126	118	116	108
Final body weight (g)	41	42	37	33	53	45	42	35
Net BW gain (g)		↑2	↓10	↓20		↓15	↓21	↓34
%Δ BW gain								
Food consumption								
% Δ in FC – wk 1		↓14	↓14	↓17		↓7	↓4	↓16

Shaded areas indicate statistically significant difference from control group.

Water Consumption: There were no drug-related effects.

Hematology: There were no drug-related effects.

Gross pathology: Although the study report states that there were no substance- or treatment-related findings, gross findings included discoloration of the adrenals in 4 of 10 high-dose males and females and involution of the thymus in 4 of 10 and 3 of 10 high dose males and females, respectively (Table 71). Other possible drug-related findings, listed below, occurred in only one high-dose male or female. Only the findings in the adrenals, eyes, and seminal vesicles were possibly correlated with histological findings at the high-dose.

Table 71. Gross findings in hamsters following 3-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	4	8	16	VC	4	8	16
n =	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Discoloration	0	0	0	4	0	0	0	4
Eyes								
Injury	0	0	0	0	0	0	0	1
Opaque/cloudy	0	0	0	0	0	0	0	1
Right	0	0	0	0	0	0	0	1
Lungs								
Enlarged	0	0	0	1	0	0	0	0
Seminal vesicles								
Focus/spot	0	0	0	1				
Skin								
Alopecia	0	0	0	0	0	0	0	1
Thymus								
Involuted	0	0	0	4	0	0	0	3
Trachea								
Foam	0	0	0	1	0	0	0	0

Histopathology: Lympho-histiocytic infiltration was noted in both control and treated animals (Table 72). Findings of note included dysspermia and tubular atrophy of the testes in all treatment groups, and seminal vesicle atrophy at the high dose (low- and mid-dose animals were not assessed). In addition, olfactory epithelial disorganization was reported at the mid- and high-doses and olfactory epithelial necrosis was observed in one high-dose male. Other notable findings included prostatic atrophy, a pancreatic granuloma at the high dose, and hyperemia of the adrenal glands in one high-dose male and female. A number of histologic effects were also observed in the eye, especially in females. Since low- and mid-dose animals were not examined, it is not possible to definitively assess the drug-relatedness of the findings.

This study was performed primarily for dose-ranging purposes for a carcinogenicity study. Maximum tolerated doses of 16 mg/kg and 8 mg/kg were identified in male and female hamsters, respectively. The target organs of toxicity include the male reproductive organs, the nasal cavity, the eye, and possibly the prostate, adrenals and pancreas. These conclusions are identical to those proposed in the review of the report summary submitted previously.

Table 72. Histopathology changes in hamsters following 3-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	4	8	16	VC	4	8	16
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Cortical fat vacuoles	0	NA	NA	1(.3)	0	NA	NA	0
Hyperemia/congestion	0	NA	NA	1(.6)	0	NA	NA	1(.14)
Medullary atrophy	0	NA	NA	1(.04)	0	NA	NA	0
Hemorrhage	0	NA	NA	0	0	NA	NA	1(.2)
Harderian glands								
Chronic inflammation	2(.5)	NA	NA	7(1)	4(.9)	NA	NA	8(1)
Eyes								
Iritis	0	NA	NA	0	0	NA	NA	3(.6)
Uveitis	1(.2)	NA	NA	1(.4)	1(.4)	NA	NA	6(1.6)
Retinal degeneration/atrophy	1(.2)	NA	NA	0	0	NA	NA	5(1.2)
Anterior chamber hemorrhage	0	NA	NA	0	0	NA	NA	3(.6)
Periorbital blood	0	NA	NA	0	0	NA	NA	1(.2)
Lympho-histiocytic infiltration	5(.4)	NA	NA	4(.4)	6(.7)	NA	NA	6(.9)
Hemosiderin deposits	0	NA	NA	0	0	NA	NA	1(.2)
Epididymis								
Dysspermia	6(1.3)	10(3.3)	8(2.7)	8(3.0)				
Esophagus								
Peri-/epi-/esophagitis	0	NA	NA	1(.4)	0	NA	NA	0
Traumatic separation	0	NA	NA	1(.2)	0	NA	NA	0
Colon								
Goblet cell hypertrophy	0	0	0	1(.1)	0	0	0	0
Ileum								
Lymphocytic hyperplasia	0	0	0	1(.1)	0	0	0	0
Kidneys								
Tubular cell regeneration	0	NA	NA	1(.1)	0	NA	NA	2(.2)
Nasal cavity								
Olfactory disorganization	0	0	7(.4)	6(.4)	0	0	5(.4)	6(.2)
Loss of PAS-positivity in Bowman's glands	0	1(.03)	8(.9)	8(1.4)	1(.03)	1(.03)	7(.7)	10(1.8)
Olfactory single cell necrosis	0	0	0	1(.1)	0	0	0	0
Prostate								
Atrophy	0	NA	NA	3(.8)				
Liver								
Increased fat in hepatocytes(n=5)	2(.4)	NA	NA	4(.8)	1(.2)	NA	NA	3(.6)
Pancreas								
Granuloma	0	NA	NA	1(.3)	0	NA	NA	0
Seminal vesicles								
Atrophy	0	NA	NA	4(.8)				
Testes								
Tubular atrophy	8(1)	10(3.4)	9(3.4)	9(3.5)				
Stomach/forestomach								
Serosal, Lympho-histiocyt infiltr	1(.1)	NA	NA	2(.2)	1(.03)	NA	NA	1(.03)

Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe
NA: no histological assessment for this group.

CHRONC TOXICITY:

Dog, 6-month Oral (tablet) Toxicity

Report No.: 94/96 *Study No.*: CD0367 *Volume*: 1.23

Study Dates: Starting date 11/7/1995; report issued 1/8/1997
Testing Lab: Byk Gulden, Inst. Pathology and Toxicology, Barsbuttel and Hamburg, Germany
Test Article: B9302-107 (Formulation A; Batches 95-46 and 95047 for the low dose, 95048 for the mid-dose, and 95052 for the high-dose; purity > 98.3%)
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

Methods: Beagle dogs (14.1-14.6 months; 9.9-11.1 kg) were assigned to the following treatment groups:

Dose (high/low regime) (mg B9302-107/kg/day):	0	0.5/0.2	2/1	8/4
No./sex-main study	5	5	5	5
No./sex- 4 wk recovery	0	0	0	2

Each dog received a daily dose of placebo or test drug tablet for 6 months. The animals were apparently separated into two replicates, although no information was provided as to the composition of these replicates. The initial dosing regime was 0.5, 2 and 8 mg/kg. Animals of the first replicate were dosed at this level from days 1 to 15 and animals in the second replicate were dosed on day 1 only. Doses were then reduced due to the increased bioavailability observed with the tablet formulation. Dosing with 0.2, 1 and 4 mg/kg was then commenced on days 17 and day 3 in the first and second replicates, respectively. Two animals per sex at the high dose were autopsied after a 4 week recovery period. No control animals were examined after recovery. The following observations were made:

Clinical observation . . . three times daily
Body weight . . . . . days -18, -4 and 2x weekly
Food consumption . . . . daily
Water consumption . . . not assessed
Ophthalmoscopy . . . . . Week -2, -1, 26 and 30
Veterinary exam. . . . . not assessed
Physical examination . once pretest (week -1) and Weeks 25 and 29; includes assessment of pulse rate, body temperature, senses, lung percussion, and auscultation
ECG . . . . . Week -2, -1, 24 and 30
Hematology . . . . . Week -2, -1, 12, 25 and 30
Clinical chemistry . . . . Week -2, -1, 12, 25 and 30
Urinalysis . . . . . Week -1, 11, 24 and 30
Enzyme induction . . . . not assessed
Reproductive exam . . . Replicate 1: Day 157/158; Replicate 2: Day 143/144; males (morphology of gonads, libido, sperm collection, quantity, quality)
Organ weights . . . . . at sacrifice; (for specific organs see Addendum, pages 91-92).
Gross pathology . . . . . not stated

Histopathology . . . . . at sacrifice; organs/tissues from all animals, (for specific tissues/organs see Addendum, pages 91-92).

Toxicokinetics . . . . . Replicate 1: Day 1, week 13, 26 (0, 1, 2, 4, 7, 10 and 24 hours after dose administration); Replicate 2: Day 3, week 13, 26 (0, 1, 2, 4, 7, 10 and 24 hours after dose administration). Results reported separately in study 147/97 and are reviewed within the context of the toxicity study.

Results:

Mortality: One mid-dose female was sacrificed in extremis due to acute intussusception of the jejunum on day 173.

Clinical Observations: Prior to dose reduction, vomiting was observed almost daily in high dose (8 mg/kg) animals. One high dose animal did not vomit on Day 1 and showed severe ataxia, parese (seal like movements) in the hind legs, and vestibular disturbances on Day 1. The animal was removed from the study but ataxia of the right front leg and parese of the hind legs was still noted on Day 4. On Day 9 the animals hind legs were still atactic. On day 10, gait was normal. These findings led to a lowering of the doses in all groups due to the increased bioavailability of the tablet form of dosage.

After dose reduction, vomiting (1-24 hours after administration) and hypersalivation were observed in high-dose animals. Vomiting occurred throughout the study in one animal and in others primarily in the first 2 weeks at a dose of 8 mg/kg. Hypersalivation was noted in 3 animals on Days 4-9 and in 5 animals on Days 11-24. Two of these animals exhibited this symptom throughout the study.

Body Weight: No substance-related body weight changes were observed. All groups gained 2-13% from Week -1 to Week 27.

Food Intake: No substance-related changes were observed.

Physical examination: No substance-related changes were observed.

Ophthalmoscopy: The sponsor states that no substance-related changes were observed. No data was submitted to support this statement.

ECG: No substance-related changes were observed.

Hematology: The sponsor stated that no substance-related changes were observed. However, reticulocyte count was reduced in all treated males (31-47%) and females (dose-related, 6-53%) at week 25 (Table 73). In addition, leukocyte counts were mildly increased in mid- and high-dose males (13-17%). Thrombocyte counts were also slightly increased in high-dose males (19%). None of these findings were statistically significant.

Clinical Chemistry: The sponsor stated that no substance-related changes were observed. However, significant reduction (21%) in serum creatinine in high-dose males and an increase in cholesterol (34-36%) in mid- and high-dose females was noted after 25 weeks dosing (Table 73). In addition, a dose-dependent significant increase in serum AP was noted in males (31-133%) and increased in high-dose females (22%). Serum LDH was also reduced in mid- and high-dose males (21%) and treated females (23-42%). A 24% increase in phosphorous was also reported in high-dose females.

Table 73. Clinical findings in dogs administered B9302-107.

	Males			Females		
Dose (mg/kg/d)	0.2	1	4	0.2	1	4
Hematology						
Reticulocytes						
% change vs control	↓47	↓31	↓47	↓6	↓47	↓53
Leukocytes						
% change vs control	↓4	↑13	↑17	--	↑15	↑15
Thrombocytes						
% change vs control	↑2	↑7	↑19	↓15	↓10	3
Clin. Chemistry						
Creatinine						
% change vs control	↓5	↑5	↓21	↑7	↑3	↓4
Cholesterol						
% change vs control	↓3	↑11	↑5	↑8	↑36	↑34
AP						
% change vs control	↑31	↑60	↑133	↓10	↑12	↑22
LDH						
% change vs control	↑16	↓21	↓21	↓27	↓23	↓42
Phosphorous						
% change vs control	--	↑7	↑4	↑9	↑8	↑24

Shaded areas indicated statistically significant change from control values.

Urinalysis: The sponsor did not comment on urinalysis findings. An increase in urine pH was noted in high dose females (pH 5.7 in controls vs 6.4 at the high-dose).

Organ Weights: The sponsor stated that no substance-related changes were observed. However, liver weight was increased in mid- and high-dose animals, and kidney weight was increased in high-dose animals (Table 74). In addition, thyroid weight was increased in treated males (38-59%) while pancreas weight was increased in treated females (20-36%). Only the liver showed significant histopathological findings.

Table 74. Organ weight changes in dogs administered B9302-107.

	Males			Females		
Dose (mg/kg/d)	0.2	1	4	0.2	1	4
Absolute Organ Wt.						
Liver						
% Δ vs control	↑3	↑15	↑14	↑3	↑16	↑19
Kidney - left						
% Δ vs control	↓1	↑7	↑8	↑2	↑7	↑21
Kidney – right						
% Δ vs control	↓2	↑8	↑8	↓1	↑4	↑21
Thyroid – left						
% Δ vs control	↑11	↑20	↑40	↓11	no Δ	↓6
Thyroid - right						
% Δ vs control	↑41	↑38	↑59	↑3	↑18	↑9
Pancreas						
% Δ vs control	↑6	↑16	↑16	↑20	↑32	↑36

Gross Pathology: No substance-related changes were observed.

Histopathology: The primary microscopic changes included mild to moderate subacute to chronic reactive inflammatory changes in the right atrium or right auricle (Table 75). One mid-dose female and 2 high-dose males and females were affected. One female also demonstrated lympho-histiocytic infiltration following the recovery period. Other findings were related to the jejunum intussuseption (submucosal hemorrhage) in the mid-dose female that died prematurely. Other apparent drug-related effects not addressed by the sponsor included liver hyperemia/congestion at the mid- and high doses, various findings in the lung including necrosis and hemorrhage, and chronic cystitis in the urinary bladder of 2 high-dose females. A number of other findings were reported in single high-dose animals and it is unclear as to the nature of their relationship to drug treatment.

Table 75. Histopathological changes after 6-month administration in dogs.

	Males					Females				
Dose (mg/kg/d)	0	0.2	1	4	4-0*	0	0.2	1	4	4-0*
Histology n=	**5**	**5**	**5**	**5**	**2**	**5**	**5**	**5**	**5**	**2**
Adrenals										
Z. fasciculata hypertrophy	0	0	0	1	0	0	0	0	0	0
Heart – right atrium										
Pericardial hemorrhage	0	0	0	1	0	0	0	0	0	0
Myocardial hemosiderin deposit	0	0	0	1	0	0	0	0	0	0
Myxoid-chondroid degeneration	0	0	0	1	0	0	0	0	0	0
Heart – right auricle										
Lympho-histiocytic infiltration	0	0	0	1	0	0	0	1	2	1
Sub-epicardial edema	0	0	0	0	0	0	0	0	1	0
Liver										
Hyperemia/congestion	0	0	2	2	0	0	1	1	2	0
Lung										
Pleuritis	0	0	0	1	0	0	0	0	0	0
Hemorrhage	0	0	0	1	0	0	0	0	1	1
Necrosis	0	0	0	1	0	0	0	0	1	0
Purulent bronchopneumonia	0	0	0	0	0	0	0	0	1	0
Lymph nodes										
Parafollicular hyperplasia	0	0	0	1	0	0	0	0	0	0
Lymphocytic depletion	0	0	0	0	0	0	0	0	1	0
Hemosiderin deposit	0	0	0	0	0	0	0	0	1	0
Pancreas										
Apoptotic bodies	2	3	4	5	2	3	3	1	3	0
Skin										
Histiocytoma	0	0	0	0	1	0	0	0	0	0
Subepith. lympho-histiocytic infiltr.	0	0	0	0	1	0	0	0	1	0
Spinal cord										
Axonal swelling/degeneration	0	0	0	1	0	0	0	0	0	0
Spleen										
Follicular bleeding	0	0	0	1	0	0	0	0	1	0
Thyroid follicle										
Lymphocytic infiltration	0	0	0	1	0	0	0	0	1	0
Mammary gland										
Alveolarization	0	0	0	0	0	0	1	0	2	1
Parotid gand										
Heterotopic submandib gland tissue	0	0	0	0	0	0	0	0	1	0
Urinary bladder										
Chronic cystitis	0	0	0	0	0	0	0	0	2	0
Uterus										
Lympho-hystiocytic infiltration						0	0	0	1	0

* Autopsy following the recovery period.

Andrology: No significant morphological or functional alterations were noted. A statistically significant increase in eosin stained spermatozoa (indicating membrane damage) was observed in treated animals (12.4-14.4% vs 7% in controls), though the response was not dose-dependent and all mean values were within historical range (1 dog in each treatment group exceeded to maximum tolerable value). Also, the incidence of spermatozoa with double or multiple deformations was increased in mid- and high-dose animals (0.8-1% vs 0.1% in controls).

However, all animals were within the maximum tolerable value. Statistical differences in sperm count parameters were observed but are not considered to be biologically relevant.

Toxicokinetics: Systemic exposures (AUC and Cmax) to B9302-107 increased sub-proportionally with increasing dose (Table 76). The pharmacokinetic parameters remained fairly constant regardless of the study day sampled, although exposures increased at the mid and high-dose from day 86 to day 178 by 19 and 24%, respectively, indicating the potential for drug absorption. The mean elimination half-lives and tmax ranged from 2-4 hours and 1.4-4 hours, respectively. In a 6 month dog study (0.2 to 4 mg/kg B9302-107, oral),

Table 76: Pharmacokinetics of B9302-107 following 6 month oral administration in dogs.

Parameter	**Day**	**Dose group (mg/kg)**		
		0.2	1	4
$AUC_{(0\text{-}inf)}$ (µg/l.h)	3 86 178	224.5 183.3 203.7	519.5 496.8 588.5	1860.7 1597.3 1982.0
Cmax (µg/l)	3 86 178	76.6 53.1 51.3	178.2 100.9 129.1	214.7 201.0 267.1
t1/2 (h)	3 86 178	2.1 2.4 2.5	2.0 3.2 2.2	4.1 3.1 3.5
tmax (h)	3 86 178	1.8 1.9 1.4	1.4 1.6 2.0	3.8 3.5 2.5

The low-dose of 0.2 mg B9302-107/kg/day was identified as the NOAEL for this study. The target organs of toxicity included the heart, pancreas, urinary bladder in females, the lung, and the liver. The NOAEL in this study is one-tenth that observed in the 1 month oral (suspension) study reviewed previously. Target organs identified in the one month study, such as the thymus, stomach, testes and epididymides, were not identified in the current study since dosing was at lower levels than in the previous study (2, 6 and 18 mg/kg).

Summary of Toxicology: Acute toxicity studies were performed in mice and rats utilizing oral and IV dosing. In mice, a maximum non-lethal dose of 600 mg/kg and a minimum lethal dose of 1100 mg/kg were demonstrated following oral dosing. The maximum tested IV dose of 20 mg/kg did not induce lethality. Rats demonstrated greater sensitivity as the maximum non-lethal oral doses were 100 mg/kg and 400 mg/kg and the minimum lethal doses were 400 mg/kg and 700 mg/kg in females and males, respectively. The minimum lethal dose following IV dosing in rats was 16 mg/kg and the maximum non-lethal dose was 12 mg/kg. Animals in all studies exhibited clinical signs (piloerection, hunched position, ptosis, reduced activity, increased respiration rate, limpness, reduced or stagnant body weight gain, reduced food and water consumption). Gross and histological assessment of selected tissues demonstrated findings in the glandular stomach, testes and olfactory epithelium. An assessment of local drug effects on the nasal cavity of mice, hamsters, and rats showed that the parent compound B9302-107 does not

directly induce nasal toxicity. Rather, labeling of the amino-dichloropyridine moiety of B9302-107 was necessary to achieve binding. Among animals treated with [^{14}C]-B9302-107, no labeling was detected in mice, hamsters showed barely detectable labeling and rats showed heavy labeling which was concentrated in cells of the olfactory submucous glands.

Subchronic toxicity studies were performed in rats (14 day IV administration and 4 week and 4 week/3 month oral administration) and dogs (14 day IV administration and 4 week oral administration). A chromic 6 month oral study was also performed in dogs. In rats, a NOAEL was not selected due to adverse findings in the nasal cavities, reduced body weight gain and clinical observations at all doses in the 14 day intravenous study (0.5 to 2 mg/kg). The primary target organ of toxicity was the nasal cavity with greatest severity in females. Findings in other organs (pulmonary inflammation and paleness and reduced size of the thyroid) were generally of low severity. Other findings included clinical observations, reduced body weight gain and food and water consumption, increased ALAT, and urine osmolarity, calcium and chloride. In the 4 week rat oral (gavage) toxicity study (0.5, 2, and 8 mg/kg), a NOAEL was not identified since the nasal cavities were not assessed and adverse findings noted in tissues of high dose males were not assessed in lower dose groups. Deaths occurred at the high dose during weeks 3-4. Target organs of toxicity included the heart, adrenals, stomach, parathyroid, spleen, testes and epididymides, and the thymus, prostate and seminal vesicles. Recovery animals continued to show oligospermia, peritonitis and jejunal inflammation associated with serositis. Additional findings included emaciation, hemorrhage and enlargement of the stomach and small intestine, decreased absolute organ weights (spleen, seminal vesicles, prostate, thymus), and increased adrenal weight. Increases in QT time, QRS time and QαT-time were also observed in high dose animals and were not fully reversible. Other findings at the high dose included a reversible reduction in estrus events and prolongation of diestrus phase (high dose), clinical observations, reduced body weight gain (reversible) and food consumption and increased leukocyte counts, PMN, serum urea, urine osmolality, sodium, chlorine, and calcium and urine volume. In the 3-month oral (gavage) study (0.02, 0.2 and 2 mg/kg/day), the mid-dose of 0.2 mg/kg/day was selected as the NOAEL. Target organs of toxicity were the nasal cavities, testes and epididymides, and the thymus. These target organs were also identified (except for nasal cavities) in the 4-week study. However, some target organs identified after 4 weeks at 8 mg/kg (heart, spleen, adrenals, GI tract, prostate, parathyroid, seminal vesicles) were not identified in the current study at a high dose of only 2 mg/kg. Lympho-histiocytic inflammation was observed in numerous tissues/organs in high dose animals and gross findings at the high dose were noted in the epididymides, glandular stomach, testes and thyroids (not reversible). Other findings at the high dose included increased calcium excretion and decreased urine osmolality, and a reversible decrease in estrus events.

In dogs, a 14 day intravenous administration (0.01, 0.03 and 0.06 mg/kg/day) resulted in a NOAEL of 0.03 mg/kg. Target organs of toxicity included the heart, and, possibly, the ovaries and uterus, mammary glands, testes, kidneys and duodenum. A 4 week oral administration study (2, 6 and 18 mg/kg) resulted in a NOAEL of 2 mg/kg. Target organs of toxicity included the heart, thymus, testes, lung, stomach and epididymides. Primary histologic findings were noted in the heart (inflammation of myocardium and nutritive vessels in right atrium and auricle;

reversible focal neutrophilic infiltration in the right auricle), thymus (atrophy) and testes (tubular degeneration with dysspermia in one high dose animal). Similarly, gross findings were observed in the heart (bleeding and scar tissue), stomach (swollen mucosa), intestine (red spots, swollen mucosa) and liver (swollen) but were not associated with changes in organ weight. Clinical observations included vomiting, tremors, reduced body weight gain and food consumption, increased leukocyte counts, erythrocyte sedimentation rate, alkaline phosphatase, and increased urinary levels of erythrocytes, leukocytes, phosphate and kidney epithelial cells. The NOAEL for the chronic 6 month study (0.2, 1 and 4 mg/kg, tablet) was the low dose of 0.2 mg/kg and was one-tenth of that observed in the 1 month oral (suspension) study. The primary target organ of toxicity was the heart. In addition, the pancreas, lung, liver and urinary bladder (females) were identified at 6 months though not at 1 month. Conversely, targets identified at 1 month (thymus, testes and epididymides) were not identified at 6 months, due likely to the reduced dosing levels in the 6 month study. The primary histologic findings in the heart included inflammatory changes in the right atrium or right auricle (mid and high doses). Other findings included liver hyperemia/congestion, lung necrosis and hemorrhage, chronic cystitis in the urinary bladder (high-dose females), and increased incidence of spermatozoa with multiple deformations (mid- and high-dose). Other significant findings included vomiting and hypersalivation, and increased AP.

Addendum: Histopathology inventory for IND 57,883.

Study No.	194/95	128/96	129/96	113/96	81/95	38/98	14/96	159/96	68/95	94/96
Duration	1/7 day	1/7 day	1/7 day	14-d, iv	4 wk, po	3-mos, po	6-mos, po	14-d, iv	4-wk, po	6-mos, po
Species	M, rat	M, rat	M, rats	rat	rat	rat	rat	Dog	Dog	Dog
Adrenals	X			X*	X*	X*	X*	X*	X*	X*
Aorta				X	X	X	X	X	X	X
Bone marrow smear										X
Bone (femur)				X			X			
Bone (tibia)				X	X	X	X			
Bone (sternum)				X	X	X	X	X	X	X
Brain:				X*	X*	X*	X*	X*	X*	X*
Cecum							X	X		X
Cervix										X
Colon							X	X		X
Duodenum				X	X	X	X	X	X	X
Epididymis		X	X	X	X	X	X	X	X	X
Esophagus				X	X	X	X	X	X	X
Eye				X	X	X	X	X	X	X
Fallopian tube										
Fat										
Gall bladder									X	X
Gross lesions	X	X	X	X	X	X	X			
Harderian gland				X	X	X	X			
Heart	X			X*	X*	X*	X*	X*	X*	X*
Hyphophysis										
Ileum				X	X	X	X	X	X	X
Injection site	NA	NA	NA	X	NA	NA	NA	X	NA	NA
Jejunum				X	X	X	X	X	X	X
Kidneys				X*	X*	X*	X*	X	X*	X*
Lacrimal gland						X				
Larynx										
Liver				X*	X*	X*	X*	X*	X*	X*
Lungs			X	X*	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical										
Lymph nodes (LALN)				X	X			X	X	X
Lymph nodes, mandibular				X	X			X	X	?
Lymph nodes, mediastinalis				X	X			X	X	?
Lymph nodes, mesenteric				X	X	X	X	X	X	?
Mammary gland				X	X	X	X	X	X	X
Nasal cavity	X	X	X	X		X	X			X
Optic nerves										
Ovaries				X*	X*	X*	X*	X*	X*	X*
Oviduct							X			
Pancreas				X	X	X	X	X*	X*	X*
Parathyroid						X	X	X		X
Peripheral nerve							X			X
Pharynx										
Pituitary				X	X*	X*	X*	X*	X*	X*
Prostate				X	X*	X*	X*	X*	X*	X*
Rectum							X	X		
Salivary gland		X	X	X	X	X	X		X	X
Sciatic nerve				X	X	X		X	X	X
Seminal vesicles				X	X*	X*	X*			
Skeletal muscle				X	X	X	X	X	X	X
Skin				X	X	X	X	X	X	X
Spinal cord				X	X	X	X	X	X	X
Spleen				X*	X*	X*	X*	X*	X*	X*
Stomach				X	X	X	X	X	X	X
Testes	X	X	X	X*	X*	X*	X*	X*	X*	X*
Thoracic Limb								X		
Thymus				X*	X*	X*	X*	X	X	X
Thyroid				X*	X*	X*	X*	X*	X*	X*
Tongue				X	X	X	X	X	X	X
Trachea				X	X	X	X	X	X	X
Urinary bladder				X	X	X	X	X	X	X
Uterus				X*	X*	X*	X*	X*	X*	X*
Uterine horn										
Vagina				X*	X*	X*	X	X		

* Organ weight obtained

Addendum (cont'd): Histopathology inventory for IND 57,883.

Study No.	4D/98	252/98	216/98
Duration	1/7 day	3-month	3-month
Species	Hamster/mouse	Hamster	Mouse
Adrenals		X	X
Aorta		X	
Bone marrow smear			
Bone (femur)			
Bone (tibia)			
Bone (strenum)			X
Brain:		X	X
Cecum		X	
Cervix			
Colon		X	X
Duodenum		X	X
Epididymis	X	X	X
Esophagus		X	X
Eye		X	X
Fallopian tube			
Fat			
Gall bladder		X	
Gross lesions		X	X
Harderian gland		X	X
Heart		X	X
Hyphophysis			
Ileum		X	
Injection site	NA	NA	NA
Jejunum		X	X
Kidneys		X	X
Lacrimal gland		X	X
Larynx			
Liver		X	X
Lungs		X	X
Lymph nodes, cervical			
Lymph nodes (LALN)			
Lymph nodes, mandibular			
Lymph nodes, mediastinalis			
Lymph nodes, mesenteric		X	X
Mammary gland		X	X
Nasal cavity	X	X	X
Optic nerves			
Ovaries		X	X
Oviduct		X	X
Pancreas		X	X
Parathyroid		X	
Peripheral nerve		X	X
Pharynx			
Pituitary		X	
Prostate		X	X
Rectum		X	
Salivary gland			
Sciatic nerve			
Seminal vesicles		X	X
Skeletal muscle		X	X
Skin		X	X
Spinal cord		X	X
Spleen		X	X
Stomach		X	X
Testes	X	X	X
Thoracic Limb			
Thymus		X	X
Thyroid		X	X
Tongue		X	
Trachea		X	X
Urinary bladder		X	X
Uterus		X	X
Uterine horn			
Vagina		X	X

* Organ weight obtained

GENETIC TOXICOLOGY:

Mammalian bone marrow chromosome aberration test in the mouse with oral administration of B9302-107

Study/Protocol No.: 0583 *Report No.*: 92/99 *Volume*: 5.3

Study Dates:	Starting date 8/26/1998; report issued 5/3/1999
Testing Lab:	Byk Gulden, Institute of Pathology and Toxicology, Hamburg, Germany
Test Article:	B9302-107 (Lot No. AM50/126; purity = 99.8%) aqueous solution with 4 g hydroxymethylcellulose
Volume:	10 ml/kg
GLP:	Yes.
QA Report:	Yes.

Methods: B9302-107 was evaluated using the in vivo bone marrow chromosome aberration test with 6 week old NMRI mice (5/sex/dose group/time point, 18 or 42 hours). Mice were administered a single oral (gavage) dose of the test drug (100, 300 or 900 mg/kg), vehicle control (aqueous solution with 4 g hydroxymethylcellulose) or positive control (cyclophosphamide, 25 mg/kg; assessed at 18 hours only). Doses were selected based upon a range-finding experiment for the in vivo micronucleus assay which had shown 1000 mg/kg to be severely toxic in 4 female mice (ptosis, piloerection increased respiration rate, reduced activity, weight loss) and 900 mg/kg was tolerated by 7 of 8 female mice. Following dosing and ip administration of colchicine solution, bone (femur) marrow was flushed from the femur and a sample of a cell suspension was taken on slides. One hundred metaphase cells per sample were evaluated for damage such as achromatic lesions, chromatid breaks, isochromatid breaks, chromatid translocations, dicentric chromosomes, rings, and polyploid cells. The percentage of mitotic cells was calculated after counting 1000 cell nuclei per sample. Data were not evaluated for statistical significance. Criteria for a valid test and a positive response were not stated by the sponsor.

Results: One mid-dose male died and was replaced by a reserve animal. Males and females responded similarly to administration of the test substance. At 18 hours post-dosing, there were no increases in the number of damaged metaphases with or without achromatic lesions, with chromatid translocations or breakage events per cell. Similar results were observed at 42 hours post-dose, although one high-dose male did demonstrate an increase in the number of damaged metaphases without achromatic lesions (7 versus control range of 0-4), and breakage events per cell (0.1 versus control range of 0.01 to 0.05). No test substance related effects were observed with mitotic indices. The positive control substance produced expected increases in damaged metaphases and breakage events per cells.

Under the conditions of this assay, B9302-107 tested negatively in the in vivo bone marrow chromosome aberration assay. This conclusion is in concurrence with the sponsor's conclusion.

^{32}P-Postlabeling assay for detection of adduct formation by 2,6-xylidine, 4,4'-methylenebis (2-chloroaniline), 4-amino-3,5-dichloropyridine and 2-acetylaminoflourene in rat nasal mucosa, liver and testes: a pilot study.

Study/Protocol No.: R-1867 *Report No.*: 14E/99 *Volume*: 5.3

Study Dates: Starting date 5/1998; report issued 11/30/1998
Testing Lab: (b) (4)
Test Article: 2,6-xylidine (2,6-X): Lot 10910TQ; Purity = 99%
4,4'-methylenebis(2-chloroaniline) (MOCA): Lot 079424; Purity = 97%
4-amino-3,5-dichloropyridine (ADCP): Lot 10018687; Purity = 98%
2-acetylaminofluorene (2-AAF): Lot 057H0293; Purity = 98%
Volume: 5 ml/kg
GLP: No.
QA Report: No.

Methods: The purpose of this study was to identify a suitable positive control for further experiments in which Roflumilast would be administered to male Wistar rats in measuring levels of DNA adducts. Rats were exposed via intragastric instillation at selected dose levels (310 mg/kg 2,6-X, 100 mg/kg MOCA, 0.5 mg/kg ADCP; dissolved in olive oil) for 7 days. 2-acetylaminofluorene was used as the positive control since it historically gives strong positive responses in the 32P-postlabeling assay using liver DNA. Ten male rats (9 weeks old) were used (1 animals per test substance with one animal per substance used in case of technical difficulties. Animals were killed 24 hours after the final dosing and DNA was eventually extracted from liver testes and nasal mucosa tissue cells, isolated and then labeled with 32P adenosine-5'-triphosphate and T4 polynucleotide kinase. Samples were then purified and resolved using two directional thin-layer chromotography. Adducts were detected by placing the TLC plates against sensitized screens in BioRad Sample Loading Docks for 5 or 10 minutes. Radioactive areas were quantitated using a computer software package. Evaluations of chromatograms were done by comparisons of treated animals with positive and negative controls. A positive result was obtained when chromatograms from treated animals exhibited additional radioactive areas not present in controls.

Results: Standards prepared from DNA reacted with N-acetoxy-ADCP or N-hydroxy 2,6-xylidine provided reference markers to develop appropriate solvent systems to separate the adducts. Results of DNA adduct levels are given in Table 78:

Table 78: DNA adduct levels in tissue.

	Adduct levels in Tissues (adducts in 10to9 normal nucleotides		
Compound	Liver	Nasal Mucosa	Testes
STD ADCP	1301	471	944
STD 2,6-X	8143	4955	4872
2-AAF	733	ND	ND
ADCP	ND	ND	ND
MOCA	17920	237	35
2,6-X	603	1578	24

Animals treated with unmodified ADCP showed no DNA adduct formation in the liver, nasal mucosa or testes. The synthetic derivative of ADCP, N-acetoxy-ADCP, reacted with DNA to produce one major adduct and provide a chromatographic standard. Both MOCA and 2,6-X demonstrated adduct formation at varying levels in all tissues tested. As the purpose of this study was to identify a suitable positive control for further experiments with Roflumilast, 2,6-X was selected because it is a monocyclic aromatic amine, a nasal carcinogen, and gives detectable levels of DNA adducts in all tissues to be tested.

^{32}P-Postlabeling assay for detection of adduct formation by Roflumilast (B9302-107) in rat nasal mucosa, liver and testes DNA.

Study/Protocol No.: R-1866 *Report No.*: 71E/99 *Volume*: 5.3

Study Dates: Starting date 7/24/1998; report issued 5/12/1999
Testing Lab: (b) (4)
Test Article: Roflumilast (B9302-107; Lot 10910TQ; Purity = 99%) in 4% Metolose SM-15
Volume: 5 ml/kg
GLP: Yes.
QA Report: Yes.

Methods: Male Wistar rats (9 weeks old) were dosed with Roflumilast via intragastric instillation for 7 days. 2,6-X (dissolved in olive oil) was used as the positive control due to positive findings in a previous pilot study.

Dose group	# of male rats dosed	Minimum # processed for 32P-postlabeling
4% aq methylcellulose	4	3
0.5 mg/kg Roflumilast	4	3
2.5 mg/kg Roflumilast	4	3
Olive oil	2	1
310 mg/kg 2,6-X	2	1

Roflumilast doses were selected based upon previous toxicity studies in which a dose of 8 mg/kg induced mortality in a 4-week study and a dose of 2.5 mg/kg induced nasal toxicity. Animals were killed 24 hours after the final dosing and DNA was eventually extracted from liver, testes and nasal mucosa tissue cells, isolated and then labeled with 32P adenosine-5'-triphosphate and T4 polynucleotide kinase. Samples were then purified and resolved using multidirectional thin-layer chromatography. Adducts were detected by placing the TLC plates against sensitized screens in BioRad Sample Loading Docks for 5 or 10 minutes. Radioactive areas were quantitated using a computer software package. Evaluations of chromatograms were done by comparisons of treated animals with positive and negative controls. A positive result was obtained when chromatograms from treated animals exhibited additional radioactive areas not present in controls.

Results: It should be noted that only representative chromatography samples were included in the study report, and thus, the following comments are based upon the "Results and Discussion" section of the study report. No radioactive spots were found DNA samples from animals treated with Roflumilast that corresponded to adducts observed with DNA treated with synthesized N-acetoxy-ADCP. ADCP was considered to be the most likely moiety to form DNA adducts . In the pilot study, ADCP itself failed to produce DNA adducts in vivo. The positive control, 2,6-X, produced DNA adducts in the three tissues tested (highest in nasal mucosa, visible in liver, borderline in the testes) indicating the validity of the assay. No adducts were formed that could be attributed to Roflumilast (whole compound). Weak spots were noted but the locations were not consistent from pate to plate and the response was not dose-related.

The report conclusion states that no evidence of DNA adduct formation in the tissues tested was obtained following Roflumilast administration in rats under the conditions of this assay. While this is true, this study is not considered to definitively offset concerns over the potential genotoxicity of Roflumilast on its own since the positive genotoxic effects occurred in bone marrow, a tissue not assessed in the current assay.

Summary of Genetic Toxicology: Previously submitted studies (reviewed in the Pre-IND Carcinogenicity Protocol Review; dated February 2, 1999; see attachment) show that B9302-107 was negative for mutagenicity and clastogenicity in the Ames Assay, an in vitro chromosome aberration assay with human lymphocytes, an HPRT test with V79 cells, and an in vitro micronucleus test with V79 cells. However, B9302-107 was positive in the in vivo mouse micronucleus assay, inducing a low number of micronuclei in polychromatic erythrocytes after a dose of 300 mg/kg in males at 48 hours and at a dose of 900 mg/kg in males and females at 24and 48 hours. These positive findings produced concern within the Division as to the safety of the proposed clinical trial. After communicating these concerns to the sponsor, the sponsor inactivated the IND pending further assessment of these findings. The sponsor subsequently submitted an in vivo mammalian bone marrow chromosome aberration assay and a postlabeling assay for detection of adduct formation in the rat nasal mucosa, liver and testes. B9302-107 was negative in the in vivo mammalian bone marrow chromosome aberration assay and no evidence of DNA adduct formation in the tissues tested was obtained following Roflumilast administration in rats, although bone marrow was not assessed for adduct formation. Thus, following consultation with CDER's Genetic Toxicology Committee, it was concluded that the sponsor had adequately assessed concerns regarding the genotoxic potential of B9302-107 since both in vitro and in vivo chromosome aberration studies were negative. However, the sponsor will be encouraged to assess the findings of their carcinogenicity studies prior to proceeding with Phase III clinical trials.

A metabolite of B9302-107, B9202-045, tested negatively in the in vivo mouse micronucleus test at doses up to 300 mg/kg.

SPECIAL TOXICOLOGY:

The local toxicity of B9302-107 after a single intramuscular injection in the rat

Protocol No.: O427 *Report No.*: 122/96 *Volume*: 1.27

Study Dates: Starting date 6/20/96; report issued 10/18/96
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch BY217-11-1-1; purity = 96.62%)
Concentration: 0.2 mg/ml; 0.02%
Dose Volume: 0.1 ml
GLP: Yes.
QA report: Yes.

Methods: A single intramuscular administration of test compound (0.02% B9302-107 emulsion) was injected into the quadriceps muscle of one hind leg of 6 male Wistar rats (10 weeks of age; 305-345 g). The other hind leg was injected with placebo. Two additional animals received sodium chloride 0.9% in each quadriceps. Animals were killed 48 hours after injection and the muscle was evaluated macro- and microscopically.

Results: Petechial hemorrhages (largest altered areas measured 1.6-119.1 mm^3) were observed in areas surrounding the injection sites after both drug and placebo administration. Histologic evaluation identified minimal to moderate edema, necrosis and inflammatory reactions in both groups, with the placebo group showing greater severity. The reduced inflammation seen with test substance may be due to its proposed mechanism of action. The control solution (NaCl) caused no specific alterations.

The local toxicity of B9302-107 after a single intravenous, paravenous or intraarterial injection in the rabbit

Protocol No.: O428 *Report No.*: 123/96 *Volume*: 1.27

Study Dates: Starting date 6/20/96; report issued 10/18/96
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch BY217-11-1-1; purity = 96.62%)
Concentration: 0.2 mg/ml; 0.02%
Dose Volume: 0.5-1 ml
GLP: Yes.
QA report: Yes.

Methods: A single administration of test compound (B9302-107 0.02%; 0.5-1 ml) was injected into one ear of female New Zealand White rabbits (n = 3/group), either iv in the marginal ear vein, paravenous to the marginal ear vein, or intra-arterial in the central ear artery. The other ear of each animal was injected in a similar mode with the placebo emulsion. Animals were evaluated for tissue reaction for 8 days and killed on Day 9 after injection and the ears were evaluated microscopically.

Results: Following iv administration of B9302-107 0.02% or placebo in the marginal ear vein (1 ml), paravenous injection (0.5 ml), or intra-arterial injection (0.5 ml), no macroscopic differences were noted between test drug or placebo injection sites (discoloration within 0.5 to 1 hour; scabby injection sites). Microscopic findings included a minimal thrombotic inflammation in one ear treated with test drug following iv administration.

Test for sensitizing properties of B9302-107 (topical) in the guinea pig

Protocol No.: VG0354 *Report No.*: 131/95 *Volume*: 1.27

Study Dates:	Starting date 5/5/95; report issued 7/31/95
Testing Lab:	Byk Gulden, Hamburg, Germany
Test Article:	B9302-107 (Batch 46/251; purity = 98.82%)
Concentration:	0.2 mg/ml; 0.02%
Dose Volume:	0.5-1 ml
GLP:	Yes.
QA report:	Yes.

Methods: Guinea pigs (274-436 g) were pretreated twice (2 x 0.1 ml B9302-107 0.5% intracutaneously with Freund's adjuvant on Day 2; 0.03 g B9302-107 25% in vaseline topically for the second on Day 9) with test drug in order to induce hypersensitivity in the Guinea Pig Maximization Test. In week four, animals were treated a third time (0.03 g B9302-107 25% in vaseline, topical challenge). Controls received placebo with first two administrations and treated with test drug at the time of challenge.

Results: Following the challenge procedure, no animals showed sensitizing properties in the maximization test. No signs of primary irritation were observed in female guinea pigs administered 1-25% B9302-107 in vaseline when applied topically.

Summary of Special Toxicity: Tests with B9302-107 were performed to assess the local toxicity of a single intramuscular dose administration in rats, a single intravenous, paravenous or intraarterial injection in the rabbit, and sensitizing properties following a topical application in rabbits. In rats, petechial hemorrhages in areas surrounding the injection sites and minimal to moderate edema, necrosis and inflammatory reactions were observed after both drug (0.02% B9302-107 emulsion) and placebo administration, with the placebo group showing greater severity. In rabbits, iv administration in the marginal ear vein (1 ml), paravenous injection (0.5 ml), or intra-arterial injection (0.5 ml) of B9302-107 0.02% resulted in no macroscopic differences between the test drug or placebo injection sites (discoloration within 0.5 to 1 hour; scabby injection sites). Microscopic findings included a minimal thrombotic inflammation in one ear treated with test drug following iv administration. In Guinea pigs, animals showed no sensitizing properties in the maximization test following the challenge procedure and no signs of primary irritation were observed in female guinea pigs administered 1-25% B9302-107 in vaseline when applied topically.

OVERALL SUMMARY AND EVALUATION:

Pharmacology: B9302-107 showed primary inhibition activity with PDE IV receptors with minimal inhibition of PDE I-III and V receptors. Activity was not observed with other receptors. The metabolite B9302-077 also showed significant inhibition of PDE IV activity. B9302-107 exhibited a 50- to 220-fold greater potency than SB207499 in inhibiting lipopolysaccharide-induced TNF-α release. In vitro/in vivo models demonstrated vasodilatory activity, as well as inhibition of inflammatory cell and protein accumulation, reactive oxygen species release, and LTB_4 synthesis, and inhibition of agonist- or antigen-induced tracheal contraction, decreased airway conductance and dynamic compliance, decreased expiratory flow and tidal volume, and bronchoconstriction.

Safety Pharmacology: The sponsor performed studies to assess the effects of B9302-107 on cardiovascular function, hemodynamics/respiration, locomotor activity, behavior, neuromuscular function, pupil diameter stomach acid secretion, GI motility, body temperature, and renal function. Cardiovascular effects (increased HR, decreased blood pressure) were noted in rats after both oral and IV dosing. A cumulative IV injection also induced increased blood pressure, heart rate, and cardiac contractility in cats, as well as a rise in the ST-phase and increased breathing rate and respiratory minute volume. Bolus injections increased coronary flow in guinea pig Langendorff hearts. Behavioral effects following oral or IV dosing in rats and mice included reduced locomotion, activity and vigilance, stalking gait, tremor, hypernoe, segregation, and limb splay lying, head twitches, forepaw shaking, increased grooming, brown/bloody colored noses and eyes with decreased palpebral fissure, prolongation of hexobarbitol-induced loss of righting reflex and increased ethanol-induced sleeping time. Animals also exhibited increased tonic convulsions and lethality in a potentiation test and shortened time to tonic convulsions. Anticonvulsive properties in an inhibition test and protection against maximal electroshock were not noted. Other findings included increased basal acid secretion and reduced urine excretion, correlated with increased urine osmolality and Na+ and K+ excretion, in rats, and reduced intestinal motility and rectal body temperature in mice.

Pharmacokinetics: Systemic exposure to B9302-107 increased proportionally in rats, supra-proportionally in dogs, and sub-proportionally in mice. Elimination half-lives ranged from 1 to 7 hours in rats and dogs. Rats demonstrated a 5-fold increase in levels of B9202-045 compared to parent compound and a 25-fold increase in B9502-044. Hamsters were similar in terms of metabolite production although the ratios varied. Mice and dogs produced low levels of B9202-045; levels of B9502-044 were similar to parent in the mouse and only 2% in the dog. Humans also produced low levels of B9202-045 and B9502-044. Clearance in rats was 4 l/hr and volume of distribution was 5 l following IV administration; similar values were noted in dogs and females rabbits. Following multiple dose administration, systemic exposure increased sub-proportionally in dogs. Drug absorption was 32% in rats following oral/id dosing while absolute bioavailability was low in rats and female rabbits (2-4%) but increased in dogs, hamsters and mice (34-61%). The greatest levels of drug-related radioactivity were observed in the nose, kidney and liver of the rat. Only low to undetected levels were observed in the nose of mice and hamsters while the primary sites of distribution were the lungs, liver, adrenals and bone marrow.

The dominant biotransformation route in all species was N-oxidation of the parent drug (B9502-044), cleavage of the parent to yield B9202-045 which in turn was oxidized to B9502-054, or O-dealkylation of the parent compound. Administration of metyrapone, a P450 inhibitor, was demonstrated to be involved in the activation of B9502-054, the major metabolite in the rat nose. Various unidentified metabolites were also observed. In vitro studies demonstrated that B9302-107 was converted to B9202-045 or B9502-054 only by rat liver microsomes. Incubation with B9202-045 resulted in formation of B9502-054 by rat, hamster, mouse and dog olfactory and respiratory microsomes. None was produced with human microsomes indicating that B9502-054-induced nasal lesions were not a safety concern in humans. Excretion of B9302-107 related radioactivity was primarily fecal in rats, dogs, mice and hamsters following oral administration. Urinary excretion increased following intravenous administration and was the predominant route in rats and hamsters.

Acute Toxicity: In mice, a maximum non-lethal dose of 600 mg/kg and a minimum lethal dose of 1100 mg/kg were demonstrated following oral dosing; the maximum IV dose (20 mg/kg) did not induce lethality. Rats demonstrated greater sensitivity as the maximum non-lethal oral doses were 100 mg/kg in females and 400 mg/kg in males; the minimum lethal doses were 400 mg/kg in females and 700 mg/kg in males. The minimum lethal dose following IV dosing in rats was 16 mg/kg and the maximum non-lethal dose was 12 mg/kg. Animals in all studies exhibited clinical signs and gross and histological assessment of selected tissues demonstrated findings in the glandular stomach, testes and olfactory epithelium. An assessment of local drug effects on the nasal cavity showed that the parent compound B9302-107 does not directly induce nasal toxicity; labeling of the amino-dichloropyridine moiety of B9302-107 was necessary to achieve binding. In contrast to mice and hamsters, rats showed heavy labeling which was concentrated in cells of the olfactory submucous glands.

Single/repeat (7 day) oral (gavage) dose studies were performed primarily to assess nasal and reproductive organ toxicity in male Wistar rats, hamsters and mice. B9302-107 induced comparable nasal cavity lesions in the hamster, mouse and rat following a single oral dose, although lesion severity was reduced in hamsters and mice. However, lesion severity was comparable when hamsters and mice were administered B9202-045 or B9502-054 directly. B9302-107 induced male reproductive organ toxicity in rats but not in hamsters, while B9202-045 and its N-oxide induced reproductive organ lesions in hamsters but not in rats. B9202-045 or its N-oxide may, thus, be responsible for the observed nasal lesions, while B9302-107 or another metabolite may be responsible for the reproductive organ changes. In repeat dose studies, 8 mg/kg B9302-107 or 6.4 mg/kg B9202-045 or B9502-054 again induced nasal toxicity in rats; lesions in the testes/epididymides were noted in B9302-107-treated rats only. B9202-045 (0.05-6 mg/kg) induced necrosis and basal cell regeneration at all doses but the lowest.

Subchronic Toxicity: Studies were performed in rats (14 day IV administration, 4 week and 4 week/3 month oral administration) and dogs (14 day IV administration and 4 week oral administration). Screening studies were also performed in mice and hamsters. In rats, a NOAEL was not selected in a 14 day intravenous study (0.5 to 2 mg/kg) due to adverse findings in the nasal cavities, reduced body weight gain and clinical observations at all doses. The primary target organ of toxicity was the nasal cavity with greatest severity in females. In the 4 week oral

(gavage) toxicity study (0.5, 2, and 8 mg/kg), a NOAEL was not identified since the nasal cavities were not assessed and adverse findings noted in tissues of high dose males were not assessed in lower dose groups. Lethality was observed at the high dose and target organs of toxicity included the heart, adrenals, stomach, parathyroid, spleen, testes and epididymides, and the thymus, prostate and seminal vesicles. Non-reversible increases in QT time, QRS time and QαT-time were also observed at the high dose. Other significant findings included a reversible reduction in estrus events and prolongation of diestrus phase, clinical observations, reduced body weight gain and food consumption. In the 3-month study (0.02, 0.2 and 2 mg/kg/day), the mid-dose of 0.2 mg/kg/day was selected as the NOAEL. Target organs of toxicity were the nasal cavities, testes and epididymides, and the thymus. These target organs (except for nasal cavities) were identified in the 4-week study. However, some target organs identified after 4 weeks at 8 mg/kg were not identified in the current study (high dose of 2 mg/kg). Lympho-histiocytic inflammation was observed in numerous tissues/organs in high dose animals and gross findings were noted in the epididymides, glandular stomach, testes and thyroids. A reversible decrease in estrus events was also noted.

In dogs, 14 day intravenous administration (0.01, 0.03 and 0.06 mg/kg/day) resulted in a NOAEL of 0.03 mg/kg. Target organs of toxicity again included the heart, and, possibly, the ovaries and uterus, mammary glands, testes, kidneys and duodenum. A 4 week oral administration study (2, 6 and 18 mg/kg, suspension), resulted in a NOAEL of 2 mg/kg with target organs of toxicity including the heart, thymus, testes, lung, stomach and epididymides. Histologic assessment revealed indications of vasculitis. Other findings included clinical observations, reduced body weight gain and food consumption, and clinical and hematological parameters.

A 3 month screening study in mice identified target organs of toxicity as the nasal cavity, adrenal glands and spleen following oral (gavage) administration (6, 12, 18 mg/kg). In general, lesion severity was minimal. In a similar study in hamsters (4, 8 or 16 mg/kg), target organs of toxicity included male reproductive organs, the nasal cavity, the eye and, possibly, the prostate, adrenals and pancreas.

Chronic Toxicity: Six month oral studies were performed in rats and dogs. In rats (0.5, 1 and 2.5 mg/kg), a NOAEL of 0.5 mg/kg was identified. This NOAEL dose is similar to that observed in the 3 month oral study (0.2 mg/kg). Target organs of toxicity included the nasal cavity, the testes, seminal vesicles and epididymis, and, possibly, the stomach. No significant findings in the thymus, identified as a target after 3 months, were observed. One high-dose female had a mammary gland adenoma at 6 months of age. Other significant findings included a reduced number of estrus events, and non-dose-dependent increases in mean PQ, QαT, and QT times in all treatment groups. In dogs (tablet: 0.2, 1 and 4 mg/kg), the NOAEL was the low dose of 0.2 mg/kg and was one-tenth of that observed in the 1 month study. The primary target organ of toxicity was the heart. In addition, the pancreas, lung, liver and urinary bladder were identified at 6 months though not at 1 month. Targets identified at high doses after 1 month (thymus, testes and epididymides) were not identified at 6 months, likely due to reduced dosing levels in the 6 month study.

Genotoxicity: B9302-107 was negative for mutagenicity and clastogenicity in the Ames Assay, an *in vitro* chromosome aberration assay with human lymphocytes, an HPRT test with V79 cells, and an *in vitro* micronucleus test with V79 cells. However, B9302-107 was positive in the *in vivo* mouse micronucleus assay, inducing a low number of micronuclei in polychromatic erythrocytes after a dose of 300 mg/kg in males at 48 hours and at a dose of 900 mg/kg in males and females at 24and 48 hours. After concerns regarding these findings were communicated to the sponsor, the sponsor inactivated the IND pending further assessment of these findings. Subsequent evaluation showed that B9302-107 was negative in the *in vivo* mammalian bone marrow chromosome aberration assay and no evidence of DNA adduct formation in the tissues tested was obtained following Roflumilast administration in rats, although bone marrow was not assessed for adduct formation. Following consultation with CDER's Genetic Toxicology Committee, the Division concluded that the sponsor had adequately assessed concerns regarding the genotoxic potential of B9302-107 since both *in vitro* and *in vivo* chromosome aberration studies were negative. However, the sponsor will be encouraged to assess the findings of their carcinogenicity studies prior to proceeding with Phase III clinical trials.

A metabolite of B9302-107, B9202-045, also tested negatively in the *in vivo* mouse micronucleus test at doses up to 300 mg/kg.

Special Toxicology: Mild local toxicity was observed in rats following IM injection of test substance. No local effects were observed in rabbits following single IV, paravenous or intra-arterial administration and guinea pigs showed no sensitizing properties or irritation following topical application.

The sponsor proposed a 28 day clinical trial to evaluate the safety and efficacy of B9302-107 (0.5 mg, tablet) in 20 asthmatic patients. The clinical trial is supported by six-month toxicology studies in rats and dogs which produced NOAELs of 0.5 and 0.2 mg/kg, respectively. The systemic exposures at the NOAEL doses for these studies were 7-17 and 183-224 μg/l.hr in rats and dogs, respectively. Although the exposure in rats is less than that in humans at the proposed clinical dose (0.5 mg; 33-34 μg/l.hr), human AUCs were estimated for a longer time interval (AUC (0-inf) vs AUC(0-8). Thus, systemic exposure in the rat at the NOAEL dose is expected to be at least comparable to the human AUC. This level of exposure in rats and dogs provides an adequate safety margin compared to human exposure levels, especially in light of the extensive clinical experience to date. In addition, the genotoxicity issues which resulted in the sponsor inactivating the IND have been adequately resolved. Thus, the proposed clinical trial is considered to be reasonably safe to proceed.

RECOMMENDATIONS

1. The proposed clinical trial is considered to be reasonably safe to proceed.

2. The Division recommends that the sponsor evaluate the results of the preclinical carcinogenicity studies prior to initiating Phase III clinical studies.

Timothy J. McGovern, Ph.D., Pharmacologist

Comment for letter to Sponsor:

1. The Division recommends that you evaluate the results of the preclinical carcinogenicity studies prior to initiating Phase III clinical studies.

Attachments: Pre-IND Carcinogenicity Protocol Review
Minutes of Executive CAC meeting for B9302-107

Original IND 57,883
CC: HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/L. Gilbert-McClain
HFD-570/L. Jafari
HFD-570/T.J. McGovern

Attachment 1:

HFD-570 : DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Pre-IND Carcinogenicity Protocol Review

IND No. Pre-IND

Serial No. NA

Protocol Submission Date: 30 DEC 1998

Supporting Data Submitted: 03 MAR 1998
30 DEC 1998
19 JAN 1999

Review Completed: 02 FEB 1999

Reviewer: Timothy J. McGovern, Ph.D.

Information to be Conveyed to Sponsor: Yes ( ), No (4)

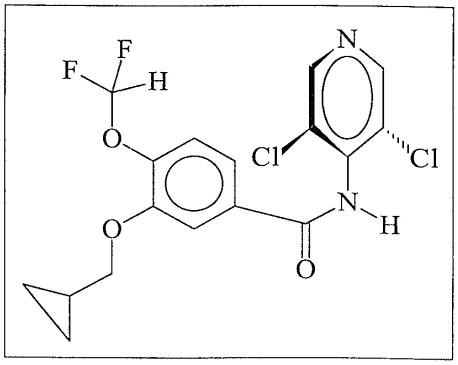
Sponsor: Byk Gulden **Representatives:** Altana, Inc.

Manufacturer: Byk Gulden

Drug Names: Roflumilast *Code Name:* B9302-107, BY217

Chemical Name: 3-Cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichlorpyrid-4-yl)-benzamid

Structure:



Molecular Weight: 403.22

Formula: $C_{17}H_{14}Cl_2F_2N_2O_3$

Related INDs/NDAs: None

Class: Phosphodiesterase IV inhibitor

Indication: Asthma

Route of Administration: Oral

Proposed Clinical Protocol: None

Previous Clinical Experience: Seven Phase I studies ranging from single oral or iv dose safety and tolerability studies, 7-day to 4-week oral dosing studies, cardiac function and effect of food intake on pharmacokinetics. Doses up to 1 mg were well tolerated in single dose studies (adverse events include headache and diarrhea). Repeated dose studies revealed a maximum tolerated dose of 0.5 mg (adverse events included headaches, back pain, liquid stool, and insomnia). Phase II studies include three 6 week trials in asthmatic subjects at a dose of 0.5 mg.

Previous Review(s), Date(s) and Reviewer(s): None

Background: A Pre-IND meeting was held with the sponsor on May 29, 1998 (see minutes of meeting). Briefly, the pharmacology/toxicology issues addressed concerned the species specific metabolism of Roflumilast by rats and the use of hamsters for carcinogenicity studies. The Agency informed the sponsor that, although it appeared that the metabolic profile of Roflumilast was unique in the rat, a definitive assessment could not be made on the species specific metabolism until "in preparation" studies included in the Pre-IND package were submitted for review along with any human PK information. In addition, the sponsor was requested to perform a toxicity study demonstrating the role of metabolism and to identify specific P450 enzymes involved in the metabolism. The sponsor was also informed that the use of hamsters for carcinogenicity studies was an issue that must be decided by the CDER Carcinogenicity Assessment Committee and that a briefing package should be submitted along with the studies indicated above. To date, the only additional studies submitted are summaries of the 3-month dose-ranging studies in mice and hamsters.

The following table summarizes the studies reviewed for this Carcinogenicity Protocol review:

Study	Res. Report #	Vol. of Pre-IND Package
Pharmacokinetics:		
Pharmacokinetics in rats following single oral doses	199/96	9
Pharmacokinetics of BY217/metabolites in hamsters, single oral doses	200/96	10
Pharmacokinetics of B9202-045 (metabolite), rats, single oral doses	35/97	11
Tissue distribution in rat, single oral and iv administration	133/95	5
Tissue distribution in mouse, single oral and iv administration	133/97	11
Metabolism of BY217 in the rat, oral, iv or id administration	141/97	11
Preliminary biotransformation in hamsters, oral and iv	197/96	9
Single/Multiple Dose Toxicology:		
Single/repeated dose toxicity in rats of BY217 and metabolite	194/95	5
Single/repeated dose toxicity in rats of BY217 and metabolites B9202-045 or B9502-054 (screening study)	128/96	8

Study	Res. Report #	Vol. of Pre-IND Package
Single/repeated dose toxicity in rats of BY217 and metabolite B9202-045 (screening study)	129/96	9
Histopathological assessment of nasal and testicular toxicity in hamsters and mice following single/multiple administration of B9302-107, B9202-045 and B9502-054	4D/98	11
Sub-Chronic Toxicity:		
Summary of results of 3-month mouse study	216/98	12/30/1998 submission
Summary of results of 3-month hamster study	251/98	12/30/1998 submission
Chronic Toxicity:		
6-month oral toxicity in Wistar rats	14/96	6
Genetic Toxicology:		
Reverse mutation assay (Ames)	127E/95	4
Chromosome aberration, in vitro, human lymphocytes	129/95	4
HPRT test in V79 cells	67/97	11
In vitro micronucleus test with V79 cells	113/97	11
In vivo micronucleus test in mice, oral administration of B9302-107	106/96	1/19/1999 submission
In vivo micronucleus test in mice, oral administration of B9202-045	106/98	1/19/1999 submission
Carcinogenicity Protocol:		
Rationale for species selection of Mouse and Hamster Carc. Studies	313P/98	12/30/1998 submission
Rationale for dose selection of Mouse and Hamster Carc. Studies	NA	12/30/1998 submission

Studies Not Reviewed in this IND: None

Studies Previously Reviewed: None

Note: Portions of this review were excerpted directly from the sponsor's submission.

PHARMACOKINETICS AND TOXICOKINETICS

Pharmacokinetics: Pharmacokinetic studies for male Sprague Dawley rats and male Syrian Golden hamsters were reviewed along with brief summaries of additional pharmacokinetic studies in mice (Report 262/97) and man (Reports 128E/97 and Report 177/98) that were submitted in the Pre-IND package of 12/30/1998. The results of the single dose pharmacokinetic studies in rats, hamsters, mice and man (7-day study) are summarized in Table 1. The exposure values for mice are back calculated from a dose of 9 mg/kg B9302-107. Systemic exposure to a single dose of B9302-107 increased proportionally with increasing dose in male Sprague Dawley rats and supra-proportionally in male Syrian golden hamsters. Exposures to the primary metabolites, the N-oxide of the parent drug (B9502-044, thought to be the primary carrier of biological activity) and B9202-045 (dichlororaminopyridine, DCAP), increased proportionally and sub-proportionally, respectively in both species. Systemic exposures to the metabolites were greater than that of the parent compound, indicating a pronounced first-pass effect. Metabolism of the parent drug did not appear to be as prevalent in the mouse as in the rat and hamster. Following drug administration, the greatest exposures in descending order were to the N-oxide of the parent drug (B9502-044), the N-oxide of DCAP (B9502-054, hamsters at 9 mg/kg, mice), DCAP and the parent drug (see Figure 1 for metabolite structures, page 45). At a dose of 1.5

mg/kg, AUC levels of B9302-107 and its N-oxide in rat serum were 16-fold and 3-fold greater, respectively, than in hamsters, but only one-half and 16-fold greater, respectively, than those in mice. Exposure to DCAP was 10-fold lower in the hamster and 48-fold lower in the mouse compared to the rat. The mouse and hamster were similar in terms of the low amounts of DCAP detected in the serum, with greater amounts of DCAP's N-oxide (B9502-054) than that found in the rat. In man, a dose of 0.5 mg resulted in relatively low levels of parent drug and high levels of the N-oxide of the parent drug (B9502-044), similar to the rat and hamster. However, similar to the mouse and hamster, very low relative levels of DCAP were observed in serum.

Table 1. Pharmacokinetics of B9302-107 and metabolites following single oral dose.

Species	**Dose (mg/kg)**	**Parameter**	**B9302-107** Roflumilast	**B9202-045** DCAP	**B9502-044** M1	**B9502-054** M2
Rat	0.5	Cmax (µg/l) Tmax (h) T1/2 (h) AUC (0-∞) (µg/l h) AUC ratio**	3.96 1.83 1.58 13.46	14.42 3.17 5.8 77.84 5.78	60.32 1.67 1.79 259.98 19.32	Not analyzed
Rat	1.5	Cmax (µg/l) Tmax (h) T1/2 (h) AUC (0-∞) (µg/l h) AUC ratio**	6.23 2.83 2.7 33.47	28.06 5.0 9.1 159.27 4.76	139.8 3.0 2.5 840.22 25.1	Below LLOQ of 50 ng/ml
Hamster	0.5	Cmax (µg/l) Tmax (h) AUC (0-8) (µg/l.h) AUC ratio**	< LLOQ of 1 ng/ml	2.261 2.43 8.05 n.a.	22.192 1.53 n.a. n.a.	Not analyzed
Hamster	1.5	Cmax (µg/l) Tmax (h) AUC (0-8) (µg/l.h) AUC ratio**	0.835 2.20 2.09	3.152 4.00 15.34 7.34	73.81 2.0 286.59 137.12	Not analyzed
Hamster	9	Cmax (µg/l) Tmax (h) AUC (0-8) (µg/l.h) AUC ratio**	11.12 3.20 31.21	15.255 3.60 70.79 2.27	586.68 1.40 1641.7 52.60	50.7 2.8 246.64 7.9
Mouse	1.5*	AUC (µg/l h) AUC ratio**	70.36	3.29 0.05	51.43 0.73	40.07 0.57
Human	0.5 mg	AUC (µg/l h) AUC ratio**	32.86	4.14 0.13	351.39 10.7	< LLOQ

n.a.: not ascertainable. * back calculated from a dose of 9 mg/kg. ** ratio of metabolite to parent drug.

When male Wistar rats were administered the metabolite DCAP (0.5-6 mg/kg) by oral gavage, systemic exposure to DCAP increased proportionally with dose (Table 2). Maximum serum concentrations of DCAP were achieved within 1.67 hours; elimination half-lives were similar for all dose groups, although a slight increase was noted at the HD due, possibly, to saturation of enzyme activity of the metabolic pathway. The maximum concentration of B9202-054, the N-oxide of DCAP, was achieved at 5.33 hours following the 6 mg/kg administration of DCAP and was present at approximately one-sixth the level of DCAP.

Table 2. Pharmacokinetics of DCAP in male Wistar rats following single oral doses.

	Dose group (mg/kg)				
	0.05	**0.5**	**1.5**	**3**	**6**
	B9202-045				
t1/2	2.7	2.4	2.5	2.7	3.3
tmax (h)	1	1	1	1.17	1.67
Cmax (µg/l)	21.698	283.1	716.3	1434.5	2770.5
AUC(0-∞) (µg/l h)	93.33	1212.3	3254.4	7860.6	17856
	B9202-054				
t1/2					NA
tmax (h)					5.33
Cmax (µg/l)					503.6
AUC(0-8) (µg/l h)					3143.5

Tissue Distribution: Sprague Dawley rats were administered radioactive B9302-107 (Roflumilast) at target doses of 1 mg/kg iv (with PEG) or 1 or 10 mg/kg, po (in a 4% methocel suspension). Following iv dosing, maximum tissue concentrations were noted within 0.5 hours with the greatest amounts detected in the liver, adrenals and fat tissue (Table 3). Nasal tissue concentrations were at their maximum at 8 hours. Following oral administration, maximum concentrations were noted within 4 and 8 hours in most tissues. The liver was again the site of highest detected radiation levels at these time points. As with iv dosing, maximum radioactivity levels in the nose were achieved later than most other tissues; 8 and 24 hours at 1 and 10 mg/kg, respectively. Systemic exposure was consistently greatest in the nose and liver; elimination half-life was generally long and consistently greatest in the nose and kidneys. At the two oral doses, AUC increased sub-proportionally to proportionally depending upon the tissue. Of special note are the high AUC tissue/AUC plasma ratios in the nose (5.8, 4.7, and 7.9 following administration of 1 mg/kg iv, and 1 and 10 mg/kg po, respectively). High radioactivity concentrations in the GI tract following iv administration indicate the presence of an enterohepatic cycle. The sponsor suggests that the parent and metabolites are largely excreted in the bile, and are subsequently reabsorbed from the intestine and then eliminated in the urine since a separate study demonstrated high recovery of radioactivity in the urine (62% of dose within 72 hours). Following oral administration, absorption was estimated to be 50 and 30% at 1 and 10 mg/kg, respectively.

Table 3. Tissue distribution following single doses of B9302-107 in Sprague Dawley rats.

Tissue	**Radioact Conc. µg.equiv/kg**			**AUC (0-168h) (µg.equivxh/g)**			**T1/2 (hr)**		
	1, iv	1, po	10, po	1, iv	1, po	10, po	1, iv	1, po	10, po
	0.5 hrs	4 hrs	8 hrs						
Liver	3.595	0.858	1.759	54.81	27.87	157.53	58	135	139
Adrenal	1.899	0.266	0.756	20.56	8.25	67.10	63	92	169
Kidney	1.519	0.392	0.990	23.65	10.32	79.94	117	152	231
Nose	1.090	0.335	1.474	69.73	26.25	286.88	192	110	346
Fat	3.651	0.421	0.715	16.52	4.54	43.05	32	11	187
Pancreas	0.932	0.256	0.438						
Parotis gland	1.200	0.161	0.367						
Mandibularis gland	1.035	0.152	0.397						
Harder's gland	1.164	0.178	0.367						
Thyroid	1.375	0.220	0.302	15.83	7.37	27.02	41	139	115
Testes	0.431	0.086	0.252						
Plasma	0.951	0.225	0.569	12.13	5.59	36.22	43	65	115

In rats, total radioactivity AUC in the nasal epithelium is 10 to 28-fold greater than other species administered 1 mg/kg labeled Roflumilast (Table 4; studies summarized in the submission of 12/30/1998). In addition, the ratio of nasal mucosa AUC to plasma AUC was also significantly greater in the rat (4.4 to 7.8-fold in rats vs 1.1 and 0.6 in hamsters and mice, respectively). A further increase of the ratio in rats was observed when DCAP was administered directly. Lack of association of radioactivity with the olfactory epithelium is, however, demonstrated in rats pre-treated with the cytochrome P450 inhibitor metyrapone prior to administration of DCAP (experiments in progress). The sponsor suggests that these results provide strong evidence that a P450-mediated activation of DCAP to its N-oxide (or follow-up metabolites) is a pre-requisite for association of radioactivity with the nasal epithelium of the rat.

Table 4. Comparison of total radioactivity in plasma or nasal mucosa following single oral administration of 1 mg/kg Roflumilast or DCAP.

Compound	Parameter	Rat		Hamster		Mouse	
		Plasma	Nasal mucosa	Plasma	Nasal mucosa	Plasma	Nasal mucosa
B9302-107	Cmax	0.23	0.47	0.09	0.05	0.10	0.05
	AUC	5.59	26.25	2.37	2.53	1.59	0.94
B9202-045	Cmax	0.64	3.22				
	AUC	5.98	121.7				

Cmax: µg equiv/ml AUC: µg equiv.h/ml

[^{14}C]-Roflumilast (1 mg/kg) was administered to male NMRI mice in a single iv or oral dose. Following iv administration, highest concentrations were observed at 5 minutes post dosing in most tissues and after 0.5 and 1 hour in fat and RBC and the lung, respectively. The highest concentrations were in the lung, liver, adrenals, fatty tissue, kidneys, and plasma (Table 5). Low concentrations in the nose even after 5 minutes. Terminal half-lives ranged from 32 hours in fat to 87 hours in liver. Roflumilast and metabolites were excreted primarily in the bile with high

recovery in feces (61% of dose) compared to urine (30% of dose). Levels of radioactivity were reduced to < 1% at 96 hrs post dose. Following oral dosing, peak concentrations were attained in most tissues after 4 hours. Highest concentrations were observed in bone marrow, adrenals, and liver. Terminal half-lives ranged from 42 hours in the liver to 88 hours in adrenals. Of special note are the low AUC tissue/AUC plasma ratios in the nose (0.5 and 0.59 following administration of 1 mg/kg iv and po, respectively) compared to those of the rat (5.75 and 4.69 following administration of 1 mg/kg iv and po, respectively). Comparison of plasma AUC following oral and iv dosing showed that ~34% of administered compound reached systemic circulation following oral dosing.

Table 5. Tissue distribution of Roflumilast in NMRI mice.

Tissue	**Radioact Conc. µg.equiv/g**		**AUC (0-96h) (µg.equivxh/g)**		**T1/2 (hr)**	
	1, iv	1, po	1, iv	1, po	1, iv	1, po
	0.08 hrs	4 hrs				
Liver	4.827	0.101	14.69	5.59	87.2	42
Adrenal	2.384	0.670	3.21	5.18	3.2	88
Kidney	1.147	0.026	3.77	1.44	40.7	60
Nose	0.566	0.047	2.24	0.94	44	71
Fat	1.047	0.038	3.67	0.98	32.5	46
Thymus	0.617	0.020	NR	NR	NR	NR
Lungs	2.340	0.028	12.54	0.98	39.9	44
Skin	0.650	0.010	NR	NR	NR	NR
Plasma	1.125	0.066	4.63	1.59	37.7	52

NR: not reported

Results of an additional distribution study (Report 22/98) were summarized in the Pre-IND package submitted 12/30/1998. The tissue exposure values listed in Table 6 for the sponsor-identified NOEL of 4 mg/kg for hamsters are extrapolated from results of a single dose of 1 mg/kg Roflumilast. The highest tissue exposure was observed in the liver. A low AUC tissue/AUC plasma ratio, similar to that found in the mouse, was determined in the nose (0.96) compared to that of the rat (4.69 at 1 mg/kg po).

Table 6. Tissue distribution of Roflumilast in the hamster.

Tissue	**Dose (mg/kg)**	**Cmax (µg.equiv/g)**	**AUC (µg.equivxh/g)**
Liver	4		54.8
Kidney	4		15.2
Nose	1	0.05	2.53
	4		10.0
Lungs	4		5.9
Plasma	1	0.09	2.37
	4		10.4

Metabolism: The metabolism of Roflumilast was investigated in male Sprague Dawley rats following a single iv dose (1 mg/kg in PEG), single oral doses of 1 and 10 mg/kg in 4% methocel, single id dose at 2 mg/kg (in PEG) and a 7-day repeated po study (2 mg/kg). Metabolite profiles were comparable regardless of administration route or dose differences

(Table 7). Three major metabolites were detected in rat plasma, the N-oxide of the parent compound (M1, B9502-044), DCAP (B202-045) and the N-oxide of DCAP (M2, B9202-054), while parent drug disappeared rapidly. The N-oxide of DCAP had previously been undetected in a single-dose pharmacokinetic assay at a dose of 1.5 mg/kg but was found at comparable levels to DCAP in this assay. Low amounts of unchanged drug after oral dosing (4-22% after 1 hour) indicates high first-pass metabolism and/or high affinity of parent drug for tissues and organs with B9502-044 being major carrier of radioactivity. In the multiple dose study, M3 increased slightly (15-25% of spotted material). Unchanged parent drug was primarily detected in the liver, kidneys and lungs and DCAP was the major metabolite. Other minor metabolites included the N-oxide of parent (M1), M3, M6 (thought to be the hydroxy derivative, B9302-077, of the parent compound), and M7 and M8 (lungs after iv dosing only). In the nose, parent compound and DCAP were the major radioactivity carriers (47-49% and 17-21%, respectively, after 0.5 hours) following oral dosing with the remainder primarily consisting of polar metabolites.

Table 7. Metabolism and excretion of B9302-107 and metabolites in Sprague Dawley rats.

					Radioactive bands (% of spotted material)									
Biologic sample	Route	Dose mg/kg	Study Duration (days)	Time after dosing (hours)	B9302-107 Roflumilast	B9202-045 DCAP	M1	M2	M3	M4	M6	M7	M8	Polar Cmpds
Plasma	PO	1	1	1	4	13	43	14	10	3				10
	PO	2	7	8	3	15	22	27	15	3	0			14
	PO	10	1	1	6	15	41	13	3	3				8
	IV	1	1	1	9	15	40	21	2	4				8
Liver	PO	1	1	1	63	18	2		4		2			6
	PO	10			59	20	3		3		nd			13
	IV	1			52	23	2		3		3			9
Lungs	PO	1	1	1	42	30	10	0				0	0	12
	PO	2	7	8	87	3	2	2		1	0			2
	PO	10	1	1	84	7	6	0				0	0	0
	IV	1	1	1	40	32	12	0				3	2	6
Kidneys	PO	1	1	1	43	20	3		8					22
	PO	10			40	18	4		0					12
	IV	1			39	32	4		6					13
Nose	PO	1	1	1	21	16	6	8	10	7				23
	PO	2	7	8	23	5	3	8	4	8				48
	PO	10	1	1	24	19	0	2	0	0				57
Excretion														
Urine	PO	2	1	0-24	0	0	0	45	6	9	0			31
	PO	2	8	0-24	0	14	0	0	3	24	15			31
	PO	10	1	4-10	0	0		63	1	1				32
	IV	1	1	4-10	0	0		45	0	26				24
Feces	PO	2	1	0-24	92	0	0	0	0	0	0			0
	PO	2	8		88	0	0	0	0	0	0			0
	PO	10	1		97	0		0	0	0	1			0
	IV	1	1		1	2		13	6	18	11			27
Bile	IV	1		4-8	2				1					89
	ID	1			2				3					84

B9302-107 (1 mg/kg) was rapidly metabolized in Syrian golden hamsters with formation of M1 (N-oxide of parent), M2 (N-oxide of DCAP), and polar compounds remaining following iv administration (Table 8). M1 decreased rapidly with time but M3 was formed. M4 was found at significant levels only at 24 hours after dosing. Only traces of DCAP were detected following dosing. Following oral administration, low amounts of parent drug and DCAP were detected in plasma, suggesting extensive first pass metabolism of the parent drug.

Table 8. Metabolism of B9302-107 (1 mg/kg, single dose) in Syrian golden hamsters.

Route of administration	Time after dosing (hr)	Radioactive bands (% of spotted material)						
		B9302-107	B9202-045	M1 B9502-044	M2 B9502-054	M3	M4	polar
iv	1	29	2	34	7	5	1	24
	4	8	4	6	15	22	3	36
	8	6	2	2	11	33	4	34
	24	0	4	0	5	24	13	37
oral	1	3	4	29	11	12	1	31
	4	2	4	16	12	23	3	33
	8	1	4	11	13	24	4	31
	24	1	2	6	9	34	7	25

The metabolites M1 and DCAP have also been identified in both mice and man, and M2 has been detected in mice. The results of these studies in mice (Report 262/97) and man (Reports 128E/97 and Report 177/98) were summarized in the Pre-IND package submitted 12/30/1998 and plasma levels are reported in the pharmacokinetics section of this review (see Table 1).

An additional study concerning in vitro metabolism of DCAP (study report 142/97) by hepatic and liver microsomes was summarized in the submission of 12/30/1998. Mouse and hamster, but not rat, hepatic microsomes convert DCAP to its N-oxide. However, rat, hamster and mouse, but not human, nasal microsomes convert DCAP to its N-oxide.

Excretion: The metabolite M2 (B9502-054) was primarily detected in male Sprague Dawley rat urine following oral (45-63%) or IV (45%) dosing (Table 7). The metabolite profile in feces following IV administration was similar to that found in urine. After hydrolysis, M3 levels increased to ~ 3-10% after oral and IV dosing, respectively). However, unchanged drug was basically the only compound detected in feces. Most radioactivity excreted with bile was in the form of polar compounds, largely present as glucuronide and/or sulfate conjugates. After hydrolysis, M3 and M6 were identified at low levels (3.7-11.1%). Only traces of parent were detected in bile and none in urine. Approximately 70% of total radioactivity was excreted in urine and 30% in feces via the bile following iv administration. The dominant routes of biotransformation were N-oxidation of parent drug, cleavage of the parent molecule to yield DCAP, N-oxidation of DCAP, or O-dealkylation of the parent compound.

Additional studies concerning urinary excretion of metabolites in different species were summarized in the submission of 12/30/1998. The sponsor states that only low amounts of B9502-054 were detected in mouse and hamster urine (study reports 224/97 and 197/96,

respectively), while none was detected in human urine (below limit of quantitation, study report 11/98). Study report 197/96 was submitted but contains no urine analysis data in hamsters.

Summary of Pharmacokinetics and Toxicokinetics

Systemic exposure to a single oral dose of B9302-107 (Roflumilast) increased proportionally while exposure to metabolites increased sub-proportionally to proportionally with increasing dose in male Sprague Dawley rats and male Syrian golden hamsters. The greatest exposures in descending order were to the N-oxide of the parent drug (B9502-044), the N-oxide of DCAP (B9502-054), B9202-045 (DCAP) and the parent drug (Table 9). Systemic exposures to the metabolites were greater than those of the parent compound in both rats and hamsters, indicating a pronounced first-pass effect. Metabolism of the parent drug was not as prevalent in the mouse. At a dose of 1.5 mg/kg, plasma AUC levels of B9302-107 and its N-oxide were greater in rats than in hamsters, but were only one-half and 16-fold greater, respectively, than in mice. Exposure to DCAP was 10-fold and 48-fold greater in the rat compared to the hamster and mouse, respectively. However, the mouse and hamster were similar in terms of the low amounts of DCAP detected, and both demonstrated greater amounts of DCAP's N-oxide compared to the rat. In man, a dose of 0.5 mg resulted in relatively low levels of parent drug and high levels of the N-oxide of the parent drug, similar to the rat and hamster. However, similar to the mouse and hamster, very low relative levels of DCAP were observed in plasma.

Table 9. Pharmacokinetics of B9302-107 and metabolites following single oral dose in rats.

Species	**Dose (mg/kg)**	**Parameter**	**B9302-107** Roflumilast	**B9202-045** DCAP	**B9502-044** M1	**B9502-054** M2
Rat	1.5	AUC (0-∞) (μg/l h) AUC ratio	33.47	159.27 4.76	840.22 25.1	Below LLOQ of 50 ng/ml
Hamster	1.5	AUC (0-8) (μg/l.h) AUC ratio	2.09	15.34 7.34	286.59 137.12	Not analyzed
Mouse	1.5*	AUC (μg/l h) AUC ratio	70.36	3.29 0.05	51.43 0.73	40.07 0.57
Human	0.5 mg	AUC (μg/l h) AUC ratio	32.86	4.14 0.05	351.39 0.73	< LLOQ

* back calculated from a dose of 9 mg/kg.

Systemic exposure to DCAP also increased proportionally when rats were directly administered the metabolite orally. Maximum concentrations were achieved within 1.67 hours and the elimination half-life was 2.5-3.5 hours. The maximum concentration of B9202-054, the N-oxide of DCAP, was achieved at 5.33 hours following administration of 6 mg/kg DCAP and was present at approximately one-sixth the level of DCAP.

Drug absorption in rats and mice was ~30-50% following oral dosing. Maximum tissue concentrations in rats were noted in the liver. Exposure was greatest in the nose and liver and high AUC tissue/AUC plasma ratios were noted in the nose (4.7-7.9). Elimination half-lives were generally long and greatest in the nose and kidneys. Highest concentrations of radioactivity in the mouse were observed in the lung, liver, adrenals, and bone marrow. Terminal half-lives ranged from 32-88 hours and low concentrations in the nose compared to the rat were detected (AUC tissue/AUC plasma ratios of 0.5-0.59). In hamsters, greatest concentrations were

again noted in the liver and relatively low levels were detected in the nose. In summarized study results, an association of radioactivity in the nasal olfactory epithelium of rats was more pronounced following the administration of [^{14}C]-DCAP than [^{14}C]-B9302-107, considered to be related to cytochrome P450 activation of DCAP to its N-oxide (or follow-up metabolites) in the nasal epithelium of the rat. These results correspond to toxicity studies showing the rat to be the most sensitive of the species tested on a mg/kg basis to nasal olfactory changes (primarily olfactory epithelial degeneration and necrosis) following DCAP administration (Table 10). The sponsor proposes that DCAP is concentrated in the nasal epithelium and converted to its N-oxide by nasal microsomes. The N-oxide is thought to be responsible for the observed nasal lesions. Mouse and hamster nasal microsomes also convert DCAP to its N-oxide. However, these species do not concentrate DCAP in the nasal epithelium as greatly as rats and, thus, exhibit a lower sensitivity to nasal toxicity than rat. In contrast, human nasal epithelial microsomes do not convert DCAP to its N-oxide form. This information, combined with the low levels of DCAP produced, indicates that the nasal lesions induced by DCAP are not a concern for humans.

Table 10. **Toxicokinetics (serum levels) and nasal olfactory epithelial lesion severity following a single oral dose of DCAP.**

Compound	Rat			Hamster			Mouse		
DCAP	AUC (µg/l h)	Cmax (µg/l)	Toxicity	AUC (µg/l h)	Cmax (µg/l)	Toxicity	AUC (µg/l.h)	Cmax (µg/l)	Toxicity
0.05 mg/kg	93	22	+	39	10	-	NA	7	-
0.5 mg/kg	1212	283	++	496	125	(+)	76	27	-
1.5 mg/kg	3254	716	+++	1589	402	+	180	82	(+)

The dominant biotransformation routes in all species tested were N-oxidation of parent drug, cleavage of the parent molecule to yield DCAP, N-oxidation of DCAP, or O-dealkylation of the parent compound. Low amounts of unchanged drug in rat plasma after oral dosing indicated high first-pass metabolism and/or high affinity of parent drug for tissues and organs with M1 the major carrier of radioactivity. DCAP was the major metabolite in the nose, liver, kidneys and lungs; minor metabolites included the N-oxide of parent (M1), M3, M6 (hydroxy derivative of parent), and M7 and M8. Similar metabolites were observed in hamsters, mice and humans, although significantly greater amounts of DCAP were detected in rat plasma than in the other species. Study summaries indicated that mouse and hamster, but not rat, hepatic microsomes convert DCAP to its N-oxide. However, rat, hamster and mouse, but not human, nasal microsomes convert DCAP to its N-oxide. Approximately 70% of total radioactivity was excreted in rat urine and 30% in feces via the bile following iv administration; M2 was primarily detected in rat urine and feces. Unchanged drug was primarily detected in feces after oral administration. Polar compounds, present as glucuronide and/or sulfate conjugates were primarily excreted with bile. Study summaries indicated that, in contrast to the rat, low amounts of M2 were detected in mouse and hamster urine, while none was detected in human urine. In mice, B9302-107 and metabolites were excreted primarily in the bile with high recovery in feces (61% of dose) compared to urine (30% of dose).

TOXICOLOGY

SINGLE/MULTIPLE DOSE TOXICITY:

Male Rats, Oral Comparative Toxicity of B9302-107 or B9202-045 following Single or Repeated Administration

Study No.: 194/95 *Vol.*: 5 of Pre-IND package

Study Dates: Starting date 10/17/95; report issued
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM 46/251; purity = 98.5%) in 4% methocel; B9202-045 (Batch AM 46/260; purity = 99.9%) in distilled water and 0.2 N HCl
Concentration: Not reported.
Volume: 10 ml/kg
GLP: Yes.
QA report: Yes.

Methods: Male Wistar rats (6 weeks old; 152-198 kg) were assigned to the following treatment groups:

Drug	B9302-107 (Roflumilast)				B9202-045 (DCAP)			
Dosing duration (days)	1		7		1		7	
Dose (mg/kg)	VC	300	0	8	0	240	0	6.4
No. of animals	5	5	5	5	5	5	5	5

Rats were exposed orally via gastric cannula for 1 or 7 days. The following observations were made:

Clinical observation . . . observed 4 times daily
Body weight . . . . . assessed daily
Gross pathology . . . . . at sacrifice (day 3 for single dose studies, day 8 for repeated dose studies)
Histopathology . . . . . at sacrifice (specific tissues/organs included nasal/paranasal cavities, adrenals, heart, testes; selected abnormalities)
Toxicokinetics . . . . . assessed in repeated dose groups only, Day -1 and Day 7 (1, 2, 3, 4, 6, 8, and 24 hours after dosing)

Results:

Mortality: None reported.

Clinical Observations: Following single-dose administration, B9302-107 induced piloerection, chromodacryorrhea, reduced activity, and a hunched position (Table 11). The metabolite induced reduced activity, hunched position and chromodacryorrhea but at a lower incidence than parent. Following repeated administration, B9302-107 again induced more frequent piloerection, hunched position and reduced activity observed than in those animals administered the

metabolite and at a similar incidence to single dose administration even though doses were significantly reduced.

Body Weight: Following single-dose administration, animals dosed with either B9302-107 or its metabolite lost weight while control animals gained 22-23 g (Table 11). Following repeated administration, both substance-treated groups experienced a reduced body weight gain compared to control. The parent compound induced greater effects than the metabolite in both cases.

Table 11. Clinical observations and body weight effects in rats.

	B9302-107		B9202-045	
Single dose administration				
Dose group (mg/kg)	VC	300	VC	240
Clinical Obs.				
Chromodacryorrhea	0	5	0	2
Hunched position	0	5	0	4
Hypersalivation	0	1	0	0
Incr Resp rate	0	3	0	0
Reduced tonus	0	4	0	0
Piloerection	0	5	0	1
Ptosis	0	2	0	0
Reduced activity	0	5	0	3
Body weight				
Net mean BW gain (g)	22	-20	23	-2
% change vs control		-190		-109
Repeated dose administration				
Dose group (mg/kg)	VC	8	VC	6.4
Clinical Obs.				
Chromodacryorrhea	0	1	0	0
Hunched position	0	5	0	4
Piloerection	0	5	0	4
Reduced activity	0	5	0	4
Body weight				
Net mean BW gain (g)	35	1	36	24
% change vs control		-97		-33

Gross pathology: Following single-dose administration, animals given B9302-107 exhibited chromodacryorrhea, reduced prostate, reduced seminal vesicles, hemorrhages, and/or ulcers in the glandular stomach, and yellow red or brown discolorations and/or activated Peyer's plaques in the intestine. Similar findings were observed in animals administered the metabolite. No substance- or treatment-related findings observed in animals administered repeated doses of parent or metabolite.

Histopathology: Moderate to severe necrosis of olfactory epithelium without reactive inflammation was observed in animals administered either the parent drug or its metabolite (Table 12). Three of five animals administered the parent compound exhibited minimal degeneration of respiratory epithelium of the nasal and paranasal cavities; submucosal or intraluminal hemorrhages were observed in 2 animals. Moderate to severe necrosis of tubular spermiogenic epithelium with intratubular giant cells was also noted in all animals administered

the parent compound while those administered the metabolite showed no evidence of these findings. Various lesions in the stomach and intestines, primarily inflammation, were observed in 1-5 animals given the parent compound.

Table 12. Histopathological changes following single administration in rats.

	B9302-107		B9202-045	
Dose (mg/kg/d)	VC	300	VC	240
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**
Resp. epith degen/necrosis	0	3	0	0
Olfactory epith degen/necrosis	0	5	0	5
Leukocytostasis	0	2	0	0
Purulent infiltration	0	1	0	0
Desorganized resp. epithelium	0	0	0	1
Lympho-histiocytic infiltration	0	1	0	0
Mucus congestion	2	5	0	5
Intraluminal blood	0	2	0	0
Eosinophilic infiltration	0	0	0	0
Sub-/intramucosal hemorrhage	0	2	0	0
Stomach n =	**4**	**5**	**2**	**2**
Gastritis	0	1	0	0
Erosion	0	1	0	0
Eosinophilic chief cells	0	0	0	2
Submucous inflammation	0	3	0	0
Submucous edema	0	3	0	0
Edema	0	1	0	0
Eosinophilic infiltration	0	2	0	0
Intestines n =	**0**	**5**	**0**	**0**
Hemorrhage		2		
Edema		5		
Eosinophilic infiltration		2		
Peri-/vasculitis, necrosis		2		
Lympho-histiocytic infiltration		2		
Intram. neutrophilic infiltration		3		
Testis n =	**5**	**5**	**5**	**5**
Tubular cell degen/necrosis	0	5	0	0
Giant cells	0	5	0	0

Following repeated administration, a mild to moderate necrosis of olfactory epithelium covered by a non-ceratinizing epithelium, indicating a reparative process, were noted in both treatment groups (Table 13). Also, a lymphohistiocytic and eosinophilic inflammatory reaction was observed. These findings were similar to those following single dosing though of lesser severity. Animals administered the parent compound also exhibited a mild to moderate atrophy of the spermiogenic epithelium which correlated with a moderate oligospermia; in two cases dysspermia was noted. Similar to the single-dose study, submucosal inflammation, edema, eosinophilic infiltration, lympho-histiocytic infiltration, or hemorrhage were also noted in the stomach and intestines of animals administered the parent drug.

Table 13. Incidence of histopathological changes following 7-day administration in rats.

	B9302-107		B9202-045	
Dose (mg/kg/d)	VC	8	VC	6.4
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**
Olf epith degen/necrosis	0	5	0	5
Desorganized resp. epithelium	0	1	0	0
Desorganized olf. epithelium	0	5	0	5
Lympho-histiocytic infiltration	0	3	0	4
Intraluminal blood	0	1	0	0
Eosinophilic infiltration	0	3	0	4
Testis n =	**5**	**5**	**5**	**5**
Atrophy of spermiogen epith	0	4	0	0
Oligospermia	0	4	0	0
Dysspermia	0	2	0	0
Giant cells	0	1	0	0

Kinetics: Although the sponsor stated that blood samples were to be taken for the purpose of assessing toxicokinetics, no data or discussion of results were included in this report.

A NOAEL could not be determined for this study since a complete histological assessment was not performed and since only one dose of test article was assessed at each timepoint. However, the parent compound did induce toxicities in the nasal cavity as well as the testis while the metabolite induced toxicity only in the nasal cavity.

Male Rats, Oral Comparative Toxicity of B9302-107, B9202-045 or B9502-054 following Single or Repeated Administration (Screening Study)

Study No.: 128/96 *Vol.*: 8

Study Dates: Starting date 7/29/96; report issued 5/27/97
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Batch AM 46/251; purity = 98.6%) in 4% methocel; B9202-045 (Batch AM 46/260; purity = 99.95%) in distilled water and 0.2 N HCl; B9302-054 (Batch AM 50/058; purity = not available) in distilled water and 0.2 N HCl
Concentration: Not reported.
Volume: 10 ml/kg
GLP: Study report was unaudited.
QA report: No.

Methods: Male Wistar rats (6 weeks old; 174-204 kg) were assigned to the following treatment groups:

Drug	Vehicle control		B9302-107		B9202-045		B9502-054	
Dosing duration (days)	1	7	1	7	1	7	1	7
Dose (mg/kg)	0	0	300	8	240	6.4	240	6.4
No. of animals	5	5	5	5	5	5	5	5

Rats were exposed orally via gastric cannula for 1 or 7 days; autopsy was performed on Days 3 and 8, respectively. The following observations were made:

Clinical observation . . .	observed 3-4 times daily
Body weight	assessed daily
Gross pathology	at sacrifice (day 3 for single dose studies, day 8 for repeated dose studies); examined tissues included GI mucosa, thyroid, adrenals
Histopathology	at sacrifice (specific tissues/organs included nasal/paranasal cavities, testes, epididymes, salivary glands, and selected abnormalities: stomach, intestine)
Toxicokinetics	assessed in repeated dose groups only, Day -1 and Day 7 (1, 2, 3, 4, 6, 8, and 24 hours after dosing)

Results:

Mortality: None reported.

Clinical Observations: Following single dose administration, animals administered B9302-107 exhibited an increased incidence of piloerection, chromodacryorrhea, reduced activity, ptosis, and hunched position compared to the other treatment groups (Table 14). Similar findings were observed following repeated administration but at a lower incidence.

Body Weight: Body weight gain was reduced in all drug treatment groups compared to control animals after either single or repeated dose administration (Table 14). Following single dose administration, mean body weight was reduced in all drug treated animals, with the greatest loss noted in the group administered the parent drug. Following repeat dose administration, mean body weight was again reduced in animals treated with the parent drug. All other groups exhibited an increased body weight. As such, the greatest reduction in body weight gain compared to controls was seen in animals treated with the parent drug and greatest effects were observed following single dose administration with significantly greater dosing.

Table 14. Clinical observations and body weight effects in rats.

Drug	Vehicle control	B9302-107	B9202-045	B9502-054
Single dose administration				
Dose (mg/kg)	0	300	240	240
Clinical Obs.				
Chromodacryorrhea	0	2	1	1
Hunched position	0	4	0	0
Hypersalivation	0	0	0	2
Incr respiratory rate	0	3	0	0
Piloerection	0	5	0	1
Ptosis	0	4	0	0
Reduced activity	0	5	0	1
Body weight				
Net mean BW gain (g)	12	-24	-12	-9
% change vs control		-300	-200	-175
Repeat dose administration				
Dose (mg/kg)	0	8	6.4	6.4
Clinical Obs.				
Chromodacryorrhea	0	0	1	0
Hunched position	0	1	0	0
Piloerection	0	1	0	1
Ptosis	0	0	0	1
Reduced activity	0	1	0	1
Body weight				
Net mean BW gain (g)	29.4	-20.2	26.8	19
% change vs control		-169	-9	-35

Gross pathology: Following single dose administration, animals administered the parent compound exhibited a reduced prostate (1of 5) and hemorrhage and/or ulcers in the glandular stomach (3 of 5). Reduced prostate was also observed in animals administered the metabolites (3 of 5, B9202-045; 1 of 5, B9502-054). Similar lesions in the glandular stomach were also observed but were of lesser severity. Following repeat dose administration, reduced prostate and intestinal morphologic deviations were noted in animals administered the parent compound.

Histopathology: Following single dose administration, animals administered either the parent compound or its metabolites exhibited moderate to severe necrosis of olfactory epithelium without reactive inflammation (Table 15). Necrotic changes were accompanied by basal cell regeneration. Slight to moderate submucosal hemorrhage was noted in 3 of 5 animals administered the parent compound and in one animal dosed with B9202-045. Moderate to severe degeneration and necrosis of tubular spermiogenic epithelium with intratubular giant cells were noted in animals administered B9302-107 only. Epididymis of all animals showed a corresponding moderate to severe hypospermia. Dysspermia, a sperm granuloma, or a dilatation of the sprem storing tubuli were detected in one animal each. Lesions in this organ system were noted in only one animal administered B9502-054. A number of lesions (ulcers, erosion, submucosal necrosis and edema, basal cell hyperplasia) of the stomach were also noted in animals administered B9302-107.

Table 15. Histopathological changes following single administration in rats.

	Veh. control	B9302-107	B9202-045	B9502-054
Dose (mg/kg/d)	0	300	240	240
Histology n=	5	5	5	5
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**
Olf epith degen/necrosis	0	5	5	5
Submucosal hemorrhage	0	3	1	0
Lympho-histiocytic infiltration	0	1	0	0
Regen of olfact. basal cells	0	5	5	5
Hyperemia/congestion	0	1	0	0
Stomach n =	**0**	**4**	**0**	**1**
Submucosal edema		3		0
Submucosal necrosis		2		0
Submucosal neutro. infiltration		3		0
Hyperplasia of basal cells		3		0
Ulcer		1		0
Erosion		1		0
Testis n =	**5**	**5**	**5**	**5**
Tubular cell degen/necrosis	1	5	0	1
Giant cells	0	4	0	1
Epididymis n =	**5**	**5**	**5**	**5**
Inflammation	1	2	0	0
Sperm granuloma	0	1	0	0
Tubule dilatation	0	1	0	0
Hypospermia	0	5	0	0
Dysspermia	0	1	0	0
Lungs n =	**4**	**5**	**5**	**4**
Hyperemia/congestion	0	1	0	0
Salivary glands n =	**5**	**5**	**5**	**5**
Calc of excretory duct lumina	0	1	0	0

Similar findings were observed following repeat dose administration as B9302-107 and metabolites induced mild to moderate disorganization and single cell necrosis of olfactory epithelium accompanied by a basal cell regeneration (Table 16). In addition, mild to moderate atrophy of the spermiogenic epithelium, mild to moderate hypospermia of the epididymis, edema and dysspermia were observed in 4, 2, 2 and 1 animals, respectively, administered B9302-107.

Table 16. Histopathological changes following repeated administration in rats.

	Veh. control	B9302-107	B9202-045	B9502-054
Dose (mg/kg/d)	0	8	6.4	6.4
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**
Olfactory degen/disorg	0	5	5	5
Olfactory epithelium necrosis	0	5	5	5
Lympho-histiocytic infiltration	0	0	0	1
Neutrophilic infiltration	0	0	0	1
Eosinophilic infiltration	0	1	0	1
Regen of olfact. basal cells	0	5	5	5
Testis n =	**5**	**5**	**5**	**5**
Tubular cell degen/necrosis	0	4	0	0
Giant cells	0	3	0	0
Epididymis n =	**5**	**5**	**5**	**5**
Inflammation	0	1	0	0
Hypospermia	0	2	0	0
Dysspermia	0	1	0	0
Edema	0	2	0	0

Kinetics: Although the sponsor stated that blood samples were to be taken for the purpose of assessing toxicokinetics, no data or discussion of results were included in this report.

A NOAEL could not be determined for this study since a complete histological assessment was not performed and since only one dose of each test article was assessed at each dosing duration. However, the parent compound did produce toxicities in the nasal cavity, testis and epididymis, and stomach, while the metabolites induced only nasal cavity toxicity.

Male Rats, Oral Toxicity of B9202-045 (DCAP) following Single or Repeated Administration (Screening Study)

Study No.: 129/96 *Vol.*: 9

Study Dates: Starting date 7/30/96; report issued 4/14/97
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9202-045 (Batch AM 46/260; purity = 99.95%) in distilled water and 0.2 N HCl
Concentration: Not reported.
Volume: 10 ml/kg
GLP: Study report was unaudited.
QA report: No.

Methods: Male Wistar rats (6 weeks old; 158-238 kg) were assigned to the following treatment groups:

Dose (mg/kg)	0		0.05		0.5		1.5		3		6	
Dosing duration (days)	1	7	1	7	1	7	1	7	1	7	1	7
No. of animals	5	5	5	5	5	5	5	5	5	5	5	5

Rats were exposed orally via gastric cannula to B9202-045 (DCAP), a metabolite of B9302-107, or vehicle control (distilled water adjusted to pH 3.3) for 1 or 7 days; autopsy was performed on Days 3 and 8, respectively. The following observations were made:

Clinical observation . . . observed 3-4 times daily
Body weight . . . . . assessed daily
Gross pathology . . . . . at sacrifice (day 3 for single dose studies, day 8 for repeated dose studies); examined tissues included GI mucosa, thyroid, adrenals
Histopathology . . . . . at sacrifice (specific tissues/organs included nasal/paranasal cavities, lung, testes, epididymes, salivary glands, and selected abnormalities: stomach, intestine)
Toxicokinetics . . . . . assessed in repeated dose groups only, Day -1 and Day 7 (1, 2, 3, 4, 6, 8, and 24 hours after dosing)

Results:

Mortality: None reported.

Clinical Observations: No treatment-related findings were reported.

Body Weight: Following single dose administration, mean body weight was slightly reduced in the high-dose animals (Table 17). A dose-dependent body weight gain reduction was observed in groups administered 1.5-6 mg/kg. Following repeat dose administration, mean body weight increased in all treatment groups. However, mean body weight gain was again dose-dependently reduced compared to control animals, although the 3 and 6 mg/kg dose groups were comparable.

Table 17. Body weight effects in rats following single dose administration of DCAP.

Dose (mg/kg)	0	0.05	0.5	1.5	3	6
Single dose administration						
Body weight						
Net mean BW gain (g)	11.8	13.8	14.0	10.8	3.4	-0.2
% change vs control		17	19	-8	-29	-102
Repeat dose administration						
Body weight						
Net mean BW gain (g)	33.6	32.6	30.8	24.2	21.8	22.6
% change vs control		-3	-8	-28	-35	-33

Gross pathology: No drug-related findings were reported.

Histopathology: Following single dose administration, olfactory degeneration was observed in the two lower dose groups at minimal severity (Table 18). However, moderate to marked necrosis was demonstrated in all animals in the three greatest dose groups. Reparative processes were also noted at the three highest doses. Minimal pulmonary inflammation was also observed at the highest dose group. Following repeated dose administration, only nasal/paranasal cavity lesions were reported. Similar to the single dose results, degeneration of the olfactory epithelium

occurred in the two lower dose groups, while necrosis occurred as the dose increased. Reparative processes were displayed by the basal cells at the four highest doses.

Table 18. Histopathological changes following single administration in rats of DCAP.

Dose (mg/kg/d)	0	0.05	0.5	1.5	3	6
Single dose administration						
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**	**5**	**5**
Olfactory degeneration	0	5 (0.5)	5 (0.6)	0	0	0
Necrosis	0	0	0	5 (2.9)	5 (4.0)	5 (4.0)
Regen hyperplas of basal cells	0	0	0	5 (1.9)	5 (4.0)	5 (4.0)
Lungs n =	**5**	**0**	**0**	**0**	**5**	**5**
Lympho-histiocytic infiltration	3 (0.3)				2 (0.3)	2 (0.55)
Eosinophilic infitration	0				0	1 (0.25)
Repeat dose administration						
Nasal/paranasal cavities n =	**5**	**5**	**5**	**5**	**5**	**5**
Olfactory degeneration	0	5 (0.9)	5 (2.4)	0	0	0
Necrosis	0	0	5 (1.75)	5 (1.8)	5 (3.0)	5 (3.0)
Regen hyperplas of basal cells	0	0	5 (2.6)	5 (3.9)	5 (4.0)	5 (4.0)

Incidence (severity). Severity scale: 1: minimal; 2: mild; 3: moderate; 4: marked.

Kinetics: Although the sponsor stated that blood samples were to be taken for the purpose of assessing toxicokinetics, no data or discussion of results were included in this report.

A NOAEL could not be determined for this study since the sponsor did not perform a complete histological assessment and since nasal cavity toxicity was noted at all doses of B9202-045 tested. However, a NOAEL of 0.5 mg/kg and 0.05 mg/kg for olfactory epithelial necrosis was identified for single and 7-day dosing, respectively. The nasal cavity was the target organ of toxicity identified in this study.

Syrian Golden hamster, Mouse, 1-7 day Oral Toxicity (Summary)
Study No.: 4D/98 *Vol.*: 11

Study Dates: Starting and report issue date not reported.
Testing Lab: Not reported.
Test Article: B9302-107, B9202-045, B9502-054 (Batch & purity not reported)
Concentration: Not reported.
Volume: Not reported.
GLP: The study was unaudited.
QA report: No.

This is a summary report of original German language reports.

The aims of these studies were to determine the pharmacokinetic profile of orally applied B9302-107, B9202-045, and B9502-054, and to perform histopathological evaluation of the nasal cavity, testes and epididymis to allow estimation of species specific differences in target organ toxicity.

Methods: Syrian golden hamsters and mice were assigned to the following treatment groups:

Species	Test substance	# of doses	Dose (mg/kg)	# of animals	Post-dose sacrifice (hr)	Organs analyzed
Hamster	B9302-107	1	9	5	3	NC, T, E
		1	9	5	24	NC, T, E
Hamster	B9302-107	7	8	10	24	NC
		1	300	10	48	NC
Hamster	B9302-107	1	300	5	72	NC, T, E
	B9202-045	1	240	5	72	NC, T, E
	B9502-054	1	240	5	72	NC, T, E
Hamster	B9202-045	1	0.05	5	72	NC, T, E
	B9202-045	1	0.5	5	72	NC, T, E
	B9202-045	1	1.5	5	72	NC, T, E
Mouse	B9302-107	1	300	5	72	NC
	B9202-045	1	240	5	72	NC
	B9502-054	1	240	5	72	NC

NC: nasal cavity T: testis E: epididymis

Results:

Nasal Cavity Histopathology: A single oral dose of 9 mg/kg B9302-107 induced no histopathological changes (3-24 hours after dosing). Similarly, repeated doses of 8 mg/kg induced no changes 24-48 hours after final dosing. In contrast, the parent drug and selected metabolites induced necrosis in the nasal cavity 72 hours after dosing (Table 19); severe in all animals treated with 240 mg/kg B9202-045 or B9502-054, but in only 2 of 5 animals administered 300 mg/kg B9302-107. However, only minimal-slight degeneration of the olfactory epithelium was noted after dosing with 0.5-1.5 mg/kg B9202-045 (2/5 or 4/5, respectively). PCNA-staining showed no significant proliferative activity in any of the analyzed dose groups.

The parent drug and selected metabolites also induced severe degeneration and/or atrophy of the olfactory epithelium 72 hours after dosing in mice. The lesion was severe in all mice treated with 240 mg/kg B9202-045 or B9502-054 but was of minimal to slight severity in animals administered 300 mg/kg B9302-107.

Table 19: Nasal cavity histopathology in Syrian golden hamsters and mice.

Species	Compound	# of doses	Dose (mg/kg)	Post-dose sacrifice (hr)	Severity of olfactory epithelium degeneration	PCNA-Reactivity
Hamster	B9302-107	1	9	3	- (5/5)	NA
		1	9	24	- (5/5)	NA
Hamster	B9302-107	7	8	24	- (5/5)	NA
		1	300	48	- (5/5)	NA
Hamster	B9302-107	1	300	72	+ (1/5), ++ (2/5), ++ (2/5)	NA
	B9202-045	1	240	72	++++ (5/5)	NA
	B9502-054	1	240	72	++++ (5/5)	NA
Hamster	B9202-045	1	0.05	72	- (5/5)	+
	B9202-045	1	0.5	72	- (3/5), + (2/5)	+
	B9202-045	1	1.5	72	- (1/5), + (3/5), ++ (1/5)	+
Mouse	B9302-107	1	300	72	+ (4/5), ++ (1/5)	NA
	B9202-045	1	240	72	++++ (5/5)	NA
	B9502-054	1	240	72	++++ (5/5)	NA

+: minimal, ++: slight, +++: moderate, ++++: severe, NA: not assessed

Testes and Epididymis Histopathology: A single dose of 9 mg/kg B9302-107 did not induce alterations in testes or epididymis tissue at 3 or 24 hours post-dose in Syrian hamsters (Table 20). However, following single dosing with greater concentrations of B9202-045 or B9502-054 (240 mg/kg), a severe degeneration of the germinative epithelium of the testes and the formation of giant cells associated with a dysspermia in the epididymis was detectable. No lesions were observed in animals administered 300 mg/kg B9302-107 or 0.05 and 1.5 mg/kg B9202-045. These tissues were juvenile in mice and, thus, were not assessed.

Table 20: Testes and epididymes histopathology in Syrian golden hamsters and mice.

Species	Compound	# of doses	Dose (mg/kg)	Post-dose sacrifice (hr)	Testes: Degeneration & Giant cell formation
Hamster	B9302-107	1	9	3	- (5/5)
		1	9	24	- (5/5)
Hamster	B9302-107	7	8	24	Tissue not submitted
		1	300	48	Tissue not submitted
Hamster	B9302-107	1	300	72	- (5/5)
	B9202-045	1	240	72	++ (2/5), +++ (2/5), ++++ (1/5)
	B9502-054	1	240	72	++ (2/5), +++ (2/5), ++++ (1/5)
Hamster	B9202-045	1	0.05	72	- (5/5)
	B9202-045	1	0.5	72	- (5/5)
	B9202-045	1	1.5	72	- (5/5)
Mouse	B9302-107	1	300	72	Juvenile, NA
	B9202-045	1	240	72	Juvenile, NA
	B9502-054	1	240	72	Juvenile, NA

+: minimal, ++: slight, +++: moderate, ++++: severe, NA: not assessed

B9302-107 induces nasal cavity lesions in the hamster and mouse similar to those observed in the rat following a single oral dose, although the lesions are of a lesser severity in the hamster and mouse at comparable doses. These lesions, additionally, occurred only 72 hour post-dose. However, administration of the metabolites B9202-045 or B9502-054 induced

similar lesion severity to that found in the rat following administration of B9302-107. Testicular toxicity showed a different pattern as B9302-107 induced toxicity in rats but not in hamsters. In contrast, direct administration of the metabolites induced lesions in hamsters but did not induce reproductive organ toxicity in male rats.

SUB-CHRONIC TOXICITY:

Mouse, 3-Month Oral Dose-Ranging Study (Summary)

Study No.: RR 216/98 *Vol.*: Pre-IND Carcinogenicity Protocol

Study Dates: Starting date not reported; report issued not reported
Testing Lab: Not reported
Test Article: B9302-107 (Batch: not reported; purity: not reported)
Concentration: Not reported.
Volume: Not reported.
GLP: The study was unaudited.
QA report: No.

Methods: Mice were assigned to the following treatment groups:

Est. total inhaled dose (mg/kg)	Veh. control	6	12	18
No. of animals/sex	10	10	10	10

Mice were exposed daily (oral gavage is assumed) to vehicle control or test drug for 3 months and the following observations were made:

Clinical observation . . . twice daily
Body weight . . . . . assessed
Food consumption . . . . assessed
Water consumption . . . assessed
Health exam . . . . . not assessed
Ophthalmoscopy . . . . . not assessed
ECG . . . . . not assessed
Hematology . . . . . Days 90/92
Clinical chemistry . . . . not assessed
Urinalysis . . . . . not assessed
Enzyme induction . . . . not assessed
Organ weights . . . . . not assessed
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice (for specific tissues/organs see Addendum, page 36)
Toxicokinetics . . . . . not assessed

Results:

Mortality: One control female and one mid-dose male were found dead in their cages on Days 12 and 55, respectively. Sponsor did not provide an explanation for these findings although the

female displayed lung discoloration with hyperemia/congestion and the male exhibited adrenal discoloration and spots on the liver and lungs (associated with intra-alveolar blood).

Clinical Observations: Alopecia was observed in a small number of males and females but does not appear to be drug-related.

Body Weight: Only slight differences in initial and final body weights were observed in treated mice (Table 21) However, a statistically significant reduction in body weight gain was reported for exposed males, although a dose-related response was not observed and the reduction was similar to the non-significant reduction observed in females. This reduction in body weight gain is not considered to be biologically significant.

Table 21. Effect of B9302-107 on body weight gain in mice.

	Males				Females			
Dose group	VC	6	12	18	VC	6	12	18
Initial body weight (g)	26	26	25	25	21	20	21	20
Final body weight (g)	32	31	30	30	24	25	25	24
Net BW gain (g)	6	5	5	5	6	7	6	7
%Δ BW gain		↓17	↓17	↓17		↑17	--	↑17

Shaded areas indicate statistically significant difference from control group.

Hematology: Reduced numbers of leukocytes in treated females and increased numbers of segmented neutrophils in treated females and mid- and high-dose males were observed (Table 22). In addition, statistically a significant reduction in lymphocytes (5-9%) and thrombocytes (7% at HD) in females was reported but the change in these parameters is not considered to be biologically significant.

Table 22. Effect of B9302-107 on body weight gain in mice.

	Males			Females		
Dose group (mg/kg)	6	12	18	6	12	18
Leukocytes %Δ vs control animals	↓6	↓8	↓19	↓19	↓33	↓44
Seg. neutrophils %Δ vs control animals	↑20	↑40	↑35	↑47	↑35	↑47

Shaded areas indicate statistically significant difference from control group.

Gross pathology: No drug-related findings were observed.

Histopathology: A systemic lympho-hystiocytic infiltration was noted in all dose groups including control animals (Table 23). Findings of note included cortical atrophy and hyperemia of the adrenal glands in high-dose males and all treated females, olfactory epithelial degeneration and necrosis were also noted in mid- and high-dose animals, and a slight increase in the incidence and severity of follicular hyperplasia of the spleen in high-dose females. In general, the severity of the observed lesions was minimal in nature.

Table 23. Histopathological changes in mice following 3-month B9302-107 administration.

	Males				Females			
Dose (mg/kg/d)	VC	6	12	18	VC	6	12	18
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Atrophy, cortical x-zone	0	0	0	5(.7)	0	5(.4)	8(.9)	8(1)
Hyperemia of inner cortex	0	0	4(.4)	1(.2)	0	4(.3)	6(.7)	9(1.3)
Harderian glands								
Lympho-histiocytic infiltration	0	NA	NA	1(.1)	1(.2)	NA	NA	1(.1)
Chromodacryorrhea	0	NA	NA	0	0	NA	NA	1(.1)
Eyes								
Uveitis	0	NA	NA	1(.3)	0	NA	NA	0
Kidneys								
Pyelonephritis	0	0	0	1(.3)	0	0	0	0
Lympho-histiocytic infiltration	4(.4)	3(.1)	4(.1)	2(.1)	5(.2)	9(.3)	7(.7)	9(.9)
Lacrimal glands								
Lympho-histiocytic infiltration	1(.03)	NA	NA	7(.4)	0	NA	NA	1(.2)
Nasal cavity								
Olfactory epithelial degeneration	0	0	0	3(.2)	0	0	4(.2)	5(.4)
Olfactory cell necrosis	0	0	1(.1)	2(.4)	0	0	1(.03)	1(.05)
Prostate								
Inflammation	0	NA	NA	1(.1)				
Thymus								
Involution	0	NA	NA	1(.2)	0	NA	NA	1(.2)
Urinary bladder								
Epithelial hyperplasia	0	0	0	1(.1)	0	0	0	0
Acute cystitis	0	0	0	1(.3)	0	0	0	0
Lympho-histiocytic infiltration	5(.1)	3(.1)	4(.1)	5(.1)	3(.1)	6(.2)	3(.1)	5(.2)
Liver								
Lympho-histiocytic infiltration	3(.1)	1(.03)	0	1(.2)	4(.2)	7(.3)	7(.6)	6(.7)
Lungs								
Lympho-histiocytic infiltration	0	1(.2)	1(.2)	0	0	1(.1)	1(.03)	2(.3)
Pancreas								
Serosal lympho-histiocyt infiltr	2(.2)	NA	NA	2(.1)	1(.3)	NA	NA	6(.4)
Lympho-histiocytic infiltration	0	NA	NA	0	0	NA	NA	1(.03)
Spleen								
Serosal Lympho-histiocyt infiltr	0	1(.03)	1(.03)	0	0	0	1(.03)	1(.1)
Follicular hyperplasia	0	0	0	0	1(.2)	0	0	2(.4)
Stomach/forestomach								
Submucosal inflammation	0	NA	NA	0	0	NA	NA	1(.1)
Submandibular glands								
Lympho-histiocytic infiltration	0	NA	NA	0	0	NA	NA	3(.1)

Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe
NA: no histological assessment for this group.

Pending submission of the full study report, a NOAEL of 6 mg/kg was identified in male mice. However, a NOAEL could not be identified in females due to histological changes in the adrenals at all doses. The target organs of toxicity were the nasal cavity and adrenal glands in both males and females.

Hamster, 3 month Dose-Ranging Study (Summary)
Study No.: 251/98 *Vol.*: Pre-IND Carcinogenicity Protocol Package

Study Dates: Starting date & report issue date not reported
Testing Lab: Not reported.
Test Article: B9302-107 (Batch not reported; purity not reported)
Concentration: Not reported.
Volume : Not reported.
GLP: The study was unaudited.
QA report: No.

Methods: Syrian Golden hamsters were assigned to the following treatment groups:

Dose (mg/kg)	Veh. control	4	8	16
No. of animals/sex	10	10	10	10

Hamsters were exposed daily (assumed to be by oral gavage) to B9302-107 for 3-months. The following observations were made:

Clinical observation . . . twice daily
Body weight . . . . . assessed
Food consumption . . . . assessed
Water consumption . . . assessed
Health exam . . . . . not assessed
Ophthalmoscopy . . . . . not assessed
ECG . . . . . not assessed
Hematology . . . . . assessed
Clinical chemistry . . . . not assessed
Urinalysis . . . . . not assessed
Enzyme induction . . . . not assessed
Organ weights . . . . . not assessed
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice (for specific tissues/organs see Addendum, page 36)
Toxicokinetics . . . . . not assessed

Results:

Mortality: Two animals died (one mid-dose and one high-dose male on Days 10 and 22, respectively) due to gavage errors. The mid-dose animal exhibited discoloration and congestion in the lungs and a watery pale or yellow exudate in the thoracic cavity. Similar findings were reported in the high-dose animal in addition to intra-alveolar blood and discoloration and congestion in the adrenals.

Clinical Observations: Not reported.

Body Weight: Body weight gain was dose-dependently reduced in both males and females (Table 24). Although the reduction was greater than or equal to 10% in all dose groups in

females and in mid- and high-dose males, only the effect in high-dose females was statistically significant (P value < 0.05, Kruskal-Wallis, two-sided Wilcoxon tests).

Table 24. Effect of B9302-107 on body weight gain in hamsters.

	Males				Females			
Dose group	VC	4	8	16	VC	4	8	16
Initial body weight (g)	77	76	77	79	73	73	74	73
Final body weight (g)	118	118	114	112	126	118	116	108
Net BW gain (g)	41	42	37	33	53	45	42	35
%Δ BW gain		↑2	↓10	↓20		↓15	↓21	↓34

Shaded areas indicate statistically significant difference from control group.

Hematology: There were no drug-related effects.

Gross pathology: The most notable gross findings included discoloration of the adrenals in 4 of 10 high-dose males and females and involution of the thymus in 4 of 10 and 3 of 10 high dose males and females, respectively (Table 25). Other possible drug-related findings occurred in only one high-dose male or female. Only the findings in the adrenals, eyes, and seminal vesicles were possibly correlated with histological findings at the high-dose.

Table 25. Gross findings in hamsters following 3-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	4	8	16	VC	4	8	16
n =	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Discoloration	0	0	0	4	0	0	0	4
Eyes								
Injury	0	0	0	0	0	0	0	1
Opaque/cloudy	0	0	0	0	0	0	0	1
Right	0	0	0	0	0	0	0	1
Lungs								
Enlarged	0	0	0	1	0	0	0	0
Seminal vesicles								
Focus/spot	0	0	0	1				
Skin								
Alopecia	0	0	0	0	0	0	0	1
Thymus								
Involuted	0	0	0	4	0	0	0	3
Trachea								
Foam	0	0	0	1	0	0	0	0

Histopathology: Lympho-histiocytic infiltration was noted in both control and treated animals (Table 26). Findings of note included dysspermia and tubular atrophy of the testes in all treatment groups, and seminal vesicle atrophy at the high dose (low- and mid-dose animals were not assessed). In addition, olfactory epithelial disorganization was reported at the mid- and high-doses and olfactory epithelial necrosis was observed in one high-dose male. Other notable findings included prostatic atrophy, a pancreatic granuloma at the high dose, and hyperemia of the adrenal glands in one high-dose male and female. A number of histologic effects were also

observed in the eye, especially in females. Since low- and mid-dose animals were not examined, it is not possible to definitively assess the drug-relatedness of the findings.

Table 26. Histopathology changes in hamsters following 3-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	4	8	16	VC	4	8	16
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Cortical fat vacuoles	0	NA	NA	1(.3)	0	NA	NA	0
Hyperemia/congestion	0	NA	NA	1(.6)	0	NA	NA	1(.1)
Medullary atrophy	0	NA	NA	1(.04)	0	NA	NA	0
Hemorrhage	0	NA	NA	0	0	NA	NA	1(.2)
Harderian glands								
Chronic inflammation	2(.5)	NA	NA	7(1)	4(.9)	NA	NA	8(1)
Eyes								
Iritis	0	NA	NA	0	0	NA	NA	3(.6)
Uveitis	1(.2)	NA	NA	1(.4)	1(.4)	NA	NA	6(1.6)
Retinal degeneration/atrophy	1(.2)	NA	NA	0	0	NA	NA	5(1.2)
Anterior chamber hemorrhage	0	NA	NA	0	0	NA	NA	3(.6)
Periorbital blood	0	NA	NA	0	0	NA	NA	1(.2)
Lympho-histiocytic infiltration	5(.4)	NA	NA	4(.4)	6(.7)	NA	NA	6(.9)
Hemosiderin deposits	0	NA	NA	0	0	NA	NA	1(.2)
Epididymis								
Dysspermia	6(1.3)	10(3.3)	8(2.7)	8(3.0)				
Esophagus								
Peri-/epi-/esophagitis	0	NA	NA	1(.4)	0	NA	NA	0
Traumatic separation	0	NA	NA	1(.2)	0	NA	NA	0
Colon								
Goblet cell hypertrophy	0	0	0	1(.1)	0	0	0	0
Ileum								
Lymphocytic hyperplasia	0	0	0	1(.1)	0	0	0	0
Kidneys								
Tubular cell regeneration	0	NA	NA	1(.1)	0	NA	NA	2(.2)
Nasal cavity								
Olfactory disorganization	0	0	7(.4)	6(.4)	0	0	5(.4)	6(.2)
Loss of PAS-positivity in Bowman's glands	0	1(.03)	8(.9)	8(1.4)	1(.03)	1(.03)	7(.7)	10(1.8)
Olfactory single cell necrosis	0	0	0	1(.1)	0	0	0	0
Prostate								
Atrophy	0	NA	NA	3(.8)				
Liver								
Increased fat in hepatocytes(n=5)	2(.4)	NA	NA	4(.8)	1(.2)	NA	NA	3(.6)
Pancreas								
Granuloma	0	NA	NA	1(.3)	0	NA	NA	0
Seminal vesicles								
Atrophy	0	NA	NA	4(.8)				
Testes								
Tubular atrophy	8(1)	10(3.4)	9(3.4)	9(3.5)				
Stomach/forestomach								
Serosal, Lympho-histiocyt infiltr	1(.1)	NA	NA	2(.2)	1(.03)	NA	NA	1(.03)

Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe
NA: no histological assessment for this group.

Pending submission of the full study report, a NOAEL could not be identified in males or females due to reproductive organ effects at all doses in males and histologic findings in the eyes of high-dose females with no assessment in low- or mid-dose animals. The target organs of toxicity include the male reproductive organs, the nasal cavity, the eye, and possibly the prostate, adrenals and pancreas.

CHRONIC TOXICITY:

Rat, 6-month Oral Toxicity

Protocols No.: CR0364 *Report No.*: 14/96 *Vol.*: 6 of Pre-IND Package

Study Dates: Starting date 7/12/95; report issue date 10/9/96
Testing Lab: Byk Gulden, Hamburg, Germany.
Test Article: B9302-107 (Batch AM/46/251; purity ~ 98%) in 4% methocel
Concentration: Not reported.
Volume: 10 ml/kg.
GLP: Yes.
QA report: Yes.

Methods: Wistar rats (6 weeks old, males 132- 211 g; females: 116-164 g) were assigned to the following treatment groups:

Dose (mg/kg)	Veh. control	0.5	1.5	2.5
No. of animals/sex – toxicity	20	20	20	20
No. of animals/sex – recovery	8	0	0	8

Rats were orally dosed daily (gavage) to B9302-107 for 6-months. A portion of animals from the control and high-dose groups were held for an additional 4-weeks to assess recovery. Doses were selected based upon a 1-month oral toxicity study in rats in which 8 of 18 males and females, each, dosed with 8 mg/kg died or were killed in extremis during the treatment period (primary histologic findings in dead animals included gastric erosion in males, lung and liver congestion, and splenic follicular atrophy). The next lowest dose (2 mg/kg) induced gastric erosions in males and single incidence of spermiogenic granuloma. The following observations were made:

Clinical observation . . .four times daily
Body weight . . . . . once pre-dose, Day 1, twice/week Weeks 1-4, once/week Weeks 5-30
Food consumption . . . . Day 1, twice/week Weeks 1-4, once/week Weeks 5-30
Water consumption . . . Day 1, Weeks 1-4, Weeks 23-26 and Weeks 27-30
Health exam . . . . . not assessed
Ophthalmoscopy . . . . . Day -7, Weeks 26 and 30
ECG . . . . . Week 19
Hematology . . . . . Weeks -1, 6, 12, 26, and 30
Clinical chemistry . . . . Weeks -1, 6, 12, 26, and 30
Urinalysis . . . . . Weeks 6, 12, 26, and 30
Vaginal smears . . . . . Weeks 15-19

Enzyme induction . . . . not assessed
Organ weights . . . . . at sacrifice (for specific tissues/organs see Addendum, page 36)
Gross pathology . . . . . at sacrifice; GI mucosa, thyroid and adrenals examined under stereomicroscope
Histopathology . . . . . at sacrifice (for specific tissues/organs see Addendum, page 36). Examination of all organs in Control and high-dose groups. Duodenum, jejunum, ileum, testes, epididymes, tibia, femur, knee-joints and nasal/paranasal cavities were examined. All animals dying before schedule and abnormalities also evaluated.
Toxicokinetics . . . . . not assessed

Results:

Mortality: One control group female died during overnight fasting for diuresis. Other deaths (2 female controls, 1 low-dose female, and one high-dose male and female) were due to prolonged narcosis during blood sampling or electrocardiogram.

Clinical Observations: At the high dose, polyuria and chromodacryorrhea were reported at slightly higher incidence than other groups but were not increased during the recovery period.

Body Weight: High dose males exhibited a slight reduction in body weight (4%) compared to control animals in the second half of the study which returned to control levels within 2 weeks after the last dosing. Female body weight was not affected by treatment.

Food consumption: All drug-treated females exhibited an increase in food consumption (6-7%) from week 5 onward but is not considered to be biologically significant.

Water Consumption: An increase in water consumption up to 30% was observed in all drug-treated groups. This trend remained in males during the recovery period.

Hematology: Increased leukocyte counts were observed in mid- and high-dose males (25 and 20%, respectively) and females (47 and 57%, respectively) at the end of the dosing period. Counts were still increased (54%) in females following the recovery period.

Clinical Chemistry: Decreased serum cholesterol were observed in high-dose males and females (17 and 24%, respectively) and triglyceride levels were reduced dose-dependently in males (31-55%) and in high-dose females (20%). No significant changes were observed following the recovery period.

Urinalysis: An dose-dependent increase in calcium levels was observed in males (60-165%) and mid- and high-dose females (75 and 68%, respectively). No alterations were observed following the recovery period.

Vaginal smears: Mid- and high-dose groups exhibited a reduced number of estrus events compared to control animals (4 and 9 mid-and high-dose animals had only 0-1 estrus events

during the 4 week treatment period and recovery period compared to none in the control group. The majority of control animals experienced 4-5 events.).

Ophthalmoscopy: No drug-related findings were observed.

Electrocardiogram: Slight but significant prolongations of mean PQ (3-10 msec, males only), QαT (2-4 msec), and QT times (2-7 msec) were detected in all drug-treatment groups. However, these findings were not dose-dependent and there was no correlating histology in the heart.

Gross pathology: Macroscopic evaluation revealed reduced adipose tissue development in high-dose animals which appeared to resolve following the recovery period.

Organ weight: Absolute organ weight changes are summarized in Table 27. The absolute adrenal weight in mid- and high dose animals was increased following the dosing period and was still increased following the recovery period (16 and 22% in males and females, respectively). Thymus weight was also decreased at in mid- and high-dose males (18 and 27%, respectively) and high-dose females (21%). In addition, slight increases in testes (18%), seminal vesicle (11%), and prostate weight (7%) were reported in high-dose males. Females exhibited increased ovary weight (23 and 31%) and reduced uterus weight (34 and 24%) in mid- and high-dose groups, respectively. After the 4 week recovery period, seminal vesicle weight was still increased 24% and uterus weight decreased by 36%.

Table 27. Absolute organ weight changes in rats after 6-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	0.5	1.5	2.5	VC	0.5	1.5	2.5
Adrenals								
% change vs control group		↑6	↑23	↑26		↑6	↑24	↑27
Thymus								
% change vs control group		↑5	↓18	↓27		--	↓5	↓21
Testes								
% change vs control group		↑11	↑6	↑18				
Seminal vesicle								
% change vs control group		↑1	↑15	↑11				
Prostate								
% change vs control group		↓10	↑12	↑7				
Ovary								
% change vs control group						↑4	↑23	↑31
Uterus								
% change vs control group						↑2	↓34	↓24

Histopathology: A non-reversible disruption of the nasal olfactory epithelium and an influx of round cells beneath the basement membrane of olfactory epithelium was observed in mid- and high-dose animals (Table 26). Also, a non-reversible increase in epididymal spermatoceles and tubular developmental defects of the testes were diagnosed in mid- and high-dose males and high-dose males, respectively. Focal erosion was reported in the stomach of 1 high-dose female and ulceration and peritonitis in one high-dose male. However, gastric findings are not considered drug related since 4 of 10 males administered 2 mg/kg for 30 days showed erosion.

One high-dose female had a mammary gland adenoma at 6 months of age. Additional findings of slightly increased incidence compared to control animals were noted in the high dose groups. However, these findings were not apparent following the recovery period.

Table 26. Histopathology changes in rats following 6-month administration of B9302-107.

	Males				Females			
Dose (mg/kg/d)	VC	0.5	1.5	2.5	VC	0.5	1.5	2.5
Epididymis n=	**20**	**20**	**20**	**20**				
Hypospermia	0	0	0	1				
Spermatocele	0	0	2	4				
Liver n=	**20**	**0**	**1**	**20**	**18**	**0**	**0**	**20**
Periacinar hepatocyt hypertrophy	8	0	0	12	0	0	0	0
Periacinar glycogen storage	2	0	0	3	0	0	0	0
Lymphocytic infiltration	3	0	0	5	0	0	0	0
Nasal cavity n=	**20**	**20**	**20**	**20**	**18**	**19**	**20**	**20**
Olfactory epithel	0	0	16	20	0	0	17	20
disorganization	0	0	19	19	0	0	15	19
Sub-olfactory epithel hypercell	**20**	**0**	**0**	**20**				
Seminal vesicles n=	0	0	0	1				
Necrosis	**5**	**0**	**0**	**5**	**5**	**0**	**0**	**5**
Spleen – Perl's n=	1	0	0	4	1	0	0	2
Mild Perl's Positive material	**20**	**4**	**5**	**20**	**18**	**1**	**1**	**20**
Stomach n=	0	0	0	1	0	0	0	0
Ulceration	0	0	0	1	0	0	0	0
Peritonitis	0	0	0	0	0	0	0	1
Focal erosion	**20**	**20**	**20**	**20**				
Testes n=	2	2	1	3				
Single tubule develop. defect	**20**	**0**	**0**	**20**	**18**	**0**	**0**	**19**
Urinary bladder n=	3	0	0	8	0	0	0	0
Dilatation	**20**	**1**	**1**	**20**	**18**	**0**	**0**	**20**
Kidney n=	10	0	0	6	0	0	0	2
Basophilic tubules	0	0	0	0	0	0	0	3
Pelvic mineralization	0	0	0	0	0	0	0	1
Papillary mineralization	**20**	**0**	**0**	**20**	**18**	**0**	**0**	**20**
Mammary gland n=	0	0	0	0	0	0	0	1
Mammary adenoma					**18**	**0**	**0**	**20**
Ovaries n=					1	0	0	2
Hemorrhage					0	0	0	1
Cysts					**18**	**0**	**0**	**19**
Vagina n=					3	0	0	5
Squam keratonizing epithelium	**20**	**0**	**0**	**18**	**16**	**0**	**0**	**20**
Thymus n=	6	0	0	8	8	0	0	16
Involution	0	0	0	0	0	0	0	1
Cysts								
Recovery								
Epididymis n=	**8**	**0**	**0**	**7**				
Spermatocele	0	0	0	1				
Testes n=	**8**	**0**	**0**	**7**				
Single tubule develop. defect **Na**	1	0	0	3				
cavity n=	**8**	**0**	**0**	**7**	**7**	**0**	**0**	**7**
Olfactory epithel disruption	0	0	0	7	0	0	0	4
Sub-olfactory epithel hypercell	0	0	0	7	0	0	0	7

A NOAEL of 0.5 mg/kg was identified in rats based upon histologic findings in the nasal cavity. The target organs of toxicity include the nasal cavity, the male reproductive organs (testes, seminal vesicles and epididymis) and, possibly, the stomach.

Summary of Toxicology

Single/repeat (7 day) oral (gavage) dose-studies were performed in male rats, hamsters and mice in order to assess the comparative toxicity of Roflumilast (B9302-107) and its metabolites. In single dose studies 300 mg/kg Roflumilast or 240 mg/kg DCAP or B9502-054 induced moderate to severe nasal toxicity (olfactory epithelial degeneration and necrosis, disorganized respiratory and olfactory epithelium, regeneration of basal cell epithelium, lympho-histiocytic infiltration and eosinophilic infiltration) in rats. Similar doses also induced severe necrosis in the nasal cavity of all hamsters and mice administered DCAP or B9502-054, but in only 2 of 5 animals administered Roflumilast. In another rat study, oral dosing of DCAP (0.05-6 mg/kg) induced minimal olfactory epithelium degeneration in the two lower dose groups (0.05 and 0.5 mg/kg) after single dosing, while moderate to marked necrosis and mild to marked basal cell regeneration was reported in the three highest dose groups. Minimal olfactory epithelium degeneration was also reported in hamsters after single dosing with 0.5 or 1.5 mg/kg DCAP. Moderate to severe lesions in the testes (tubular cell degeneration/ necrosis, Giant cells, atrophy) and epididymes (hypospermia, dysspermia, sperm granuloma, tubule dilatation) were also observed in rats administered 300 mg/kg Roflumilast only. Additional findings at this dose included lesions in the stomach and intestines. In contrast, only DCAP and B9502-054 (240 mg/kg, single) induced severe lesions in the testes of hamsters (degeneration of germinative epithelium, giant cell formation, dysspermia); doses of 0.05 and 1.5 mg/kg B9202-045 or up to 300 mg/kg B9302-107 produced no lesions. Mice were not assessed for reproductive organ changes. All treated-rats at doses of 240-300 mg/kg also experienced reduced body weight gain (109 to 300%) and clinical signs (chromodacryorrhea, hunched position, hypersalivation, increased respiratory rate, decreased tonus, piloerection, ptosis, reduced activity) which were most severe in Roflumilast-treated animals. Thus, Roflumilast induced comparable nasal cavity lesions in the hamster, mouse and rat following a single oral dose, although of lesser severity in the hamster and mouse at comparable doses. However, when administered DCAP or B9502-054 directly, lesion severity was comparable to the rat following administration of B9302-107. The pattern of male reproductive organ toxicity was different in that Roflumilast induced toxicity in rats but not in hamsters, while DCAP and its N-oxide induced lesions in hamsters but not in rats. DCAP or its N-oxide may, thus, be responsible for the observed nasal lesions, while Roflumilast or another metabolite may be responsible for the reproductive organ changes noted in rats.

In repeat dose (7 day) studies, 8 mg/kg Roflumilast or 6.4 mg/kg DCAP or B9502-054 induced mild to moderate nasal toxicity in rats. Lesions in the testes/epididymis (mild to moderate atrophy of spemiogenic epithelium, oligo-, dys-, or hyperspermia, edema, Giant cells) were noted in Roflumilast-treated rats only. Body weight gain was reduced (9-169%), but most severely in Roflumilast-treated animals; clinical signs were comparable among treatment groups. Gastrointestinal toxicity was not observed with repeated dosing. In another rat study, oral dosing of DCAP (0.05-6 mg/kg) induced mild to moderate necrosis and basal cell regeneration at all

doses but the lowest. Reduced body weight gain was noted at doses > 0.05 mg/kg (3-35%) after repeated dosing.

Study summaries for 3-month dose-ranging studies in mice and hamsters were submitted to support dose selection for carcinogenicity protocols. In mice, orally (gavage) administered Roflumilast (6, 12, 18 mg/kg) induced olfactory epithelial degeneration and necrosis in mid- and high-dose animals, cortical atrophy and hyperemia of the adrenal glands in high-dose males and all treated females, and slightly increased incidence and severity of splenic follicular hyperplasia in high-dose females. In general, lesion severity was minimal. Reduced leukocyte numbers in treated females and increased numbers of segmented neutrophils in treated females and mid- and high-dose males were also observed. The target organs of toxicity were the nasal cavity and adrenal glands. In hamsters, dysspermia and tubular atrophy of the testes in all groups, and seminal vesicle atrophy at the high dose (low- and mid-dose animals not assessed) were noted following 3-month oral administration of 4, 8 and 16 mg/kg. In addition, olfactory epithelial disorganization was reported at the mid- and high-doses and necrosis was observed in one high-dose male. Other findings included prostatic atrophy, a pancreatic granuloma (high dose), adrenal gland hyperemia in one high-dose male and female, and various findings in the eye, especially in females. Low- and mid-dose animals were not examined, thus, it is not possible to definitively assess the significance of these latter findings. Body weight gain was dose-dependently reduced and, although the reduction was greater than or equal to 10% in all females (15-34%) and mid- and high-dose males (10 and 20%, respectively), only the effect in high-dose females was statistically significant. The target organs of toxicity include the male reproductive organs, the nasal cavity, the eye, and possibly the prostate, adrenals and pancreas.

In a 6-month oral toxicity study in rats (0.5, 1.5, and 2.5 mg/kg Roflumilast), a non-reversible disruption of the nasal olfactory epithelium and an influx of round cells beneath the basement membrane was observed at the mid- and high doses. A non-reversible increase in epididymal spermatoceles and tubular developmental defects of the testes were also diagnosed in mid- and high-dose males and high-dose males, respectively. One high-dose female had a mammary gland adenoma at 6 months of age. Slightly increased findings, including focal gastric erosion, peritonitis and ulceration, were noted in the high dose groups, but were not apparent following the recovery period. At the high dose, the incidence of polyuria and chromodacryorrhea were slightly increased during the dosing period. Other findings included increased leukocyte counts (mid- and high-dose), reversible decreases in serum cholesterol (high-dose) and triglyceride levels in males and high-dose females, reversible increases in urine calcium levels in males and mid- and high-dose females, a reduced number of estrus events (mid- and high-dose groups), slight non-dose dependent increases in mean PQ, QαT, and QT times in all treatment groups, and a reversible reduction of adipose tissue development in high-dose animals. A NOAEL of 0.5 mg/kg was selected due to histologic findings in the nasal cavity. Target organs of toxicity include the nasal cavity, the testes, seminal vesicles and epididymis, and, possibly, the stomach.

Addendum: Histopathology inventory for Pre-IND Roflumilast.

Study No.	194/95	128/96	129/96	4D/98	252/98	216/98	14/96
Duration	1/7 day	1/7 day	1/7 day	1/7 day	3-month	3-month	6-month
Species	M, rat	M, rat	M, rats	Hamster/mouse	Hamster	Mouse	rat
Adrenals	X				X	X	X*
Aorta					X		X
Bone marrow smear							
Bone (femur)							X
Bone (tibia)							X
Bone (strenum)						X	X
Brain:					X	X	X*
Cecum					X		X
Cervix							
Colon					X	X	X
Duodenum					X	X	X
Epididymis		X	X	X	X	X	X
Esophagus					X	X	X
Eye					X	X	X
Fallopian tube							
Fat							
Gall bladder					X		
Gross lesions	X	X	X		X	X	X
Harderian gland					X	X	X
Heart	X				X	X	X*
Hyphophysis							
Ileum					X		X
Injection site	NA	NA	NA	NA	NA	NA	NA
Jejunum					X	X	X
Kidneys					X	X	X*
Lacrimal gland					X	X	
Larynx							
Liver					X	X	X*
Lungs			X		X	X	X*
Lymph nodes, cervical							
Lymph nodes (LALN)							
Lymph nodes, mandibular							
Lymph nodes, mediastinalis							
Lymph nodes, mesenteric					X	X	X
Mammary gland					X	X	X
Nasal cavity	X	X	X	X	X	X	X
Optic nerves							
Ovaries					X	X	X*
Oviduct					X	X	X
Pancreas					X	X	X
Parathyroid					X		X
Peripheral nerve					X	X	X
Pharynx							
Pituitary					X		X*
Prostate					X	X	X*
Rectum					X		X
Salivary gland		X	X				X
Sciatic nerve							
Seminal vesicles					X	X	X*
Skeletal muscle					X	X	X
Skin					X	X	X
Spinal cord					X	X	X
Spleen					X	X	X*
Stomach					X	X	X
Testes	X	X	X	X	X	X	X*
Thoracic Limb							
Thymus					X	X	X*
Thyroid					X	X	X*
Tongue					X		X
Trachea					X	X	X
Urinary bladder					X	X	X
Uterus					X	X	X*
Uterine horn							
Vagina					X	X	X

* Organ weight obtained

GENETIC TOXICOLOGY

Reverse Mutation Assay using Salmonella typhimurium and Escherichia coli with B9302-107

(b) (4) *Study/Protocol No.*: 951059 *Report No.*: 127E/95 *Volume*: 4

Study Dates: Starting date 3/17/95; report issued 7/28/95
Testing Lab: (b) (4)
Test Article: B9302-107 (Lot No. AM46/234; purity = 99.7%) in DMSO
GLP: Yes.
QA Report: Yes.

Methods: This study is an Ames Assay using plate incorporation (experiment I) and the pre-incubation test (experiment II) in which 3 plates per dose were analyzed. B9302-107 was assayed ± metabolic activation by S9 liver microsomal fraction. The following strains and positive controls were used:

Strain	Positive Controls Without S9 (μg/plate)		Positive Controls W/S9 (μg/plate in Exp I/Exp II)	
TA 98	4-nitro-o-phenylene-diamine	(10)	2-aminoanthracene	(2.5/1)
TA 100	Sodium azide	(10)	2-aminoanthracene	(2.5/1)
TA 1535	Sodium azide	(10)	2-aminoanthracene	(2.5/1)
TA 1537	4-nitro-o-phenylene-diamine	(10)	2-aminoanthracene	(2.5/1)
WP2	Methyl methane sulfonate	(5 μl/plate)	2-aminoanthracene	(2.5/1)
WP2 uvrA	Methyl methane sulfonate	(5 μl/plate)	2-aminoanthracene	(2.5/1)

A pre-experiment toxicity and plate incorporation mutation induction test was performed with strains TA 98 and TA 100 with 8 concentrations of B9302-107 (3.3 to 5000 μg/plate; 3 plates each). Toxicity was detected by a reduction in the number of revertants, a clearing or diminution of the background lawn or by the degree of survival of treated cultures. In the main experiments, the following concentrations were tested: 33.3, 100, 333.3, 1000, 2500, and 5000 μg/plate. Three plates were used for each strain and dose level. Tests were positive if a dose-related and reproducible increase (2-fold increase in reversions for strain TA 100; 3-fold increase in strains TA 1535, 1537, 98 and WP 2 and WP uvrA) in the number of revertants occurs, and a substantial and reproducible increase for at least one test concentration.

Results: In the pre-experiment, no toxicity or increase in mutation induction was observed. There was also no indication of mutagenic activity for B9302-107 in either of the main experiments. Positive control groups exhibited expected mutagenic activity. Thus, B9302-107, at concentrations up to 5000 μg/plate, displayed no mutagenic potential under the conditions of this assay in agreement with the sponsor's conclusion.

In vitro gene mutation test (HPRT test) using Chinese hamster cell line V79

Study/Protocol No.: 0426 *Report No.*: 67/97 *Volume*: 11

Study Dates: Starting date 6/11/96; report issued 4/29/97
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Lot No. AM50/039; purity = 99.83%) in DMSO
GLP: Yes.
QA Report: Yes.

Methods: B9302-107 (1.25 to 150 μmol/l in DMSO) was evaluated for its potential to induce forward mutations in Chinese hamster V79 cells (1 million cells per culture) following a 4 hour treatment period with and without Aroclor 1254-induced rat liver preparation and co-factors (S9 mix). Doses of greater than 20 μmol/l resulted in precipitation. Therefore, in the main experiment, doses of 1.25 to 20 μmol/l were investigated in triplicate for cultures without S9 mix and in quadruplicate with S9 mix. Positive controls included ethyl methanesulfonate (8000 μmol/l without S9 mix) and dimethylbenzanthracene (60 μmol/l with S9 mix). The criteria for a positive response included a reproducible concentration-related increase in mutant frequency, and a reproducible positive response for at least one of the test substance concentrations.

Results: There was no significant reduction in plating efficiency at any dose level in the presence or absence of S9 mix. There was also no significant increase in mutant frequency at any dose level tested under either test condition. Thus, the results indicate that B9302-107 was non-mutagenic under the conditions of this mutation assay, in concurrence with the sponsor's conclusion.

Chromosome aberration assay with human lymphocytes in vitro

Study/Protocol No.: 0333 *Report No.*: 129/95 *Volume*: 4

Study Dates: Starting date 1/13/95; report issued 7/14/95
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: B9302-107 (Lot No. AM46/234; purity = 100.7 and 99.7%) in DMSO
GLP: Yes.
QA Report: Yes.

Methods: B9302-107 at 75 to 150 μmol/l (selected on the basis 50% suppression of mitotic activity) was tested in duplicate cultures of human lymphocytes from male and female blood donors. In test 1, the cultures were treated with 1, 10, 25, 50, 75, 100, 125, and 135 μmol B9302-107 /l for 24 hours without addition of S9 mix. Based on results of Test 1, the second experiment without S9 mix was performed with 75, 100, 125 and 150 μmol B9302-107 /l and a treatment time of 4 hours. For Test 2 experiments with S9 mix, 75, 100, 125 and 150 μmol/l and a treatment time of 4 hours was used. Sampling times (time from end of treatment to harvest time) were for 24 or 48 hours. The same procedures were used for the positive controls methyl methanesulfonate (200 μmol/l) and cyclophosphamide (10 μmol/l). Once harvested, cells were mounted and stained. One hundred metaphases per culture were examined for achromatic

lesions, chromatid and isochromatid breaks and exchange aberrations, and the mitotic index was calculated. A Fisher's exact test was used for statistical evaluation of treatment groups versus the concurrent solvent control group. However, no criteria for a valid assay or a positive result were provided.

Results: In tests without S9 mix, the mitotic index was reduced to about 56% after 125 mol/l compared to concurrent solvent control. The mitotic indices in test with S9 mix were reduced to 87% after 125 and 58% after 100 μmol/l. Under these test conditions, no significant increases in the incidence of damaged metaphases without achromatic lesions were found. Positive controls were found to exert chromosome-breaking action. Thus, B9302-107 was not clastogenic in this in vitro chromosome aberration assay, in concurrence with the sponsor's conclusion.

In vitro micronucleus test using Chinese hamster cell line V79

Study/Protocol No.: 0480 *Report No.*: 113/97 *Volume*: 11

Study Dates:	Starting date 4/2/97; report issued 6/17/97
Testing Lab:	Byk Gulden, Hamburg, Germany
Test Article:	B9302-107 (Lot No. AM50/126; purity = 100.7 and 99.7%) in DMSO
GLP:	Yes.
QA Report:	Yes.

Methods: B9302-107 (1 to 20 μg/ml; up to the lowest precipitating concentration) was evaluated for its in vitro clastogenic and aneugenic potential in V79 Chinese hamster cells. Duplicate cell cultures were treated with test substance approximately 16 hours after seeding with and without S9 liver microsomal fraction of Aroclor-treated rats. Treatment times were 21 hours without S9 mix or 3 hours, with an 18 hour recovery period, with S9 mix. Positive controls were 0.0087 and 0.0117 μg colcemid/ml in cultures without S9 mix and 3.16 μg cyclophosphamide/ml in cultures with S9 mix. Following treatment, the mitotic cells were shaken off, resuspended, fixed on slides and stained. A total of 1000 cells per experimental group were counted for incidence of micronuclei. A positive result was determined if the incidence of micronuclei was at least 3 times higher than concurrent controls, a clear dose-response relationship was observed and if the results were reproducible.

Results: Treatment with B9302-107 did not induce an increased incidence of micronuclei in the presence or absence of S9 mix. The positive controls did result in expected increases. Thus, the results indicate that B9302-107 was negative under the conditions of this Micronucleus Assay up to a dose of 20 μg/ml, in concurrence with the sponsor's conclusion.

In vivo micronucleus test in the mouse with oral administration of B9302-107
Study/Protocol No.: MM0415 *Report No.*: 106/96 *Volume*: Pre-IND package

Study Dates:	Starting date 4/24/1996; report issued 8/15/1996
Testing Lab:	Byk Gulden, Hamburg, Germany
Test Article:	B9302-107 (Lot No. AM50/039; purity = 99.8%) aqueous solution with 4 g hydroxymethylcellulose
Volume:	10 ml/kg
GLP:	Yes.
QA Report:	Yes.

Methods: B9302-107 was evaluated in the in vivo micronucleus assay with NMRI mice (5/sex/dose group/time point, 24 or 48 hours). Mice were administered a single oral (gavage) dose of the test drug (100, 300 or 900 mg/kg), vehicle control (aqueous solution with 4 g hydroxymethylcellulose) or positive control (cyclophosphamide, 25 mg/kg). A range-finding experiment had shown 1000 mg/kg to be severely toxic in 4 female mice (ptosis, piloerection increased respiration rate, reduced activity, weight loss) and 900 mg/kg was tolerated by 7 of 8 female mice. Following dosing, bone (femur) marrow was flushed and smears were taken on slides. Two thousand polychromatic erythrocytes per animal were then evaluated for incidence of micronuclei and 1000 red blood cells per animal were counted for determination of the ratio of polychromatic to all erythrocytes. Evaluation took place at 24 and 48 hours post-dosing with the exception of the positive control group (24 hours only). Data was evaluated for statistical significance using the Jonckheere-Terpstra test. For a valid test, the positive control must induce a statistically significant response relative to the solvent control and the corresponding median should not be less than 5 (24 hour sampling) or 3 (48 hour sampling) micronucleated polychromatic erythrocytes and the median of the solvent control group (pooled over sex) should not exceed 4 (24 hour sampling) or 2 (48 hour sampling) micronucleated polychromatic erythrocytes. The following criteria were established for a positive response: a statistically significant result ($p<0.05$; critical dose has to be included as the high dose) and the median of the dose which has given a statistically significant response, should not be less than 5 (24 hour sampling) or 3 (48 hour sampling) micronucleated polychromatic erythrocytes.

Results: One mid-dose female died in both the 24 hour and 48 hour groups. Males and females responded similarly to administration of the test substance. Positive responses were observed following administration of B9302-107 in high dose animals at 24 hours and in mid-dose males and high dose animals at 48 hours (Table 27). Low- and mid-dose females did exhibit a median of 3, however, the response was not statistically significant at the mid-dose (statistical results were not reported for low-dose females). The ratio of polychromatic erythrocytes to all red blood cells was also reduced at the high-dose at both time points in males and females. The positive control produced expected results.

Table 27. Results of micronucleus assay in mice following dosing with B9302-107.

24 hour sampling period: # of cells with micronuclei										
	Females					Males				
	Vehicle control	B9302-107 (mg/kg)			Pos. Control (mg/kg)	Vehicle control	B9302-107 (mg/kg)			Pos. Control (mg/kg)
	0	100	300	900	25	0	100	300	900	25
	1	1	3	4	22	1	2	2	3	28
	1	2	3	5	23	1	2	3	4	29
	2	3	4	5	28	2	2	3	5	33
	2	3	4	6	35	3	3	3	6	35
	3	4		6	37	3	3	4	8	41
Median	2.0	3.0	3.5	5.0	28.0	2.0	2.0	3.0	5.0	33.0
≥ 5	no	no	no	yes	yes	no	no	no	yes	yes
p-value		0.191[1]	0.011[2]	0.001[3]	0.004[4]		0.341[3]	0.04[2]	0.001[1]	0.004[4]
Mean PCE/ERY ratio	0.64	0.57	0.57	0.52	0.66	0.68	0.63	0.63	0.52	0.68

48 hour sampling period: # of cells with micronuclei										
	Females					Males				
	Vehicle control	B9302-107 (mg/kg)				Vehicle control	B9302-107 (mg/kg)			
	0	100	300	900		0	100	300	900	
	1	2	2	3		1	1	2	4	
	2	2	3	4		1	2	3	4	
	2	3	3	4		2	2	4	4	
	3	3	3	5		2	3	4	4	
	3	4		7		2	3	5	7	
Median	2.0	3.0	3.0	4.0		2.0	2.0	4.0	4.0	
≥ 3	no	yes	yes	yes		no	no	yes	yes	
p-value		NA	0.157[2]	0.003[3]			0.198[1]	0.003[2]	<0.001[3]	
Mean PCE/ERY ratio	0.63	0.57	0.54	0.52		0.63	0.57	0.58	0.51	

1: Jonckheere-Terpstra Test for the doses: 0 and 100 mg/kg
2: Jonckheere-Terpstra Test for the doses: 0, 100 and 300 mg/kg
3: Jonckheere-Terpstra Test for the doses: 0, 100, 300 and 900 mg/kg
4: Wilcoxon Test (one-sided): vehicle versus positive control

Under the conditions of this assay, B9302-107 tested positively in the in vivo micronucleus assay by inducing an increase in micronuclei in polychromatic erythrocytes of mouse bone marrow This conclusion is in concurrence with the sponsor's conclusion.

In vivo micronucleus test in the mouse with oral administration of B9202-045
Study/Protocol No.: MM0535 *Report No.*: 106/98 *Volume*: Pre-IND package

Study Dates:	Starting date 2/9/1998; report issued 5/5/1998
Testing Lab:	Byk Gulden, Hamburg, Germany
Test Article:	B9202-045 (Lot No. AM46/260; purity = 99.97%) in aqueous solution
Volume:	10 ml/kg
GLP:	Yes.
QA Report:	Yes.

Methods: B9202-045 was evaluated in the in vivo micronucleus assay with NMRI mice (5/sex/dose group/time point, 24 or 48 hours). Mice were administered a single oral (gavage) dose of the test drug (30, 100 or 300 mg/kg), vehicle control (aqueous solution) or positive control (cyclophosphamide, 25 mg/kg). A range-finding experiment had shown 1000 mg/kg to be severely toxic in 4 female mice (convulsions, lying stretched out, increased respiration rate) and 1 animal died within 6 hours after dosing. Three were sacrificed in extremis. Doses of 750 mg/kg and 500 mg/kg resulted in sacrifices in extremis and severe signs of toxicity, respectively. 300 mg/kg was tolerated by 4 female mice over a 5 day observation period. Following dosing in the current study, bone (femur) marrow was flushed and smears were taken on slides. Two thousand polychromatic erythrocytes per animal were then evaluated for incidence of micronuclei and 1000 red blood cells per animal were counted for determination of the ratio of polychromatic to all erythrocytes. Evaluation took place at 24 and 48 hours post-dosing with the exception of the positive control group (24 hours only). Data was evaluated for statistical significance using the Jonckheere-Terpstra test. The positive control group was compared to the negative control group (24 hour only) using the one-sided Wilcoxon test. For a valid test, the positive control must induce a statistically significant response relative to the solvent control and the corresponding median should not be less than 5 (24 hour sampling) or 3 (48 hour sampling) micronucleated polychromatic erythrocytes and the median of the solvent control group (pooled over sex) should not exceed 3 (24 hour sampling) or 2 (48 hour sampling) micronucleated polychromatic erythrocytes. The following criteria were established for a positive response: a statistically significant result ($p<0.05$; critical dose has to be included as the high dose) and the median of the dose which has given a statistically significant response, should not be less than 5 (24 hour sampling) or 3 (48 hour sampling) micronucleated polychromatic erythrocytes.

Results: Treatment with B9202-045 did not induce an increased incidence of micronuclei in treated mice at either time point compared to vehicle control animals. However, the results from the vehicle control animals did not meet the criteria for a valid assay as the median number of cells with micronuclei were greater than or equal to four or three at 24 or 48 hours after dosing, respectively (Table 28). The ratio of polychromatic erythrocytes to all red blood cells was reduced in mid- and high-dose males at both time points and in high-dose females at 48 hours. The positive controls resulted in expected increases in the median number of cells with micronuclei.

Table 28. Results of micronucleus assay in mice following dosing with B9202-045.

24 hour sampling period: # of cells with micronuclei										
	Females					Males				
	Vehicle control	B9302-107 (mg/kg)			Pos. Control (mg/kg)	Vehicle control	B9302-107 (mg/kg)			Pos. Control (mg/kg)
	0	30	100	300	25	0	30	100	300	25
	0	0	1	1	29	2	3	2	1	39
	2	2	2	2	30	4	4	2	2	49
	4	6	3	3	32	4	4	4	4	51
	4	6	4	3	41	5	4	5	5	51
	7	8	4	4	44	6	6	6	7	71
Median	4.0	6.0	3.0	3.0	32.0	4.0	4.0	4.0	4.0	51.0
≥ 4	yes	yes	no	no	yes	yes	yes	yes	yes	yes
p-value				>0.05[1]	0.004[2]				>0.05[1]	0.004[2]
Mean PCE/ERY ratio	0.72	0.69	0.70	0.67	0.62	0.73	0.72	0.58	0.57	0.67

48 hour sampling period: # of cells with micronuclei									
	Females					Males			
	Vehicle control	B9302-107 (mg/kg)				Vehicle control	B9302-107 (mg/kg)		
	0	30	100	300		0	30	100	300	
	3	2	1	0		2	2	2	1	
	3	3	2	1		4	3	3	2	
	3	3	3	2		5	4	3	4	
	4	3	5	4		5	5	7	5	
	5	4	9	8		7	7	7	5	
Median	3.0	3.0	3.0	2.0		5.0	4.0	3.0	4.0	
≥ 3	yes	yes	yes	no		yes	yes	yes	yes	
p-value				>0.05[1]					>0.05[1]	
Mean PCE/ERY ratio	0.68	0.79	0.64	0.59		0.71	0.68	0.60	0.59	

[1]: Jonckheere-Terpstra Test for the doses: 0, 100, 300 and 900 mg/kg
[2]: Wilcoxon Test (one-sided): vehicle versus positive control

The validity of the study as a whole is in question due to the greater than expected numbers of cells with micronuclei observed in vehicle control animals. The sponsor did not repeat the study since the overall results were negative, the criteria for validity were very strict, and biological variability should be taken into account. This reviewer agrees that the study needs not be repeated since the criterion for mean number of micronuclei for vehicle control animals was only marginally exceeded and since values for treated animals were either equal to or less than the those reported for vehicle control animals. Thus, the results indicate that B9202-045 was

negative under the conditions of this in vivo Micronucleus Assay up to a dose of 300 mg/kg, in concurrence with the sponsor's conclusion.

Summary of Genetic Toxicology

B9302-107 was negative for mutagenicity and clastogenicity in the Ames Assay, an in vitro chromosome aberration assay with human lymphocytes, an HPRT test with V79 cells, and an in vitro micronucleus test with V79 cells. However, B9302-107 tested positive in the in vivo micronucleus test in mice, inducing a low number of micronuclei in polychromatic erythrocytes after a dose of 300 mg/kg in males at 48 hours and at a dose of 900 mg/kg in males and females at 24 and 48 hours. The metabolite, B9202-045 (DCAP), tested negatively in the in vivo mouse micronucleus test at doses up to 300 mg/kg. The sponsor stated that the origin of the micronuclei in the in vivo mouse micronucleus assay with B9302-107 is unclear since the human lymphocyte assay gave no indication of a chromosome breaking effect. It was supposed that the micronuclei were a result of a stimulation of erythropoiesis in bone marrow or of an aneugenic potential, which was ruled out by negative findings in the in vitro micronucleus test. The sponsor should further evaluate the positive findings in the in vivo micronucleus assay.

CARCINOGENICITY PROTOCOLS

1. Proposal for Use of the Hamster Rather than the Rat for Carcinogenicity Study:

Table 9 (page 10) summarizes the comparative pharmacokinetic profiles of Roflumilast and metabolites in the relevant species following single oral dose administration. Approximately 30-50% absorption of Roflumilast has been reported. Enzyme induction and plasma protein binding by Roflumilast have not been evaluated. Maximum plasma levels were attained within 3.5 hours in rats and hamsters and no evidence of drug accumulation was observed in rats over 7 days. At comparable doses, the amount of Roflumilast present in rat serum was only one-half that observed in mice, but 16-fold greater than in hamsters, respectively. The major metabolite detected in rats, hamsters, mice and humans is the N-oxide of the parent drug (B9502-044, M1), thought to be the primary carrier of biological activity. At comparable doses, this metabolite was present in rat serum at 3- and 16-fold greater levels than in hamsters and mice, respectively. Other significant metabolites include DCAP, and the N-oxide of DCAP (B9502-054, M2). See Figure 1 for the structures of these metabolites. Serum levels of DCAP, a metabolite thought to be involved in induction of nasal olfactory epithelial lesions, were also detected at significantly greater levels in rats than in mice, hamsters and humans; a 10- to 46-fold increase compared to mice and hamsters, respectively. In contrast, serum levels of the N-oxide of DCAP were significantly greater in mice than in rats (hamster not analyzed at this dose). The differences in serum metabolite levels are thought to be due to the rapid breakdown of DCAP to its N-oxide (B9502-054, M2) by liver microsomes in hamsters and mice, but not in rats, while in humans only very low levels of DCAP are produced. All species exhibit significant metabolism of Roflumilast, and high levels of the N-oxide of the parent drug. However, relatively low levels of DCAP are observed in hamsters, mice and humans compared to rats.

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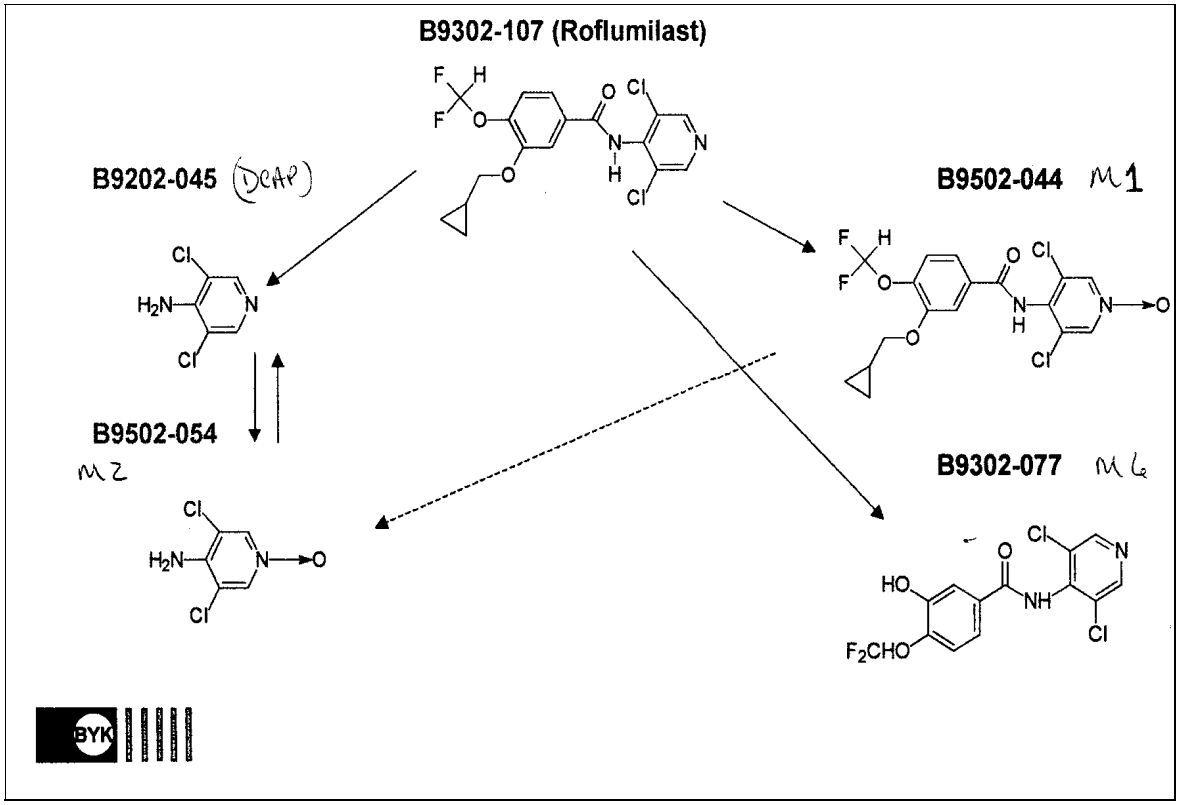
Figure 1: The primary metabolites of Roflumilast.



In rats, total radioactivity AUC in the nasal epithelium is 10 to 28-fold greater than other species administered 1 mg/kg labeled Roflumilast (Table 4, page 6). In addition, the ratio of nasal mucosa AUC to plasma AUC was also significantly greater in the rat (4.4 to 7.8-fold in rats vs 1.1 and 0.6 in hemasters and mice, respectively). A further increase of the ratio in rats was observed when DCAP was administered directly. The data indicate that rats selectively concentrate drug-related material in the nasal cavity epithelium. These results also correspond to toxicity studies showing the rat to be the most sensitive of the species tested on a mg/kg basis to nasal olfactory changes (primarily olfactory epithelial degeneration and necrosis) following administration of DCAP (Table 10, page 11). The sponsor proposes that DCAP is concentrated in the nasal epithelium and converted to its N-oxide by nasal microsomes. The N-oxide is thought to be responsible for the observed nasal lesions. Mouse and hamster nasal microsomes have also been shown to convert DCAP to its N-oxide, but these species do not concentrate DCAP in the nasal epithelium to the degree found in rats and, thus, exhibit a lower sensitivity to DCAP than the rat in terms of nasal toxicity on a mg/kg basis. In contrast, human nasal epithelial microsomes do not convert DCAP to its N-oxide form. This information, combined with the low levels of DCAP produced in humans, indicates that the nasal lesions induced by DCAP are not a concern for humans.

The sponsor proposes to use the hamster, rather than the rat, for its carcinogenicity study due to the unique metabolic profiling and handling of metabolites in the rat and the resulting increased nasal toxicity. The sponsor suggests that the lesions in the nasal cavity would be the dose

limiting factor for a 24-month carcinogenicity study in rats. Thus, they propose that the lower sensitivity of the hamster to nasal olfactory toxicity, compared to the rat on a mg/kg basis, allows for increased dosing of Roflumilast and overall greater systemic exposure to drug-related material.

Based upon the information provided, the reviewer concurs with the sponsor that the rat does uniquely metabolize Roflumilast compared to the hamster by producing excessive levels of DCAP and selectively concentrating DCAP in the nasal epithelium. However, the results of single and 7-day dosing studies in rats and hamster (Table 29), a 3-month dose-ranging study in hamsters and a 6-month toxicity study in rats demonstrated similar target organs of toxicity in the two species. In addition, Table 29 shows that, while comparable doses of Roflumilast induce slightly increased nasal toxicity in the rat compared to the hamster in 7-day studies, the systemic exposure to Roflumilast and DCAP are greater in the rat at doses approximating the MTD in both species in long-term studies. The exposure to the N-oxide of Roflumilast, considered a primary carrier of biological activity, is comparable at these doses. By combining the nasal mucosa AUC data in Table 4, extrapolated to relevant doses, and the toxicity findings in Table 10, it appears that similar degrees of severity in nasal cavity lesions are produced at nasal AUC levels of 65.
63 μg/l.hr in the rat and 20.24 in the hamster. Thus, on the basis of nasal mucosa exposure to administered drug material, the hamster shows greater sensitivity than the rat. Furthermore, the goal of a carcinogenicity study is to assess responses based upon systemic exposure to the administered drug (and, in this case, its proposed biologically active metabolite, B9502-044). Thus, based upon the results of a 3-month hamster study and a 6-month rat study, summarized in the following section, and the limited pharmacokinetic data available (single-dose except for human, 7-day), the rat appears more likely to receive the greatest systemic exposure to the parent compound and active metabolite at doses approximating the MTD in each species.

Table 29. Comparative toxicities and pharmacokinetics for rats and hamsters.

					Estimated plasma AUC levels (μg/l.hr)			
Species	**Dose** (mg/kg)	**# of doses**	**Nasal Toxicity**	**Repro Toxicity**	**Roflumilast**	**DCAP**	**M1**	**M2**
Rat	8	7	Mild to moderate necrosis	Mild to moder-ate atrophy of spermiogenic epithelium/ oligospermia/ dysspermia	178	849	4481	NA
Hamster	8	7	none	Not assessed	27.7	63	1459	219
Rat	2.5*	180	Olfactory epithelial disorganization; hypercellularity	Seminal vesicle necrosis	55.8	265	1400	NA
Hamster	8*	90	Olfactory disorganization; Single cell necrosis	Seminal vesicle atrophy; testicular atrophy	27.7	63	1459	219

*Dose approximates MTD.

2. Carcinogenicity Protocols:

The sponsor's carcinogenicity study proposals are based upon data from 3-month oral gavage study summaries in Syrian Golden hamsters and mice. The doses proposed by this reviewer for a rat carcinogenicity study are based upon a 6-month oral gavage study. The dose-ranging and proposed carcinogenicity studies utilize the oral gavage formulation. The review of these study summaries, the 6-month toxicity study in rats, and the pharmacokinetic, toxicity and genotoxicity studies for B9302-107, has been provided as an attachment. An evaluation of the sponsor's carcinogenicity study proposals follows and is also incorporated within the formal review.

2a. Summary of 3-month Hamster Study and Proposed Protocol:

2.a.1 3-month hamster study:

The 3-month hamster oral gavage study assessed doses of 4 and 8 mg/kg for the low- and mid-doses, respectively, with a high-dose of 16 mg/kg. One mid-dose and one high-dose male died due to gavage error. Body weight gain was dose-dependently reduced in both males and females, although only the effect in high-dose females was statistically significant. The primary target organs of toxicity were the nasal olfactory epithelium (disorganization, necrosis at the MD and HD), the testes (tubular atrophy, all doses), seminal vesicles (atrophy, HD) and the epididymis (dysspermia, all doses). Nasal olfactory findings were observed at the mid- and high-dose, while male reproductive findings were noted at all assessed doses. Other findings in organs such as the prostate (atrophy), the pancreas (granuloma), the eye, and the adrenal glands (hyperemia, cortical fat vacuoles, medullary atrophy, hemorrhage). were of generally low severity and were primarily observed at the high dose, although low- and mid-dose animals were not assessed. Pertinent results are summarized in Table 30.

Table 30. Roflumilast-related findings in hamsters following 3-month administration.

	Males				Females			
Dose (mg/kg/d)	0	4	8	16	0	4	8	16
Mortality			1/10	1/10				
Body weight gain								
%Δ vs control group		↑2	↓10	↓20		↓15	↓21	↓34
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Nasal cavity								
Olfactory disorganization	0	0	7(.4)	6(.4)	0	0	5(.4)	6(.2)
Loss of PAS-positivity in Bowman's glands	0	1(.03)	8(.9)	8(1.4)	1(.03)	1(.03)	7(.7)	10(1.8)
Olfactory single cell necrosis	0	0	0	1(.1)	0	0	0	0
Purulent infiltration	0	0	0	0	0	0	0	1(.03)
Seminal vesicles								
Atrophy	0	NA	NA	4(.8)				
Testes								
Tubular atrophy	8(1)	10(3.4)	9(3.4)	9(3.5)				
Epididymis								
Dysspermia	6(1.3)	10(3.3)	8(2.7)	8(3.0)				

Shaded areas indicate a significant difference from vehicle controls.
Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe
NA: no histological assessment for this group.

2.a.2. Review of Proposed Carcinogenicity Protocol.

The proposed high-dose of 4 mg/kg in a methylcellulose suspension for the hamster carcinogenicity study is expected to result in a B9302-107 AUC of approximately 13.9 μg/l.hr based on a 1-day pharmacokinetic study (Table 31). The predicted AUC is approximately four-tenths of the predicted highest human AUC. The primary metabolite in both humans and hamsters is the N-oxide of the parent drug (B9502-044), considered to be the predominant carrier of biological activity. The proposed high-dose of 4 mg/kg is expected to result in a B9302-044 AUC of 729.6 μg/l.hr based on a 1-day pharmacokinetic study. The predicted AUC is approximately 2.1-fold of the predicted highest human AUC. The sponsor proposed the high-dose of 4 mg/kg due to significant reduction in body weight gain of high-dose females and since they felt that the MTD for males was "in the range of" 4 mg/kg based on histological findings of comparable severity in the testes/epididymes at all doses tested.

Table 31. Proposed dose-selection for the hamster carcinogenicity study.

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.25	1	4
Predicted AUC of B9302-107 (μg/l hr)*	0.35	1.4	13.9
Multiple of human AUC**	0.01	0.04	0.42
Predicted AUC of B9502-044 (μg/l hr)*	47.8	191.1	729.6
Multiple of human AUC***	0.14	0.54	2.08

* AUC estimates based upon 1-day PK study extrapolated from dose of 1.5 mg/kg (for proposed doses 0.25 and 1 mg/kg) and 9 mg/kg (for proposed dose of 4 mg/kg).

** Based upon AUC of 32.86 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days

The sponsor's proposed high-dose of 4 mg/kg, would be acceptable for females only should the hamster be considered an appropriate species for the carcinogenicity testing of Roflumilast. The reduced body weight gain in females, though significant only at the high-dose, was 21% at the mid-dose and would likely increase with increasing duration. Thus, a high-dose of 4 mg/kg should be acceptable for females. For males, however, it is questionable as to whether the histologic findings in the testes/epididymes, though of a moderate to severe nature, are likely to affect the survivability of the animals. Therefore, body weight gain should be used, in addition to the nasal olfactory necrosis found at the high dose, to determine the high-dose for males. The dose of 16 mg/kg induced a non-significant reduction in body weight gain of 20% and may affect survivability in a chronic study. Therefore, a dose of 8 mg/kg should be selected as the high dose in males based upon reduced body weight gain and nasal olfactory necrosis. A dose of 8 mg/kg would provide an estimated exposure ratio of 0.94 compared to the human exposure of Roflumilast and a ratio of 4.2 compared to the human exposure of B9502-044.

2.b. Summary of 6-month Rat Study and Reviewer's Proposed Dose-selection:

2.b.1 6-month rat study:

A 6-month oral (gavage) toxicity study was performed in Wistar rats with Roflumilast (0.5, 1.5, and 2.5 mg/kg/day). Dose selection for this study was based upon a 1-month study in which numerous high-dose animals (8 mg/kg) died during the treatment period; the next lowest dose (2 mg/kg) induced gastric erosions in males and a single incidence of spermiogenic granuloma.

Results of the 6-month study are summarized in Table 32. A non-reversible disruption of the nasal olfactory epithelium and an influx of round cells beneath the basement membrane of the olfactory epithelium were observed in mid- and high-dose animals. Also, a non-reversible increase in epididymal spermatoceles (mid- and high-dose males) and tubular developmental defects of the testes were diagnosed (high-dose males). One high-dose female had a mammary gland adenoma at 6 months of age. Additional findings of slightly increased incidence compared to control animals, including focal gastric erosion, peritonitis and ulceration, hematologic and clinical chemistry findings, and slight increases in ECG parameters were noted in the high dose groups, were not apparent following the recovery period. The gastrointestinal findings were not considered to be drug related since 4 of 10 males administered Roflumilast for 30 days showed gastric erosion and only 1 female exhibited this finding in the 6-month study.

Table 32. Summary of Roflumilast-related findings in rats following 6-month administration.

	Males				Females			
Dose (mg/kg/d)	0	0.5	1.5	2.5	0	0.5	1.5	2.5
Mortality	0/28	0/20	0/20	1/28	3/28	0/20	1/20	1/28
Body weight change %Δ vs control group		↑2	↓1	↓4		↑7	↑5	↑9
Histology								
Epididymis n=	**20**	**20**	**20**	**20**				
Hypospermia	0	0	0	1				
Spermatocele	0	0	2	4				
Nasal cavity n=	**20**	**20**	**20**	**20**	**18**	**19**	**20**	**20**
Olfactory epithel disorganization	0	0	16	20	0	0	17	20
Sub-olfactory epithelial hypercellularity	0	0	19	19	0	0	15	19
Seminal vesicles n=	**20**	**0**	**0**	**20**				
Necrosis	0	0	0	1				
Testes n=	**20**	**20**	**20**	**20**				
Single tubule developmental defect	2	2	1	3				
Recovery								
Epididymis n=	**8**	**0**	**0**	**7**				
Spermatocele	0	0	0	1				
Testes n=	**8**	**0**	**0**	**7**				
Single tubule developmental defect	1	0	0	3				
Nasal cavity n=	**8**	**0**	**0**	**7**	**7**	**0**	**0**	**7**
Olfactory epithelial disruption	0	0	0	7	0	0	0	4
Sub-olfactory epithel hypercellularity	0	0	0	7	0	0	0	7

2.b.2. Reviewer's Recommendation for Dose-selection in Rat Carcinogenicity Study:

Based upon the results of the 6-month rat study, a high dose for a rat carcinogenicity study should be 2.5 mg/kg, since none of the findings of the 6-month study are considered to be serious enough to reduce animal survival and since a dose of 8 mg/kg resulted in high mortality in a 1 month study. The proposed high-dose of 2.5 mg/kg in a methylcellulose suspension for the rat carcinogenicity study results in an estimated B9302-107 AUC of 55.78 µg/l.hr (Table 33) based upon linear extrapolation from a 1-day pharmacokinetic study in Sprague Dawley rats. The predicted AUC is ~ 1.7-fold of the predicted highest human AUC. The primary metabolite in both humans and rats is the N-oxide of the parent drug (B9502-044), considered to be the predominant carrier of biological activity. The proposed high-dose of 2.5 mg/kg results in an

estimated B9302-044 AUC of 1400 μg/l.hr based upon linear extrapolation from a 1-day pharmacokinetic study. The predicted AUC is approximately 4-fold of the predicted highest human AUC.

Table 33. Proposed dose-selection for a rat carcinogenicity study.

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.5	1.5	2.5
Predicted AUC of B9302-107 (μg/l hr)*	13.46	33.47	55.78
Multiple of human AUC**	0.4	1.0	1.7
Predicted AUC of B9502-044 (μg/l hr)*	259.98	840.22	1,400
Multiple of human AUC***	0.7	2.4	4.0

* AUC estimates based on 1-day PK study for doses of 0.5 mg/kg and 1.5 mg/kg.

** Based upon AUC of 32.86 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days

In comparing the 3-month hamster and 6-month rat studies, it is apparent that the systemic exposure to Roflumilast is approximately 4-fold greater in the rat compared to the hamster at doses estimated to be the MTD in each species. Similarly, exposure to the N-oxide of Roflumilast is estimated to be 2-fold greater in female rats and comparable in males at the respective MTDs. In addition, hamsters appear to be more sensitive to DCAP exposure in the nasal cavity than the rat, although the rat concentrates more of the metabolite in this region. Also, the hamster and mouse demonstrate a similar metabolic profile of Roflumilast and the mouse has already been selected for testing. Finally, a greater historical data base is available for the rat. It is, thus, recommended that the rat be utilized for carcinogenic assessment rather than the hamster. It is also recommended that a high dose of 2.5 mg/kg Roflumilast be administered in the 24-month rat carcinogenicity study.

2c. Summary and Evaluation of Mouse Carcinogenicity Proposal:

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.5	1.7	6
Predicted AUC B9302-107 (μg/l·hr)*	23.5	79.7	281.44
Multiple of human AUC**	0.7	2.4	8.6
Predicted AUC B9502-044 (μg/l·hr)*	17.1	58.3	205.7
Multiple of human AUC***	0.05	0.17	0.59

* AUC estimates based upon 1-day PK studies in mice.

** Based upon AUC of 32.86 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days

The sponsor proposes a carcinogenicity study in B6C3F1 mice by the oral gavage route, using a methylcellulose suspension at a high dose of 6 mg/kg. The proposal is based upon a 3-month oral gavage study in mice at doses of 6, 12, and 18 mg/kg. One control female and one mid-dose male were found dead in their cages on Days 12 and 55, respectively. Although, the sponsor did not provide an explanation for these findings, they did not appear to be drug-related. Body weight gain, although statistically reduced in all treated males, does not appear to be dose-related and changes in actual weight compared to control groups were minimal. The primary target organs of toxicity were the nasal cavity (olfactory epithelial degeneration and epithelial necrosis,

mid- and high-dose) and the adrenals (cortical zone atrophy and hyperemia of the inner cortex). In addition, splenic follicular hyperplasia was noted in two high-dose females and lympho-histiocytic infiltration was a fairly systemic finding, usually of minimal severity. All histologic findings were of minimal severity. Additional findings included decreased leukocyte numbers in females and increased segmented neutrophils in males and females which did not correlate with any histological findings. A summary of the results pertaining to the selection of the MTD are found in Table 34.

Table 34. Summary of Roflumilast-related findings in mice following 3-month administration.

	Males				Females			
Dose group	VC	6	12	18	VC	6	12	18
Body weight gain								
Net BW gain (g)	6	5	5	5	6	7	6	7
%Δ BW gain		↓17	↓17	↓17		↑17	--	↑17
Histology n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Adrenal								
Atrophy, cortical x-zone	0	0	0	5(.7)	0	5(.4)	8(.9)	8(1)
Hyperemia of inner cortex	0	0	4(.4)	1(.2)	0	4(.3)	6(.7)	9(1.3)
Nasal cavity								
Olfactory epithel degeneration	0	0	0	3(.2)	0	0	4(.2)	5(.4)
Olfactory cell necrosis	0	0	1(.1)	2(.4)	0	0	1(.03)	1(.05)
Spleen								
Serosal Lympho-histiocyt infiltr	0	1(.03)	1(.03)	0	0	0	1(.03)	1(.1)
Follicular hyperplasia	0	0	0	0	1(.2)	0	0	2(.4)

Shaded areas indicate a significant difference from vehicle controls.
Incidence (severity). Severity Scale: 1: minimal 2: mild 3: moderate 4: severe

The proposed high-dose of 6 mg/kg for the mouse carcinogenicity study results in an estimated B9302-107 AUC of 422.2 μg/l.hr based upon linear extrapolation from a 1-day pharmacokinetic study. The predicted AUC is approximately 8.6-fold of the predicted highest human AUC. The primary metabolite in both humans and mice is the N-oxide of the parent drug (B9502-044), considered to be the predominant carrier of biological activity. The proposed high-dose of 6 mg/kg results in an estimated B9502-044 AUC of 308.6 μg/l.hr based upon linear extrapolation from a 1-day pharmacokinetic study. The predicted AUC is approximately 0.6-fold of the predicted highest human AUC. The proposed high dose was selected by the sponsor based upon the histological findings in the nasal olfactory epithelium and the adrenals. The sponsor considers that these findings indicate that the MTD has been achieved.

In the reviewer's opinion, although the sponsor has identified toxicity in the target organs, it is questionable as to whether these changes would alter the animal's normal life span due to the minimal severity reported at even the highest dose tested. However, slight increases in olfactory epithelium necrosis in high-dose animals suggests a dose-related response. Therefore, a high dose of 12 mg/kg could be selected, since a progressive necrosis could influence survivability over the course of a chronic study. The proposed high-dose of 12 mg/kg results in an estimated B9302-107 AUC of 563 μg/l.hr based upon linear extrapolation from a 1-day pharmacokinetic study in mice. The predicted AUC is ~ 17-fold of the predicted highest human AUC.

OVERALL SUMMARY AND EVALUATION

Roflumilast has been developed as a PDE IV inhibitor for the treatment of asthma. A dose of 0.5 mg is expected to be the marketed dose. The sponsor has proposed two carcinogenicity protocols in hamsters and mice and has requested Agency concurrence for use of the hamster in place of the mouse and, also, for the doses selected in their proposed protocols. Pharmacokinetic and single/repeat dose toxicology studies, study summaries for 3-month dose-ranging studies in hamsters and mice and a 6-month toxicity study in rats were reviewed in order to assess the use of the hamster and the carcinogenicity study protocols submitted by the sponsor.

Pharmacokinetics: Systemic exposure to a single oral dose of B9302-107 (Roflumilast) increased proportionally while exposure to metabolites increased sub-proportionally to proportionally in male rats and male Syrian golden hamsters. The greatest exposures in descending order were to the N-oxide of the parent drug, the N-oxide of DCAP, DCAP and the parent drug, as exposures to metabolites were greater than those of the parent compound. Serum AUC levels of B9302-107 and its N-oxide were greater in rats than in hamsters, but were only one-half and 16-fold greater, respectively, than in mice. Exposure to DCAP was 10-fold and 48-fold greater in the rat compared to the hamster and mouse, respectively. However, the mouse was similar to the hamster in terms DCAP and DCAP's N-oxide levels. In man, a dose of 0.5 mg produced low levels of parent drug and high levels of its N-oxide, similar to the rat and hamster. However, very low relative levels of DCAP were observed in serum. Exposure to DCAP also increased proportionally in rats administered DCAP orally. Drug absorption in rats and mice was ~30-50% following oral dosing. Exposure was greatest in the nose and liver and high AUC tissue/AUC plasma ratios were noted in the nose (4.7-7.9). An association of radioactivity in the nasal olfactory epithelium of rats was more pronounced following the administration of [^{14}C]-DCAP than [^{14}C]-B9302-107, considered to be related to cytochrome P450 activation of DCAP to its N-oxide (or follow-up metabolites) in the nasal epithelium. Highest concentrations of radioactivity in the mouse and hamster were observed in the lung, liver, adrenals, and bone marrow and low concentrations in the nose compared to the rat were detected. Single dose toxicity studies have shown the rat to be the most sensitive of the species tested on a mg/kg basis to nasal olfactory changes (primarily olfactory epithelial degeneration and necrosis) following DCAP administration. The sponsor proposes that DCAP is concentrated in the nasal epithelium and converted to its N-oxide, thought to be responsible for the observed nasal lesion, by nasal microsomes. Mouse and hamster nasal microsomes also convert DCAP to its N-oxide but do not concentrate DCAP in the nasal epithelium. Human nasal epithelial microsomes do not convert DCAP to its N-oxide form. Thus, DCAP exposure should not be a concern for humans. The dominant biotransformation routes in all species tested were N-oxidation of parent drug, cleavage of the parent molecule to yield DCAP, N-oxidation of DCAP, or O-dealkylation of the parent compound. Low amounts of unchanged drug in rat plasma after oral dosing indicated high first-pass metabolism and/or high affinity of parent drug for tissues and organs with M1 the major carrier of radioactivity. DCAP was the major metabolite in the nose, liver, kidneys and lungs of rats only. Study summaries indicated that mouse and hamster, but not rat, hepatic microsomes convert DCAP to its N-oxide. Approximately 70% of total radioactivity was excreted in rat urine and 30% in feces via the bile following iv administration; M2 was primarily detected in rat urine and feces. Unchanged drug was primarily detected in

feces after oral administration. Low amounts of M2 were detected in mouse and hamster urine, in contrast to the rat, while none was detected in human urine. In mice, B9302-107 and metabolites were excreted primarily in the bile with high recovery in feces (61% of dose) compared to urine (30% of dose).

Single/Repeat Dose Toxicity: Single/repeat (7 day) oral (gavage) dose-studies were performed in male Wistar rats, hamsters and mice. In single dose studies 300 mg/kg Roflumilast or 240 mg/kg DCAP or B9502-054 induced moderate to severe nasal toxicity in rats. Similar doses also induced severe necrosis of all hamsters and mice administered DCAP or B9502-054, but in only 2 of 5 animals administered Roflumilast. Oral dosing with DCAP (0.05-6 mg/kg) induced minimal olfactory epithelium degeneration at low doses (0.05 and 0.5 mg/kg), while necrosis and basal cell regeneration occurred at higher doses. Minimal olfactory epithelium degeneration was also reported in hamsters at 0.5 or 1.5 mg/kg DCAP. Lesions in the testes and epididymes were also observed in rats administered 300 mg/kg Roflumilast only. Additional findings at this dose included lesions in the stomach and intestines. In contrast, only DCAP and B9502-054 (240 mg/kg, single) induced lesions in the testes of hamsters; lower doses of B9202-045 or up to 300 mg/kg B9302-107 produced no lesions. All treated-rats at doses of 240-300 mg/kg also experienced reduced body weight gain and clinical signs which were most severe in Roflumilast-treated animals. Thus, Roflumilast induced comparable nasal cavity lesions in the hamster, mouse and rat following a single oral dose, although lesion severity was reduced in hamsters and mice. However, lesion severity was comparable when hamsters and mice were administered DCAP or B9502-054 directly. Roflumilast induced male reproductive organ toxicity in rats but not in hamsters, while DCAP and its N-oxide induced reproductive organ lesions in hamsters but not in rats. DCAP or its N-oxide may, thus, be responsible for the observed nasal lesions, while Roflumilast or another metabolite may be responsible for the reproductive organ changes. In repeat dose studies, 8 mg/kg Roflumilast or 6.4 mg/kg DCAP or B9502-054 induced nasal toxicity in rats; lesions in the testes/epididymis were noted in Roflumilast-treated rats only. Body weight gain was reduced most severely in Roflumilast-treated animals and clinical signs were comparable among treatment groups. Gastrointestinal toxicity was not observed with repeated dosing. Oral dosing of DCAP (0.05-6 mg/kg) induced necrosis and basal cell regeneration at all doses but the lowest and reduced body weight gain was noted at doses > 0.05 mg/kg after repeated dosing.

Subchronic Toxicity: Study summaries for 3-month dose-ranging studies in mice and hamsters were submitted. In mice, orally (gavage) administered Roflumilast (6, 12, 18 mg/kg) induced olfactory epithelial degeneration and necrosis, cortical atrophy and hyperemia of the adrenal glands, and increased incidence/severity of splenic follicular hyperplasia in high-dose females. In general, lesion severity was minimal. Reduced leukocyte numbers and increased segmented neutrophil numbers were also observed. The target organs of toxicity were the nasal cavity and adrenal glands. In hamsters, dysspermia and tubular atrophy of the testes, and seminal vesicle atrophy were noted following oral administration of 4, 8 or 16 mg/kg. In addition, olfactory epithelial disorganization and necrosis were reported. Other findings of unclear significance included prostatic atrophy, a pancreatic granuloma, adrenal gland hyperemia, and various findings in the eye. Body weight gain was dose-dependently reduced and, although only the effect in high-dose females was statistically significant. The target organs of toxicity include the

male reproductive organs, the nasal cavity, the eye, and possibly the prostate, adrenals and pancreas.

Chronic Toxicity: In a 6-month oral toxicity study in rats (0.5, 1.5, and 2.5 mg/kg Roflumilast), a non-reversible disruption of the nasal olfactory epithelium and an influx of round cells beneath the basement membrane, as well as a non-reversible increase in epididymal spermatoceles and tubular developmental defects of the testes, were observed. One high-dose female had a mammary gland adenoma at 6 months of age. Slightly increased findings, including focal gastric erosion, peritonitis and ulceration, were noted in the high dose groups, but were not apparent following the recovery period. At the high dose, the incidence of polyuria and chromodacryorrhea were slightly increased during the dosing period. Other findings included increased leukocyte counts, reversible decreases in serum cholesterol and triglyceride levels, reversible increases in urine calcium levels, a reduced number of estrus events, slight non-dose dependent increases in mean PQ, QαT, and QT times in all treatment groups and a reversible reduction of adipose tissue development. A NOAEL of 0.5 mg/kg was selected due to histologic findings in the nasal cavity. Target organs of toxicity include the nasal cavity, the testes, seminal vesicles and epididymis, and, possibly, the stomach.

Genetic Toxicology: B9302-107 was negative for mutagenicity and clastogenicity in the Ames Assay, an in vitro chromosome aberration assay with human lymphocytes, an HPRT test with V79 cells, and an in vitro micronucleus test with V79 cells. However, B9302-107 tested positive in the in vivo micronucleus test in mice. The metabolite, B9202-045 (DCAP), tested negatively in the in vivo mouse micronucleus test.

Carcinogenicity Protocol:

1. Proposal for Use of the Hamster Rather than the Rat for Carcinogenicity Study:

Enzyme induction and plasma protein binding by Roflumilast have not been evaluated. Maximum plasma levels were attained within 3.5 hours in rats and hamsters and no evidence of drug accumulation was observed in rats over 7 days. At comparable doses, the amount of Roflumilast present in rat serum was only one-half that observed in mice, but 16-fold greater than in hamsters, respectively. The major metabolite detected in rats, hamsters, mice and humans is the N-oxide of the parent drug (M1), thought to be the primary carrier of biological activity. At comparable doses, this metabolite was present in rat serum at 3- and 16-fold greater levels than in hamsters and mice, respectively. Other significant metabolites include DCAP, and the N-oxide of DCAP (M2). Serum levels of DCAP, a metabolite thought to be involved in induction of nasal olfactory epithelial lesions, were also detected at significantly greater levels in rats than in mice, hamsters and humans; a 10- to 46-fold increase compared to mice and hamsters, respectively. In contrast, serum levels of the N-oxide of DCAP were significantly greater in mice than in rats (hamster not analyzed at this dose). In rats, total radioactivity AUC in the nasal epithelium is 10 to 28-fold greater than other species administered 1 mg/kg labeled

Roflumilast. In addition, the ratio of nasal mucosa AUC to plasma AUC was also significantly greater in the rat and increased further DCAP was administered directly. These results correspond to toxicity studies showing the rat to be the most sensitive of the species tested on a mg/kg basis to nasal olfactory changes following administration of DCAP. The sponsor proposes that DCAP is concentrated in the nasal epithelium and converted to its N-oxide, thought to be responsible for the observed nasal lesions, by nasal microsomes. Mouse and hamster nasal microsomes have also been shown to convert DCAP to its N-oxide, but these species do not concentrate DCAP in the nasal epithelium to the degree found in rats. Since human nasal epithelial microsomes do not convert DCAP to its N-oxide form only low levels of DCAP are produced in humans, nasal lesions induced by DCAP are not a concern for humans.

The sponsor proposes to use the hamster for its carcinogenicity study due to the unique metabolic profiling and handling of metabolites in the rat and the resulting increased nasal toxicity. The sponsor suggests that the lesions in the nasal cavity would be the dose limiting factor for a 24-month carcinogenicity study in rats. Thus, they propose that the lower sensitivity of the hamster to nasal olfactory toxicity, compared to the rat on a mg/kg basis, allows for increased dosing of Roflumilast and overall greater systemic exposure to drug-related material. Based upon the information provided, the reviewer concurs with the sponsor that the rat does uniquely metabolize Roflumilast compared to the hamster by producing excessive levels of DCAP and selectively concentrating DCAP in the nasal epithelium. However, the results of single dose through 6-month toxicity studies in rats and hamsters demonstrate similar target organs of toxicity in the two species. In addition, the systemic exposure to Roflumilast and DCAP are greater in the rat at doses approximating the MTD in both species in long-term studies and exposure to the N-oxide of Roflumilast, considered a primary carrier of biological activity, is comparable. Nasal mucosa AUC and toxicity data suggest that similar degrees of lesion severity in nasal cavity are produced at greater nasal AUC levels in the rat. Thus, on the basis of nasal mucosa exposure to administered drug material, the hamster shows greater sensitivity than the rat. Furthermore, the goal of a carcinogenicity study is to assess responses based upon systemic exposure to the administered drug (and, in this case, its proposed biologically active metabolite, B9502-044). Thus, based upon the results of a 3-month hamster study and a 6-month rat study, summarized in the following section, and the limited pharmacokinetic data available (single-dose except for human, 7-day), the rat appears more likely to receive the greatest systemic exposure to the parent compound and active metabolite at doses approximating the MTD in each species.

Summary and evaluation of Sponsor-proposed hamster carcinogenicity protocol.

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.25	1	4
Predicted AUC of B9302-107 (µg/l hr)*	0.35	1.4	13.9
Multiple of human AUC**	0.01	0.04	0.42
Predicted AUC of B9502-044 (µg/l hr)*	47.8	191.1	729.6
Multiple of human AUC***	0.14	0.54	2.08

* AUC estimates based upon 1-day PK study extrapolated from dose of 1.5 mg/kg (for proposed doses 0.25 and 1 mg/kg) and 9 mg/kg (for proposed dose of 4 mg/kg).

** Based upon AUC of 32.86 µg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 µg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

The sponsor proposes a carcinogenicity study in Syrian golden hamsters at a high dose of 4 mg/kg. In a 3-month hamster oral gavage study (4, 8 and 16 mg/kg) one mid-dose and high-dose male died (gavage error). The sponsor proposed the high-dose of 4 mg/kg due to significant reduction in body weight gain of high-dose females and since they felt that the MTD for males was "in the range of" 4 mg/kg based on histological findings of comparable severity in the testes/epididymes at all doses tested. The sponsor's proposed high-dose would be acceptable for females only should the hamster be considered an appropriate species for the carcinogenicity testing of Roflumilast. The reduced body weight gain in females, though significant only at the high-dose, was 21% at the mid-dose and would likely increase with increasing duration. Thus, a high-dose of 4 mg/kg should be acceptable for females. A dose of 4 mg/kg is expected to be approximately 0.4-fold and 2.1-fold of the predicted highest human AUC of parent drug and B9502-044, respectively. For males, however, it is questionable as to whether the histologic findings in the testes/epididymes are likely to affect the survivability of the animals. Therefore, body weight gain should be used, in addition to the nasal olfactory necrosis, to determine the high-dose for males. A dose of 8 mg/kg should be selected as the high dose in males based upon reduced body weight gain and nasal olfactory necrosis. This dose would provide estimated exposure ratios of 0.94 and 4.2 compared to the human exposure of Roflumilast and B9502-044, respectively.

Proposed (by reviewer) rat carcinogenicity protocol.

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.5	1.5	2.5
Predicted AUC of B9302-107 (μg/l hr)*	13.46	33.47	55.78
Multiple of human AUC**	0.4	1.0	1.7
Predicted AUC of B9502-044 (μg/l hr)*	259.98	840.22	1,400
Multiple of human AUC***	0.7	2.4	4.0

* AUC estimates based on 1-day PK study for doses of 0.5 mg/kg and 1.5 mg/kg.

** Based upon AUC of 32.86 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days

A 6-month oral (gavage) toxicity study was performed in Wistar rats with Roflumilast (0.5, 1.5, and 2.5 mg/kg/day). Dose selection for this study was based upon a 1-month study in which numerous high-dose animals (8 mg/kg) died during the treatment period; the next lowest dose (2 mg/kg) induced gastric erosions in males and a single incidence of spermiogenic granuloma. A non-reversible disruption of the nasal olfactory epithelium and influx of round cells beneath the basement membrane of the olfactory epithelium were observed in mid- and high-dose animals. Also, a non-reversible increase in epididymal spermatoceles (mid- and high-dose males) and tubular developmental defects of the testes were diagnosed (high-dose males). One high-dose female had a mammary gland adenoma at 6 months of age. Additional findings of slightly increased incidence compared to control animals, including focal gastric erosion, peritonitis and ulceration, hematologic and clinical chemistry findings, and slight increases in ECG parameters were noted in the high dose groups, were not apparent following the recovery period.

Based upon the results of the 6-month rat study, a high dose for a rat carcinogenicity study should be 2.5 mg/kg, since none of the findings of the 6-month study are considered to be serious

enough to reduce animal survival and since a dose of 8 mg/kg resulted in high mortality in a 1 month study. The proposed high-dose of 2.5 mg/kg in a methylcellulose suspension for the rat carcinogenicity study results in an estimated B9302-107 AUC of 55.78 μg/l.hr, ~ 1.7-fold of the predicted highest human AUC. The proposed high-dose of 2.5 mg/kg results in an estimated AUC of B9302-044, the primary active metabolite in both humans and rats, of 1400 μg/l.hr, approximately 4-fold of the predicted highest human AUC.

In comparing the 3-month hamster and 6-month rat studies, it is apparent that the systemic exposure to Roflumilast is approximately 4-fold greater in the rat compared to the hamster at doses estimated to be the MTD in each species. Similarly, exposure to the N-oxide of Roflumilast is estimated to be 2-fold greater in female rats and comparable in males at the respective MTDs. In addition, hamsters appear to be more sensitive to DCAP exposure in the nasal cavity than the rat, although the rat concentrates more of the metabolite in this region. Also, the hamster and mouse demonstrate a similar metabolic profile of Roflumilast and the mouse has already been selected for testing. Finally, a greater historical data base is available for the rat. It is, thus, recommended that the rat be utilized for carcinogenic assessment rather than the hamster. It is also recommended that a high dose of 2.5 mg/kg Roflumilast be administered in the 24-month rat carcinogenicity study.

Summary and Evaluation of Mouse Carcinogenicity Proposal:

	Low	Mid	High
Proposed oral gavage dose (mg/kg)	0.5	1.7	6
Predicted AUC B9302-107 (μg/l·hr)*	23.5	79.7	281.44
Multiple of human AUC**	0.7	2.4	8.6
Predicted AUC B9502-044 (μg/l·hr)*	17.1	58.3	205.7
Multiple of human AUC***	0.05	0.17	0.59

* AUC estimates based upon 1-day PK studies in mice.

** Based upon AUC of 32.86 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days.

*** Based upon AUC of 351.39 μg/l hr in healthy volunteers dosed with 0.5 mg B9302-107 for 7 days

The sponsor proposes a carcinogenicity study in B6C3F1 mice by the oral gavage route, using a methylcellulose suspension at a high dose of 6 mg/kg. The proposal is based upon a 3-month oral gavage study in mice at doses of 6, 12, and 18 mg/kg. One control female and one mid-dose male were found dead in their cages but did not appear to be drug-related. Reduced body weight gain was not dose-related and changes in actual weight compared to control groups were minimal. The primary target organs of toxicity were the nasal cavity (olfactory epithelial degeneration and epithelial necrosis, mid- and high-dose) and the adrenals (cortical zone atrophy and hyperemia of the inner cortex). In addition, splenic follicular hyperplasia was noted in high-dose females and systemic lympho-histiocytic infiltration of minimal severity was reported. All histologic findings were of minimal severity. Additional findings included decreased leukocyte numbers and increased segmented. The proposed high dose of 6 mg/kg was selected by the sponsor based upon the histological findings in the nasal olfactory epithelium and the adrenals. The sponsor considers that these findings indicate that the MTD has been achieved. In the

reviewer's opinion, although the sponsor has identified toxicity in the target organs, it is questionable as to whether these changes would alter the animal's normal life span. However, slight increases in olfactory epithelium necrosis in high-dose animals suggests a dose-related response. Therefore, a high dose of 12 mg/kg could be selected, since a progressive necrosis could influence survivability over the course of a chronic study. The proposed high-dose of 12 mg/kg results in an estimated B9302-107 AUC of 563 µg/l.hr based upon linear extrapolation from a 1-day pharmacokinetic study in mice. The predicted AUC is ~ 17-fold of the predicted highest human AUC.

RECOMMENDATIONS

1. The reviewer disagrees with the sponsor in terms of species selection for the carcinogenicity study based upon the unique metabolic and transport characteristics observed in the rat. The rat is the recommended species over the hamster due to the increased systemic exposure to Roflumilast and its N-oxide observed in the Sprague Dawley rat based upon extrapolation from single dose pharmacokinetic data at the estimated MTDs for each species. However, if additional pharmacokinetic data from Wistar rats indicates otherwise, the hamster could be the species of choice.

2. The reviewer recommends a high dose of 2.5 mg/kg in male and female rats due to a lack of serious findings in the 6-month study and since a dose of 8 mg/kg induced increased mortality in a 1-month study.

3. The reviewer does not concur with the sponsor on a high-dose in mice of 6 mg/kg as a maximum tolerated dose. The proposed high dose was selected by the sponsor based upon the findings in the nasal olfactory epithelium and the adrenals. Although the sponsor considers that these findings indicate that the MTD has been reached, it is questionable as to whether these findings would significantly influence animal survival in a chronic study. However, a slight increase in olfactory epithelium necrosis in high-dose males and females suggests a dose-related response. Therefore, the dose of 12 mg/kg could be selected as the high dose, since a progressive necrosis could influence survivability over a chronic study. This recommendation is made on the condition that studies summarized in the Sponsor's Carcinogenicity Protocol package are submitted and are found to be in agreement with the submitted summaries.

4. For rats, the low dose of 0.5 mg/kg was selected since this dose was determined to be the NOAEL in a 6-month study. The mid-dose of 1.5 mg/kg is justified on the basis of providing an adequate determination of dose-response. In mice, the low and mid-doses proposed by the sponsor have been increased to 1 and 6 mg/kg in order to provide an adequate dose-response for the high-dose of 12 mg/kg.

5. The above recommendations are pending the CAC's concurrence.

6. The sponsor should address the positive findings observed in the in vivo micronucleus assay in mice following administration of Roflumilast.

Timothy J. McGovern, Ph.D., Pharmacologist

CC: HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/D. Hilfiker
HFD-570/T.J. McGovern

Executive CAC
February 9, 1999

Committee: Joseph DeGeorge, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-900, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Alternate Member
Joseph Sun, Ph.D., HFD-570, Team leader
Timothy McGovern, Ph.D., HFD-570, Presenting Reviewer
Adele Seifried, Project Manager

Author of Draft: Timothy McGovern

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

IND #: Pre-IND
Drug Name: Roflumilast (BY217)
Sponsor: Byk Gulden (represented by Altana)

Roflumilast is a PDE IV inhibitor, and is intended for the treatment of asthma. The sponsor sought Agency concurrence with their proposal to use the hamster rather than the rat for carcinogenicity testing due to the unique metabolic profile of Roflumilast and nasal cavity toxicity found in the rat. In addition, the sponsor sought concurrence for dose selection in hamster and mouse studies. The anticipated clinical dose is 0.5 mg per day. Roflumilast was negative in the Ames Assay, an in vitro human lymphocyte chromosome aberration assay, an HPRT test with V79 cells, and an in vitro micronucleus test with V79 cells, but was positive in the in vivo micronucleus test in mice. The metabolite, B9202-045 (dichloroaminopyridine, DCAP), tested negatively in the in vivo mouse micronucleus test.

Selection of hamster for carcinogenicity testing.

The sponsor proposed using the hamster in place of the rat for carcinogenicity testing with Roflumilast based upon comparative metabolic profiling in which significantly greater serum levels of the metabolite DCAP were detected in the rat versus the hamster, mouse or humans. The sponsor also indicated that the rat was inappropriately sensitive to the nasal toxicity of Roflumilast. The committee discussed the merits of these considerations and the overall comparative systemic exposure data that was available. Several issues were apparent. 1) Although systemic exposure to parent and the primary active metabolite(s) at testable doses appeared greater in the rat than the hamster (thus potentially favoring use of the rat), the exposure data were not from the rat stain used in the toxicity studies. 2) The AUC data were not calculated based on integration over the same time period; the hamster AUC was based on integration of 1/3 the time period used for the rat. 3) Using the available data and comparing exposures resulting in nasal toxicity, it was not clear that the nasal toxicity was in fact due solely to DCAP and its N-oxide. 4) In subchronic toxicity studies, hamster demonstrated similar nasal toxicity at

approximately similar exposures as observed in the rat, indicating that the rat is not likely to be more sensitive, although the toxicity in the rat was observed at overall lower doses than in the hamster. 5) The profile of exposure to parent drug and metabolites in hamster (and mouse) was more similar to humans than appeared to be the case with the available rat data. Although not based on reduced sensitivity to the local toxicity, the hamster was considered an acceptable alternative to the rat in testing the carcinogenic potential of Roflumilast.

Dose selection for hamster carcinogenicity studies.

The sponsor proposed doses of 0.25, 1 and 4 mg/kg/day by oral gavage. The doses for the proposed 18-month study were selected based upon determination of the MTD from a 3-month oral gavage study in hamsters (doses of 0, 4, 8, and 16 mg/kg). Body weight gain was reduced by 10 and 20% in mid- and high-dose males, respectively, and 15, 21 and 34% in low-, mid- and high-dose females, respectively. Only the effect in high-dose females was statistically significant. Nasal olfactory epithelium disorganization was observed at the mid- and high doses, but lesion severity was not convincing. Also, increased incidence and severity of male reproductive organ effects were noted at all treatment doses, however, these effects are not considered to represent an MTD that, if exceeded, would reduce the life expectancy of the animals. The findings used by the sponsor to select the MTD, primarily male reproductive organ effects at all doses and reduced body weight gain at the mid-dose in females, were not viewed by the Committee to be of a type or consistency at the proposed doses to be the basis of the MTD. Doses of 16 mg/kg in males and 8 mg/kg in females were considered by the committee to be the MTD, since body weight gain was significantly decreased at the high dose of 16 mg/kg in females in the 3-month study. It was noted that there is not available data to address the percentage reduction in body weight gain in three month studies that would be detrimental to hamster survival or could be anticipated to adversely impact progression of tumors. There was also concern for the availability of contemporary historical data should tumors be observed in the carcinogenicity study and it was noted that historically, carcinogenicity studies in hamsters were of 24 months duration.

Dose selection for mouse carcinogenicity studies.

The sponsor proposed doses of 0.5, 1.7 and 6 mg/kg/day by oral gavage. The doses for the 24-month study were selected based upon determination of the MTD from a 3-month oral gavage study in mice (doses of 0, 6, 12 and 18 mg/kg/day). Adrenal atrophy and hyperemia were noted at all doses in females and at doses of $\geq$ 12 mg/kg in males. In addition, nasal olfactory epithelial degeneration and olfactory cell necrosis were noted at the mid- and high-doses. However, the MTD selected by the sponsor, based upon histological findings in the adrenal gland and nasal cavity, was not considered by the Committee to be of a severity to adversely influence animal survival. Rather, doses of 18 mg/kg in males and 12 mg/kg in females were considered the MTD due to the increased incidence and severity of the adrenal and nasal lesions in the 3-month study.

Executive CAC Recommendations and Conclusions:

Hamster study:

1) The committee concurs with the sponsor's proposal to use the hamster for carcinogenicity testing rather than the rat.

2) The committee did not concur with the sponsor's proposed doses for the carcinogenicity in hamster. Concurrence could be offered on doses of 16, 8 and 4 mg/kg in males and 8, 3, and 1 mg/kg in females based primarily on consistent effects on body weight gain in the 3-month study. This concurrence is contingent upon agreement with the sponsor's summarized evaluation of findings submitted in carcinogenicity protocol package of 12/30/1998 following submission to and review by the agency of the final reports for the dose-ranging studies.

3) Since it is currently unclear as to what percentage reduction in body weight gain is detrimental to hamster survival or would adversely impact progression of tumors, the high doses may need to be adjusted during the carcinogenicity study in the event severe toxicity is observed. The sponsor is encouraged to discuss the need for dose adjustment with the agency.

4) The sponsor is encouraged to include an additional control group other than vehicle control unless sufficient contemporary historical data are available to the sponsor.

5) The standard duration for hamster carcinogenicity studies is 24 months. If overall effects on survival are observed, the sponsor is encouraged to contact the agency prior to premature study termination.

Mouse Study:

6) The CAC could not offer concurrence with the proposed doses, but could concur with doses of 18, 6 and 2 mg/kg in males and 12, 6, and 2 mg/kg in females. This concurrence is contingent upon agreement with the sponsor's summarized evaluation of findings submitted in carcinogenicity protocol package of 12/30/1998 following submission to and review by the agency of the final reports for the dose-ranging studies.

2/11/99

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:/
/Division File HFD-570, Pre-IND Roflumilast
/HFD-570/McGovern/Sun/Hilfiker
/ASeifried, HFD-024

Appendix 2

Pharmacology and Toxicology Review No. 2
by Dr. Timothy McGovern Completed on May 29, 2001
In IND 57,883

HFD-570 : DIVISION OF PULMONARY AND ALLERGY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review #2

IND No.	57,883	**Serial No.**	000	**Submission Date**:	16 FEB 1999
			007		29 JUN 1999
			035		28 APR 2000

Reviewer: Timothy J. McGovern, Ph.D. **Review Completed:** 29 MAY 2001

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

Sponsor: Byk Gulden Pharmaceuticals through Altana, Inc. (local agent)

Drug Names: Roflumilast *Code Name:* BY 217, B9302-107

Chemical Name: 3-cyclopropylmethoxy-4-difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide

Class: Selective phosphodiesterase 4 (PDE_4) inhibitor

Indication: Asthma

Route of Administration: Oral (tablet)

Proposed Clinical Protocol: (b) (4)

(b) (4)

Previous Clinical Experience: The sponsor has performed Phase 1 and 2 clinical trials in both the United States, Europe and South Africa.

Previous Review(s), Date(s) and Reviewer(s):

Review Type	Date of Submission(s)	Reviewer	Date of Review
Original Review	February 16-June 29, 1999	McGovern	July 24, 2000

Background: The sponsor submitted a 3-month juvenile rat study to support a single dose clinical trial in pediatric patients 6 years or older. Although the Division normally requires juvenile animal studies for trials including patients under 2 years of age, this study was reviewed

due to concerns over male reproductive organ toxicity associated with this drug. Reproductive toxicity studies were submitted in the Original IND package and are reviewed currently.

The following table summarizes the studies submitted and reviewed in this review:

Preclinical Studies Submitted and Reviewed in this IND:

Study	Submission #	Res. Report #	Vol.
Multiple Dose Toxicology:			
The toxicity of B9302-107 after oral administration to juvenile rats for 3-months	035	62/99	10.1
General Toxicology:			
Expert reports:			
Metabolism and pharmacokinetics of Roflumilast in animals	007	20G/99	5.6
Assessment of non-clinical toxic effects of roflumilast on nasal mucosa	007	86D/99	5.6
Comment on the induction of micronuclei in bone marrow erythrocytes of mice after oral administration of roflumilast	007	88/99	5.6
Assesment of genotoxic potential	007	89E/99	5.6
Carcinogenicity:			
Response to CAC meeting minutes.	007		5.5
Reproductive Toxicology:			
Influence of B9302-107 (po) on male and female fertility in rats. Effects on early embryo-fetal development.	000	19/97	1.24
Effects of B9302-107 (po) on embryo-fetal development in rats.	000	8/96	1.25
Effects of B9302-107 (po) on embryo-fetal development in rabbits.	000	191/95	1.25

Studies Not Reviewed in this IND: The following studies were not reviewed due to the proposed administration route of the drug. The current route is via oral administration. Should the sponsor propose to study the inhalation route in clinical trials, these studies should be reviewed.

Study	Submission #	Res. Report #	Vol.
(b) (4)			

Studies Previously Reviewed: None.

Note: Portions of this review were excerpted directly from the sponsor's submission.

TOXICOLOGY:

MULTIPLE-DOSE TOXICITY:

Oral administration of B9302-107 to juvenile rats for 3 months with 8 week recovery

Study No.: WR0589 *Report No.*: 62/99 *Volume*: 10.1

Study Dates: Starting date 9/2/1998; report issued 2/8/2000
Testing Lab: Byk Gulden, Hamburg, Germany
Test Article: BYK33043 (Batch AM50/126; purity = 99.79%) in suspension with 4% methocel and Med Antifoam C
Concentration: 0.2-0.8 mg/ml
Dose Volume: 1 ml/kg/day
GLP: The study summary was accompanied by a signed GLP statement.
QA report: Yes.

Note: Although the sponsor refers to this study as a juvenile rat study, juvenile studies should assess rats at an age of 1-2 weeks of age at study initiation.

Methods: Wistar rats (3 weeks old; 27-60 g) were assigned to the following treatment groups:

Dose (mg/kg/day):	0	0.2	0.5	0.8
No./sex 3 month study	10	10	10	10
No./sex 8-week recovery	8	0	0	8

Each rat received a daily oral (gastric cannula) dose of vehicle or test drug for 3-months. Autopsies were performed immediately following the dosing periods and 8-weeks following the dosing period. The following observations were made:

Clinical observation . . . 4 times daily
Body weight . . . . . days -1, 1; 2x/week
Food consumption . . . . day 1; 2x/week
Water consumption . . . day 1; 2x/week
Ophthalmoscopy . . . . . days 76, 132
ECG . . . . . days 70, 126
Hematology . . . . . days 29, 87, 143
Clinical chemistry . . . . days 29, 87, 143
Urinalysis . . . . . days 86/87, 142/143
Enzyme induction . . . . not performed
Vaginal smears . . . . . days 20-52, 111-136
Organ weights . . . . . at sacrifice; (for specific organs see Addendum, pages 6-7)
Gross pathology . . . . . at sacrifice
Histopathology . . . . . at sacrifice; all organs/tissues from vehicle control and high-dose groups, (for specific tissues/organs see Addendum, pages 6-7). In low- and mid-dose groups as well as recovery animals, nasal/paranasal cavities, lung, liver, heart, thyroids, testes, and epididymis were examined as well as additional target organs identified in the high-dose group and any gross changes.
Toxicokinetics . . . . . not assessed.

Results:

Mortality: There was no drug-related mortality, although single deaths occurred due to the gavage procedure in the first week in all but the highest dose group. One high-dose recovery female died due to ether narcosis for blood sampling at the end of the study.

Clinical Observations: No substance-related effects were observed.

Body Weight: No significant treatment-related effects were observed although high-dose females demonstrated a 9-13% increase in body weight gain following the dosing and recovery periods compared to control values.

Food Intake: No substance-related effects were observed.

Water consumption: Water consumption was increased 11-20% in high-dose animals throughout the dosing period and 19-32% in high-dose females during the recovery period.

Hematology: Leukocyte levels were increased 22-25% in high-dose females during the dosing period (Table 1). Levels were still increased by 31% after the recovery period.

Clinical Chemistry: No substance-related effects were noted.

Urinalysis: Urine chlorine levels were reduced by 21-35% in high-dose animals after 3 months dosing (Table 1). Potassium levels were reduced by 30% in high-dose females. These findings were reversible in females but not in males (chlorine levels reduced by 40%).

Table 1. Clinical pathology findings in juvenile rats following 3-month administration.

	Males				Females			
Dose (mg/kg/d)	0.2	0.5	0.8	0.8-0	0.2	0.5	0.8	0.8-0
Hematology								
Leukocytes								
%Δ vs control group	↑3	↑10	↑14	↓11	↑18	↑23	↑22	↑31
Urinalysis								
Chlorine								
%Δ vs control group	↑4	↓7	↓21	↓40	↑8	↓5	↓33	↑31
Potassium								
%Δ vs control group	↓3	↓2	↓9	↓30	↑7	↑2	↓27	↓9

Shaded areas indicate a significant difference from control values.

Vaginal Smears: No substance-related effects were noted.

Ophthalmoscopy: No substance-related effects were noted.

Electrocardiogram: No substance-related effects were noted.

Organ Weights: No substance-related effects were noted.

Gross Pathology: Intestinal discoloration was noted in one high-dose female but was not observed following the recovery period.

Histopathology: Centrilobular hypertrophy of the hepatocellular cytoplasm was noted in one mid- and high-dose male and two high-dose females (Table 2). In addition, myocardial degeneration was noted in one high-dose male. Other findings were indicative of inflammation and were of low severity. Findings in the liver, lungs and epididymis were not fully reversible.

Table 2. Histopathological changes following 3-month oral Roflumilast administration in rats.

	Males				Females			
Dose (mg/kg/d)	0	0.2	0.5	0.8	0	0.2	0.5	0.8
Liver n=	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
-lympho-histiocytic infiltration	1(.1)	0	7(.7)	7(1)	3(.3)	4(.5)	4(.4)	6(.8)
-centrilobular hypertrophy	0	0	1(.1)	1(.1)	0	0	0	2(.2)
-apoptosis	0	0	0	1(.1)	0	0	0	2(.2)
-vascular edema	0	0	0	1(.4)	0	0	0	0
Heart	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
-myocardial degeneration	0	0	0	1(.1)	0	0	0	0
Lungs	**10**	**1**	**2**	**10**	**10**	**0**	**1**	**10**
-lympho-histiocytic infiltration	7(1.5)	0	1(1.5)	9(1.7)	7(.9)	0	0	8(1.5)
-interstitial pneumonia	1(.3)	0	0	3(.7)	0	0	0	1(.3)
-BALT-activation	4(1.1)	0	0	7(1.3)	1(.2)	0	0	6(1.1)
Spleen	**10**	**3**	**1**	**10**	**10**	**0**	**1**	**10**
-hemosiderosis	0	0	0	3(.5)	1(.1)	0	0	3(.3)
-chronic perisplenitits	0	0	0	0	0	0	0	1(.3)
Epididymis	**10**	**10**	**10**	**10**				
- lympho-histiocytic infiltration	2(.2)	2(.3)	2(.4)	4(.5)				
Recovery – 8 weeks	**8**			**8**	**8**			**8**
Liver								
-lympho-histiocytic infiltration	2 (0.3)			6 (1)	7 (0.9)			7 (1.1)
-apoptosis	0			2 (0.3)	0			0
Epididymis								
-lympho-histiocytic infiltration	3 (0.5)			6 (0.9)				
Lungs	**0**			**1**	**1**			**2**
-lympho-histiocytic infiltration				1 (2.0)	0			2 (1.5)

Severity scale: 1: minimal; 2: mild; 3: moderate; 4: marked.

NOAELs of 0.2 mg/kg in males and 0.5 mg/kg in females were identified. The sponsor suggested that no drug-related findings were noted. Target organs of toxicity included the liver, epididymis, heart, lungs and spleen. The NOAELs in this study are comparable to that found in the previous 3-month adult oral toxicity study in rats (0.2 mg/kg; next highest dose was 2 mg/kg; see Original IND Review). There was no increased sensitivity in terms of male reproductive organ toxicity compared to the previous study. However, some differences were noted in other target organs of toxicity. The liver and lungs were not previously identified in adult studies and may indicate an increased sensitivity in slightly younger animals. The identified target organs in the 3-month adult study (nasal cavities, testes, epididymis, and the thymus) were not identified in juvenile animals with the exception of the epididymis. This result may be due to reduced dosing levels in the present study. Although the heart and spleen were not identified in the 3-month adult study, they were identified in a 4-week study which utilized doses up to 8 mg/kg. This finding may indicate an increased sensitivity in younger rats for these organs.

Addendum: Histopathology inventory for IND 57,883.

Study No.	194/95	128/96	129/96	113/96	81/95	38/98	14/96	159/96	68/95	94/96
Duration	1/7 day	1/7 day	1/7 day	14-d, iv	4 wk, po	3-mos, po	6-mos, po	14-d, iv	4-wk, po	6-mos, po
Species	M, rat	M, rat	M, rats	rat	rat	rat	rat	Dog	Dog	Dog
Adrenals	X			X*	X*	X*	X*	X*	X*	X*
Aorta				X	X	X	X	X	X	X
Bone marrow smear										X
Bone (femur)				X			X			
Bone (tibia)				X	X	X	X			
Bone (sternum)				X	X	X	X	X	X	X
Brain:				X*	X*	X*	X*	X*	X*	X*
Cecum							X	X		X
Cervix										X
Colon							X	X		X
Duodenum				X	X	X	X	X	X	X
Epididymis		X	X	X	X	X	X	X	X	X
Esophagus				X	X	X	X	X	X	X
Eye				X	X	X	X	X	X	X
Fallopian tube										
Fat										
Gall bladder									X	X
Gross lesions	X	X	X	X	X	X	X			
Harderian gland				X	X	X	X			
Heart	X			X*	X*	X*	X*	X*	X*	X*
Hyphophysis										
Ileum				X	X	X	X	X	X	X
Injection site	NA	NA	NA	X	NA	NA	NA	X	NA	NA
Jejunum				X	X	X	X	X	X	X
Kidneys				X*	X*	X*	X*	X	X*	X*
Lacrimal gland						X				
Larynx										
Liver				X*	X*	X*	X*	X*	X*	X*
Lungs			X	X*	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical										
Lymph nodes (LALN)				X	X			X	X	X
Lymph nodes, mandibular				X	X			X	X	?
Lymph nodes, mediastinalis				X	X			X	X	?
Lymph nodes, mesenteric				X	X	X	X	X	X	?
Mammary gland				X	X	X	X	X	X	X
Nasal cavity	X	X	X	X		X	X			X
Optic nerves										
Ovaries				X*	X*	X*	X*	X*	X*	X*
Oviduct							X			
Pancreas				X	X	X	X	X*	X*	X*
Parathyroid						X	X	X		X
Peripheral nerve							X			X
Pharynx										
Pituitary				X	X*	X*	X*	X*	X*	X*
Prostate				X	X*	X*	X*	X*	X*	X*
Rectum							X	X		
Salivary gland		X	X	X	X	X	X		X	X
Sciatic nerve				X	X	X		X	X	X
Seminal vesicles				X	X*	X*	X*			
Skeletal muscle				X	X	X	X	X	X	X
Skin				X	X	X	X	X	X	X
Spinal cord				X	X	X	X	X	X	X
Spleen				X*	X*	X*	X*	X*	X*	X*
Stomach				X	X	X	X	X	X	X
Testes	X	X	X	X*	X*	X*	X*	X*	X*	X*
Thoracic Limb								X		
Thymus				X*	X*	X*	X*	X	X	X
Thyroid				X*	X*	X*	X*	X*	X*	X*
Tongue				X	X	X	X	X	X	X
Trachea				X	X	X	X	X	X	X
Urinary bladder				X	X	X	X	X	X	X
Uterus				X*	X*	X*	X*	X*	X*	X*
Uterine horn										
Vagina				X*	X*	X*	X	X		

* Organ weight obtained

Addendum (cont'd): Histopathology inventory for IND 57,883.

Study No.	62/99	4D/98	252/98	216/98
Duration	3-mos	1/7 day	3-month	3-month
Species	Rat, juvenile	Hamster/mouse	Hamster	Mouse
Adrenals	X*		X	X
Aorta	X		X	
Bone marrow smear				
Bone (femur)				
Bone (tibia)	X (T)			
Bone (strenum)	X			X
Brain:	X*		X	X
Cecum	X		X	
Cervix				
Colon	X		X	X
Duodenum	X		X	X
Epididymis	X	X	X	X
Esophagus	X		X	X
Eye	X		X	X
Fallopian tube				
Fat				
Gall bladder			X	
Gross lesions	X		X	X
Harderian gland	X		X	X
Heart	X*		X	X
Hyphophysis				
Ileum	X		X	
Injection site	NA	NA	NA	NA
Jejunum	X		X	X
Kidneys	X*		X	X
Lacrimal gland	X		X	X
Larynx				
Liver	X*		X	X
Lungs	X*		X	X
Lymph nodes, cervical				
Lymph nodes (LALN)				
Lymph nodes, mandibular				
Lymph nodes, mediastinalis				
Lymph nodes, mesenteric	X		X	X
Mammary gland	X		X	X
Nasal cavity	X	X	X	X
Optic nerves				
Ovaries	X*		X	X
Oviduct	X		X	X
Pancreas	X		X	X
Parathyroid	X		X	
Peripheral nerve			X	X
Pharynx				
Pituitary	X*		X	
Prostate	X*		X	X
Rectum	X		X	
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X*		X	X
Skeletal muscle	X		X	X
Skin	X		X	X
Spinal cord	X		X	X
Spleen	X*		X	X
Stomach	X		X	X
Testes	X*	X	X	X
Thoracic Limb				
Thymus	X*		X	X
Thyroid	X*		X	X
Tongue	X		X	
Trachea	X		X	X
Urinary bladder	X		X	X
Uterus	X*		X	X
Uterine horn				
Vagina	X*		X	X

* Organ weight obtained

GENERAL TOXICOLOGY:

Four Expert Reports were submitted by the sponsor as part of their reactivation package following the sponsor's withdrawal of the IND due to Division concerns regarding the potential genotoxicity of Roflumilast. The topics of the reports included metabolism and pharmacokinetics of Roflumilast, non-clinical toxic effects of Roflumilast on the nasal mucosa, induction of micronuclei in bone marrow erythrocytes of mice and an assessment of genotoxic potential. The former three reports were produced by Byk Gulden staff and the latter report was produced by (b) (4) . The report on metabolism and pharmacokinetics summarized study reports which have previously been reviewed and focused on the production of metabolites responsible for nasal toxicity. The second report summarized previously reviewed toxicity studies demonstrating rodent-specific nasal toxicity due to metabolites of Roflumilast and concluded that there was no relationship between the results of the genotoxicity studies and the nasal toxicity since no DNA adducts were formed in nasal tissue. The third and fourth reports summarize the results of previously reviewed genotoxicity assays performed with Roflumilast, including a positive finding in an in vivo mouse micronucleus assay. A published study, which suggested that errors in the process of enucleation or differentiation of erythrocytes could be a possible explanation for a higher incidence of micronuclei in an in vivo micronucleus test, was also summarized. The focus of the reports is to demonstrate a lack of correlation of the genotoxicity studies with the nasal toxicity observed in rodents.

Overall, the Expert Reports submitted by the sponsor provide an adequate summary of the data generated in individual study reports. However, the sponsors focus is on demonstrating a lack of correlation between genotoxicity and rodent-specific nasal toxicity. The Division's concerns, in contrast, stemmed from positive findings in an in vivo mouse micronucleus assay which demonstrated potential genotoxicity in bone marrow tissue. Thus, the sponsor's arguments were not relevant to the Division's concern. However, negative findings in an in vivo mouse bone marrow assay relieved concerns regarding the genotoxic potential of Roflumilast following consult with the Center Genotoxicity Committee.

REPRODUCTIVE TOXICOLOGY:

Oral (gavage) fertility and early embryo-fetal development study of B9302-107 in rats
Report No.: 19/97 *Protocol No.*: RR0414 *Volume*: 1.24

Study Dates: Starting date 4/19/1996; report issued 9/25/1997
Testing Lab: Byk Gulden Institute of Pathology and Toxicology, Hamburg, Germany
Test Article: B9302-107 (Batch# AM46/251; purity = 98.57%) in 0.4% methylcellulose
Concentration: 0.02-0.18 mg B9302-107/ml
Dose Volume: 10 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

The protocol for this study was not reviewed by the Division.

Methods: Wistar Crl: (Wi)WU BR rats (males: 6 weeks old; 158-209 g; females: 12 weeks old; 173-239 g) were assigned to the following treatment groups:

Dose (mg/kg/day)	0	0.2	0.6	1.8
No. of rats/sex/group	28	28	28	28

Male rats were orally administered vehicle or B9302-107 via stomach tube for 70 days prior to mating, during the mating period (up to 3 weeks) until proof of pregnancy, and continued up to their own autopsy. Female rats were dosed for 14 days prior to mating and throughout the mating period (up to 3 weeks) and until day 15 pc. Mating attempts continued until spermatozoa appeared in vaginal smear or 3 weeks at the most. Males which did not copulate or the females which were not fertile despite evidence of spermatozoa were again mated with untreated females/males. The following observations were made:

Clinical observation . . . 1 time daily
Body weight . . . . . Males: 2x per week. Females: 2x per week days 1-14, 1x per week thereafter.
Food consumption . . . . 2x per week except during mating.
Males . . . . . pre-coital interval (# of nights to mate), copulation index (# of females assumed mated / # of females paired), autopsy, spermatology (in cases of disturbed fertility, histology of testes and epididymis, prostate and seminal vesicles; motility and count of sperm); histology of nasal and paranasal cavities, testes, epididymis
Females until autopsy . Vaginal smear and estrus cycle
Autopsy of dams . . . . . gravid uterus weight, fertility rate, implantation rate, preimplantation loss, postimplantation loss, living fetuses
Fetuses . . . . . Assessed for behavior and appearance, determination of sex, body weight, external, visceral and skeletal changes.
Statistics . . . . . descriptive characteristics, Kruskall Wallis test, Jonckheere-Terpstra test, Fisher test, Cochran-Armitage test, Ulemann test.

Results:

Mortality: One high-dose male died on day 82. The cause of death was stated as renal failure as a cystic kidney showing reddish fluid and dilated urethras was noted. The relationship of this death to drug-treatment is unclear.

Clinical Observations: No significant drug-related findings were noted.

Body Weight: Mid- and high-dose females demonstrated an increased body weight gain of 38 and 75% after the first two weeks of dosing (Table 3). This finding was not statistically significant. From days 15-20 PC, high-dose dams demonstrated a 14% decrease in body weight gain. Earlier time points showed comparable gains to control animals.

Gravid uterus weight was also reduced by 8 and 20% at the mid- and high-doses, respectively.

Table 3: Summary of effects on body weight gain (%).

Dose (mg/kg)	0.2	0.6	1.8
Male – body weight gain (days 0-100)	↓3	↓1	↓2
Female – body weight gain (days 0-16)	no Δ	↑38	↑75
Female – body weight gain (days 15-20, PC)	↑2	↓6	↓14
Gravid uterus weight	↑3	↓8	↓20

Food Intake: Food consumption was reduced by 9% in high-dose dams after the first week of dosing but recovered in the second week. No significant findings were noted in males.

Estrus cycle: No drug-related effects were observed.

Reproductive parameters: The pre-coital interval was similar between the groups. Copulation rate and fertility rate was significantly decreased in the 1.8 mg/kg group (Table 4). One high-dose female showed total abortions and another showed total resorptions. In addition, pre- and post-implantative losses were increased at the mid- and high-doses. The number of living fetuses was significantly reduced at the high-dose.

Table 4: Summary of effects on reproductive parameters.

Dose (mg/kg)	0	0.2	0.6	1.8
Copulation rate (%)	100	100	96.4	78.6
Fertility rate (%)	89.3	100	92.9	64.3
Pre-implantation loss (%)	10.6	11.6	21.5	17.5
Post-implantation loss (%)	4.7	5.5	14.8	30.7
Live fetuses	284	323	258	140

Shaded areas represent significant difference from control values.

Necropsy: Sperm count was reduced by 7% in high-dose males; sperm motility was not significantly affected. Testicular weight was increased up to 18% at the high-dose (Table 5). Histologic findings were noted in the nasal cavities, testes and epididymis.

Table 5. Autopsy findings in rats.

	Males				Females			
Dose (mg/kg/d)	0	0.2	0.6	1.8	0	0.2	0.6	1.8
Organ weight Testes - % Δ from control		↑3	↑9	↑18				
Nasal cavities n=	**28**	**28**	**28**	**27**	**28**	**28**	**28**	**28**
-olfactory cell degeneration	0	20(.75)	25(.93)	23(1.33)	0	12(.43)	24(1)	28(2.11)
-olfactory disorganization	0	0	16(.57)	26(2.11)	0	0	8(.29)	28(2.8)
-basal cell hyperplasia								
pale flat cells	0	0	0	16(.8)	0	0	0	8(.33)
basophilic cells	0	0	0	20(.86)	0	0	0	27(1.07)
-intraluminar cell. debris	0	0	0	5(.19)	0	0	0	0
-subepith neutrophil infiltr	0	0	0	3(.28)	0	0	0	0
Testes	**28**	**28**	**28**	**28**				
-tubular atrophy	1(.07)	4(.06)	3(.16)	8(.57)				
Epididymis	**28**	**28**	**28**	**28**				
-inflammation	0	1(.04)	0	3(.15)				
-sperm granuloma	0	0	0	4(.38)				
-hypospermia	0	0	0	1(.14)				
-dysspermia	0	0	1(.11)	1(.04)				

Severity scale: 1: minimal; 2: mild; 3: moderate; 4: marked.

Fetal effects: Mean litter weight was significantly reduced at the high dose. Slight increases in the incidence of liver hemorrhage, cavernous lung and incomplete ossification of certain structures were noted primarily at the high-dose (Table 6).

Table 6: Summary of effects on fetuses.

Dose (mg/kg)	0	0.2	0.6	1.8
Litter weight - % Δ from control		↑5	↓10	↓21
Fetal defects				
Liver hemorrhage - % of fetuses	0.7	0	2.4	3
% of litters	4	0	7.7	13.3
Cavernous lung - % of fetuses	0	0.6	0.8	4.5
% of litters	0	3.6	3.8	13.3
Inc Oss Manubrium- % of fetuses	0.7	0.6	3	4.1
% of litters	4	3.6	7.7	12.5
Inc Oss 1st sternal vertebral body				
% of fetuses	2.7	3.6	6	8.2
% of litters	16	14.3	23.1	31.3
Inc Oss 3rd sternal vertebral body				
% of fetuses	0.7	3	3	8.2
% of litters	4	14.3	11.5	31.3

Inc Oss = incomplete ossification

Control pairing: One mid-dose male and 6 high-dose males failed to produce sperm during the first mating trial with treated females. All high-dose males but one were able to mate with untreated females. Corresponding treated females that revealed no positive vaginal

smear after the first mating trial were mated again with untreated males. Four of six females of the high-dose group remained without positive vaginal smear.

Two control males, one mid-dose male and two high-dose males produced a positive vaginal smear during the first mating trial although the females were empty at autopsy. The males were again mated with untreated females and all produced a positive vaginal smear.

Key study observations: The NOAEL for fertility effects was the mid-dose of 0.6 mg/kg while the NOAEL for embryo-fetal development was the low-dose of 0.2 mg/kg. A NOAEL for general toxicity findings was not identified due to findings in the nasal cavities at all doses tested. Other effects at the high-dose included increased pre- and post-implantation loss, and live fetuses, and male reproductive organ toxicity. The general findings have been identified in previous toxicity studies.

Study for effects on embryo-fetal development (Segment II) in the rat
Report No.: 8/96 *Study No.*: TR0355 *Volume*: 1.25

Study Dates: Starting date 5/22/1995; report issued 6/17/1996
Testing Lab: Byk Gulden Institute of Pathology and Toxicology, Hamburg, Germany
Test Article: B9302-107 (Batch# AM46/251; purity = 98.5-98.8%) in 0.4% methylcellulose
Concentration: 0.02-0.18 mg B9302-107/ml
Dose Volume: 10 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

The protocol for this study was not reviewed by the Division.

Methods: Crl:(Wi)WU BR female rats (12 weeks old; 166-235 g) were assigned to the following treatment groups:

Dose (mg/kg/day)	0	0.2	0.6	1.8
No. of females	27	27	26	29

Each female rat was placed with a breeder male on a one-to-one basis until positive evidence of mating was observed. Female rats in which copulation was confirmed received a daily oral dose of vehicle or test drug via stomach tube once daily on days 6 through 15 of gestation in order to assess its effects on dams, fetuses and offspring. The following observations were made:

Dams:
Clinical observation . . . daily examination of mated females
Body weight . . . . . Days 0, 6-15, and 20 of gestation
Food consumption . . . . daily
Vaginal smear . . . . . morning after overnight mating
Necropsy . . . . . mated females sacrificed on gestation day 20; gravid uterus weight

Reproduction parameters . . . . . determination of implantation rate, preimplantation loss, postimplantation loss, fetuses (live/dead), and resorptions.

Fetuses (F_1):

Behavior and appearance . . . . . motility, respiration, circulation, irregularities, sex determination, body weight

Fetal morphology . . . . . external, gross visceral changes and skeletal examination.

Statistical analysis: Kruskal-Wallis test, Mann-Whitney-Wilcoxon test, Jonckheere-Terpstra test.

Results:

Dams:

Mortality: No unscheduled deaths occurred.

Clinical Observations: No drug-related clinical observations were noted.

Body Weight: Maternal body weight gain in high-dose dams was reduced by 20% compared to control animals from day 6 to day 15 of gestation. The low- and mid-dose groups were comparable to controls and no differences were observed from days 15 to 20.

Food Intake: Food consumption in high-dose dams was reduced by 15% compared to control animals from day 6 to day 15 of gestation. The low- and mid-dose groups were comparable to controls. High-dose animals demonstrated an 9% increase in consumption from days 15 to 20.

Necropsy: No differences in gravid uterus weight were noted.

Reproduction Parameters: A slight increase in abortions was noted in the high-dose group (Table 7). Also, the percentage of corpora lutea was increased which was followed by an increase in pre-implantation loss. Though not statistically significant, an increasing trend was noted for post-implantation loss (12.5% at high-dose versus 8.2% in control group).

Table 7: Summary of effects on reproductive parameters.

	Dose (mg/kg)			
Observation	0	0.2	0.6	1.8
Abortions	1	0	1	2
Corpora lutea #	12.2	13.1	13.4	15.2
% of control value		↑7%	↑10%	↑25%
Preimplantation loss (%)	22.2	24.9	18	32.9
Post-implantation loss (%)	8.2	9.3	7.1	12.5
Early deaths	13	15	12	24
Late deaths	6	4	8	10

Shaded area indicates statistically significant difference from control value.

Fetuses (F1):
Behavior and appearance: Motility, sex ratio, behavior and fetal weights were not significantly affected by drug treatment.

Skeletal and visceral examination: An increased incidence of incomplete ossification of the intraparietal, parietal and supraoccipital bones were seen in fetuses of the mid- and high-doses (Table 8).

Table 8: Summary of effects on skeletal variations in fetuses: total (%)

	Dose (mg/kg)			
Observation	0	0.2	0.6	1.8
(# fetus/litter)	129/27	130/27	135/26	138/29
Incomplete ossification of skull bones:				
Interparietal	53/23	49/18	59/22	89/28
Parietal	17/11	20/11	34/15	46/23
Supraoccipital	113/25	115/27	116/26	130/29

Shaded area indicates statistically significant difference from control value.

Key study observations: Developmental effects (malformations) were not observed at doses up to 1.8 mg/kg while skeletal variations were noted at doses of 0.6 mg/kg and above. A slight increase in abortions was noted at the high-dose. Though not statistically significant, an increasing trend was noted for post-implantation loss.

Study for effects on embryo-fetal development in rabbits (po)
Report No.: 191/95 *Study No.*: TK0351 *Volume*: 1.25

Study Dates: Starting date 5/20/1995; report issued 3/21/1996
Testing Lab: Byk Gulden Institute of Pathology and Toxicology, Hamburg, Germany
Test Article: B9302-107 (Batch# AM46/251; purity = 98.6-98.8%) in 0.4% methylcellulose
Concentration: 0.02-0.08 mg B9302-107/ml
Dose Volume: 10 ml/kg/day
GLP: The study was accompanied by a signed GLP statement.
QA report: Yes.

The protocol for this study was not reviewed by the Division.

Methods: Female Himalaya rabbits (9 months old; ~ 3 kg) were assigned to the following treatment groups:

Nominal Dose (mg/kg/day)	0	0.2	0.4	0.8
No. of copulated females	15-16	15-16	15-16	15-16

Females were mated with untreated males; day of mating was designated as Day 0 of pregnancy. Females in which copulation was confirmed received a daily dose of vehicle or test drug by gastric intubation (gavage) once daily on days 6 through 18 of gestation. Dose selection was based upon results of a dose-ranging study in which female rabbits

demonstrated reduced food consumption at doses of 1 mg/kg or greater. The following observations were made:

Dams:

Clinical observation . . . daily
Body weight . . . . . Days 0, from day 6 daily after mating.
Food consumption . . . daily
Necropsy . . . . . mated females sacrificed on gestation day 29; gravid uterus weight
Reproduction parameters . . . . . determination of implantation rate, preimplantation loss, postimplantation loss, fetuses (live/dead), and resorptions.

Fetuses (F_1):

Behavior and appearance . . . . . motility, respiration, circulation, irregularities, sex determination, body weight
Fetal morphology . . . . . external, gross visceral changes and skeletal examination.
Statistical analysis: Kruskal-Wallis test, Mann-Whitney-Wilcoxon test, Jonckheere-Terpstra test.

Results:

Dams:

General signs: Blood on the bedding was noted in 2-3 animals in drug-treated groups. Reduced feces were noted in all groups but was most pronounced in the high-dose group. All animals were sacrificed on schedule.

Body weight: High-dose animals demonstrated a loss in mean body weight gain of 0.06 kg compared to controls which lost 0.03 kg during days 6-12 of pregnancy. Body weight gains were comparable at later time points. Low- and mid-dose animals were not affected.

Food consumption: Mean food consumption was reduced (28-30%) in high-dose animals from gestation days 6-12 and 13-18.

Necropsy: Mean gravid uterus weights were comparable among groups.

Reproduction Parameters: One female in each of the low- and mid-dose groups showed total abortion (Table 9). Post-implantation loss was increased at the high-dose although the finding was not statistically significant.

Table 9: Effects of B9302-107 on reproductive parameters following oral administration.

	Dose (mg/kg)			
Parameter	0	0.2	0.4	0.8
Females with abortion	0/15	1/15	1/16	0/16
Post-implantation loss (%)	19.5	14.5	20.7	25.1

Fetuses:

Behavior and appearance: Motility, sex ratio, behavior and fetal weights were not significantly affected by drug treatment.

Fetal Gross/Skeletal observations: A number of findings were noted in only one high-dose fetus and are of unclear relationship to the study drug (Table 10). An increase in missing spontaneous respiration observed at the high dose is of lowered concern when combined with the incidence of partly missing respiration. The combined incidences are comparable among dose groups.

Table 10: Effects of B9302-107 on fetal morphology.

	Dose (mg/kg)			
Parameter	0	0.2	0.4	0.8
Liver hemorrhage – moderate (%fetus/% litter)	0/0	0/0	0/0	1.3/6.3
Ascites (%fetus/% litter)	0/0	0/0	0/0	1.3/6.3
Spontaneous respiration missing (%fetus/% litter) Spontaneous respiration partly missing (%fetus/% litter)	1.5/6.7 20/53.3	1.2/7.1 13.6/50	1.2/6.7 28/66.7	5.1/18.8 17.9/43.8
Manubrium to xiphisternum – assymetrically shaped (%fetus/% litter)	0/0	0/0	0/0	1.3/6.3
First sternal vertebral body – assymetrically shaped (%fetus/% litter)	0/0	0/0	0/0	1.3/6.3
3^{rd} and 4^{th} sternal vertebral bodies – fused (%fetus/% litter)	0/0	0/0	0/0	2.6/12.5

Key study observations: Developmental effects were not observed at doses up to 0.8 mg/kg.

Summary of Reproductive Toxicology Studies: Oral fertility studies with B9302-107 in rats, and embryo-fetal developmental toxicity studies in rats and rabbits were performed. In the fertility study (0.2, 0.6, and 1.8 mg/kg), treatment-related effects included reduced body weight gain (14%) from days 15-20 post coitum and reduced food consumption (9%) in high-dose dams, male reproductive organ toxicity (testicular tubular atrophy and epididymal spermiogenic granuloma, hypospermia and dysspermia) and nasal cavity toxicity (olfactory cell degeneration, basal cell hyperplasia). Gravid uterus weight was also reduced (8 and 20%) at the mid- and high-doses. Copulation rate (21%) and fertility rate (25%) were significantly decreased at the high-dose. One high-dose female showed total abortions and another showed total resorptions. In addition, pre- and post-implantative losses were increased (7-10% and 10-26%, respectively) at the mid- and high-doses. The number of living fetuses was significantly reduced at the high-dose. Sperm count was reduced by 7% in high-dose males and corresponded with a dose-dependent increase in testicular weight (up to 18%) at the high-dose; sperm motility was not significantly affected. In control pairing, one mid-dose male and 6 high-dose males failed to produce sperm during the first mating trial with treated females. Mean litter weight was significantly reduced (21%) at the high dose. Slight increases in the incidence of liver hemorrhage, cavernous lung and incomplete ossification of certain structures were noted primarily in high-dose pups. The NOAEL for fertility effects was the mid-dose of 0.6 mg/kg while the NOAEL for embryo-fetal development was the low-dose of 0.2 mg/kg. A NOAEL for general toxicity findings was not identified due to findings in the nasal cavities at all doses tested.

In the rat embryo-fetal development study (0.2, 0.6, 1.8 mg/kg), there were no skeletal or visceral malformations although skeletal variations were noted at the mid- and high-doses (incomplete ossification of skull bones). In rabbits (0.2, 0.4, 0.8 mg/kg), findings included clinical signs in all groups, body weight loss (0.006 kg), reduced food consumption (28-30%), and a slight increase in post-implantation loss at the high dose. No drug-related gross or visceral malformations or variations were observed. No drug-related developmental effects were noted at doses up to 0.8 mg/kg.

CARCINOGENICITY DOSE SELECTION:

On February 9, 1999, the Executive CAC met to discuss dose selection for 2 year carcinogenicity studies with B9302-107 in mice and hamsters based upon 3-month range finding studies in these species. The committee concluded that doses of 2, 6 and 18 mg/kg should be tested in male mice and 2, 6 and 12 mg/kg should be tested in female mice. The sponsor had proposed doses of 0.5, 1.7 and 6 mg/kg by oral gavage. In hamsters, the committee recommended doses of 4, 8 and 16 mg/kg in males and 1, 3 and 8 mg/kg in females. The sponsor had proposed doses of 0.25, 1 and 4 mg/kg by oral gavage.

The sponsor submitted a response to the Executive CAC meeting minutes in submission 007 (June 29, 1999, volume 5.5) and the submission included revised study protocols. In the mouse study, the sponsor included the doses recommended by the Executive CAC and, in addition, incorporated a low dose of 0.5 mg/kg in both males and females. In the hamster study, the sponsor included doses of 0.25, 1, 4 and 8 mg/kg. Although this dosing strategy incorporates the recommendations of the Executive CAC for female hamsters, the sponsor chose not to incorporate the high dose of 16 mg/kg in male hamsters. Overall, the changes implemented by the sponsor in the revised study protocols incorporate the Executive CAC recommendations with the exception of the high-dose of 16 mg/kg in male hamsters. Thus, the validity of the carcinogenicity study in male hamsters may become a review issue once the study reports are submitted.

OVERALL SUMMARY AND EVALUATION:

A 3-month juvenile rat study was performed and submitted by the sponsor in support of a clinical trial in subjects 6 years of age and above consisting of two dose administrations (100 and 250 μg) with a 14-day wash-out period between doses. Although the Division typically requires juvenile studies to support dosing in populations of under 2 years of age, the juvenile rat study was reviewed in this case primarily to assess whether younger populations demonstrated increased male reproductive organ sensitivity to the study drug since B9302-107 has been shown to induce male reproductive organ toxicity (testes, epididymis) in adult studies. In juvenile rats orally dosed with 0.2-0.8 mg/kg, target organs of toxicity included the liver, epididymis, heart, lungs and spleen. The NOAELs in the juvenile study are comparable to that found in the 3-month adult oral toxicity study in rats (0.2 mg/kg; next highest dose was 2 mg/kg; see Original IND Review). Although no increase in sensitivity in

terms of male reproductive organ toxicity was observed in the juvenile rat study, some differences were noted in other target organs of toxicity. The liver and lungs were not previously identified in adult studies and may indicate an increased sensitivity in juvenile animals. The identified target organs in the 3-month adult study (nasal cavities, testes, epididymis, and the thymus) were not identified in juvenile animals with the exception of the epididymis. However, the high-dose in the juvenile study (0.8 mg/kg) was less than that used in the adult study (2 mg/kg). Although the heart and spleen were not identified in the 3-month adult study, they were identified in a 4-week study which utilized doses up to 8 mg/kg. This finding may indicate an increased sensitivity in juvenile rats for these organs. Overall, the findings of this study support the conduct of the proposed clinical trial.

The sponsor also performed oral fertility studies with B9302-107 in rats, and embryo-fetal developmental toxicity studies in rats and rabbits. In the fertility study (0.2, 0.6, and 1.8 mg/kg), fertility was impaired as indicated by reduced copulation and fertility rates and sperm count. Individual high-dose females showed total abortions and total resorptions. In addition, pre- and post-implantative losses were increased at the mid-dose and above and number of living fetuses and mean litter weight were reduced at the high dose. Slight increases in liver hemorrhage, cavernous lung and incomplete ossification of certain structures were noted primarily in high-dose pups. Treatment-related effects on general toxicity included male reproductive organ toxicity. Gravid uterus weight was also reduced at the mid- and high-doses. The NOAEL for fertility effects was 0.6 mg/kg; the NOAEL for embryo-fetal development was 0.2 mg/kg. In embryo-fetal development studies, administration of up to 1.8 mg/kg in rats and 0.8 mg/kg in rabbits during the period of organogenesis produced no evidence of major developmental toxicity although signs of embryotoxicity were observed. Skeletal variations in rat fetuses included incomplete ossification of skull bones at doses of 0.6 mg/kg and above.

The sponsor replied to the minutes of the Executive CAC concerning dose-selection for their proposed 2-year carcinogenicity studies in mice and hamsters. Overall, the changes implemented by the sponsor in the revised study protocols incorporate the Executive CAC recommendations with the exception of the high-dose of 16 mg/kg in male hamsters; the sponsor included doses of 0.25, 1, 4 and 8 mg/kg for males and females. Thus, the validity of the carcinogenicity study in male hamsters may become a review issue once the study reports are submitted.

RECOMMENDATIONS:

1. The proposed clinical trial is considered to be reasonably safe to proceed in patients 6 years of age or older.

2. The results of the reproductive toxicology studies should be incorporated into the label once the NDA is submitted.

Timothy J. McGovern, Ph.D., Pharmacologist

IND 57,883
CC: HFD-570/Division File
HFD-570/C.J. Sun
HFD-570/L. Gilbert-McClain
HFD-570/L. Jafari
HFD-570/T.J. McGovern

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Timothy McGovern
5/29/01 10:07:03 AM
PHARMACOLOGIST

Joseph Sun
6/1/01 08:28:26 AM
PHARMACOLOGIST
I concur.

Appendix 3

Pharmacology and Toxicology Review No. 3
by Dr. Timothy McGovern Completed on January 30, 2003
In IND 57,883

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND number: 57,883
Review number: 3
Sequence number/date/type of submission: 096/March 12, 2001/Protocol amendment; RD
115/July 12, 2001/New protocol; Tox. information
124/August 23, 2001/Tox. Information
137/October 29, 2001/Tox. Information
143/November 28, 2001/Tox. Information
157/February 27, 2002/PC, GC
162/April 12, 2002/PC

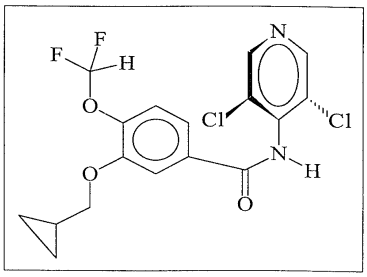
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Byk Gulden Pharmaceuticals, Hamburg, Germany
Manufacturer for drug substance: Byk Gulden Pharmaceuticals, Hamburg, Germany

Reviewer name: Timothy J. McGovern, Ph.D.
Division name: Pulmonary and Allergy Drug Products
HFD #: 570
Review completion date: January 30, 2003

Drug:

Trade name: Roflumilast
Generic name (list alphabetically): NA
Code name: B9302-107; BY 217
Chemical name: 3-cyclopropylmethoxy-4difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide
CAS registry number: NA
Mole file number: NA
Molecular formula/molecular weight: $C_{17}H_{14}Cl_2F_2N_2O_3$/403.2

Structure:



Relevant INDs/NDAs/DMFs: None

Drug class: PDE4 inhibitor

Indication: Asthma/COPD

Clinical formulation:

Clinical Formulation B:

Components	**Amount/tablet type (mg)**
Micronized BY 217	0.25 or 0.5
Lactose monohydrate	(b) (4)
Maize starch	
Polyvidone (b) (4)	
Magnesium stearate	
Total:	

Route of administration: Oral

Proposed clinical protocols:

(b) (4)

Submission 157: Phase 3 Protocol BY217/M2-110 (Draft): Comparison of treatment with 250 µg roflumilast versus 500 µg roflumilast versus placebo over 24 weeks in patients with chronic obstructive pulmonary disease (COPD).
Duration of dosing: 24-weeks
Subjects: 1000 COPD patients; age ≥ 45 years old
Objective: efficacy related to pulmonary function, exacerbation rate, quality of life, symptoms, and use of rescue medication

(b) (4)

Previous clinical experience: The sponsor has performed or has ongoing clinical trials in Europe and the US. Currently, long-term trials at doses up to 250 µg/day are underway in the US.

Previous reviews:

Review Type	Date of Submission(s)	Reviewer	Date of Review
Original Review	February 16-June 29, 1999	McGovern	July 24, 2000
Review #2	February, 1999-April, 2000	McGovern	May 29, 2001

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

Introduction and drug history: Roflumilast is a PDE4 inhibitor that is under development for the indications of asthma and COPD. During the course of development, the primary non-clinical issues identified include nasal toxicity and male reproductive organ toxicity. At an EOP2 meeting for the asthma indication and a Pre-IND meeting for the COPD indication (both held on in October 5, 2000), the Division raised concerns that the systemic exposure levels (AUC) for roflumilast and its metabolite (roflumilast N-oxide, B9502-044) at a NOAEL dose from a 6-mos study in rats do not provide an adequate safety margin for humans at the maximum proposed clinical dose of 0.5 mg. The Division advised that the sponsor further evaluate the TK data at the NOAEL dose in rats, or reduce dosing in the clinical trials to provide human exposure to one half of that observed at the NOAEL dose. Toxicities of concern in the rat were nasal toxicity and male reproductive organ toxicity. The sponsor was informed that they should provide a rationale to explain why the rat is not an appropriate model from its total toxicity profile perspective and to justify the use of another rodent species. In addition, safety margins provided by studies in dogs alone may not be sufficient to support the proposed clinical program since the rat was currently considered the most sensitive species.

A follow-up teleconference was held in June 2001 to continue discussions concerning the development program. Of note, the sponsor was informed that the Division agreed that the observed nasal toxicity in rodent species is not of relevance to humans due to a demonstrated uniqueness in the local handling of the drug by rodents. In addition, the Division informed the sponsor that there is currently not convincing evidence to support the sponsor's claim that the male reproductive toxicity findings are rat specific based on clear treatment-related effects on male reproductive organs in the 3-mos hamster study, tubular degeneration at the high dose of 18 mg/kg in the four week toxicity study in dogs, and the Division's experience with other PDE4 inhibitors. The issue of species specificity notwithstanding, the sponsor's proposed mechanism by which roflumilast induces the male reproductive organ toxicity in rats has not been conclusively defined and remains speculative. The sponsor was informed that assuming the final report of a 6-mos rat study supports the interim summary that a NOAEL of 0.8 mg/kg was observed, a mean AUC of 30.3 µg.hr/ml provides a 0.9-fold safety ratio compared to the maximum proposed human dose. The Division expects a 2-fold animal to human safety ratio based on AUC with this type of toxicity. The sponsor was informed that dosing up to 250 µg in a clinical setting would be supported assuming the report of the 6-mos rat study were in agreement with the submitted summary. The sponsor agreed to submit the data from the 6-mos rat study, as well as a detailed report of the histopathology re-evaluation of the 3-mos hamster study and a dose-range finding study in monkeys.

An EOP2 meeting for the COPD indication was held on December 6, 2001. At this time the sponsor was informed that the current non-clinical database supports clinical dosing up to 250 µg in younger female subjects. However, this dose is not supported in males due to spermiogenic findings in the 12-mos dog study with roflumilast N-oxide. In addition, the data in rats and dogs do not support dosing at 250-500 µg in older patients who would be enrolled in the COPD trial due to increased systemic exposure in older patients. The NOAEL dose in the 12-mos dog study with roflumilast N-oxide was identified as 0.1 mg/kg. The sponsor was requested to submit historical data to support their claim that the observed spermiogenic findings fall within normal range. The sponsor indicated that ongoing studies included a 4-mos dose-ranging study in monkeys, a 6-mos mouse study and a 4-week study in healthy volunteers and asked if these data would be helpful in answering the indicated safety concerns. The Division indicated that additional data might be helpful but the most sensitive species (the rat in this case) would be considered in determining the available safety factors.

To date, the sponsor has provided a re-evaluation of systemic exposure data in rats to cover a 0-24 hour timeframe, the 6-mos rat study, the 4-week dose-rang finding study in monkeys, historical data in dogs concerning spermiogenic alterations. These data, as well as the numerous additional studies listed below, are reviewed currently to assess the non-clinical support for proposed clinical studies in asthmatics and COPD patients up to 500 µg.

Studies reviewed within these submissions:

Study	Report #	Serial #:	Vol.
Pharmacokinetics and Toxicokinetics:			
Re-evaluation of serum/plasma AUC of roflumilast and metabolites in animals with estimation of 0-24 hours values	NA	096	18.1
12-months toxicokinetics of B9302-107 in the dog following oral administration at three different dose levels.	4/2001	096	18.1
4-week toxicokinetics of B9502-044 in the dog following oral administration at three different dose levels.	5/2001	096	18.2
4-week toxicokinetics of B9502-044 in the rat following oral administration at three different dose levels.	27/2001	096	18.2
4-week toxicokinetics of B9302-107 in the rat following oral administration at dose levels of 0.5 and 1.5 mg/kg.	31/2001	096	18.2
Toxicokinetics of B9302-107 in the cynomolgus monkey in an escalating DRF study, followed by a 4-week fixed dose oral toxicity study.	182/2001	137	26.6
Toxicokinetics of B9302-107 in the hamster in a 3-month DRF-study following oral administration at different dose levels.	147/99	096	18.5
12-months toxicokinetics of B9502-044 in the dog following oral administration at 4 different dose levels	160/2001	137	26.5
Sub-Chronic Toxicology:			
7-day endocrine toxicity study in rats treated orally with B9302-107	244/2000	096	18.4
Histopathological assessment of testes and epididymides of rats of the 7-days endocrine toxicity study with B9302-107	21/2001	096	18.3
The toxicity of B9502-044 after oral administration in the rat for 4-weeks	116/99	096	18.3
Toxicity of B9502-044 in beagle dogs following oral administration for 4 weeks.	33/99	096	18.4
Overview of historical data for male reproductive organs in Wistar rats at Byk Gulden since 1994	NA	115	1
Escalating dose range finder followed by a 4-week fixed dose oral (gavage) toxicity study in the adult cynomolgus monkey with B9302-107	95/2001	124	1
Hamster, 3 month toxicity study, p.o.: Peer review of histological sections of testes and epididymides and revisions to original report	GH0562	115	1

Chronic Toxicology:			
The toxicity of B9302-107 after oral administration to rats for 6 months	CR0695	096	18.4
The toxicity of B9302-107 after oral administration to rats for 6 months (Interim report #2; histopathology data)	CR0695	115	1
Toxicology of B9302-107 in beagle dogs following administration for 12 months	132/2000	137	26.1
52-week chronic toxicity study of roflumilast N-oxide (B9502-044) by oral administration to beagle dogs (Final report)	13001/00	157	33.2
Summary of the results concerning spermiograms of dogs treated with the test compound B9302-107 or B9502-044.	22/2001	096	18.4
Peer review of histological finding in the testes and epididymides of rats, dogs, mice and hamsters treated orally with B9302-107 or B9502-044.	20/2001	096	18.4
Testicular toxicity of B9302-107 in rats: relevance to man	25/2001	096	18.5
Carcinogenicity:			
Preliminary results of the mouse and hamster studies.		143	1

Studies not reviewed within this submission: None

TABLE OF CONTENTS - PHARMACOLOGY/TOXICOLOGY REVIEW

PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOKINETICS/TOXICOKINETICS:

Pharmacokinetics:

Rat: The oral (gavage) pharmacokinetics of B9302-107 and its metabolite B9502-044 in rats following 4-week oral administration (Study 31/2001) of B9302-107 in 4% methocel are summarized in Table 1. Systemic exposure levels (AUC) were measured via HPLC and MS-MS detection (LLOQ = 0.504 ng/ml). Exposures to B9302-107 increased supra-proportionally with increasing dose while Cmax increased sub-proportionally. The elimination half-life and the tmax were between 1-3 hours. Exposure to B9502-044 increased supra-proportionally; AUC levels were 20-29-fold greater than those of B9302-107. The tmax values were comparable to those for B9502-044 while elimination half-lives tended to be slightly greater. Exposure levels on Day 28 were reduced by 40% for B9302-107 and 16% for B9502-044 compared to Day 1.

Table 1. Toxicokinetics of B9302-107 and metabolite B9502-044 following 4-week oral administration of B9302-107 in rats.

Dose (mg/kg)				
AUC (µg/l*hr)	B9302-107		B9502-044	
	Day 1*	Day 28**	Day 1*	Day 28**
0.5	12.67	11.67	267.38	246.81
1.5	54.21	32.44	1104.44	927.45
Cmax (µg/l)				
0.5	4.38	3.7	71.31	67.32
1.5	9.12	7.71	124.94	161.88
T1/2 (hr)				
0.5	1.62	1.84	2.21	3.13
1.5	1.08	2.78	4.03	3.43
Tmax (hr)				
0.5	1.08	1.25	1.08	1
1.5	1.92	1.25	1.17	1

* Day 1: AUC (0-inf); **Day 28: AUC (0-24hr) NA: Not available

The oral (gavage) pharmacokinetics of B9502-044 and its metabolite B9302-107 in rats following 4-week oral administration (Study 27/2001) of B9502-044 in 4% methocel are summarized in Table 2. Systemic exposure levels (AUC) were measured via HPLC and MS-MS detection (LLOQ = 0.5 ng/ml). Exposures to B9502-044 increased supra-proportionally with increasing dose while Cmax increased proportionally. The elimination half-life was 2-4 hours and the tmax was 1-1.5 hours. Exposure to the B9302-107 increased proportionally; AUC levels were < 2% of those observed with B9502-044. The tmax values were comparable to those for B9502-044. Exposure levels were 30-90% greater on Day 28 compared to Day 1 at the low and mid-doses while values were comparable at the high-dose.

Table 2. Toxicokinetics of B9502-044 and metabolite B9302-107 following 4-week oral administration of B9502-044 in rats.

Dose (mg/kg)				
AUC (µg/l*hr)	B9502-044*		B9302-107**	
	Day 1	Day 28	Day 1	Day 28
0.4	168.48	319.09	NA	5.97
1.2	538.49	704.02	3.18	4.74
3.6	2829.93	2713.55	11.03	14.33
Cmax (µg/l)				
0.4	54.79	79.10	0.87	4.02
1.2	115.30	159.27	0.84	1.64
3.6	363.37	507.94	2.28	3.3
T1/2 (hr)				
0.4	2.23	3.58	NA	NA
1.2	3.21	2.6	NA	NA
3.6	3.12	3.78	NA	NA
Tmax (hr)				
0.4	1.08	1.0	1	1.67
1.2	1.08	1.0	1.38	1.27
3.6	1.0	1.58	4.17	2.83

* Day 1: AUC (0-inf); Day 28: AUC (0-24hr) ** AUC (0-8 hr)
NA: Not available

Dog: The pharmacokinetics of B9302-107 and metabolites in dogs following 52-week oral administration (Study 4/2001) of B9302-107 are summarized in Table 3. Systemic exposure levels (AUC) were comparable between Day 1 and weeks 13 and 52. Exposures to B9302-107 increased with increasing dose although the increase was sub-proportional. Similar results were observed with Cmax. The elimination half-life was ~ 4-6 hours and the tmax was 1-2 hours. Exposure to the metabolites also tended to increase in a sub-proportional manner and elimination half-life and tmax were comparable to the parent drug.

Table 3. Pharmacokinetics of B9302-107 and metabolites following 52-week oral administration in dogs.

Dose (mg/kg)	B9302-107			B9502-044			B9202-045		
AUC* (µg/l*hr)									
	Day 1	Wk 13	Wk 52	Day 1	Wk 13	Wk 52	Day 1	Wk 13	Wk 52
0.2	180.1	222.3	159.5	23.4	11.2	15.1	7.3	9.6	7.8
0.6	462.3	521.9	510.1	53.5	65.5	65.9	27.2	30.7	28.7
2	1015.3	1213.5	918.9	80.9	104.3	53.6	56.9	58.5	51.3
Cmax (µg/l)									
0.2	44.2	51.1	38.1	2.9	2.2	2.7	1.3	1.3	1.4
0.6	101.5	130.3	98.5	8.2	15.3	10.7	3.4	4.0	4.4
2	171.3	227.9	179.3	9.3	15.8	12.8	5	8.1	7.7
T1/2 (hr)									
0.2	4.7	5.4	5.4	6.3	3.2	5.7	3.9	4.3	3.2
0.6	5.3	5.5	6.2	5.1	6.6	4.2	5.6	5.0	5
2	4.2	4.3	5.1	9.3	4.5	5.2	5.2	4.3	3.8
Tmax (hr)									
0.2	1	1.6	1.1	1.2	1.8	1.4	1.3	2.1	2
0.6	1.1	1.1	1.4	1.5	1.57	2.2	1.8	2.2	2.3
2	1	1.5	1.6	1	1.6	1.8	1.4	2.1	1.9

* Day 1 (0-inf); Wk 13 and 52 (0-24 hr)

The pharmacokinetics of B9502-044 as gelatin capsules (5% suspensions in methocel) and its metabolite B9302-107 in dogs following 4-week oral administration (Study 5/2001) of B9502-044 are summarized in Table 4. Systemic exposure levels (AUC) were measured via liquid/liquid extraction, HPLC and MS-MS detection (LLOQ = 0.1 ng/ml). Exposures to B9502-044 increased supra-proportionally with increasing dose; similar results were observed with Cmax. The elimination half-life was < 1 hour and the tmax was ~ 1 hour. Exposure to the B9302-107 also increased with dose; AUC levels were 15-44% of those observed with B9502-044. The elimination half-life and tmax were greater than values for B9502-044. Exposure levels were generally consistent on Days 1 and 25 although levels dropped 8-30% at the high dose.

Table 4. Pharmacokinetics of B9502-044 and metabolite B9302-107 following 4-week oral administration of B9502-044 in dogs.

Dose (mg/kg)	Analyte			
AUC* (µg/l*hr)	B9502-044		B9302-107	
	Day 1	Day 25	Day 1	Day 25
0.6	27.1	21.6	4.23**	5.88**
1.2	82.75	97.19	36.8	26.17
2.4	349.21	247.88	84.57	78.53
Cmax (µg/l)				
0.6	37.75	31.01	2.44	2.8
1.2	61.09	68.21	2.94	3.1
2.4	281.27	167.28	9.54	11.8
T1/2 (hr)				
0.6	0.51	0.67	8.91	NA
1.2	0.58	0.54	6.10	6.6
2.4	0.57	0.7	4.86	6.37
Tmax (hr)				
0.6	1.08	1.08	1.92	2.25
1.2	1.08	1.25	2.42	2.17
2.4	0.83	1.08	2.92	2.42

* Day 1: AUC (0-inf); Day 25: AUC (0-24hr) ** AUC (0-6 hr)
NA: Not available

The pharmacokinetics of B9502-044 and the metabolite B9302-107 following 52-week oral administration (Study 160/2001) in dogs are summarized in Table 5. Systemic exposure to B9502-044 increased supra-proportionally with increasing dose. Levels of B9302-107 were only 5-10% of the levels of N-oxide. Significant drug accumulation was evident at the highest dose. The elimination half-life was ~ 0.5 to 1.5 hours for B9502-044 and 2-6 hours for B9302-107. The tmax was 1 hour for B9502-044 and 1.5 to 4 hours for B9302-107.

Table 5. Pharmacokinetics of B9502-044 and metabolites following 52-week oral administration in dogs.

	Analyte: B9502-044				Analyte: B9302-107			
Dose (mg/kg)	0.1	0.4	0.8	1.2	0.1	0.4	0.8	1.2
Day 1								
AUC (0-∞) (µg/lxhr)	11.01	50.96	125.75	180.28	NA	NA	4.18	6.36
C max (µg/l)	9.25	43.46	90.68	130.93	0.25	0.43	1.17	1.55
T-half (hr)	0.47	0.39	0.54	1.28	NA	NA	2.2	2.6
Tmax (hr)	0.65	0.8	1.05	0.8	2.1	1.56	2.4	2.4
Week 13								
AUC (0-∞) (µg/lxhr)	13.4	63.69	159.14	352.94	NA	2.31	6.05	15.13
C max (µg/l)	15.92	64.16	114.44	304.13	0.24	0.62	1.22	3.04
T-half (hr)	0.41	0.56	0.88	1.24	NA	3.83	3.89	3.43
Tmax (hr)	0.5	0.75	1.1	0.75	1.36	3.8	2.4	2.1
Week 46								
AUC (0-∞) (µg/lxhr)	14.58	54.01	152.93	482.59	NA	1.25	11.97	41.3
C max (µg/l)	13.12	45.49	115.48	261.91	0.27	0.76	1.36	4.82
T-half (hr)	0.46	0.51	0.69	1.47	5.51	2.2	5.76	5.2
Tmax (hr)	0.6	0.8	0.9	1.21	1.25	0.9	1.6	3.32

NA: not ascertainable, LLOQ = 0.1 µg/l for both compounds

Hamsters: The pharmacokinetics of B9302-107 (oral gavage, 10 ml/kg) and metabolites in hamsters (n=10/sex/dose) following 3-month dose-range finding study performed for carcinogenicity study (Study 147/99) are summarized in Table 6. B9302-107 and B9502-044 by HPLC with fluorescence, LLOQ = 1 and 5 µg/l, respectively; B9202-045 with GC/MS LLOQ = 0.41 µg/l; B9502-054 with LC MS LLOQ = 6 µg/l. Pronounced metabolism of B9302-107 was noted as levels of B9502-044 were higher than the parent compound by 23- to 38-fold. Levels of B9502-054 were higher by 7- to 10-fold over parent while levels of B9502-045 were 1.2- to 2-fold over parent. Exposure to all compounds increased with dose in a generally proportional manner. Maximum concentrations were noted from 1.5-4.5 hours at the low and mid-doses and 3-7 hours at the high dose.

Table 6. Pharmacokinetics of B9302-107 and metabolites following 3-month (days 87/88) oral administration in hamsters.

Dose (mg/kg)	B9302-107	B9502-044	B9202-045	B9502-054
$AUC_{0\text{-}8hr}$ (µg/l*hr)				
4	25.11	584.6	46	177.7
8	38.82	1485.8	84.2	405.6
16	106.3	2790.8	124.5	773.7
Cmax (µg/l)				
4	9.36	149.6	8.4	34.6
8	9.6	344.4	15.1	80.6
16	27.3	634	27	157
Tmax (hr)				
4	2.6	1.5	2.9	2.6
8	3.8	1.4	4.6	3.9
16	4.1	3.2	7	5

Monkey: Cynomolgus monkeys (2/sex/dose group) were initially administered B9302-107 in a dose-escalating fashion (1, 0.5 and 0.7 mg/kg, oral gavage). Administration was once daily for 5 days at each dose with a 2 or 9 day washout period. An additional 2/sex/dose group were then administered 0.5 mg/kg po for 28 days. Table 7 summarizes the kinetic data for these two phases of dosing. Systemic exposure to B9302-107 increased as the dose increased from 0.5 to 0.7 mg/kg. However, exposure on day 5 at 1 mg/kg was comparable to that on day 26 at 0.7 mg/kg. Exposure to B9502-044, the N-oxide of the parent, was approximately 4- to 8-fold greater than the parent compound during the escalation phase and 3-fold greater than the parent compound during the fixed dose phase. Significant drug accumulation was noted with increasing duration of exposure. Exposure to B9202-045 (ADCP) was significantly less (approximately 8-15%) than that of the parent compound. The N-oxide of ADCP (B9502-054) was not detectable (LLOQ = 6 µg/l). Tmax of the parent compound and its N-oxide were generally about 1-3 hours and 4-5 hours, respectively. The elimination half-life of the parent compound and its N-oxide were generally about 5-6 hours and 6-12 hours, respectively, with the N-oxide's half-life increasing with dose.

Table 7. Toxicokinetics of B9302-107 and metabolites following administration of escalating doses and 28 day administration of B9302-107 in monkeys.

Dose (mg/kg)		Analyte			
$AUC_{0\text{-}24\ hr}$ (µg/l*hr)		B9302-107	B9502-044	B9202-045	B9502-054
1	Day 1	320.6*	1977.1	NA	NA
	Day 5	810.8	6152.7	101.46	NA
0.5	Day 19	494.2	2347.5	77.01	NA
0.7	Day 26	789.4	3042	NA	NA
0.5	Day 28, fixed dose phase	1232.1	3482.2	96.81	NA
Cmax (µg/l)					
1	Day 1	60.33	146.45	2.65	NV
	Day 5	106.94	581	5.02	NV
0.5	Day 19	83.85	220.71	4.016	NV
0.7	Day 26	74.53	200.11	NA	NA
0.5	Day 28, fixed dose phase	117.01	251.9	6.56	NV
T1/2 (hr)					
1	Day 1	5.6	11.7	NA	NA
	Day 5	15.1	11.6	NA	NA
0.5	Day 19	5.25	6.27	NA	NA
0.7	Day 26	6.1	8.5	NA	NA
0.5	Day 28, fixed dose phase	5.7	6.8	NA	NA
Tmax (hr)					
1	Day 1	1.13	4	8.6	NA
	Day 5	2.75	5	13.6	NA
0.5	Day 19	1.25	5	6.5	NA
0.7	Day 26	1.67	5.33	NA	NA
0.5	Day 28, fixed dose phase	2.3	5	4	NA

* Day 1: AUC (0-inf) NA: Not ascertained NV: no value

Re-evaluation of Serum/Plasma AUC of Roflumilast and metabolites in animals with estimation of 0-24 hr values (Appendix 2 of Volume 18.1)**:**

During an EOP2 meeting with the sponsor **(**October, 2000**)**, one approach to resolve issues of inadequate safety margins for proposed Phase 3 clinical trials was to re-examine kinetic data in animals that had been estimated at 0-8 hours after dosing to determine exposure estimates at 0-24 hr after dosing. The sponsor re-evaluated data from a 6-month rat study (study 97/96) and a 3-month hamster study (147/99) with B9302-107, originally calculated for 0-8 hours after dosing. In addition, the sponsor provided a single-dose mouse study (262/97), and 26 week and 52-week studies in dogs (studies 147/97 and 4/2001) for comparative safety assessments, although re-calculations of AUC were not performed since previous calculations provided daily coverage. The newly calculated exposure data for B9302-107 are summarized in Tables 8-11. The trapezoidal rule was used to calculate the AUC 8-24 hr, whereby all concentration values below the limit of quantitation (<LLOQ) were set to zero. The AUC 8-24 hr was added to the previously calculated AUC 0-8hr. In the rat, the new calculations for systemic exposure during the 0-24 hour period demonstrated an increase of 60-100% compared to the previous estimates for 0-8 hours. In the hamster, the 0-24 hour period calculations for systemic exposure were

increased by 70-155% compared to estimates for 0-8 hours. The sponsor also calculated the geometric mean of the AUC0-24hr for days 91 and 184 in the rat to achieve a more reliable estimate of the mean of the AUC.

Table 8. Re-calculated B9302-107 exposure levels.

Species	Dose (mg/kg)	AUC(0-8 hr)		AUC µg.hr/L			
Rat		Day 91	Day 184	Day 91*	Day 184*		Geometric mean****
	0.5 1.5	15.92 27.5	9.72 37.17	24.81 48.28	19.08 66.7		21.76 56.75
Mice				Day 1**			
	0.5 1.5 9			27.18 75.19 290.28			
Hamster			Day 87	Day 87*			
	4 8 16		25.11 38.82 106.3	44.4 88.91 271.43			
Dog				Wk 13	D 178	Wk 52*	Geom. Mean****
	0.2 0.6 1 2 4			222 521.9 1214 	204 589 1982	159.5 510.1 918.9 	188.3 515 1056 2112***

* AUC (0-24 hr)
** AUC (0-inf)
*** linearly extrapolated by the sponsor from dose of 2 mg/kg
****Geometric mean obtained from resulting individual AUCs 0-24hr in each dosage/day group.

Table 9. Re-calculated B9502-044 exposure levels in mice, hamsters and dogs.

Species	Dose** (mg/kg)	AUC(0-8 hr)		AUC* µg.hr/L	
Mice				Day 1	
	9			315.92	
Hamster			Day 87	Day 87	
	4 8 16		584.6 1485.8 2790.8	918.54 2781.63 6103.71	
Dog				Wk 13	Wk 52
	0.2 0.6 2 4*** 4****			11.2 65.5 104.3 208.6 	15.1 65.9 53.6 107.2 83.8

* AUC (0-24 hr)
** B9302-107
*** AUC values linearly extrapolated by the sponsor from dose of 2 mg/kg
**** AUC value extrapolated by the reviewer based on 20% increase in AUC value from a dose of 0.6 to 2 mg/kg; geometric mean AUC value from weeks 13 and 52 (74.8 µg.h/ml) at dose of 2 mg/kg used for extrapolation

Table 10. Re-calculated B9502-045 exposure levels in hamsters and dogs.

Species	Dose** (mg/kg)	AUC(0-8 hr)		AUC µg.hr/L	
Hamster			Day 87	Day 87*	
	4 8 16		46 84.2 124.5	93.25 186.76 358.4	
Dog				Wk 13*	Wk 52*
	0.2 0.6 2 4*** 4****			9.6 30.7 58.5 107.6	7.8 28.7 51.3 102.6 84

* AUC (0-24 hr)
** B9302-107
*** extrapolated by sponsor from dose of 2 mg/kg
**** AUC value extrapolated by the reviewer based on 89% increase in AUC value from a dose of 0.6 to 2 mg/kg; geometric mean AUC value from weeks 13 and 52 (54.8 µg.h/ml) at dose of 2 mg/kg used for extrapolation

Table 11. Re-calculated B9502-054 exposure levels in hamsters and dogs.

Species	Dose** (mg/kg)	AUC(0-8 hr)		AUC µg.hr/L	
Hamster			Day 87	Day 87*	
	4 8 16		177.7 405.6 773.7	293.11 873.07 1865.98	
Dog				Wk 13*	Wk 52*
	0.2 0.6 2			LLOQ LLOQ LLOQ	LLOQ LLOQ LLOQ

* AUC (0-24 hr)
** B9302-107

PK/TK summary: Systemic exposure to B9302-107 in rats following 4-week oral dosing with B9302-107 increased supra-proportionally with increasing dose while Cmax increased sub-proportionally. The elimination half-life and the tmax were between 1-3 hours. Exposure to the metabolite B9502-044 increased supra-proportionally; AUC levels were 20- to 29-fold greater than those of B9302-107. The tmax values were comparable to those for the metabolite B9502-044 while elimination half-lives tended to be slightly greater. Exposure levels on Day 28 were reduced by 40% for B9302-107 and 16% for B9502-044 compared to Day 1. Exposures to B9502-044 following 4-week oral administration of B9502-044 increased supra-proportionally with increasing dose in rats while Cmax increased proportionally. The elimination half-life was 2-4 hours and the tmax was 1-1.5 hours. Exposure to the B9302-107 increased proportionally; AUC levels were < 2% of those observed with B9502-044. The tmax values were comparable to those for B9502-044. Exposure levels were 30-90% greater on Day 28 compared to Day 1 at the low and mid-doses while values were comparable at the high-dose.

In dogs, 52-week oral administration of B9302-107 resulted in exposures to B9302-107 that increased sub-proportionally with increasing dose with no indication of drug accumulation. The elimination half-life was ~ 4-6 hours and the tmax was 1-2 hours. Exposure to the metabolites

also tended to increase in a sub-proportional manner and elimination half-life and tmax were comparable to the parent drug. Following oral administration of B9502-044 for 4 or 52 weeks, exposures to B9502-044 increased supra-proportionally with increasing dose. The elimination half-life was 0.5 to 1.5 hours and the tmax was ~ 1 hour. Exposure to the B9302-107 also increased with dose; AUC levels were 15-44% of those observed with B9502-044 in the 4-week study but only 5-10% in the 52-week study. The elimination half-life and tmax (2-6 hours and 1.5 to 4 hours, respectively) were greater than values for B9502-044.

The kinetics in hamsters following a 3-month oral administration demonstrated pronounced metabolism of B9302-107 as levels of B9502-044 were higher than the parent compound by 23- to 38-fold. Levels of B9502-054 were higher by 7- to 10-fold over the parent compound while levels of B9502-045 were 1.2- to 2-fold over the parent compound. Exposure to all compounds increased with dose in a generally proportional manner. Maximum plasma concentrations were noted from 1.5-7.

The kinetics in cynomolgus monkeys during an oral escalating dose study with B9302-107 demonstrated increasing systemic exposure to B9302-107 as the dose increased from 0.5 to 0.7 mg/kg. However, exposure on day 5 at 1 mg/kg was comparable to that on day 26 at 0.7 mg/kg. Exposure to B9502-044 was approximately 4- to 8-fold greater than the parent compound during the escalation phase and 3-fold greater than the parent compound during the fixed dose phase at 0.5 mg/kg. Significant drug accumulation was noted with increasing duration of exposure. Exposure to B9202-045 was significantly less than that of the parent compound. The N-oxide of ADCP was not detected. Tmax of the parent compound and its N-oxide were generally about 1-3 hours and 4-5 hours, respectively. The elimination half-life of the parent compound and its N-oxide were generally about 5-6 hours and 6-12 hours, respectively.

Re-evaluation of systemic exposures in rats and hamsters to provide estimates for 0-24 hour exposure demonstrated significant increases in the systemic exposure estimates (60-100% in rats and 70-155% in hamsters).

PK/TK conclusions: The kinetics of B9302-107 in rats and dogs following repeated oral dosing are similar to results observed previously in that exposure increased proportionally in rats and sub-proportionally in dogs. In rats, exposure to the metabolite B9502-044 was significantly greater than the parent compound while systemic exposure in dogs was primarily to the parent compound. A 3-month oral administration to B9302-107 in hamsters resulted in exposure to B9502-044 that were 23- to 38-fold higher than the parent compound. Levels of B9502-054 were higher by 7- to 10-fold over the parent compound while levels of B9502-045 were 1.2- to 2-fold over the parent compound. Exposure to all compounds increased with dose in a generally proportional manner. An oral dose-escalation study in monkeys also demonstrated greater exposure to the metabolite B9502-044 (3 to 8-fold) compared to parent compound. Following administration of B9503-044, exposure to B9502-044 in rats increased supra-proportionally with increasing dose; exposure to the B9302-107 increased proportionally but was < 2% of B9502-044. In dogs, exposures to B9502-044 increased supra-proportionally with increasing dose. Exposure to the B9302-107 also increased with dose; AUC levels were 15-44% of those observed with B9502-044 in the 4-week study but only 5-10% in the 52-week study.

II. GENERAL TOXICOLOGY:

Study title: 7-days endocrine toxicity study in rats treated orally with B9302-107

Key study findings: This study was designed to determine the effects of administration of B9302-107 on the hormone status in rats. High-dose females exhibited increased levels of FSH, LH and progesterone, and decreased levels of 17β-estradiol. Fewer high-dose animals were observed to be in estrus cycle. Males exhibited a dose-related decrease in testosterone, corticosterone and progesterone. High dose males lost body weight during the administration period and high dose females exhibited a reduced body weight gain.

Study no: 244/2000
Volume #, and page #: 18.4, 115
Conducting laboratory and location: (b) (4)

Date of study initiation: December 1999
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: B9302-107, batch 298 569, not provided
Formulation/vehicle: Suspension in 4% methocel prepared fresh weekly

Methods:
Dosing:

Species/strain: Rat/Wistar WU [Crl: (WI) WU BR]
#/sex/group or time point (main study): 10-12 males, 40-42 females
Satellite groups used for toxicokinetics or recovery: None
Age: 17 weeks
Weight: 205-342 g
Doses in administered units: 0, 0.5, 2.5 mg/kg
Route, form, volume, and infusion rate: PO, suspension, 5 ml/kg

Observations and times:

Clinical signs:	once daily
Body weights:	beginning and end of study
Food consumption:	not assessed
Ophthalmoscopy:	not assessed
EKG:	not assessed
Hematology:	a blood sample for hormone determination (testosterone, estradiol, progesterone, luteinizing hormone, corticosterone, follicle stimulating hormone, adrenocortical hormone, prolactin) was taken prior to autopsy, levels determined by radio-immunoassay
Clinical chemistry:	not assessed
Urinalysis:	not assessed
Gross pathology:	at necropsy
Organs weighed:	not assessed

Histopathology:	Uterus, vagina, ovaries and cervix were assessed for determination of the estrus cycle on day 7
Toxicokinetics:	not assessed
Other:	determination of estrus cycle based upon vaginal smear and histopathology

Results:

Mortality: The study text reports that one high-dose male and one control female were killed in moribund condition due to perforation of the esophagus which occurred during dose administration. Dates of death were not reported. The data table, however, indicates that a female in the low-dose group rather than the control group exhibited a perforated esophagus.

Clinical signs: No drug related findings were noted.

Body weights: High dose males lost 21.2 g after 7 days treatment while control animals gained 4.1 g. Body weight gain was also reduced by 75% in high-dose females compared to control females.

Gross pathology: No treatment-related effects noted by the sponsor. However, one male and female from the high dose group exhibited red spots on the thymus. A high-dose female also exhibited red area(s) on the stomach.

Estrus cycle: Fewer high-dose animals were observed to be in estrus cycle during the course of the study. The low-dose group did not appear to be affected. The sponsor notes that this duration of study is too short to conduct a conclusive analysis.

Hormone data: A significant dose-related decrease in testosterone was noted in males (Table 12). Decreases in corticosterone and progesterone were also noted although the changes were not statistically significant. These changes were not associated with histological reproductive organ changes (see review for study 21/2001 below). Changes assessed without consideration of the sexual cycle phase in females were restricted to the high dose and included increased progesterone and FSH and decreased 17β-estradiol. When relating the data to the estrus cycle, only high-dose animals were affected. Decreased mean estradiol (80%) was found on proestrus, increased mean LH (25%) and FSH (2.5-fold) on metestrus, the latter of which was also elevated on diestrus (3.5-fold).

Table 12. Effects on hormone levels following 7-day administration of B9302-107 in rats.

	Dose group (mg/kg)			
	Males		Females	
Hormone	0.5	2.5	0.5	2.5
Corticosterone				
% change vs control	↓31	↓45	↑16	↓36
Testosterone				
% change vs control	↓76	↓82		
Progesterone				
% change vs control	↓57	↓77	↑1	↑88
Follicle-stimulating hormone				
% change vs control	↑12	↑3	↓14	↑41
17β-estradiol				
% change vs control			↓32	↓69

Summary of individual study findings: In an assessment of B9302-107-related effects on hormone levels, females administered 2.5 mg/kg orally exhibited increased levels of FSH, LH and progesterone, and decreased levels of 17β-estradiol. Males exhibited a dose-related decrease in testosterone, corticosterone and progesterone. Fewer high-dose animals were observed to be in estrus cycle. High dose males lost body weight during the administration period and high dose females exhibited a reduced body weight gain.

Study title: Histopathological assessment of testes and epididymides of rats of the 7-days endocrine toxicity study with B9302-107 (Ref. Study FhG 03G 99032; Report No. 244/2000)

Key study findings: This study was designed to determine effects of decreased serum testosterone levels reported in Report No. 244/2000 on the testes and epididymides. No findings that were characteristic of testosterone deficiency were observed in rats administered 0.5 and 2.5 mg/kg B9302-107, po, for 7 consecutive days. However, tubular dilatation of the testes was observed at the high dose.

Study no: 21/2001
Volume #, and page #: 18.3, 1
Conducting laboratory and location: (b) (4)

Date of study initiation: December 1999
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: B9302-107, batch 298 569, purity not provided
Formulation/vehicle: Suspension in methocel

Methods:
Dosing:

Species/strain: Rat/Wistar WU [Crl: (WI) WU BR]
#/sex/group or time point (main study): 10-12 males
Satellite groups used for toxicokinetics or recovery: None

Age: 17 weeks
Weight: not reported
Doses in administered units: 0, 0.5, 2.5 mg/kg
Route, form, volume, and infusion rate: PO, suspension, 5 ml/kg

Observations and times:

Clinical signs: not assessed
Body weights: not assessed
Food consumption: not assessed
Ophthalmoscopy: not assessed
EKG: not assessed
Hematology: not assessed
Clinical chemistry: not assessed
Urinalysis: not assessed
Gross pathology: not assessed
Organs weighed: not assessed
Histopathology: evaluation of testes and epididymides in all dose groups; for evaluation of fat content, one testis of each of 5 animals of the 0 and 2.5 mg/kg groups was processed for Sudan VII b stain.
Toxicokinetics: not assessed
Other: not applicable

Results:

Histopathology: The dose of 2.5 mg/kg induced mild dilatation of testicular tubules in most animals (Table 13). No effects on seminiferous epithelium of the testicular tubules or on the number and morphology of leydig cells could be detected. In addition, no effects on the epididymides were detected. No differences in Sudan VII b staining for fat in the testes were observed between control and high-dose animals. Thus, no changes characteristic of testosterone deficiency were detected in testes and epididymides.

Table 13: Histopathologic findings following 7-day administration of B9302-107.

	Dose Group (mg/kg)		
	0	0.5	2.5
Tubular dilatation of testes -mild/unilateral	0/10	0/10	9/12
Leydig cell morphology	0/10	0/10	0/12
Sudan VII stain	0/5	Not assessed	0/5

Summary of individual study findings: No findings that were characteristic of testosterone deficiency were observed in rats administered 0.5 and 2.5 mg/kg B9302-107, po, for 7 consecutive days. However, tubular dilatation of the testes was observed at the high dose.

Study title: The toxicity of B9502-044 after oral administration in the rat for 4 weeks

Key study findings:
- Two high-dose female deaths were associated with GI inflammation.
- A NOAEL was not identified since findings in the prostate, uterus and seminal vesicle at the high dose were not examined in lower dose groups.
- The primary target organs of toxicity were the male reproductive organs, GI tract, thymus and uterus.
- The QT interval was increased in all female treatment groups compared to control.
- Exposures to B9502-044 increased supra-proportionally while exposure to B9302-107 was < 2% the parent; elimination half-life was 2-4 hours

Study no: 116/99
Volume #, and page #: 18.3, 9
Conducting laboratory and location: Byk Gulden, Hamburg, Germany
Date of study initiation: December 1998
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: B9502-044, batch # Am54/049/002, 98.5%
Formulation/vehicle: suspension in 4% methocel

Methods:
Dosing:

Species/strain: Rats/Wistar
#/sex/group or time point (main study): 10
Satellite groups used for toxicokinetics or recovery: 6/sex/dose group for TK; 8 sex/group for recovery (control and HD only)
Age: 6 weeks
Weight: 111-218 g
Doses in administered units: 0.4, 1.2, 3.6 mg/kg
Route, form, volume, and infusion rate: oral (via gastric tube), 4 ml/kg

Observations and times:

Clinical signs:	4 times daily
Body weights:	2 times weekly, day 30 and day 58
Food consumption:	2 times weekly, day 30 and day 58
Ophthalmoscopy:	pretest, days 28 and 56
EKG:	Sponsor's methodology did not indicate that ECG was assessed in this study. However, the data tables included ECG data taken on day 22. Time of the reading was not indicated.
Hematology:	pretest, days 29 and 57
Clinical chemistry:	pretest, days 29 and 57
Urinalysis:	days 28/29 and 56/57
Gross pathology:	at necropsy on day 30 and day 58 (recovery). GI mucosa, thyroid and adrenals assessed under stereomicroscope
Organs weighed:	at necropsy, for specific organs see Appendix on page 55.

Histopathology:	at necropsy; for specific organs see Appendix on page 55. All organs from all animals in the control and high-dose groups were examined. Testes, epididymis, heart, kidneys, adrenals and thyroids/parathyroids from the mid- and low-dose groups were examined as well as changes at the high dose. In recovery groups, the nasal/paranasal cavities including the olfactory bulbus were evaluated.
Toxicokinetics:	Animals bled on days 1/2 and 28/29; sampling times were pre-dose, 1, 2, 3, 4, 8, and 24 hrs after administration
Other:	water consumption measured on days 1, 2 times weekly and days 30 and 58

Results:

Mortality: Two high-dose females were killed in moribund condition on day 17 and day 25. The primary histological findings were inflammatory changes (ileum, jejunum, pancreas).

Clinical signs: Clinical observations occurred only in high-dose animals and were noted in the last week of treatment in males and the second to last week of treatment for females (Table 14). One recovery female displayed emaciation on day 31 only.

Table 14. Clinical observations following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)							
	Males				Females			
	0	0.4	1.2	3.6	0	0.4	1.2	3.6
Piloerection	0	0	0	2	0	0	0	3
Ptosis	0	0	0	2	0	0	0	1
Emaciation	0	0	0	2	0	0	0	5
Hunched position	0	0	0	2	0	0	0	3
Swollen abdomen	0	0	0	0	0	0	0	3

Body weights: Body weight gain in high-dose males was reduced by 25% on day 28 and 38% in (recovery animals only) on day 30 although the finding was statistically significant only in the satellite group. The finding was reversible.

Food consumption: Mean food consumption was reduced by 11-13% in high-dose animals in weeks 1 and 4 of dosing. Food consumption in high-dose recovery animals was increased by 6% compared to control values during the recovery period. Water consumption was increased in mid- and high-dose males and all female treatment groups (3-27%). Water consumption remained increased during the recovery period.

Ophthalmoscopy: No treatment-related effects were noted.

Electrocardiography: No treatment-related effects were noted in males. QT interval was increased in drug-treated females (76-80 msec vs 71 msec in controls). Heart rate was also significantly decreased (378-396 bpm vs 431 bpm in controls). No corresponding microscopic changes were observed in the heart.

Hematology: Males and females demonstrated increased leukocyte and segmented neutrophil levels primarily at the high dose (Table 15). Slight reductions in lymphocyte levels were also noted. All findings were reversible. These findings correspond with inflammation noted in the GI tract of high-dose animals.

Table 15. Clinical pathology changes following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)					
	Males			Females		
	0.4	1.2	3.6	0.4	1.2	3.6
Leukocytes % change vs control	↑1	↑5	↑19	↑21	↑21	↑76
Segmented neutrophils % change vs control	no Δ	↑50	↑425	no Δ	no Δ	↑420
Lymphocytes % change vs control	↑1	↓1	↓17	↑1	↑1	↓22

Clinical chemistry: Reversible changes included increased levels of serum urea (19-40%) in HD animals and serum AP (36%) in HD females, and decreased AP levels (13%) in HD males. Serum cholinesterase was reduced by 35% in HD females at the end of the dosing period and was still reduced by 17% after recovery period.

Urinalysis: A number of reversible changes were noted following urinalysis assessment. Reduced pH was noted in treated males (6.5-6.6 vs 7 in control males), and osmolality was increased by 44% in HD males. Levels of Na increased by 39% and 70% in MD and HD males, respectively, and 69-193% in MD and HD females. Urine Ca levels increased by 82% and 321% in MD and HD males, and 92% and 232% in MD and HD females. Urine Cl increased in HD males (37%) and females (62%).

Vaginal smears: Fewer estrus events (30%) were observed in the high-dose group during the dosing period compared to control animals. This finding was reversible.

Organ weights: Reversible changes in organ weights at the highest dose included increased adrenal weights (49% and 38% in males and females, respectively), decreased thymus weight (30% and 42% in males and females, repsectively) and increased testes weight (28%; 15% at end of recovery period). Though not statistically significant, absolute prostate and seminal vesicle weights were reduced 21% and 18%, respectively, at the high dose. Relative (to body weight) analysis was performed only with the liver, kidneys, heart lungs, spleen and brain and showed no treatment-related effect.

Gross pathology: Table 16 summarizes the gross findings following 4-week administration of B9502-044. Findings were at the highest doses and included small prostate, seminal vesicles and uterus, involuted thymus, swollen intestine. The findings were not observed in the recovery animals.

Table 16. Gross changes in rats following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)											
	Males						Females					
	0	0-0	0.4	1.2	3.6	3.6-0	0	0-0	0.4	1.2	3.6	3.6-0
n=	10	8	10	10	10	8	10	8	10	10	7	8
Pancreas												
discoloration	0	0	0	0	1	0	0	0	0	0	0	0
edema	0	0	0	0	0	0	0	0	0	0	2	0
Prostate												
Smaller	0	0	0	0	2	0						
Seminal ves.												
Smaller	0	0	0	0	2	0						
Spleen												
Layer	0	0	0	0	1	0	0	0	0	0	0	0
Testes												
Smaller	0	0	0	0	0	1						
Thymus												
Involuted	0	0	0	0	1	0	0	0	0	0	1	0
Injury	0	0	0	0	0	0	0	0	0	0	1	0
Smaller	0	0	0	0	0	0	0	0	0	0	1	0
Intestine												
Dilated	0	0	0	0	0	0	0	0	0	0	1	0
Edema	0	0	0	0	0	0	0	0	0	0	1	0
Swollen	0	0	0	0	2	0	0	0	0	0	1	0
Uterus												
Smaller							0	0	0	0	1	0

Histopathology: Microscopic findings included slight to marked inflammation of the stomach, intestines (ileum and jejunum) and the pancreas (Table 17). Male reproductive organ effects were noted at the high dose and included spermiogenic effects in the epididymides that were non-reversible, tubular dilatation of the testes and slight to marked atrophy of the prostate and seminal vesicles. Atrophy of the uterus and thymus was also noted.

Table 17. Histopathology changes in rats following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)											
	Males						Females					
	0	0-0	0.4	1.2	3.6	3.6-0	0	0-0	0.4	1.2	3.6	3.6-0
n=	10	8	10	10	10	8	10	8	10	10	10	8
Epididymides												
Spermatocele	0	0	0	0	3	4						
oligospermia	0	0	0	0	0	1						
Peri-epi infla	0	0	0	0	1	0						
Ileum												
Inflammation	0	0		0	0	0	0	0		0	2	0
Jejunum												
Inflammation	0	0		0	2	0	0	0		0	5	0
Kidneys												
Caliceal mineraliz	0	0	0	0	1	0	0	0	0	0	1	0
Pancreas												
Inflammation	0	0		0	1	0	0	0		0	3	0
Prostate												
Atrophy	0	0			2	0						
Seminal ves												
Atrophy	0	0			2	0						
Stomach												
Congestion	0	0			1	0	0	0			0	0
Hyperkeratos	0	0			1	0	0	0			0	0
Inflammation	0	0			1	0	0	0			0	0
Testes												
Tub dilatation	0	0	0	0	9	0						
Tub atrophy	0	0	0	0	0	1						
Thymus												
Atrophy	0	0	0	0	3	0	0	0	0	0	7	0
Uterus												
atrophy							0	0			2	0

Toxicokinetics: B9502-044 exposure increased supra-proportionally with increasing dose while Cmax increased proportionally (Table 18). The elimination half-life was 2-4 hours and the tmax was 1-1.5 hours. B9302-107 exposure increased proportionally; AUC levels were < 2% of those observed with B9502-044. The tmax values were comparable to those for B9502-044. Drug accumulation was observed at the two lower doses as exposure levels were 30-90% greater on Day 28 compared to Day 1; values were comparable at the high-dose.

Table 18. Toxicokinetics of B9502-044 and metabolite B9302-107 following 4-week oral administration in rats.

Dose (mg/kg)				
AUC (µg/l*hr)	B9502-044*		B9302-107**	
	Day 1	Day 28	Day 1	Day 28
0.4	168.48	319.09	NA	5.97
1.2	538.49	704.02	3.18	4.74
3.6	2829.93	2713.55	11.03	14.33
Cmax (µg/l)				
0.4	54.79	79.10	0.87	4.02
1.2	115.30	159.27	0.84	1.64
3.6	363.37	507.94	2.28	3.3
T1/2 (hr)				
0.4	2.23	3.58	NA	NA
1.2	3.21	2.6	NA	NA
3.6	3.12	3.78	NA	NA
Tmax (hr)				
0.4	1.08	1.0	1	1.67
1.2	1.08	1.0	1.38	1.27
3.6	1.0	1.58	4.17	2.83

* Day 1: AUC (0-inf); Day 28: AUC (0-24hr) ** AUC (0-8 hr)
NA: Not available

Summary of individual study findings: Two high-dose females died during the course of the study; deaths were associated with GI inflammation. A NOAEL could not be determined from this study since findings in the prostate, uterus and seminal vesicle at the high dose were not examined in lower dose groups. The sponsor, however, concluded that the NOAEL was the low-dose of 1.2 mg/kg. The primary target organs of toxicity were the male reproductive organs, GI tract (stomach and intestines), thymus and uterus. The QT interval was increased in all female treatment groups compared to control, and body weight gain was significantly reduced in high-dose males. Various clinical signs were noted at the high dose, urinary electrolytes were increased in association with altered osmolality and estrus cycling was disrupted in high-dose females. Exposures to B9502-044 increased supra-proportionally with increasing dose while Cmax increased proportionally; elimination half-life was 2-4 hours. Exposure to the B9302-107 increased proportionally; AUC levels were < 2% of those observed with B9502-044.

Sponsor response to Division concern of male reproductive organ effects in 4-week oral dog study expressed in Teleconference of June 6, 2001:
Division concern: One reason expressed as to why the observed male reproductive organ findings are rat-specific is that the four-week toxicity study in dogs (68/95) demonstrated an increased incidence of testicular tubular degeneration and dysspermia at the high dose of 18 mg/kg, po. Specifically, one of three high dose animals demonstrated these responses while none of the animals in the other groups did. The sponsor acknowledged the presence of these findings.

Sponsor response: In submission 157 (dated February 27, 2002; Attachment 3), the sponsor states that histology sections of the testes and epididymides of this animal were reevaluated in a peer review performed on all section of all testes and epididymides of animals treated with roflumilast or its N-oxide (discussion 20/2001, submission 096). The reviewer stated that the testicular

finding in the dog was not treatment-related as it was unilateral and focal with only a small number (< 5) tubule cross sections affected; changes that are known to occur spontaneously in purpose-bred beagle dogs.[1] This reference cites an incidence of 3 cases of mild to severe hypospermatogenesis out of 11 control animals (27%) aged 8-11 months (similar to the age of the animals in the 4-week toxicity study) and 15 of 50 animals (30%) aged 8-20 months. The changes "occurred throughout the testes and were characterized by inadequate and/or reduced proportions of germ cells, tubular shrinkage, and Sertoli cell prominence."
The sponsor also provided historical control data used in repeat dose toxicity studies conducted since 1994 at Byk Gulden Institute of Pathology and Toxicology. The sponsor provided information from 27 studies ranging in duration from 2 weeks to 12 months. Animal numbers per study ranged from 2-5 males. In ten of the studies, 1-2 animals were diagnosed with testicular tubular degeneration with a per study incidence of 20-67% and an overall incidence from all 27 studies of 13% (13/100).
In terms of the epididymal dysspermia, the sponsor states that terminology differs slightly among various pathologists and that desquamated cells and spermiogenic disturbance are synonymous with dysspermia. Of the 27 studies referenced, none reported epididymal dysspermia. However, in 4 studies, 2-4 animals were diagnosed with desquamated cells with a per study incidence of 67-100% and an overall incidence from all 27 studies of 11% (11/100).

Evaluation of sponsor's response: Based upon the submitted historical data and referenced literature publication, the male reproductive organ effects noted in the 4-week oral toxicity study in dogs (see Original IND review dated July 24, 2000) is not considered to be treatment related. However, the NOAEL for this study remains at 2 mg/kg due to toxicities in the heart, thymus, lung, gastrointestinal tract and pancreas (females).

Study title: The toxicity of B9502-044 after oral administration in beagle dogs for 4 weeks

Key study findings:
- 2 high dose males died; days 8 and 21
- Clinical signs included tremor, vomiting and hypersalivation
- Histopathology at the high dose revealed cardiac (myocarditis of the right atrium and inflammation/ degeneration of the left ventricle of one high-dose female) and male reproductive organ toxicity (prostate atrophy)
- The NOAEL is the mid-dose of 1.2 mg/kg B9502-044; associated with systemic exposures of 97 µg/l.hr B9502-044 and 26 µg/l.hr B9302-107.

[1] Rehm, S. (2000). Spontaneous testicular lesions in purpose-bred beagle dogs. Toxicol. Pathol. 28(6), 782-787.

Study no: 33/99
Volume #, and page #: 18.4, 322
Conducting laboratory and location: Byk Gulden, Hamburg, Germany
Date of study initiation: December 1998
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: B9502-044, batch # Am54/049/002, > 98.5%
Formulation/vehicle: suspension in 4% methocel

Methods:
Dosing:

Species/strain: Beagle dogs
#/sex/group or time point (main study): 3 (control, LD and MD), 5 F/6M HD (main study)
Satellite groups used for toxicokinetics or recovery: 1-2 (recovery)
Age: 15-22 months
Weight: 9.4-17 kg
Doses in administered units: 0.6, 1.2, 2.4 mg/kg
Route, form, volume, and infusion rate: oral, gelatinous capsule, 0.5 ml/kg

Observations and times:

Clinical signs:	daily
Body weights:	pre-dose, 2 times weekly
Food consumption:	daily
Ophthalmoscopy:	pretest, week 4
EKG:	pre-dose: twice prior to study initiation, weeks -2, -1, 2, 4, 8; 2 hr after dosing: weeks 2 and 4
Hematology:	pretest, weeks 1, 5 and 8
Clinical chemistry:	pretest, weeks 1, 5 and 8
Urinalysis:	weeks 4 and 7
Gross pathology:	at necropsy on day 29-31 and day 57 (recovery).
Organs weighed:	at necropsy, for specific organs see Appendix on page 55.
Histopathology:	at necropsy; for specific organs see Appendix on page 55. All organs from all animals in the control and high-dose groups were examined. Nasal and paranasal cavities, testes, epididymis, heart, and adrenals from the mid- and low-dose groups were examined as well as changes at the high dose.
Toxicokinetics:	Animals bled on days 1 and 24/25; sampling times were pre-dose, 0.5, 1, 2, 3, 4, 6, and 24 hrs after administration
Other:	Physical exam assessing pulse, body temperature, senses, muscle tone, reflexes, vision, hearing, CNS, ANS, percussion (lungs, auscultation (lung, heart, heart rate, abdomen), weeks – 1, 4 and 8

Results:

Mortality: Two high-dose males were killed in extremis on days 8 and 21 due to continuous vomiting and deteriorating general condition.

Clinical signs: Tremor, vomiting and hypersalivation increased with dose in both incidence in individual animals and number of animals affected (1 low-dose female, 2 mid-dose males and 10 high-dose animals).

Body weights: No treatment-related changes in body weight gain were noted.

Food consumption: No treatment-related changes were noted.

Physical exam: No treatment-related effects were noted on body temperature, pulse rate and respiratory rate.

Ophthalmoscopy: The sponsor reports that no treatment-related effects were noted; no data was submitted.

Electrocardiography: The QRS interval, measured 2 hours after dosing during week 2, was significantly increased in high dose males (61 msec vs 46 msec in controls). This parameter was still increased during week 4 (60 msec vs 48 msec in controls) although the finding was not significant. QT prolongation was not observed.

Hematology: No treatment-related changes were noted.

Clinical chemistry: No treatment-related changes in mean values were noted. One high-dose animal killed before schedule displayed increased serum urea and ALT and AP.

Urinalysis: No treatment-related changes were noted.

Organ weights: Absolute thyroid weight was increased 22-30% in high-dose males (not statistically significant); absolute testes weight was increased 24-28% in high-dose males (not statistically significant).

Gross pathology: The two animals killed in extremis showed massive bleeding at the right auriculum.

Histopathology: Cardiac toxicity was noted in two high-dose males (Table 19). Although these two animals were part of the recovery group, they were sacrificed in moribund condition on days 8 and 21 of dosing. Findings included a moderate to severe myocarditis of the right atrium and were characterized by moderate to severe lympho-histiocytic and purulent infiltration with hemorrhage. The changes were accompanied by a moderate-to-severe activation of mesenchymal connective tissue, reflected by fibroblastic proliferation and de-novo synthesis of juvenile capillaries. The surviving recovery animal did not display this effect. The sponsor states that this finding is the result of an exaggerated pharmacodynamic effect. Since only 2 of 11 animals demonstrated this effect, a responder/non-responder effect rather than a dose-dependent

effect may have caused these lesions. Reversible findings were also noted in the left ventricle of one high-dose female (inflammation, degeneration).

Reversible reproductive organ effects of generally low severity were also noted in high-dose males and included prostatic atrophy and fibrosis, testicular tubular cell degeneration, and epididymal dysspermia. Dr. (b) (4) performed a peer review of the testes and epididymides findings. The tubular degeneration noted in one animal (small severity) was reclassified as minimal degeneration, and the dysspermia (small severity) that was observed in the same animal was reclassified as unilateral degenerate cells. The sponsor states that these findings are spontaneous in Beagle dogs of this age. The historical information reviewed above indicates that testicular and epididymal findings are not treatment related.

Table 19. Histopathology changes in dogs following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)									
	Males					Females				
	0	0.6	1.2	2.4	2.4-0	0	0.6	1.2	2.4	2.4-0
n=										
Liver	**3**	**1**	**0**	**3**	**2**	**3**	**0**	**0**	**3**	**2**
Lym-histio infiltr	1(0.3)	1(1)		2(0.7)	1(0.5)	3(1)			2(0.7)	0
Hepat pigment deposits	0	0		1(0.3)	0	0			1(0.3)	0
Kupfer pigment deposits	0	0		1(0.3)	0	1(0.3)			3(1)	0
Heart, atrial, right	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Acute myocarditis	0	0	0	0	2(2.7)	0	0	0	0	0
Myocardial fibrosis	0	0	0	0	1(0.7)	0	0	0	0	0
Mycard degeneration	0	0	0	0	2(1.7)	0	0	0	0	0
Hemorrhage	0	0	0	0	2(2)	0	0	0	0	0
Heart, ventricle, left	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**
Hyperemia/congestion	0	0	0	0	0	0	0	0	1(0.7)	0
Myocar lymph infiltr	0	0	0	0	0	0	0	0	1(0.3)	0
Mycard degeneration	0	0	0	0	0	0	0	0	1(0.3)	0
Pancreas	**3**	**0**	**0**	**3**	**2**	**3**	**0**	**0**	**3**	**0**
Apoptosis	0			1(0.3)	1(1)	1(0.3)			1(0.3)	0
Prostate	**3**	**0**	**0**	**3**	**2**					
Atrophy	0			1(1)	0					
Fibrosis	1(0.7)			2(1.3)	0					
Testes	**3**	**3**	**3**	**3**	**3**					
Tubular cell degenerat.	0	0	0	1(0.7)	0					
Epididymis	**3**	**3**	**3**	**3**	**3**					
Dysspermia	0	0	0	1(1)	0					
Gastric fundus	**3**	**0**	**0**	**3**	**2**	**3**	**0**	**0**	**3**	**0**
Erosion	0			0	1(1)	0			0	
Uterus						**3**	**0**	**0**	**3**	**0**
Gland.-cyst hyperplasia						0			1(1)	

Toxicokinetics: The pharmacokinetics of B9502-044 and its metabolite B9302-107 in dogs following 4-week oral administration of B9502-044 are summarized in Table 20 (results reported in Study 5/2001, see Pharmacokinetics/Toxicokinetics section of review). Exposures to B9502-044 increased supra-proportionally with increasing dose; similar results were observed with Cmax. Exposure to the B9302-107 also increased with dose; AUC levels were 15-44% of those observed with B9502-044. Exposure levels were generally consistent on Days 1 and 25 although levels dropped 8-30% at the high dose.

Table 20. Pharmacokinetics of B9502-044 and metabolite B9302-107 following 4-week oral administration.

Dose (mg/kg)				
AUC* (µg/l*hr)	B9502-044		B9302-107	
	Day 1	Day 25	Day 1	Day 25
0.6	27.1	21.6	4.23**	5.88**
1.2	82.75	97.19	36.8	26.17
2.4	349.21	247.88	84.57	78.53
Cmax (µg/l)				
0.6	37.75	31.01	2.44	2.8
1.2	61.09	68.21	2.94	3.1
2.4	281.27	167.28	9.54	11.8

* Day 1: AUC (0.inf); Day 25: AUC (0-24hr) ** AUC (0-6 hr)
NA: Not available

Summary of individual study findings: A NOAEL of the mid-dose of 1.2 mg/kg B9502-044 was observed. The primary target organs included the heart and male reproductive organs (prostate). The NOAEL dose is associated with systemic exposures of 97 µg/l.hr B9502-044 and 26 µg/l.hr B9302-107.

Study title: Escalating dose range finder followed by a 4-week fixed dose oral (gavage) toxicity study in the adult cynomolgus monkey with B9302-107

Key study findings:

- Primary findings included clinical signs, reduced body weight, and numerous microscopic findings that cannot be definitively attributed to the test substance due to lack of background information with control animals.
- A NOAEL was not identified in this study due to inadequate animal numbers and possible drug-related findings at the doses that were tested. A control group was not employed to distinguish between background and drug-related findings.

Study no: 95/2001
Volume #, and page #: 24.1, 2
Conducting laboratory and location: (b) (4)
Date of study initiation: December, 2000
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: B9302-107, batch # 298569, ?%
Formulation/vehicle: suspension in 4% hydroxypropylmethyl cellulose

Methods: An escalating dose ranging study was initially performed to evaluate the tolerability of the test article when administered orally by gavage at different dose levels for five days each with 2 or 9 days of recovery between each dosing period. The toxicity of repeated administration at the obtained dose level was then assessed using the control animals for 4 weeks.

Dosing:

Species/strain: Cynomolgus monkeys
#/sex/group or time point (main study): 2 dose range finding; 2 (main study)
Satellite groups used for toxicokinetics or recovery: none
Age: 4-8 years old
Weight: 2.6-6.8 kg
Doses in administered units: Dose ranging: the same animals were administered 1 mg/kg days 1-5, 0.5 mg/kg days 15-19, 0.7 mg/kg days 22-26, control animals were administered 10 ml/kg vehicle over the same days. Fixed dose phase: 0.5 mg/kg for 28 days
Route, form, volume, and infusion rate: oral (gavage), suspension, 10 ml/kg

Observations and times:

Clinical signs:	2 x daily
Body weights:	pre-dose, days 1, 5, 8, 12, 15, 19, 22 and 26 during escalating dose range phase, once weekly during the fixed dose phase and at necropsy
Food consumption:	daily during escalating dose range phase, once weekly during the fixed dose phase and at necropsy
Ophthalmoscopy:	not assessed
EKG:	not assessed
Hematology:	pretest, at end of escalating dose range phase, during week 4 of the fixed dose phase
Clinical chemistry:	pretest, at end of escalating dose range phase, during week 4 of the fixed dose phase
Urinalysis:	weeks 4 and 7
Gross pathology:	Drug-treated animals from the dose escalation phase were sacrificed at the end of the escalating dose range-finding phase. The remaining animals were sacrificed on the day following 4 weeks of daily administration of the fixed dose.
Organs weighed:	at necropsy, for specific organs see Appendix on page .
Histopathology:	at necropsy; for specific organs see Appendix on page . All organs were examined from all animals.

Toxicokinetics:	Animals bled on days 1, 5, 19 and 26 of the escalating dose range phase, and day 28 of the fixed dose phase; sampling times were 0, 0.5, 1, 2, 4, 6, 8 and 24 hrs after dosing
Other:	Hormone analysis (testosterone, luteinizing hormone, FSH) in all male animals three times pre-dose and weekly during the fixed dose phase Spermatogenesis staging performed after necropsy.

Results:

Mortality: None

Clinical signs: Escalating dose phase: Sluggishness, emesis and lack of feces were reported after administration of 1 mg/kg. Emesis and lack of feces were reported after administration of 0.7 mg/kg. Fixed dose phase: Emesis was observed especially during the first 2 weeks of dosing. One animal exhibited soft feces and diarrhea.

Body weights: Escalating dose phase: Animals administered 1 mg/kg lost 0.2 to 0.4 kg while control animals gained 0.1 kg or maintained weight. Similarly, animals administered 0.7 mg/kg and females dosed with 0.5 mg/kg lost weight. Fixed dose phase: Males and females lost weight progressively throughout the study with totals of 0.75 kg (males) and 0.2 kg (females). Lack of control animals precluded a comparison.

Food consumption: Escalating dose phase: Food consumption was reduced in treated animals compared to control animals with reductions of up to 70% at 1 mg/kg, 54% at 0.7 mg/kg and 33% at 0.5 mg/kg. Animals administered 1 mg/kg lost 0.2 to 0.4 kg while control animals gained 0.1 kg or maintained weight. Similarly, animals administered 0.7 mg/kg and females dosed with 0.5 mg/kg lost weight. Fixed dose phase: Mean weekly food consumption increased with each week. Lack of control animals precluded a comparison.

Hormone Analysis: Only two animals were used for this analysis. Testosterone levels were stable from pre-dose to week 4 in male number 102 while pre-dose levels of 41-123 nmol/L were reduced to 16 nmol/L by week 4. A similar pattern was observed with luteinizing hormone (pre-dose levels of 40-69 IU/L reduced to 5.6 IU/L in male 101). No effect on follicle stimulating hormone was observed.

Hematology: No definitive treatment-related changes were noted although the limited number of animals (2/sex/group) precluded an adequate assessment. Increased creatinine kinase levels (32%) were noted in males during the fixed dose phase compared to controls from the escalating dose phase.

Clinical chemistry: No definitive treatment-related changes were noted although the limited number of animals (2/sex/group) precluded an adequate assessment.

Urinalysis: No definitive treatment-related changes were noted although the limited number of animals (2/sex/group) precluded an adequate assessment.

Organ weights: Organ weight effects due to drug treatment could not be detected since comparison to control animals was not performed; control animals from the escalating phase were used for drug treatment for the fixed dose phase.

Gross pathology: No significant findings were noted following the fixed dose phase of dosing (28 days with 0.5 mg/kg). One male (202) of the escalating dose phase displayed severe skin lesions at different locations. Additional findings included innerventricular adhesion combined with enlargement of several organs (adrenals, liver, sacral lymph nodes, spleen) and adhesion of the right kidney and left testis. The sponsor stated that the cause of skin lesions was likely due to an infection of the blood sampling sites which spread to other skin areas and eventually to the affected internal organs.

Histopathology: Comparison to control animals was not performed in this study, thus the true drug-relatedness of any findings is not possible. The findings in the fixed dose phase at 0.5 mg/kg and the escalating dose phase are summarized below (Table 21). The sponsor states that the adrenal, heart, liver, lung spleen, testes and trachea of male 202 (escalating dose phase) were due to severe skin lesions and not a toxicological finding.

Table 21. Histopathology changes in monkeys following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)	
	Males	Females
Fixed dose phase	0.5	0.5
n=	**2**	**2**
Adrenal		
mineralization	1	0
Heart		
Inflammatory foci	0	1
Kidney		
Cortical mineralization	1	0
Inflammatory cell foci	1	2
Capsular fibrosis	1	0
Liver		
Hepatocyte vacuolation	1	1
Inflammatory cell foci	1	1
Lung		
Pigment	2	2
Congestion	0	1
Mesenteric lymph node		
Sinus histiocytosis	1	0
Nasal cavity		
Acute inflammation	0	1
Lymphocyte foci	0	1
Ovary		
Mineralization		1
Prostate		
Inflammatory cell foci	1	
Thymus involution	1	1
Escalating dose phase	1/0.5/0.7	1/0.5/0.7
Adrenal		
Mineralization	1	2
Edema	1	0
Extramedulalry hemopoiesis	1	0

Cortical hypertrophy	1	0
Cecum		
Hemorrhage	0	1
Duodenum		
Hemorrhage	0	1
Erosion	1	1
Heart		
Inflammatory foci	1	1
Necrosis	1	0
Hemorrhage	1	0
Thrombus	1	0
Myocarditis	1	0
Ileum		
Goblet cell hyperplasia	1	0
Kidney		
Basophilic tubules	0	1
Cortical mineralization	2	0
Papillary mineralization	1	0
Inflammatory cell foci	1	2
Capsular fibrosis	1	0
Liver		
Vasculitis	1	0
Basophilic deposits	1	1
Lung		
Pigment	2	1
Pleuritis	1	1
Pneumonitis	1	0
Bronchiolo-alveolar hyperplasia	1	0
Skin+subcutis		
necrosis	1	0
abscess	1	0
Spleen		
Lymphoid atrophy	2	0
Congestion	1	0
Splenitis	1	0
Stomach		
Hemorrhage	0	2
Erosion	0	1
Decreased chief cells	1	1
Testis		
Tubular atrophy	2	
Subacute inflammation	1	
Capsular fibrosis	1	
Thymus		
Cyst	0	2
Involution	2	1
Thyroid		
Cyst	1	0
Lymphocyte foci	1	0
Ectopic salivary gland	1	0

Toxicokinetics: Results from study report 182/2001 (see pharmacokinetics section for greater detail). Table 22 summarizes the kinetic data for the two phases of dosing. Systemic exposure to B9302-107 increased as the dose increased from 0.5 to 0.7 mg/kg. However, exposure on day 5 at 1 mg/kg was comparable to that on day 26 at 0.7 mg/kg. Exposure to B9502-044, the N-oxide of the parent, was approximately 4- to 8-fold greater than the parent compound during the escalation phase and 3-fold greater than the parent compound during the fixed dose phase. Significant drug accumulation was noted with increasing duration of exposure. Exposure to B9202-045 (ADCP) was significantly less (approximately 8-15%) than that of the parent compound. The N-oxide of ADCP (B9502-054) was not detectable (LLOQ = 6 µg/l).

Table 22. Toxicokinetics of B9302-107 and metabolites following administration of escalating doses and 28 day administration of B9302-107.

Dose (mg/kg)		Analyte			
$AUC_{0\text{-}24\ hr}$ (µg/l*hr)		B9302-107	B9502-044	B9202-045	B9502-054
1	Day 1	320.6*	1977.1	NA	NA
	Day 5	810.8	6152.7	101.46	NA
0.5	Day 19	494.2	2347.5	77.01	NA
0.7	Day 26	789.4	3042	NA	NA
0.5	Day 28, fixed dose phase	1232.1	3482.2	96.81	NA

* Day 1: AUC (0-inf) NA: Not ascertained NV: no value

Spermatogenesis staging: The spermatogenic stages were present and complete in the right and left testis of both animals in the fixed dose phase at a dose of 0.5 mg/kg. In the animals from the escalating dose phase, spermatogenic stages were present and complete in the right testis of one but staging was not possible in the left due to focal tubular atrophy. In the second animal, spermatogenic stages were present and complete in the right and left testis; however, many tubules showed disturbed spermatogenesis and could not be staged.

Summary of individual study findings: A NOAEL was not identified in this study due to inadequate animal numbers and possible drug-related findings at all doses. A control group was not employed to distinguish between background and drug-related findings. Primary findings included clinical signs, reduced body weight, and numerous microscopic findings that cannot be definitively attributed to the test substance due to the lack of background information with control animals.

Chronic toxicology:
Study title: The toxicity of B9302-107 after oral administration to rats for 6 months

Key study findings:
- Study at an oral (gastric tube) dose of 0.8 mg/kg performed to clarify NOAEL dose since original 6-month study resulted in NOAEL of 0.5 mg/kg.
- NOAEL for 6-month oral administration is 0.8 mg/kg as no definitive treatment-related findings were observed. This dose is associated with an AUC of 35.4 µg.hr/ml; extrapolated from re-assessed PK data for 6-month kinetic data at doses of 0.5 and 1.5 mg/kg.

Study no: CR0695
Volume #, and page #: 18.4, 213
Conducting laboratory and location: Byk Gulden, Hamburg, Germany
Date of study initiation: March 2000
GLP compliance: The report included a signed GLP report
QA report: yes ( ) no (✔)
Drug, lot #, radiolabel, and % purity: B9302-107, batch # 298569, 99.9%
Formulation/vehicle: suspension in 4% methocel

Methods:
Dosing:

Species/strain: Rats/Wistar
#/sex/group or time point (main study): 20
Satellite groups used for toxicokinetics or recovery: 8/sex/dose group used for recovery
Age: 6 weeks
Weight: 116-224 g
Doses in administered units: 0, 0.8 mg/kg
Route, form, volume, and infusion rate: oral (via gastric tube), 1 ml/kg

Observations and times:

Clinical signs:	4 times daily
Body weights:	1-2 times weekly
Food consumption:	1-2 times weekly
Ophthalmoscopy:	pretest, weeks 26, 30
EKG:	week 19
Hematology:	pretest, weeks 6, 13, 26, 30
Clinical chemistry:	pretest, weeks 6, 13, 26, 30
Urinalysis:	weeks 6, 13, 26, 30
Gross pathology:	at necropsy on weeks 27 and 31 (recovery). Thyroid and adrenals assessed under stereomicroscope
Organs weighed:	at necropsy, for specific organs see Appendix on page 55.
Histopathology:	at necropsy; for specific organs see Appendix on page 55. Liver, heart, lungs, adrenals, uterus, vagina, ovaries, oviducts, testes, epididymis, nasal cavities, and thyroids/parathyroids and abnormalities from all animals were examined. Evaluation performed by (b) (4)

Toxicokinetics:	not assessed
Other:	water consumption measured on days 1, 2 times weekly

Results:

Mortality: Two control animals died due to ether narcosis for blood sampling.

Clinical signs: No treatment-related effects were noted.

Body weights: No treatment-related effects were noted.

Food/water consumption: No treatment-related effects were noted in relation to food consumption. Water consumption tended to be increased in males treated with B9302-107 (~14%) and remained increased during the recovery period.

Ophthalmoscopy: Results not reported.

Electrocardiography: No treatment-related effects were noted.

Hematology: Males and females demonstrated increased leukocyte numbers (23-36%) which remained slightly elevated following the recovery period (12%).

Clinical chemistry: No treatment-related effects were noted.

Urinalysis: Urine calcium levels were increased in males (124%) and females (52%). This increase was no longer apparent following the recovery period.

Vaginal smears: No treatment-related effects were noted.

Organ weights: Absolute testes weight was slightly increased (21%) in males treated with B9302-107. This increase was no longer apparent following the recovery period.

Gross pathology: The sponsor reported in the original study submission that no substance-related findings were noted. However, no data was submitted to support the conclusion.

In submission 115, the sponsor provided further information although the sponsor's macroscopic evaluation was not submitted. A list of macroscopic findings was submitted to laboratory performing histopathologic evaluation with the request that only those changes in the tissues sent for histopathologic evaluation be entered. The findings on the list included red or white focus/spot on the lung, kidney abnormality (not explained), involuted thymus (Table 23). Kidney findings were also noted in recovery animals, as was enlarged thyroid. These findings are not considered to be drug-related since they were not observed in a previously performed 6-month study in Wistar rats at doses up to 2.5 mg/kg (Study 14/96; see Original IND review).

Table 23. Gross findings in rats following 6-month administration of roflumilast.

Finding	Dose (mg/kg)			
	0		0.8	
	Male	Female	Male	Female
n=	19	20	20	20
Lungs White focus/spot Red focus/spot	 0 0	 0 0	 0 0	 1 1
Kidneys - abnormality	 0	 0	 0	 2
Thymus - involuted	0	0	0	1
Recovery				
n=	8	7	8	8
Thyroids - enlarged	0	0	2	0
Kidneys – abnormality dilated	 0 0	 0 0	 0 0	 2 1

Histopathology: In the original study submission (interim report, submission 096), the sponsor submitted a summary of histopathology data but did not submit data. The sponsor reported that no substance-related findings were noted.

Following a request for the histopathology data, the sponsor submitted the data (submission 115) in a histopathology report performed by (b) (4) Generally, a slightly increased incidence in findings in the adrenals, lungs, kidneys, uterus and thymus of minimal to slight severity (Table 24) are not considered to be drug-related as the previous 6-month study in Wistar rats did not show effects at doses up to 2.5 mg/kg.

The epididymides, testes, and nasal cavities have previously been identified as target organs of toxicity in rats. Three of nineteen control males and four of twenty treated males demonstrated testicular seminiferous tubular atrophy of minimal to slight severity. One of these treated males showed minimal, degenerate spermatogenic cells in the epididymis. Testicular seminiferous tubular atrophy (minimal) was observed in 1 of 8 control and treated animals, respectively, following the recovery period. The latter animal and one additional treated animal again demonstrated degenerate spermatogenic cells in the epididymis. The testicular findings are not considered to be drug-related as the incidence and severity was similar in control and treated animals and since historical data submitted by the sponsor shows that tubular degeneration was observed in 1 to 4 of 20 untreated control Wistar rats (data from 1994 on). The epididymal findings are considered to be secondary to the testicular findings.

In the nasal cavity, one treated male produced atrophy/disorganisation of the olfactory epithelium associated with an inflammatory exudate in the lumen. Additionally, transitional epithelial hyperplasia and inflammation were observed; study report associated this as a reactive atrophy of exogenous cause (e.g. inhaled bedding). The atrophy/disorganisation may be due to the drug treatment but is not considered relevant to human safety assessment. The nasal findings were not noted in recovery animals.

Table 24. Histopathology findings following 6-mos administration of roflumialst.

Finding	Dose (mg/kg)			
	0		0.8	
	Male	Female	Male	Female
n=	19	20	20	20
Adrenals				
Cort hypertrophy/vacuolation (minimal)	0	0	0	1
Focal cortical vacuolation (minimal)	0	0	0	1
Epididymides				
Prominent degenerate spermatogenic cells (minimal)	0	0	1	0
Lungs				
Alveolitis (minimal)	0	0	4	2
Aggregations of alv macroph (minimal)	0	0	2	2
Foamy alveolar macrophages (minimal)	0	3	0	5
(slight)	1	0	0	0
Nasal cavity				
Atrophy/disorg of olf epith (slight)	0	0	1	0
Inflammatory exudate in lumen (slight)	0	0	1	0
Transitional epith hyperplasia and inflammation (minimal)	0	0	2	0
(slight)	0	0	1	0
Prominent inflam in wall of nasolacrimal duct (present)	0	0	1	0
Testes				
Seminiferous tubular atrophy (subcapsular) (minimal)	1		2	
(slight)	1		0	
Seminiferous tubular atrophy (minimal)	0		1	
(slight)	1		1	
Kidneys – pelvic calculus(i) (slight)				1
(moderate)				1
Uterus – luminal dilatation (minimal)		1		2
(slight)		3		5
Thymus – involution/atrophy (slight)				1
Recovery				
n=	8	7	8	8
Epididymides				
Prominent degenerate spermatogenic cells (minimal)	0	0	2	0
Lungs				
Alveolitis (minimal)	0	0	0	2
Foamy alveolar macrophages (minimal)	2	1	2	2
(slight)	0	0	2	0
Testes				
Seminiferous tubular atrophy (subcapsular) (minimal)	1		0	
Seminiferous tubular atrophy (minimal)	0		1	
Kidneys – pelvic calculus(i) (moderate)				2
Uterus – luminal dilatation (minimal)		1		1
(slight)		0		3

Toxicokinetics: This parameter was not assessed.

Summary of individual study findings: No definitive drug-related findings were noted at the dose tested (0.8 mg/kg). Thus, the NOAEL for roflumilast for 6-month administration in rats is 0.8 mg/kg. This dose is associated with an AUC of 35.4 µg.hr/L; extrapolated from re-assessed PK data for 6-month kinetic data at doses of 0.5 and 1.5 mg/kg.

Study title: Toxicity of B9302-107 in beagle dogs following administration for 12 months

Key study findings:

- A NOAEL of 0.6 mg/kg was identified due to cardiac hemorrhage and hemosiderin deposit and splenic erythropoiesis at the high dose of 2 mg/kg.
- The NOAEL dose correlates with an AUC of 462-522 µg/l*hr.

Study no: 132/2000
Volume #, and page #: 26.1
Conducting laboratory and location: Byk Gulden, Institute of Pathology and Toxicology, Hamburg, Germany
Date of study initiation: April 1999
GLP compliance: Yes
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: Batch No. 298569, NA, 99.95%
Formulation/vehicle: Suspension with 4% methocel with 2-3 drops Med Antifoam C/200 ml

Methods (unique aspects)**:**
Dosing:

Species/strain: Beagle dogs
#/sex/group or time point (main study): 5
Satellite groups used for toxicokinetics or recovery: 2/sex at the high dose only used for 4-week recovery
Age: 11.5-11.8 months
Weight: 9.3-14.9 kg
Doses in administered units: 0 (vehicle), 0.2, 0.6, 2 mg/kg/d
Route, form, volume, and infusion rate: Oral (gelatinous capsules), 0.5 ml/kg/d, NA

Observations and times:

Clinical signs:	daily, pre-dose and during the morning
Body weights:	day -11, -4, 1, 4; 1x/week
Food consumption:	daily
Ophthalmoscopy:	weeks -1, 52, 56
EKG:	weeks -2, -1; 2 hrs after administration on weeks 4, 52 and 56
Hematology:	weeks -2, -1, 4, 52 and 56
Clinical chemistry:	weeks -2, -1, 4, 52 and 56
Urinalysis:	weeks 52 and 56
Gross pathology:	at autopsy weeks 53 and 57
Organs weighed:	at autopsy, heart, liver, kidneys, brain, thyroid, adrenals, pancreas, testes, ovaries, uterus, spleen, lungs, prostate

Histopathology: for specific organs/tissues, see Addendum on page 55. Examination of all organs in animals of control and high-dose groups and the heart, testes, epididymides, adrenals, nasal cavity of animals in the low-, mid- and recovery high-dose animals.
Toxicokinetics: Day 1, weeks 13 and 52; sampling at pre-dose and 1, 2, 4, 7, 10 and 24 hours post-administration; results reported separately
Other:
Physical exam including pulse rate, body temperature, senses, muscle tone, reflexes, vision, hearing, CNS, ANS, pulmonary percussion, ascultation: weeks -1, 4, 26, 52 and 56
Hormone determination: weeks -2, -1, 4, 52 and 56. Samples kept for possible future examination.

Results:

Mortality: No deaths were observed.

Clinical signs: Clinical observations included vomiting, diarrhea and hypersalivation, the most prominent of which was vomiting (Table 25). The number of animals affected and the incidence rate increased for vomiting in the two highest dose groups.

Table 25. Summary of clinical observations.

Clinical observation	Dose (mg/kg)			
	0	0.2	0.6	2
Vomiting	M: 1 F: 1	M: 2 F: 1	M: 4 F: 5	M: 6 F: 7
Diarrhea	M: 0 F: 0	M: 1 F: 0	M: 1 F: 1	M: 2 F: 0
Hypersalivation	M: F:	M: 0 F: 0	M: 0 F: 1	M: 3 F: 2

Body weights: No drug-related changes were observed.

Food consumption: One mid-dose female exhibited reduced food consumption on 11 days between dosing days 3 and 43, consuming between 8 and 91% of the offered food. From there on, consumption was generally 100%. One high-dose male also exhibited reduced food consumption (68-85% of offered food) on 8 days during dosing days 124 and 137.

Physical Exam: No drug-related changes were observed.

Ophthalmoscopy: The sponsor reported that no drug-related changes were observed. However, no data was submitted to support the conclusion.

Electrocardiography: Two low-dose animals (one male and one female) demonstrated increased QT interval (24-30 msec and 30-50 msec, respectively) at week 4, 26 and 52 compared to pre-treatment measurements (weeks -1 and -2). Likewise, one mid-dose male demonstrated a 40 msec increase in QT interval at week 52 only; heart rate was reduced by 40 beats per minute.

No significant effects were noted at the high-dose. Mean values for each dose-group were comparable to the control group at each time point. No histological findings were noted in the animals that demonstrated prolonged QT interval.

Hematology: Mean reticulocyte numbers were reduced by 70% after 4 weeks in high-dose males. Levels were still reduced at week 26, though not statistically significant. By week 52, no change compared to control values was noted. Otherwise, no other significant changes were observed. Recovery data was not submitted.

Clinical chemistry: No drug-related changes were observed. Lactate dehydrogenase levels were increased (66% at week 26) in one high-dose female.

Urinalysis: No drug-related changes were observed.

Organ weights: The only significant observation was an increased relative (to body weight) spleen weight in high-dose females of 41%. Absolute spleen weight was also increased by 51% in this group but was not significant ($p = 0.111$). Recovery data was not submitted. Erythropoiesis was noted microscopically in the spleen.

Gross pathology: In the heart, 2-3 mm blood cysts were noted in one high-dose male and female. This finding was not noted in the recovery animals. Discoloration, swelling and spotting of various regions of the stomach was reported but had no dose-dependency in terms of incidence. No correlating microscopic findings were observed in the GI tract.

Histopathology: The primary drug-related microscopic finding was observed in the heart and included minimal focal epicardial hemosiderin deposits at the right auricle concurrent with minimal to mild hemorrhages in high-dose animals (Table 26). The hemorrhages appeared to be reversible. An increased incidence of minimal to moderate oligospermia was observed in high-dose animals from one set of epididymis while the other set demonstrated a similar incidence in the control group (mild severity).

Table 26. Microscopic observation following 52-week dosing of roflumilast in dogs.

	Dose (mg/kg)									
	Male					Female				
	C	0.2	0.6	2	2-0	C	0.2	0.6	2	2-0
Histopathology										
Adrenal	5	5	5	5	2	5	5	5	5	2
Cortical round cell infiltr	0	0	0	0	0	0	0	0	2	0
Epididymis 1	5	5	5	5	2					
Oligospermia	1	1	1	4	0					
Epididymis 2	5	5	5	5	2					
Oligospermia	3	2	1	3	0					
Heart, auricle, right	5	5	5	5	2	5	5	5	5	2
Hemorrhages	1	0	0	2	0	0	1	0	2	0
Hemosiderin deposits	0	0	0	3	2	1	0	0	2	2
Mononuclear cell infiltr	0	0	2	2	0	0	0	1	0	0

Spleen	5	0	0	5	0	5	0	0	5	0
Erythropoiesis	1			2		2			5	

(b) (4) Dr. K Tuch of Byk Gulden performed a peer review of the male reproductive organ findings. The peer review determined that one control and low-dose animal originally determined to have oligospermia were normal (Table 27). In addition, 2 high-dose animals originally diagnosed with oligospermia were considered normal under peer review. Thus, given the high incidence of the findings in control animals in the original review of the data and the lack of an increase between control and high-dose animals in the peer review, the findings are not considered to be drug-related. The findings were not observed in the recovery animals.

Table 27. Peer-reviewed observations following 52-week dosing of roflumilast in dogs.

	Dose (mg/kg)				
	Male				
	C	0.2	0.6	2	2-0
Epididymis n=	5	5	5	5	2
Oligospermia	2	1	1	2	0

Toxicokinetics: The pharmacokinetics of B9302-107 and metabolites in dogs following 52-weeks oral administration (Study 4/2001) of B9302-107 are summarized in Table 28. Systemic exposure levels (AUC) were comparable between Day 1 and weeks 13 and 52. Exposures to B9302-107 increased with increasing dose although the increase was sub-proportional. Similar results were observed with Cmax. The elimination half-life was ~ 4-6 hours and the tmax was 1-2 hours. Exposure to the metabolites also tended to increase in a sub-proportional manner and elimination half-life and tmax were comparable to the parent drug. Evaluation of B9502-054 could not be performed as plasma concentrations were below the lower limit of quantitation (not reported).

Table 28. Pharmacokinetics of B9302-107 and metabolites in dogs.

Dose (mg/kg)	B9302-107			B9502-044			B9202-045		
AUC* (µg/l*hr)									
	Day 1	Wk 13	Wk 52	Day 1	Wk 13	Wk 52	Day 1	Wk 13	Wk 52
0.2	180.1	222.3	159.5	23.4	11.2	15.1	7.3	9.6	7.8
0.6	462.3	521.9	510.1	53.5	65.5	65.9	27.2	30.7	28.7
2	1015.3	1213.5	918.9	80.9	104.3	53.6	56.9	58.5	51.3
Cmax (µg/l)									
0.2	44.2	51.1	38.1	2.9	2.2	2.7	1.3	1.3	1.4
0.6	101.5	130.3	98.5	8.2	15.3	10.7	3.4	4.0	4.4
2	171.3	227.9	179.3	9.3	15.8	12.8	5	8.1	7.7
T1/2 (hr)									
0.2	4.7	5.4	5.4	6.3	3.2	5.7	3.9	4.3	3.2
0.6	5.3	5.5	6.2	5.1	6.6	4.2	5.6	5.0	5
2	4.2	4.3	5.1	9.3	4.5	5.2	5.2	4.3	3.8
Tmax (hr)									
0.2	1	1.6	1.1	1.2	1.8	1.4	1.3	2.1	2
0.6	1.1	1.1	1.4	1.5	1.57	2.2	1.8	2.2	2.3
2	1	1.5	1.6	1	1.6	1.8	1.4	2.1	1.9

* Day 1 (0-inf); Wk 13 and 52 (0-24 hr)

Summary of individual study findings: A NOAEL of 0.6 mg/kg was identified due to cardiac findings and splenic erythropoiesis. The NOAEL dose correlates with an AUC of 462-522 µg/l*hr). The Sponsor also concluded that the NOAEL is 0.6 mg/kg although they suggest that the cardiac findings are species-specific and not relevant to humans.

Study title: 52-week chronic toxicity study of roflumilast N-oxide (B9502-044) by oral administration to beagle dogs

Key study findings:

- Roflumilast N-oxide (B9502-044), a metabolite of Roflumilast, was tested in dogs since only low levels are observed in plasma when dogs are administered Roflumilast directly.
- A NOAEL of the high-dose of 1.2 mg/kg was identified; no definitive target organs of toxicity were noted.
- The NOAEL correlates with systemic exposure of 352-483 µg/l*hr.

Study no: 13001/00
Volume #, and page #: 33.2, 1
Conducting laboratory and location: (b) (4)
Date of study initiation: April 2000
GLP compliance: An unsigned report was included.
QA report: yes ( ) no (✔)
Drug, lot #, radiolabel, and % purity: B9502-044, Batch No. Z128/103/001, NA, 100.2%
Formulation/vehicle: Suspension with 4% methocel with 2-3 drops Med Antifoam C/200 ml

Methods (unique aspects)**:**
Dosing:

Species/strain: Beagle dogs
#/sex/group or time point (main study): 5
Satellite groups used for toxicokinetics or recovery: 2/sex at the high dose only used for 4-week recovery
Age: 6.5 months
Weight: 5.6-8.8 kg
Doses in administered units: 0 (vehicle), 0.1, 0.4, 0.8 and 1.2 mg/kg; 0.2, 0.8, 1.6 and 3.2 mg/ml/; 0.02, 0.08, 0.16 and 0.24%; dosing occurred 2 hours prior to feeding.
Route, form, volume, and infusion rate: Oral (gelatinous capsules), 0.5 ml/kg/d, NA

Observations and times:

Clinical signs:	multiple times daily
Body weights:	weekly
Food consumption:	daily
Ophthalmoscopy:	weeks -2, 52 and 56
EKG:	weeks -2, -1, 4, 26, 52 and 56
Hematology:	weeks -2, -1, 4, 26, 52 and 56

Clinical chemistry: weeks -2, -1, 4, 26, 52 and 56
Urinalysis: weeks -2, -1, 4, 26, 52 and 56
Gross pathology: at autopsy weeks 52 and 56
Organs weighed: at autopsy, adrenals, brain, epididymis, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroid and parathyroid
Histopathology: for specific organs/tissues, see Addendum on page 55.
Toxicokinetics: Day 1, weeks 13 and 46; sampling at pre-dose and 0.5, 1, 2, 3, 4, 6 and 24 hours post-administration; results reported separately
Other:
Andrologic exam including gonad morphology and manually stimulated ejaculation to test for sperm quantity, motility and morphology: days 219/220 and 337/338

Results:

Mortality: No deaths were observed.

Clinical signs: Clinical observations included vomiting, hypersalivation, tremor and reduced motility and occurred primarily in the upper-mid-dose and high-dose groups. The incidence increased with dose and time. Emesis was usually noted within 2 hours of dosing. Sporadic occurrences were noted in the control and lower dose groups. Hypersalivation was noted in the two- mid-dose groups from weeks 17-19 onward and in high-dose animals from week 13 onward. Tremor and reduced motility were observed in most high-dose animals from weeks 41 onward. The mid-dose groups exhibited these findings from week 47 onward. All symptoms subsided during the 4-week recovery period.

Body weights: No drug-related changes were observed in males. At the end of the dosing period, upper- mid and high-dose females exhibited an 8-11% decrease in body weight (non-significant).

Food consumption: Food consumption was not significantly affected by drug administration. The report also states that water consumption was not affected although no data was provided to support the conclusion.

Ophthalmoscopy: No drug-related changes were observed.

Electrocardiography: No definitive changes in QT interval were noted although high data variability was evident. Most changes were directed downward rather than increased. Mean arterial systolic blood pressure was increased in all high-dose animals at week 26 when compared to week –1 (mean increase equals 21% vs week -1; 11% increase when compared to control animals at week 26). This increase was still evident at week 52 (mean increase of 22%; 20% when compared to control values at week 52). The pressure was reduced by 20 and 48% in the two recovery males. A similar effect was noted in females at week 52 only (mean increase of 21% vs week -1; 29% when compared to control values at week 52). The pressure was reduced in the two recovery females by 20 and 36%.

Hematology: No drug-related changes were observed.

Clinical chemistry: No drug-related changes were observed.

Urinalysis: No drug-related changes were observed.

Organ weights: Absolute spleen weight was increased in HD males by 34% (ns) and absolute prostate weight was decreased by 22% at the high dose. In females, absolute left ovary weight was decreased by 34% at the high dose; no change noted in the right ovary. In addition, decreased left thyroid weight was reported at all doses (26%, 37%, 27% and 46%, respectively). Decreased right thyroid weight was observed only at the high dose (22%). No changes in relative body weight were noted.

Gross pathology: No definitive drug-related findings were noted. Areas of lung discoloration were observed in all dose groups. No correlating microscopic changes were noted. The two high-dose recovery females also demonstrated scarred induration at the right cranial lobe and several dark red foci.

Histopathology: A slight increase in the incidence of minimal liver pigmentation was observed in high-dose animals (Table 29). One high-dose recovery female demonstrated yellow-brown hepatocelluar pigment (grade 1) at necropsy.

Table 29. Microscopic observation following 52-week dosing with B9502-044 in dogs.

	Dose (mg/kg)									
Histopathology	Male					Female				
	C	0.1	0.4	0.8	1.2	C	0.1	0.4	0.8	1.2
Liver	5	5	5	5	5	5	5	5	5	5
Pigment										
Minimal	1	0	1	1	2	1	0	2	1	4
Slight	0	0	0	0	0	1	0	0	0	0

Toxicokinetics: Data taken from study report 160/2001: “12-months toxicokinetics of B9502-044 in the dog following oral administration at four different dose levels”. Systemic exposure to B9502-044 increased supra-proportionally with increasing dose (Table 30). Levels of B9302-107 were 5-10% of the levels of the N-oxide B9502-044. Drug accumulation was evident at the highest dose.

Table 30. Summary of kinetic data following administration of B9502-044 in dogs.

	Analyte: B9502-044				Analyte: B9302-107			
Dose (mg/kg)	0.1	0.4	0.8	1.2	0.1	0.4	0.8	1.2
Day 1								
AUC (0-∞) (µg/lxhr)	11.01	50.96	125.75	180.28	NA	NA	4.18	6.36
C max (µg/l)	9.25	43.46	90.68	130.93	0.25	0.43	1.17	1.55
T-half (hr)	0.47	0.39	0.54	1.28	NA	NA	2.2	2.6
Tmax (hr)	0.65	0.8	1.05	0.8	2.1	1.56	2.4	2.4
Week 13								
AUC (0-∞) (µg/lxhr)	13.4	63.69	159.14	352.94	NA	2.31	6.05	15.13
C max (µg/l)	15.92	64.16	114.44	304.13	0.24	0.62	1.22	3.04
T-half (hr)	0.41	0.56	0.88	1.24	NA	3.83	3.89	3.43
Tmax (hr)	0.5	0.75	1.1	0.75	1.36	3.8	2.4	2.1
Week 46								
AUC (0-∞) (µg/lxhr)	14.58	54.01	152.93	482.59	NA	1.25	11.97	41.3
C max (µg/l)	13.12	45.49	115.48	261.91	0.27	0.76	1.36	4.82
T-half (hr)	0.46	0.51	0.69	1.47	5.51	2.2	5.76	5.2
Tmax (hr)	0.6	0.8	0.9	1.21	1.25	0.9	1.6	3.32

NA: not ascertainable, LLOQ = 0.1 µg/l for both compounds

Andrology: In the first examination of ejaculate parameters, a significant increase in neck alterations was observed in the upper-mid and high-dos groups (Table 31). The high mean values were caused by one individual ejaculate in each of the two groups (UMD values: 2.5, 6.5, 5.5, 34, 1.5; HD values: 2.5, 4, 6.5, 1, 32.5, 1.5, 2.5) due to high percentages of spermatozoa with a cytoplasmic droplet, representing incomplete or delayed epididymal sperm maturation. An increase was also noted during the second assessment at the three upper doses but was only close to statistical significance at the low mid-dose, suggesting that the effect is an individual property and/or age-related epididymal immaturity. Single animals in each group demonstrated increased neck alterations (25, 33 and 42.5%). During the second examination, an increase in eosin stained spermatozoa was noted at the high dose. The report stated that a negative drug-effect could not be excluded, although two dogs at the high-dose did not exhibit an increase and a rise of > 5% was observed in individual dogs of other groups, which points to intra-individual variation of plasma membrane integrity. The range in control animals was 4-13% vs 10-19% in high-dose animals. No effects were noted in the parameters length of testis, width of testis, ejaculate volume, total sperm number, motile spermatozoa, morphologically altered spermatozoa, acrosome alterations, head alterations, midpiece alterations, endpiece alterations, and double and multiple deformations.

Table 31. Assessment of ejaculate parameters following 52-week dosing in dogs.

	Dose (mg/kg)				
	Male				
	C	0.1	0.4	0.8	1.2
Andrology					
1st assessment					
Neck alterations (%)	1.2	2.8	5.8	10	7.2
2nd assessment					
Neck alterations (%)	1.6	3.1	7.3	9.3	7.3
Eosin stained spermatozoa (%)	9.2	9.8	9.3	11.4	14

Shaded area indicates significant difference from control values.

In the EOP2 meeting held December 6, 2001, the sponsor presented data indicating no treatment-related finding related to eosin staining. An action item from this meeting was that the sponsor would check their data for the source of the discrepancy between the study report and their presented data. In the submission of February 27, 2002 (SN 157) the sponsor states that the discrepancy was due to their reference to the first sperm analysis only while the Agency referred to both analyses. During the EOP2 meeting, the Agency indicated that the NOAEL for this study was identified as 0.1 mg/kg in males due to the spermatogenic findings. In response, the sponsor provided historical data on semen quality of three groups of dogs bred for experimental studies in submission 157 dated February 27, 2002 (Attachment 2). In 12 to 16-month old dogs (n=15 control dogs from a toxicity study; sexually inexperienced; six ejaculation from each dog) the mean % of neck alterations ranged from 0.8 to 7.7% while the intra-individual ranges varied from 0 to 22% for individual ejaculations. The mean of 89 ejaculations in the 15 dogs was 2.7%. Eosin stained spermatozoa varied from 4.1 to 10.9 with intra-individual ranges of 1.5 to 30%; overall mean of 6.7%.

In a second group of 21 sexually inexperienced dogs (13-21 months; 1-2 ejaculations per dog) the mean % of neck alterations ranged from 0.5 to 20.8% while the intra-individual ranges varied from 0.5 to 27.5% for individual ejaculations. The overall mean was 3%. Eosin stained spermatozoa varied from 4.5 to 18; overall mean of 10.6%.

A third group of 9 dogs (9-25 months old; 5 ejaculates per dog) used for studies on semen preservation and breeding purposes demonstrated mean % of neck alterations ranging from 2.7 to 11.8% while the intra-individual ranges varied from 0 to 19.5% for individual ejaculations. The overall mean was 5.1%. Eosin stained spermatozoa varied from 3.8 to 9; overall mean of 6%.

Based on the submitted historical data, the sponsor notes high intra- and inter-individual variability of all ejaculate parameters. The sponsor concludes that the NOAEL should be 1.2 mg/kg in males based on the submitted historical data. It is agreed that the increase in eosin stained spermatozoa observed at the high dose in the second assessment is within the historical control range (up to 30%) and is not a definitive drug-related effect. Although the increased magnitude in neck alterations observed in the first and second assessment exceeds the range of values in the historical data, the drug-related nature of the observed findings is questionable since the increased means are due to only one animal in each group and there is no increase in response with increasing dose.

Summary of individual study findings: A NOAEL of the high-dose of 1.2 mg/kg B9502-044 was identified as no definitive target organs of toxicity were observed. The NOAEL correlates with a systemic exposure of 352-483 µg/l*hr.

Study title: Summary of results concerning spermiograms of dogs treated with the test compound B9302-107 or B9502-044.

Study no: 22/2001
Volume #, and page #: 18.4, 175
Conducting laboratory and location: Byk Gulden, Hamburg, Germany
Date of study initiation: see relevant study reports
GLP compliance: The report included a signed GLP report
QA report: yes (✔) no ( )
Drug, lot #, radiolabel, and % purity: see review of study reports
Formulation/vehicle: tablet/capsule

Semen samples were collected from dogs during the 6-month toxicity study with B9302-107 (study 94/96 reviewed in Review # 1, dated July 24, 2000) and 12-month study with B9502-044 (study 13001/01; interim samples taken at weeks 31-32).

Methods:
Dosing:

Species/strain: Male beagle dogs
#/sex/group or time point (main study): 5-7 males
Satellite groups used for toxicokinetics or recovery: NA
Age:
Weight:
Doses in administered units: 0, 0.2, 1, 4 mg/kg B9302-107; 0, 0.1, 0.4, 0.8, 1.2 mg/kg B9502-044
Route, form, volume, and infusion rate: oral tablet (B9302-107); capsules (B9502-044)

Observations and times:

Clinical signs:	not assessed
Body weights:	not assessed
Food consumption:	not assessed
Ophthalmoscopy:	not assessed
EKG:	not assessed
Hematology:	not assessed
Clinical chemistry:	not assessed
Urinalysis:	not assessed
Gross pathology:	not assessed
Organs weighed:	not assessed
Histopathology:	not assessed
Toxicokinetics:	not assessed
Other:	adspection and palpation of the scrotum, testes and epididymides, measurement of testicular length and width, rectal palpation of prostate gland and collection and examination of one ejaculate per dog.

Results:
Histopathology: B9302-107 (study 94/96): These results were initially reviewed in the original IND review. As concluded previously, no significant morphological changes were observed with the testes. Similarly, no significant changes in ejaculate volume, % progressively motile spermatozoa, % morphologically altered spermatozoa, head alterations, % acrosome alterations, % neck alterations, % midpiece alterations, % endpiece alterations, or total sperm number. The percentage of eosin stained spermatozoa (indicative of membrane damage) was significantly increased in B9302-107-treated animals although the finding was not dose-related (14.4, 12.6, 12.4% vs 7 % in controls). An increase in the incidence of spermatozoa with double or multiple deformations was noted in MD and HD groups (1 and 0.8% vs 0.1% in controls). However, all animals were within the maximum tolerable value. The sponsor concluded that a relationship to test substance is doubtful since the percentage of eosin staining was below the tolerable maximum value of 15% reported by (b) (4) (1994), based on 243 fertile males of different breeds ranging from 1 to 32% eosin stained spermatozoa, and only single animals in each treatment group exceed the maximum tolerable level (16-19%). Further the sponsor concluded that a dose-dependent influence of the test substance could not be detected with regard to acrosome and midpiece alterations or the double and multiple deformations since the mean values did not markedly differ from those found in fertile dogs of different breeds (b) (4) et al, 1994).

B9502-044 (study 13001/00): At the interim assessment (weeks 31-32), a 5x to 7.3x increase in % neck alterations at UMD and HD was observed due to single animals in each group. The length and width of the testes were unaffected. Non statistically significant reductions in ejaculate volume (35%) at the HD, and total sperm number (11-28%) at all doses were noted. The parameters % progressively motile sperm, % eosin stained sperm, % morphologically altered spermatozoa, % acrosome alterations, % head alterations, midpiece and endpiece alterations, and double and multiple deformations were unaffected. These findings were in agreement with those of the interim sacrifice data reported in the above review of the final study report.

Although the sponsor concluded that no treatment-related effects were noted, it is pointed out that findings were observed at the second assessment of spermiogenic parameters in the 52-week study with B9502-044 (see study review above). These findings included an increase in neck alterations that was only statistically significant at the mid-dose, suggesting an individual effect and/or age-related epididymal immaturity. In addition, a significant increase in eosin stained spermatozoa was noted at the high dose. The study report stated that a negative drug-effect could not be excluded. However, two dogs at the high-dose did not exhibit an increase and a rise of > 5% was observed in individual dogs of other groups, which points to intra-individual variation of plasma membrane integrity.

Peer review of histological finding in the testes and epididymides of rats, dogs, mice and hamsters treated orally with B9302-107 and B9502-044 (20/2001)

Sections of all testes and epididymides (no prostate or seminal vesicle) of animals out of 13 studies (Rats: 4 wk, 3 mos, 3 mos, 6 mos, 6 mos, Seg I with B9302-104, 4 wk with B9502-044; Dogs: 4 wk, 6 mos, 12 mos with B9302-107, 4 wk with B9502-044; hamster and mice: 3 mos with B9302-107) were reviewed. Different pathologists originally read the studies over time. A single pathologist reassessed the sections in order to harmonize terminologies and to achieve a more accurate overall assessment. The report states that the results of the review were reported with the original diagnosis on an individual animal basis for each study. Where review findings were different from the original, Drs. (b) (4) and K. Tuch (Byk Gulden) reviewed the sections and consensus was obtained. It is noted that the original diagnoses for the individual animals were not included.

The following is a summary of the sponsor's reassessment and comparison with the Agency's review of the original study reports; sponsor and Agency assessments are provided for each species tested.

Rat:

Sponsor assessment: Toxicity was noted only in the rat. Findings included epididymal sperm granulomas, and an increased incidence of oligospermia. Testicular changes appeared as tubular dilatation, increased tubular degeneration/atrophy and/or disturbed spermiogenesis. NOEL levels for the studies in rats ranged from 0.2-0.8 mg/kg B9302-107 and 1.2 mg/kg B9502-044. The highest dose of B9302-107, which produced no effects, was 0.8 mg/kg (6 mos); the lowest dose, which produced findings, was 1.2 mg/kg. Thus, the NOAEL is between 0.8 mg/kg and 1.2 mg/kg. The highest dose of B9502-044 tested producing no effects was 0.4 mg/kg (4 wk); the lowest dose, which produced findings, was 1.2 mg/kg. Thus, the NOAEL is between 0.4 mg/kg and 1.2 mg/kg.

4 week rat study with B9302-107 (0.5, 2, 8 mg/kg): Results are summarized below (Table 32); the sponsor states that the increased tubular degeneration (atrophy) is possibly treatment-related only at 8 mg/kg.

Table 32. Histopathology changes in rats following 4-week administration.

Observed signs	Dose group (mg/kg)					
	0	0-0	0.5	2	8	8-0
n=	10	8	10	10	10	8
Epididymides						
Sperm granuloma	0	0	0	1	3	3
Oligospermia	increased at 8 mg/kg, non-reversible					
Testes						
Tubular dilatation - minimal	0	0	0	2	4	0
Disturbed spermiogenesis	0	0	0	0	3	2
Tubular atrophy - minimal to marked	0	0	1*	2	3	3

* - marked

3 month rat study with B9302-107 (0.02, 0.2, 2 mg/kg): Results are summarized below; the no treatment related findings in testes reported.

Table 33. Histopathology changes in rats following 3-month administration.

Observed signs	Dose group (mg/kg)					
	0	0-0	0.02	0.2	2	2-0
n=	8	8	8	8	8	8
Epididymides						
Sperm granuloma	0	0	0	0	2	1

3 month rat study with B9302-107 (0.2, 0.5, 0.8 mg/kg, report not submitted): No treatment related findings reported in reproductive organs.

6 month rat study with B9302-107 (0.5, 1.5, 2.5 mg/kg): Results are summarized below; no "clear-cut" treatment-related findings in testes reported.

Table 34. Histopathology changes in rats following 6-month administration.

Observed signs	Dose group (mg/kg)					
	0	0-0	0.5	1.5	2.5	2.5-0
n=	20	8	20	20	20	8
Epididymides						
Sperm granuloma	0	0	0	1	4	1

6 mos rat with B9302-107 (0.8 mg/kg): No treatment related findings. In agreement with report reviewed above.

Rat Segment I reproductive toxicology study with B9302-107 (0.2, 0.6, 1.8 mg/kg): Results are summarized below; increase in tubular atrophy in testes not considered of toxicological significance since severity was minimal to slight, incidence not reported.

Table 35. Histopathology changes in rats in Segment 1 study.

Observed signs	Dose group (mg/kg)			
	0	0.2	0.6	1.8
n=	28	28	28	28
Epididymides				
Sperm granuloma	0	0	0	3
Testes				
Tubular dilatation	0	0	0	1
Tubular atrophy	0	0	0	increased

Agency review of original study reports in rats:
4 week (0. 0.5, 2, 8 mg/kg): mortality at the HD; Epididymis: oligospermia 8/17 HD; aspermia 1/17 HD, granuloma 6/17 HD; Testes: spermiogenic disturbance 1/10 MD and 8/17 HD; prostatic and seminal vesicle: atrophy 3/9 HD

3 months: (0.02, 0.2, 2 mg/kg): Testes: atrophy 3/8 HD (minimal to moderate severity); Epididymis: oligo/aspermia (4/8 HD), spermatocele (2/8 HD); findings in epididymis were not fully reversible.

3 months: (0.2, 0.5, 0.8 mg/kg): report not submitted to or reviewed by the Agency.

6 months (0.5, 1.5, 2.5 mg/kg): Epididymis: hypospermia (1/20 HD), spermatocele (2/20 MD, 4/20 HD), seminal vesicle: necrosis (1/20 HD)

6 months (0.8 mg/kg): No histologic findings observed; conclusion in agreement with the sponsor's evaluation.

4 week rat study with B9502-044 (0, 0.4, 1.2, 3.6 mg/kg): The sponsor's evaluation was generally consistent with the original Agency review, although there were slight changes in numbers, and lack of testicular atrophy and epididymal findings (Table 36). The NOEL for the testes finding was reduced from 1.2 to 0.4 mg/kg; the NOEL for epididymis is consistent.

Table 36. Histopathology changes in rats following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)					
	0	0-0	0.4	1.2	3.6	3.6-0
n=	10	8	10	10	10	8
Epididymides						
Sperm granuloma	0	0	0	0	3	4
Testes						
Tubular dilatation (min)	0	0	0	2	8	0
Agency review						
Epididymides						
Spermatocele					3	4
Oligospermia					0	1
Inflammation					0	1
Prostate Atrophy					2	0
Seminal Ves. Atrophy					2	0
Testes						
Tubular dilatation					9	0
Tubular atrophy					0	1

Dogs:

Sponsor's assessment:

4-weeks (2, 6, 18 mg/kg): no treatment-related changes were noted, 1/3 HD animals with minimal tubular degeneration with a few degenerate cells in the epididymal ducts

6 months (0.2, 1, 4, mg/kg): no treatment related changes were noted, (1/5 MD, 1/5 HD and 1/2 HD-Recovery showed minimal to slight focal tubular degeneration) treated with B9302-107.

12 months (0.2, 0.6, 2 mg/kg): no treatment-related changes. Dogs from control and treatment groups showed minimal focal tubular degeneration/atrophy and/or minimal number of giant cells in seminiferous tubules. Slight to minimal oligospermia was reported in occasional dogs.

Agency review of original reports in dogs:
4-week:
Testes: tubular degeneration (1/3 HD, reversible)
Epididymis: dysspermia (1/3 HD, reversible)

6 mos: no treatment-related findings

12 mos study: no treatment-related findings

4 week study in dogs with B9502-044: (0.6, 1.2, 2.4 mg/kg): The sponsor's assessment was that no treatment-related findings were observed although one high-dose dog exhibited minimal focal tubular degeneration and a few degenerate cells in epididymal ducts unilaterally. These findings are consistent with the Agency review of the data (Table 37). Other findings included increased testes weight (24-28%, HD), and smaller prostate (2/3 HD) and seminal vesicle (2/3 HD) size.

Table 37. Histopathology changes in dogs following 4-week administration of B9502-044.

Observed signs	Dose group (mg/kg)					
	0	0-0	0.6	1.2	2.4	2.4-0
n=	3	0	3	3	3	3
Epididymides						
Degenerate cells in ducts	0	0	0	0	1	0
Testes						
Focal tubular dilatation (min)	0	0	0	0	1	0
Agency review n=	3	3	3	3	3	2
Epididymides						
Dysspermia	0	0	0	0	1	0
Testes						
Tubular cell degen	0	0	0	0	1	0
Prostate n=	3	0	0	0	3	2
Atrophy	0				1	0
Fibrosis	1				2	0

Hamster:
Sponsor reassessment:
No treatment-related changes were noted in hamsters (3 months, 4, 8, 16 mg/kg: minimal to marked arrest of spermiogenesis in majority of hamsters from control and all treatment groups, *incidence not reported*). The findings are associated with evidence of varying degrees of oligospermia in the respective epididymis.

Agency review of original report in hamsters:
Epididymis: Increased incidence (8-10 vs 6/10 control) and severity (2.7-3.3 vs 1.3 control) of dysspermia at all doses
Prostate and Seminal vesicle: increased incidence and severity of atrophy at HD (3-4/10 HD, 0.8 severity; LD and MD not assessed)
Testes: increased incidence (9-10 vs 8 in control), and severity (3.4-3.5 vs 1 in control) in tubular atrophy (all doses).

Mouse:
Sponsor reassessment and Agency review: no treatment-related findings.

Overall Assessment: In the meeting held in December 2001, the Agency requested that the sponsor submit the individual animal evaluations and rationale for the change in the above noted findings from the original study reports to their reassessment. This information would allow for a definitive review of the issues related to male reproductive organ toxicity in multiple species. That information has not been submitted to date.

Expert statement on testicular toxicity of roflumilast and its relevance to man (Report 25/2001).

Based on the sponsors' review of the data, effects noted in rats (focal testicular atrophy and epididymal sperm granuloma), were not noted in mice and dogs, or hamsters. Thus, it is questionable as to whether findings in the rat bear clinical significance to man. The following is a summary of the sponsor's submitted rationale:

A review of the testicular organization and control of function was provided. In rats seminiferous tubules are loosely arranged and do not anastomose, and peritubular cells form 1-2 layers. In humans tubuli are highly contorted and can anastomose, peritubular cells are stratified around tubules and form up to 6 concentrical layers separated by collagen layers.

The following points were noted:
♦ In rats only 10% of normal intratesticular androgen concentrations are sufficient to support (in conjunction with FSH) fully normal germ cell production[2] and ~ 30% of normal intratesticular androgen concentrations are sufficient to fully support germ cell production even when FSH secretion is suppressed[3]. In primates, 30-50% normal intratesticular androgen concentrations with low FSH fails to support spermatogenesis and is associated with severely reduced sperm numbers or even lack of sperm production[4,5]. The findings suggest that FSH is important for

[2] Cunningham, GR, Huckins C. 1979. Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. Endocrinology 105:177-186.
[3] Sharpe RM. 1994. Regulation of spermatogenesis. In: The Physiology of Reproduction (Knobil E, Neil JD, eds). Raven Press, New York, pp 1363-1434.
[4] Weinbauer GF, Gockeler E, Nieschlag E. 1988. Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone (GnRH) antagonist-treated non-human primate (*Macaca fascicularis*). J Clin Endocrinol Metab. 67: 284-290.

primate spermatogenesis whereas rat spermatogenesis is more dependent on testosterone levels.

♦ Withdrawal of trophic hormones in rats leads to the appearance of degenerating spermatocytes and spermatids in stage VII seminiferous tubules[3] or a multifocal lesion[6,7]. Degenerating spermatocytes have been observed in stage VII seminiferous tubules within 3 days after selective elimination of Leydig cells and testosterone[8]. In primates and man, gonadotropin withdrawal acutely and specifically affects spermatogonial proliferation[9]; gonadotropin withdrawal initially affects less advanced germ cells (spermatogonia) in primates. Thus, germ cell targets responding to complete reproductive hormone loss are substantially different in monkeys and men compared to the rat.

♦ Physiologic significance of GnRH system is currently unknown, although some evidence supports specificity to the rat and has been implicated in rat-specific testicular toxicity of GnRH agonists[10]. Rat Leydig cells have functional binding sites for GnRH[11], while evidence is conflicting in primates. In vitro incubation of human Leydig cells with GnRH agonist failed to affect human chorionic gonadotropin (hCG) induced testosterone production (hCG binds to LH receptor and stimulates Leydig cell production)[12,13].

♦ In rats, LH receptors noted on Leydig cells, and LH and hCG binding sites have been demonstrated on endothelial cells of testicular microvasculature[14]. High doses of hCG (100 IU) or exposure to GnRH agonists induce spermatogenic damage, inflammation like changes and formation of edema of interstitial area[15,16]. The effects have been related to initial (2-6 hrs after injection) decreased blood flow caused by hCG-induced precapillary constrictions. LH first

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[5] Weinbauer GF, Schlatt S, Walter V, Nieschlag E. 2001. Testosterone-induced inhibition of spermatogenesis is more related to suppression of FSH rather than testicular androgen levels in the cynomolgus monkey model (*Macaca fascicularis*). J Endocrinol. 168:1-14.

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[7] Kerr JB, Millar M, Maddocks S, Sharpe RM. 1993. Stage dependent changes in spermatogenesis and Sertoli cells in relation to the onset of spermatogenic failure following withdrawal of testosterone. Anat Rec 235:547-559.

[8] Bartlett JMS, Kerr JB, Sharpe RM. 1986. The effect of selective destruction and regeneration of rat Leydig cells on the intratesticular distribution of testosterone and morphology of the seminiferous epithelium. J Androl 7:240-253.

[9] Weinbauer GF, Nieschlag E. 1999. Testicular physiology of primates. In: Reproduction in Nonhuman Primates (Weinbauer GF, Korte R, eds). Waxman Verlag, Munster, pp 13-26.

[10] Wang NG, Sundaram K, Pavlou S, Rivier J, Vale W, Bardin CW. 1983. Mice are insensitive to the antitesticular effects of leutenizing hormone-releasing hormone agonists. Endocrinology 112:331-335.

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[12] Rajfer J, Sikka SC, Swerdoff RS. 1987. Lack of a direct effect of gonadotropin hormone-releasing hormone agonist on human testicular steroidogenesis. J Clin Endocrinol Metab 64:62-67.

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decreases and later increases testicular blood flow and causes PMN accumulation[17,18]. Similar effects have been noted in mice although with no PMN accumulation[19]. Within 12-24 hours after exposure to hCG or GnRH agonist in the rat, the seminiferous epithelium collapses[17,20]. Administration of 100 IU to rats caused seminiferous tubule degeneration within 1 week, with maximal damage by week 4 and partial recovery by week 12[21].

Exposure to high LH activity or hCG does not cause testicular damage in primates; no information as to effects in humans. Administration of 250 IU hCG (alternate days for 4 wk) produced no discernible effect on testicular morphology in juvenile monkeys and no leukocyte accumulation or infiltration, although blood flow was not measured[22]. Similarly, no interstitial pathology was observed in adult monkeys exposed to hCG for 16 days[23]. Exposure to recombinant LH had no effect in monkeys.[24] Clinically, hCG treatment or high LH via administration of human menopausal gonadotropin is used to successfully induce spermatogenesis in hypogonadotropic hypogonadism[25,26]; no evidence of testicular effects although it is unclear that histopathology assessments were performed. Administration of hCG to healthy males yielded no evidence of adverse effects on spermatogenesis as judged from sperm counts[27].

In rats, exposure to high levels of LH/hCG induce pathological changes of the interstitial and tubular compartment, while in humans Leydig cells do not react to LH[28]. In rats, GnRH agonists provoke focal and irreversible testicular damage, which is not observed in dogs, monkeys or men[29].

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The following summarizes a discussion of Roflumilast-related male reproductive organ toxicity:
Mouse: No substance-related testicular toxicity was noted.
Hamster: Observed atrophy in testicular epithelium was not substance-related
Dog: A 6-mos study revealed no substance-related effects. Seminal parameters were not affected in a 1-year study with Roflumilast N-oxide.
Rat: Various studies demonstrated adverse effects including focally disturbed spermiogenesis, tubular atrophy, and tubular dilatation of the testes, sperm granuloma, oligozoospermia, and degenerating cells in the epididymis, reduction of serum testosterone levels and serum progesterone.

A possible mechanism for findings includes the reduction/prevention of cAMP degradation. Roflumilast probably provokes a condition of hyperstimulation of Leydig cells, Sertoli cells and endothelial cells since G-protein coupled receptors are present. LH hyperstimulation of rat testes is associated with focal tubular atrophy of rat testes, but not seen in mice, dogs, monkeys and humans. Therefore, Roflumilast-associated tubular atrophy appears to be rat-specific and does not bear relevance to man.

Roflumilast reduced testosterone levels and progesterone levels in male rats within 7 days with no effect on FSH and LH levels. Unaltered LH but reduced testosterone suggest direct effect of drug on testicular Leydig cell testosterone production. Since progesterone levels were also non-signficantly lowered, an inhibitory effect likely involves steroidogenic steps prior to progesterone formation. Reduced testosterone levels would provide an explanation for reduced sperm count, copulation rates and fertility in Segment I rat study. Rat spermatogenesis is sensitive to androgen deficiency, leading to focal germ cell degeneration and disturbed spermiogenesis. Effects on spermiogenesis were also noted in a 4-week roflumilast study.

The relationship of focal tubular atrophy to testosterone reduction is questionable. Long term androgen deficiency causes uniform suppression of spermatogenesis; theophylline lowered testosterone and caused tubular atrophy while caffeine and theobromine elevated testosterone but also induced tubular atrophy. A direct relationship of testosterone and spermatogenic damage is unlikely.

LH is sensitive to alterations in testosterone. Elimination or inhibition of testosterone provokes an increase in LH within 3-5 days in male rats. The unaffected LH secretion in the 7-day rat study (244/2000) is explained if the reduction of testosterone levels occurred only after several days of drug treatment. Unaltered LH with decreased testosterone could also be due to hyperstimulation of hypothalamic-pituitary GnRH-gonadotropin circuit. Inhibition of PDE4 activity in vitro in GT1 neuronal cells has been reported to cause stimulation of GnRH secretion. Exposure to high GnRH suppresses LH beta-subunit secretion rather than that of the alpha subunit. The beta-subunit confers biological activity of the LH molecule, and testosterone levels could be lowered with the LH immunoactivity retained if the LH immunoassay used mainly detects the alpha-subunit.

In terms of the relevancy of the preclinical findings to man, the report concludes that roflumilast-associated testicular toxicity is relegated to the rat model only. The physiology and endocrinology of the rat is different from man. Rat testes are particularly vulnerable to increased

exposure of cAMP-mediated effects like PDE4 inhibitors. Vulnerability is rat specific by comparison to numerous reports dealing with testicular hyperstimulation. Hence, rat testicular toxicity is not considered relevant to man.

However, the report recommends that the influence of Roflumilast on spermatogenesis in man be investigated. If one assumes similar degree of focal tubular atrophy to that observed in rat, precipitous and substantial alterations of sperm numbers and other semen parameters would not be expected. Human ejaculate parameters are highly variable and the spermatogenic process requires about 70 days in man. It is difficult to detect minor changes of spermatogenesis using semen parameters; depending on site of lesion, spermatogenic defects may become apparent only after 10 weeks. The report recommends analysis of relevant hormones for assessment of testicular function, which are more sensitive than semen analysis; endocrine diagnosis should include LH, testosterone, FSH, and inhibin B. FSH and inhibin B provide very sensitive measures of impaired spermatogenesis and testes toxicity.

Evaluation of Expert report: During the teleconference of June 2001, the sponsor was informed that, although their proposed mechanism of action for the effects of roflumilast on male reproductive organ toxicity in rats may be accurate, there is currently insufficient evidence to demonstrate the actual mechanism of action. In addition, the sponsor has not demonstrated that the mechanism related to roflumilast-induced toxicity is not relevant to humans.

Toxicology summary: Studies were performed in rats (7-day endocrine and male reproductive organ histology, 6-month oral study with B9302-107 and a 4-week oral study with the metabolite B9502-044), dogs (a 4-week oral study with B9502-044 and one-year studies with B9302-107 and B9502-044) and monkeys (4 week dose-ranging study with B9302-107). In rats increased levels of FSH, LH and progesterone, and decreased levels of 17β-estradiol were observed after 7-days dosing with the high dose (2.5 mg/kg) and fewer high-dose animals were observed to be in estrus cycle. Males exhibited a dose-related decrease in testosterone, corticosterone and progesterone. No findings that were characteristic of testosterone deficiency were observed in male rats administered B9302-107 for 7 consecutive days, although tubular dilatation of the testes was observed at the high dose. The chronic study in rats identified a NOAEL of 0.8 mg/kg; a previous 6-month oral study had demonstrated a NOAEL of 0.5 mg/kg with the next highest dose (1.5 mg/kg) producing nasal cavity, male reproductive organ and stomach toxicity. This dose is associated with an AUC of 35.4 μg.hr/ml; extrapolated from re-assessed PK data for 6-month kinetic data at doses of 0.5 and 1.5 mg/kg. A 4-week study in rats with B9502-044 produced deaths associated with GI inflammation in two high-dose females (3.6 mg/kg). A NOAEL was not identified in this study since findings in the prostate, uterus and seminal vesicle at the high dose were not examined in lower dose groups. The primary target organs of toxicity were the male reproductive organs, GI tract, thymus and uterus. The QT interval was increased in all female treatment groups compared to control.

The sponsor addressed the male reproductive organ findings in a 4-week oral dog study (68/95) which demonstrated an increased incidence of testicular tubular degeneration and dysspermia at the high dose of 18 mg/kg, po. Based on the sponsor's submitted historical data and referenced literature publication, the male reproductive organ effects noted in the 4-week oral toxicity study

in dogs are not considered to be treatment related. However, the NOAEL for this study remains at 2 mg/kg due to toxicities in the heart, thymus, lung, gastrointestinal tract and pancreas (females). In a 12-month oral study (0.2, 0.6, 2 mg/kg) a NOAEL of 0.6 mg/kg was identified due to cardiac findings and splenic erythropoiesis. Other findings included clinical signs (vomiting, diarrhea, hyaperslaivation) and reduced relative spleen weight. The NOAEL dose correlates with an AUC of 462-522 μg/l*hr).

Four-week and 12-month oral studies were performed in dogs with B9502-044. In the 4-week study (0.6, 1.2, 2.4 mg/kg), 2 high dose males died on days 8 and 21. Histopathology at the high dose revealed cardiac (myocarditis of the right atrium and inflammation/ degeneration of the left ventricle of one high-dose female) and male reproductive organ toxicity (prostate atrophy, fibrosis). Clinical signs included tremor, vomiting and hypersalivation. The NOAEL is the mid-dose of 1.2 mg/kg B9502-044. A similar NOAEL was identified in a 12-month oral study with B9502-044(0.1, 0.4, 0.8, 1.2 mg/kg); no definitive target organs of toxicity were noted in the study although slight liver pigmentation was noted at the high dose. The cardiac and prostate effects noted in the 4-week study at 2.4 mg/kg were not evident. Similar clinical signs were noted. The NOAEL correlates with systemic exposure of 352-483 μg/l*hr.

In monkeys, the primary findings in a dose-ranging study (4-5 day administration of 3 doses ranging from 0.5 to 1 mg/kg followed by a 28-day dosing period at 0.5 mg/kg) included clinical signs, reduced body weight, and numerous microscopic findings that cannot be definitively attributed to the test substance due to lack of background information with control animals. A NOAEL was not identified in this study due to inadequate animal numbers and possible drug-related findings at the doses that were tested.

Toxicology conclusions: Studies performed with B9302-107 in rats and dogs demonstrate a NOAEL of 0.8 mg/kg and 0.6 mg/kg, respectively, for chronic dosing. Studies performed with B9502-044 demonstrated similar toxicity profiles to B9302-107. A NOAEL was not identified in monkeys for short-term dosing (28 days).

Histopathology Inventory for IND # 57,883.

Study No.	194/95	128/96	129/96	113/96	81/95	38/98	14/96	159/96	68/95	94/96
Duration	1/7 day	1/7 day	1/7 day	14-d, iv	4 wk, po	3-mos, po	6-mos, po	14-d, iv	4-wk, po	6-mos, po
Species	M, rat	M, rat	M, rats	rat	rat	rat	rat	Dog	Dog	Dog
Adrenals	X			X*	X*	X*	X*	X*	X*	X*
Aorta				X	X	X	X	X	X	X
Bone marrow smear										X
Bone (femur)				X			X			
Bone (tibia)				X	X	X	X			
Bone (sternum)				X	X	X	X	X	X	X
Brain:				X*	X*	X*	X*	X*	X*	X*
Cecum							X	X		X
Cervix										X
Colon							X	X		X
Duodenum				X	X	X	X	X	X	X
Epididymis		X	X	X	X	X	X	X	X	X
Esophagus				X	X	X	X	X	X	X
Eye				X	X	X	X	X	X	X
Fallopian tube										
Fat										
Gall bladder									X	X
Gross lesions	X	X	X	X	X	X	X			
Harderian gland				X	X	X	X			
Heart	X			X*	X*	X*	X*	X*	X*	X*
Hyphophysis										
Ileum				X	X	X	X	X	X	X
Injection site	NA	NA	NA	X	NA	NA	NA	X	NA	NA
Jejunum				X	X	X	X	X	X	X
Kidneys				X*	X*	X*	X*	X	X*	X*
Lacrimal gland						X				
Larynx										
Liver				X*	X*	X*	X*	X*	X*	X*
Lungs			X	X*	X*	X*	X*	X*	X*	X*
Lymph nodes, cervical										
Lymph nodes (LALN)				X	X			X	X	X
Lymph nodes, mandibular				X	X			X	X	?
Lymph nodes, mediastinalis				X	X			X	X	?
Lymph nodes, mesenteric				X	X	X	X	X	X	?
Mammary gland				X	X	X	X	X	X	X
Nasal cavity	X	X	X	X		X	X			X
Optic nerves										
Ovaries				X*	X*	X*	X*	X*	X*	X*
Oviduct							X			
Pancreas				X	X	X	X	X*	X*	X*
Parathyroid						X	X	X		X
Peripheral nerve							X			X
Pharynx										
Pituitary				X	X*	X*	X*	X*	X*	X*
Prostate				X	X*	X*	X*	X*	X*	X*
Rectum							X	X		
Salivary gland		X	X	X	X	X	X		X	X
Sciatic nerve				X	X	X		X	X	X
Seminal vesicles				X	X*	X*	X*			
Skeletal muscle				X	X	X	X	X	X	X
Skin				X	X	X	X	X	X	X
Spinal cord				X	X	X	X	X	X	X
Spleen				X*	X*	X*	X*	X*	X*	X*
Stomach				X	X	X	X	X	X	X
Testes	X	X	X	X*	X*	X*	X*	X*	X*	X*
Thoracic Limb								X		
Thymus				X*	X*	X*	X*	X	X	X
Thyroid				X*	X*	X*	X*	X*	X*	X*
Tongue				X	X	X	X	X	X	X
Trachea				X	X	X	X	X	X	X
Urinary bladder				X	X	X	X	X	X	X
Uterus				X*	X*	X*	X*	X*	X*	X*
Vagina				X*	X*	X*	X	X		

* Organ weight obtained

Addendum (cont'd): Histopathology inventory for IND 57,883.

Study No.	62/99	4D/98	252/98	216/98
Duration	3-mos	1/7 day	3-month	3-month
Species	Rat, juvenile	Hamster/mouse	Hamster	Mouse
Adrenals	X*		X	X
Aorta	X		X	
Bone marrow smear				
Bone (femur)				
Bone (tibia)	X (T)			
Bone (strenum)	X			X
Brain:	X*		X	X
Cecum	X		X	
Cervix				
Colon	X		X	X
Duodenum	X		X	X
Epididymis	X	X	X	X
Esophagus	X		X	X
Eye	X		X	X
Fallopian tube				
Fat				
Gall bladder			X	
Gross lesions	X		X	X
Harderian gland	X		X	X
Heart	X*		X	X
Hyphophysis				
Ileum	X		X	
Injection site	NA	NA	NA	NA
Jejunum	X		X	X
Kidneys	X*		X	X
Lacrimal gland	X		X	X
Larynx				
Liver	X*		X	X
Lungs	X*		X	X
Lymph nodes, cervical				
Lymph nodes (LALN)				
Lymph nodes, mandibular				
Lymph nodes, mediastinalis				
Lymph nodes, mesenteric	X		X	X
Mammary gland	X		X	X
Nasal cavity	X	X	X	X
Optic nerves				
Ovaries	X*		X	X
Oviduct	X		X	X
Pancreas	X		X	X
Parathyroid	X		X	
Peripheral nerve			X	X
Pharynx				
Pituitary	X*		X	
Prostate	X*		X	X
Rectum	X		X	
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X*		X	X
Skeletal muscle	X		X	X
Skin	X		X	X
Spinal cord	X		X	X
Spleen	X*		X	X
Stomach	X		X	X
Testes	X*	X	X	X
Thoracic Limb				
Thymus	X*		X	X
Thyroid	X*		X	X
Tongue	X		X	
Trachea	X		X	X
Urinary bladder	X		X	X
Uterus	X*		X	X
Vagina	X*		X	X

* Organ weight obtained

Study	116/99 – 4-wk with B9502-044 (po)	95/2001 – 4 week (po)	33/99- 4 wk (po) with B9502-044	132/2000 – 52 week (po)	13001/00 – 52 wk (po) with B9502-044
Species	Rat	Monkey	Dog	Dog	Dog
Adrenals	X*	X*	X*	X*	X*
Aorta	X	X	X	X	X
Bone Marrow smear	X		X	X	X
Bone (femur)	X		X		X
Brain	X*	X*	X*	X*	X*
Cecum	X	X	X	X	X
Cervix				X	X
Colon	X	X	X	X	X
Duodenum	X	X	X	X	X
Epididymis	X	X*	X	X	X*
Esophagus	X	X	X	X	X
Eye	X	X	X	X	X
Fallopian tube					
Gall bladder		X	X		X
Gross lesions	X	X		X	X
Harderian gland	X				
Heart	X*	X*	X*	X*	X*
Ileum	X	X	X	X	X
Injection site	NA	NA	NA	NA	NA
Jejunum	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*
Lachrymal gland	X				X
Larynx					X
Liver	X*	X*	X*	X*	X*
Lungs	X*	X*	X*	X*	X*
Lymph nodes, cervical					
Lymph nodes mandibular		X		X	X
Lymph nodes, mesenteric	X	X			X
Mammary Gland	X	X	X	X	X
Nasal cavity	X	X	X	X	X
Optic nerves					X
Ovaries	X*	X*	X*	X*	X*
Pancreas	X	X	X*	X*	X
Parathyroid	X	X*	X	X	X*
Peripheral nerve					
Pharynx					X
Pituitary	X*	X	X	X	X*
Prostate	X*	X	X*	*	X*
Rectum	X	X	X	X	X
Salivary gland		X	X		X
Sciatic nerve	X	X	X	X	X
Seminal vesicles	X*	X			X
Skeletal muscle	X	X	X	X	X
Skin	X	X	X	X	X
Spinal cord	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*
Sternum		X			
Stomach	X	X	X	X	X
Testes	X*	X*	X*	X*	X*
Thymus	X*	X*	X	X	X
Thyroid	X*	X*		X*	X*
Tongue	X	X	X	X	X
Trachea	X	X	X	X	X
Urinary bladder	X	X	X	X	X
Uterus	X*	X	X*	X*	X
Vagina	X	X		X	X
Zymbal gland					
Standard List					

X, histopathology performed
*, organ weight obtained

V. CARCINOGENICITY:

Carcinogenicity summary: In submission 143 dated November 28, 2001, the sponsor submitted preliminary results from their oral (gavage) carcinogenicity studies in mice and hamsters. Mice were reported to have no neoplastic findings. However, neuroepithelial tumors were found in the nasal cavities of some of the hamsters treated with high doses (8 mg/kg/day; see table below). The tumors appeared late in the study, were small and did not metastasize. The sponsor indicates that the appearance of the tumors is expected on the basis the nasal toxicity of roflumilast metabolites observed in rodents. The dose of 8 mg/kg produced disorganization of the olfactory epithelium and loss of PAS staining intensity in submucosal Bowman's glands in the 3-month dose-range finding study. Nasal toxicity is considered by the sponsor to be an epigenetic cause for tumor development. The sponsor suggests that nasal toxicity is not relevant to humans due to the following reasons: the findings that human microsomes do not show metabolic activity for the oxidation of ADCP to ADCP-N oxide, the oxidation of the ADCP N-oxide to the proposed reactive oxo-intermediate is due to species-specific cytochrome P-450 isoenzyme not present in humans, and nasal toxicity was not observed in dogs or monkeys. The sponsor notes the Division's agreement with their position of the specificity of the nasal toxicity to rodents in a teleconference held on June 6, 2001.

Dose (mg/kg/d)	0	0 Vehicle	0.25	1	4	8
Animals Males/Females	60/60	60/60	60/60	60/60	60/60	60/60
NT, Males (n)	1	0	0	0	(1*)	1
NT, Females (n)	0	0	0	0	0	4
Total	1	0	0	0	(1*)	5

NT: neuroepithelial tumors in nasal cavities

The sponsor requested Agency feedback on the carcinogenicity findings during the EOP2 meeting held on December 6, 2001. At that meeting, the Agency informed the sponsor that the findings are considered likely related to the rodent-specific metabolism of roflumilast and are not likely to be relevant to huimans (see meeting minutes). However, the Carcinogenicity Assessment Committee following submission and review of the final study report will provide a final determination on the relevancy of the findings.

Carcinogenicity conclusions: The nasal neuroepithelial tumors observed in the two-year hamster study are not likely relevant to humans but are probably due to the rodent-specific metabolism of roflumialst. The Carcinogenicity Assessment Committee following submission and review of the final study report will provide a final determination on the relevancy of the findings.
Recommendations for further analysis: None at this time.

Labeling Recommendations: None at this time.

VI. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:
Systemic exposure to B9302-107 generally increases proportionally in rats, and sub-proportionally in dogs and mice. Elimination half-lives ranged from 1 to 7 hours in rats and dogs. Rats demonstrate a 5-fold increase in levels of B9202-045 compared to parent compound and a 25-fold increase in B9502-044. In rats, exposure to the metabolite B9502-044 was significantly greater than the parent compound while systemic exposure in dogs was primarily to the parent compound. A 3-month oral administration to B9302-107 in hamsters resulted in exposure to B9502-044 that was 23- to 38-fold higher than the parent compound. Levels of B9502-054 were higher by 7- to 10-fold over the parent compound while levels of B9502-045 were 1.2- to 2-fold over the parent compound. Exposure to all compounds increased with dose in a generally proportional manner. An oral dose-escalation study in monkeys also demonstrated greater exposure to the metabolite B9502-044 (3 to 8-fold) compared to parent compound. Following administration of B9503-044, exposure to B9502-044 in rats increased supra-proportionally with increasing dose; exposure to the B9302-107 increased proportionally but was < 2% of B9502-044. In dogs, exposures to B9502-044 increased supra-proportionally with increasing dose. Exposure to the B9302-107 also increased with dose; AUC levels were 15-44% of those observed with B9502-044 in the 4-week study but only 5-10% in the 52-week study. Hamsters are similar in terms of metabolite production although the ratios vary. Mice and dogs produce low levels of B9202-045; levels of B9502-044 are similar to parent in the mouse and only 2% in the dog. Humans also produce low levels of B9202-045 and B9502-044. Drug absorption is 32% in rats with oral/id dosing while absolute bioavailability is low in rats and rabbits (2-4%) and higher in dogs and mice (34-64%). Highest levels of drug-related radioactivity are observed in the nose, kidney and liver of the rat. Low to undetected levels are observed in the nose of mice and hamsters, while the primary sites of distribution are the lungs, adrenals and bone marrow. The dominant biotransformation route in all species is N-oxidation of the parent to form B9502-044, or cleavage of the parent to form B9202-045, which in turn is oxidized to B9502-054, or dealkylation of the parent. Excretion is primarily fecal following oral administration; urinary excretion increased following IV administration.

General toxicology studies were performed in, dogs, mice, hamsters and monkeys. In rats increased levels of FSH, LH and progesterone, and decreased levels of 17β-estradiol were observed after 7-days dosing with the high dose (2.5 mg/kg) and fewer high-dose animals were observed to be in estrus cycle. Males exhibited a dose-related decrease in testosterone, corticosterone and progesterone. No findings that were characteristic of testosterone deficiency were observed in male rats administered B9302-107 for 7 consecutive days, although tubular dilatation of the testes was observed at the high dose. A 4-week toxicity study (0.5, 2 and 8 mg/kg) produced lethality at the high dose; target organs included the heart, adrenals, stomach, parathyroid, spleen, testes and epididymis, and the thymus, prostate and seminal vesicles. Other findings included non-reversible changes in QT time, QRS time at the high dose, reduction of estrus events and prolongation of diestrus phase, and clinical signs, reduced body weight and food consumption. A NOAEL was not identified due to lack of assessment of the nasal cavity. A 3-month study (0.02, 0.2, 2 mg/kg) also identified similar target organs (testes, epididymis, and thymus) as well as the nasal cavity. The NOAEL was identified as 0.2 mg/kg. Chronic studies in rats identified a NOAEL of 0.8 mg/kg with the next highest dose (1.5 mg/kg) inducing nasal

cavity and male reproductive organ toxicity (spermatocele in the epididymis). The dose of 0.8 mg/kg is associated with an AUC of ~ 35 µg.hr/L; extrapolated from re-assessed PK data for 6-month kinetic data at doses of 0.5 and 1.5 mg/kg. Since the nasal cavity toxicity is considered to be irrelevant to humans, the female NOAEL could be increased to 1.5 mg/kg with an associated AUC of 67 µg.hr/L. A 4-week study in rats with B9502-044 produced deaths associated with GI inflammation in two high-dose females (3.6 mg/kg). A NOAEL was not identified in this study since findings in the prostate, uterus and seminal vesicle at the high dose were not examined in lower dose groups. The primary target organs of toxicity were the male reproductive organs, GI tract, thymus and uterus. The QT interval was increased in all female treatment groups compared to control.

A 4-week oral study in dogs (2, 6, 18 mg/kg) resulted in a NOAEL of 2 mg/kg with target organs including the heart, pancreas (females), thymus, testes, lung, stomach and epididymis; there were also indications of vasculitis. The sponsor addressed the male reproductive organ findings which demonstrated an increased incidence of testicular tubular degeneration and dysspermia at the high dose of 18 mg/kg, po. Based on the sponsor's submitted historical data and referenced literature publication, the male reproductive organ effects noted in the 4-week oral toxicity study in dogs are not considered to be treatment related. However, the NOAEL for this study remains at 2 mg/kg due to the other organ toxicities. A six-month oral study (0.2, 1 and 4 mg/kg) identified a NOAEL of 0.2 mg/kg and the primary target organ was the heart. In a 12-month oral study (0.2, 0.6, 2 mg/kg) a NOAEL of 0.6 mg/kg was identified again due to cardiac findings and splenic erythropoiesis. Other findings included clinical signs (vomiting, diarrhea, hypersalivation) and reduced relative spleen weight. The NOAEL dose correlates with an AUC of ~ 510 µg/l*hr at week 52. Four-week and 12-month oral studies were performed in dogs with B9502-044. In the 4-week study (0.6, 1.2, 2.4 mg/kg), 2 high dose males died on days 8 and 21. Histopathology at the high dose revealed cardiac (myocarditis of the right atrium and inflammation/degeneration of the left ventricle of one high-dose female) and male reproductive organ toxicity (prostate atrophy, fibrosis). Clinical signs included tremor, vomiting and hypersalivation. The NOAEL is the mid-dose of 1.2 mg/kg B9502-044. A similar NOAEL was identified in a 12-month oral study with B9502-044 (0.1, 0.4, 0.8, 1.2 mg/kg); no definitive target organs of toxicity were noted in the study although slight liver pigmentation was noted at the high dose. The cardiac and prostate effects noted in the 4-week study at 2.4 mg/kg were not evident although similar clinical signs were noted. The NOAEL correlates with systemic exposure of ~ 483 µg/l*hr at week 52.

Three-month oral screening studies in mice (6, 12, 18 mg/kg) and hamsters (4, 8, 16 mg/kg) were performed for dose ranging purposes for carcinogenicity studies. The identified target organs included the nasal cavity and adrenal glands (both), spleen (mouse), and male reproductive organs, eye, prostate, and pancreas (hamster). As indicated in the Executive CAC minutes of February 9, 1999, maximum tolerated doses of 18 and 12 mg/kg were identified in male and female mice, respectively, and 16 and 8 mg/kg in male and female hamsters, respectively.

In monkeys, a dose-ranging study (4-5 day administration of 3 doses ranging from 0.5 to 1 mg/kg followed by a 28-day dosing period at 0.5 mg/kg) produced clinical signs, reduced body weight, and numerous microscopic findings that cannot be definitively attributed to the test substance. A NOAEL was not identified in this study.

General Toxicology Issues:

Discussions have been ongoing with the sponsor concerning the adequacy of the non-clinical database to support proposed clinical trials. Of specific concern was the adequacy of the data to support a clinical dose of 500 µg/day. The following table summarizes the NOAEL doses in chronic animal toxicity studies with associated exposure levels to roflumilast and its primary metabolite B9502-044. Data in monkeys is not considered in thi sevaluation due to the short-term nature (28 days) of the administration. The expected human exposure levels at proposed clinical doses of 125, 250 and 500 µg are also summarized in various age groups. Of note, exposure to roflumilast in humans has been demonstrated to increase with age; data are not available for the metabolite B9202-044. In addition, the Division informed the sponsor that a 2-fold animal to human exposure ratio for asthmatic patients should be provided since the findings of reproductive organ toxicity are difficult to monitor in the clinical setting and have potential adverse effects in the indicated population.

Species	Age	Study duration	Dose	B9302-107 AUC (µg.hr/L)	B9202-044 AUC (µg.hr/L)
Human	Young	-	125 µg 250 µg 500 µg	8.8 17.5 34.9	88 175 351
	45-65	-	125 µg 250 µg 500 µg	10.1 20.2 40.4	101* 202* 406*
	Over 65	-	125 µg 250 µg 500 µg	13.3 26.7 53.2	133* 267* 534*
Rat	-	6-mos	NOAEL = 0.8 mg/kg (M) = 1.5 mg/kg (F)	35.4** 67	494*** 927
Dog	-	12-mos	B9302-107 NOAEL = 0.6 mg/kg	522	66
	-	12-mos	B9202-044 NOAEL = 1.2 mg/kg	41	483

Linear PK has been observed in humans within 100-500 µg dose range (communication by Dr. Young Moon Choi).
*: exposure to B9202-044 assumed to increase with age in a similar fashion to B9302-107
**: extrapolated from 6-mos kinetic data at doses of 0.5 and 1.5 mg/kg
***: extrapolated from 4-week kinetic data at doses of 0.5 and 1.5 mg/kg

The table below summarizes the relevant animal to human exposure (AUC) ratios using the rat as the most sensitive species:

Age group (years)	Dose	Rat to human exposure (AUC) ratio	
		Roflumilast	Roflumilast N-oxide
Young	250 µg 500 µg	M: 2 /F: 3.9 M: 1 /F: 2	M: 2.8 /F: 5.3 M: 1.4 /F: 2.6
45-65	250 µg 500 µg	M: 1.8 /F: 3.3 M: 0.9 /F: 1.7	M: 2.5 /F: 4.6 M: 1.2 /F: 2.3
65 & over	250 µg 500 µg	M: 1.3 /F: 2.5 M: 0.7 /F: 1.3	M: 1.9 /F: 3.5 M: 0.9 /F: 1.7

(b) (4)

(b) (4)

A determination was made at an internal team meeting that a 1-fold safety margin based upon systemic AUC levels would be adequate to support clinical dosing in COPD patients with adequate information in the patients Informed Consent. This determination was based upon the severity of the disease, the available alternative treatments and the age of the intended population. Using a 1-fold safety margin based upon systemic AUC levels, doses up to 500 µg in males aged 65 and under are supported. In males over 65 years of age, the animal data supports dosing up to 250 µg. Dosing up to 500 µg roflumilast in females of all ages is supported.

Of note, the sponsor is currently conducting a clinical trial to assess the spermiogenic effects of roflumilast in humans. The sponsor has been previously informed that negative findings in this clinical trial would alleviate the Division's concerns related to male reproductive organ toxicity.

Recommendations:

(b) (4)

The sponsor's proposed trial in COPD patients is considered to be reasonably safe to proceed in females of all age groups and males 65 years or younger. However, the nonclinical data support dosing only up to 250 µg per day in males over the age of 65 due to the observed increased systemic exposure in this population.

Submission of the ongoing clinical trial to assess male reproductive organ effects of roflumilast could provide adequate support for the proposed clinical trials should they allay concerns identified in the animal studies.

Labeling with basis for findings: None at this time

Reviewer signature: _____
Timothy J. McGovern, Ph.D.

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

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Timothy McGovern
1/30/03 08:56:15 AM
PHARMACOLOGIST

Joseph Sun
2/4/03 05:33:53 PM
PHARMACOLOGIST
I concur.

Appendix 4

Pharmacology and Toxicology Review No. 5
by Dr. Luqi Pei Completed on June 27, 2007
In IND 57,883

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND Number:	57,883
Review Number :	5
Sequence number/date/submission type:	235/ 26-SEP-2003 /IT 254/ 09-JAN-2005 / IT
Information to the Sponsor:	No.
Sponsor/or Agent:	Altana
Manufacturer of the Drug Substance:	N/A
Reviewer Name:	Luqi Pei, Ph.D.
Division Name:	Pulmonary and Allergy Products
Review Completion Date:	June 27, 2007

Drug:

Trade Name:	N/A
Generic Name:	Roflumilast
Code Name:	BY 217, B9302-107
Chemical Name:	3-cyclopropylmethoxy-4difluoromethoxy-N-(3,5-dichloropyrid-4-yl)-benzamide
CAS Register Number:	N/A
Mole File Number:	N/A
Molecular Form and Weight:	N/A

Relevant IND/NDAs: N/A

Drug Class: PDE_4 inhibitor

Intended clinical population: Asthma and COPD

Route of Administration: Oral (tablets)

Clinical Formulations: Each tablet contains 0.25 or 0.5 mg micronized BY 217, (b) (4) lactose monohydrate, (b) (4) maize starch, (b) (4) Polyvidone (b) (4) magnesium stearate

Proposed Clinical Protocol: None.

Previous Human Experience: The drug is currently in phase 3 development for the indication of asthma and COPD. Roflumilast doses in clinical trials are 0.25 or 0.5 mg once a day.

Studies Submitted and Reviewed in the Review: None.

2-Year Oral [Gavage] Study with B9302-107 in B6C3F1 mice. Submitted on September 26, 2003 (draft) and January 9, 2004 (final). Vol. 65.1, p1.

2-Year Oral [Gavage] Study with B9302-107 in Hamsters. Submitted on September 26, 2003. Vol. 59.1, p65.

2-Year Oral [Gavage] Study with B9302-107 in Hamsters. Submitted on September 26, 2003. Vol. 59.11, p1.

Studies Submitted but Not Reviewed in this Review: None.

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

Drug History:

Roflumilast is currently in phase 3 development. The drug is intended for the indications of asthma and COPD. Roflumilast doses in clinical trials are 0.25 or 0.5 mg once a day in both indications.

The sponsor completed three 2-year traditional carcinogenicity bioassays of roflumilast in mice and hamsters. Reports of these studies were submitted on September 26, 2003 and on January 9, 2004. The Executive CAC reviewed the protocols and results of roflumilast carcinogenicity studies on February 9, 1999 and May 10, 2005, respectively. The committee determined that the carcinogenicity of roflumilast had been adequately evaluated. Minutes for the Executive CAC reviews are attached to the review. Dr. Ted Guo of Biometrics II completed the statistical analysis of the tumor data on May 16, 2005.

The Division evaluated the relevance of nasal tumors of roflumilast in hamsters to humans for the last four years. On November 21, 2001, the sponsor reported, in its preliminary findings, the observation of an increased incidence of nasal tumors in female hamsters receiving 8 mg/kg/day of roflumilast. The Division subsequently had 2 meetings with the sponsor to discuss the finding. The first meeting occurred on December 6, 2001 as a part of an End-of-Phase 2 meeting. The Division informed the sponsor that "the nasal tumors observed in hamsters are considered likely to be related to the nasal toxicity found in rodents. However, further review, as well as consultation with the Agency's Carcinogenicity Assessment Committee, is needed to confirm that the tumor findings in hamsters are irrelevant to humans." The Division requested the sponsor to state in the Informed Consent Form that the carcinogenicity potential for roflumilast was being evaluated. In response to the request, the Sponsor included the following statement in the Informed Consent Form:

(b) (4)

- Source: January 10, 2003 submission

The March 3, 2003 meeting was held to discuss the sponsor's request to delete in the (b) (4) As part of the evaluation, the Division requested additional information that included tumor incidence tables. The evaluation found that there appeared to be dose-related increases in the incidence of tumors in other organs (i.e., uterus, forestomach and larynx), in addition to the nasal cavity. The Division was concerned about the additional

findings (b) (4). The Division informed the sponsor that the available evidence was inconclusive to support (b) (4) The determination was based on the paucity of the data, the additional tumor findings, and the failure to comply with Executive CAC recommendations regarding the completed study (i.e., The Executive CAC recommended a top roflumilast of 16 and 8 mg/kg/day in males and females, respectively. The completed study had a top roflumilast dose of 8 mg/kg/day in both sexes). Responding to the comment of failing to comply with the executive CAC recommendation, the sponsor indicated that it was performing a supplemental study to the initial study and results of this supplemental study should be available soon.

Both studies have been completed and submitted. The Executive CAC has reviewed these studies. The committee concluded that only the nasal tumor was a treatment related effect and that the tumor may not be relevant to humans. The primary rationale was the difference in roflumilast metabolism between rodents and humans. The rodent nasal epithelium produces a reactive intermediate, ADCP-N-oxide while humans do not. Internal deliberation on the issue was documented in Dr. Timothy McGovern's review dated January 30, 2003.

The evaluation of the relevance of the tumorigenic findings in hamsters has been complicated by the selection of the most relevant species in the carcinogenicity studies. The Executive CAC evaluated the species selection and the acceptability of hamster for the carcinogenicity assessment of the drug during the review of study protocol in 1999. Roflumilast causes rather prevalent nasal lesions in rodent species, but the hamster appears less susceptible than rats regarding the nasal injuries. Concerned that the nasal lesions could prohibit prolonged dosing in rats, the Executive CAC accepted the hamster in place of the rat for carcinogenicity testing with roflumilast. The conclusion was "based upon comparative metabolic profiling in which significantly greater serum levels of the metabolite DCAP were detected in the rat versus the hamster, mouse or humans" and "the greater similarity of the metabolic profile of Roflumilast in hamsters to humans". Despite the effort to minimize nasal lesions in the carcinogenicity study, nasal tumors were found in the hamsters in the 2-year bioassays.

Pfizer was a member of the roflumilast development team. Pfizer recently exited the program (b) (4)

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2.6.2 PHARMACOLOGY

No new data were submitted. Roflumilast dilates the airway by raising the intracellular cAMP level in smooth muscle cells in the respiratory tract. Roflumilast is a phosphodiesterase IV (PDE_4) inhibitor. Located primarily in the respiratory system, phosphodiesterase IV hydrolyzes cAMP, a second intracellular messenger. cAMP relaxes the smooth muscle cells in the respiratory tract via a complex chemical cascade reaction. The inhibition of PDE4 would result in bronchial dilation and be beneficial to asthma and COPD patients.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

No data were submitted.

2.6.4 PHARMACOKINETICS/ TOXICOKINETICS

No new data were submitted. Previous submissions indicate that orally administered roflumilast is well absorbed. About 30 – 50% of the drug is absorbed in mice, rats, hamsters and dogs. Peak plasma drug levels are reached 1 – 3 hours after the oral administration. Plasma concentrations of roflumilast generally increase proportionally to oral dose while the AUCs increase supra-proportionally with the dose. Systemic exposure of roflumilast is primarily eliminated through urine. Approximately 70% and 30% of total intravenously administered radioactivity is excreted through urine and feces in rats. Highest drug concentrations were detected in the nose, live and kidney in rats.

Roflumilast is rapidly metabolized by cytochrome P450 enzymes to several metabolites. These metabolites are roflumilast-N-oxide (B9502-044), and 9302-077 and amino-dichloro-pyridine (ADCP or B9202-045). ADCP is further metabolized to ADCP-N-oxide by CYP2G1, an enzyme found in the epithelium of rodent nasal cavities. Figure 1 (next page) presents the metabolic pathways of roflumilast. Table 2 (below) summarizes the AUC levels of these compounds in mice, hamsters and humans. Roflumilast-N-oxide is the predominant metabolite across species. The mice and hamster data were from the carcinogenicity studies while human data were from the sponsor's summary. Both ADCP and ADCP-N-oxide were present in hamsters while only ADCP was detected in humans.

Table 1. AUCs of Roflumilast and its metabolites in Mice, Hamsters and Humans

Species	Roflumilast (mg/kg/day, PO)	Plasma AUC (μg.h/L) [a]			
		Roflumilast	Roflumilast-N-oxide	ACDP	ACDP-N-oxide
Mouse	12 mg/kg/day	663	2,145		
Hamster [b]	4 mg/kg/day	30.4	1,204	101	345
	8 mg/kg/day	69	3,073	223	821
Human	500 μg/patient /day	32.9	351.4	4.1	

a. Source: Table 5.3-1, 06-SEP-04 Amendment, vol. 59.1, page 23.
b. From Study Report 7/2002. See table 13, page 17 of this review.

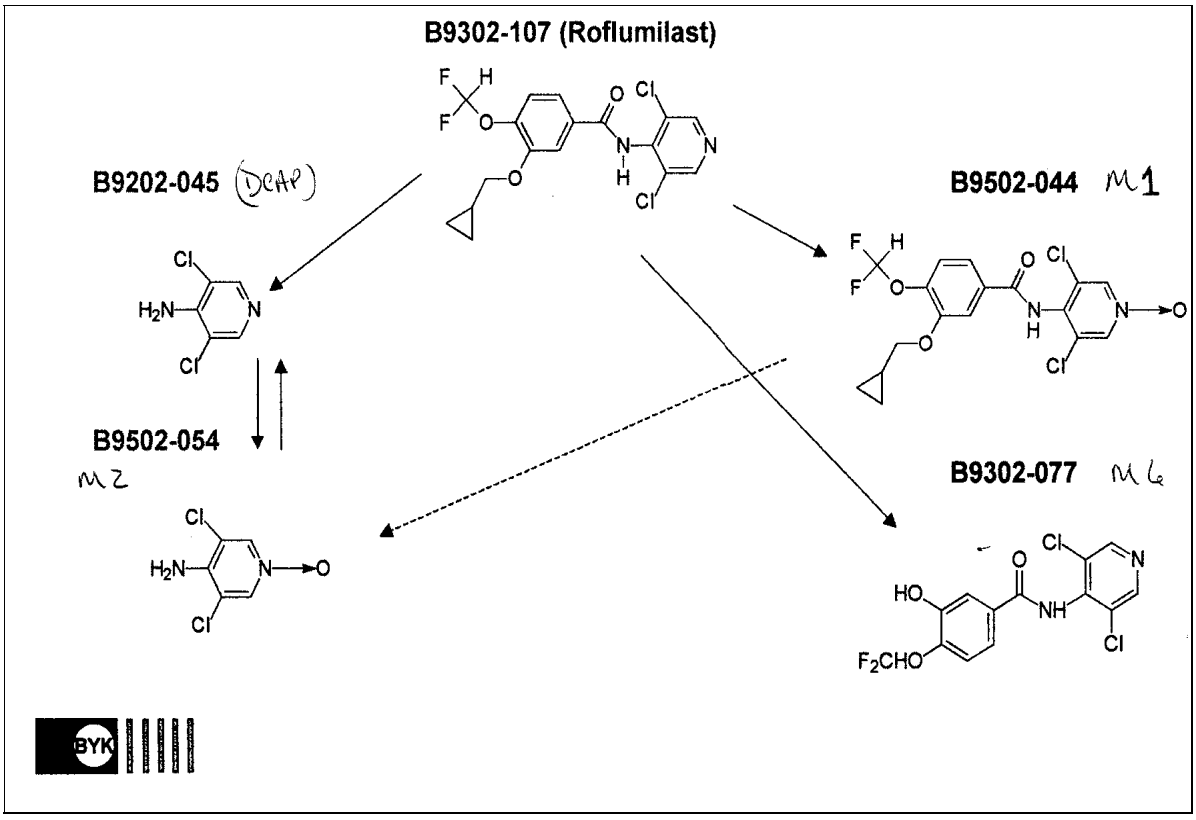

Figure 1.Metabolic pathways of roflumilast.

2.6.6 TOXICOLOGY

2.6.6.1 Overall Summary

Genetic toxicity

Roflumilast tests positively in an *in vivo* mouse micronucleus test, but negatively in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosome aberration assay in human lymphocytes, *in vitro* HPRT test with V79 cells, an *in vitro* micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and *in vivo* mouse bone marrow chromosome aberration assay.

In the *in vivo* mouse micronucleus test, roflumilast induces micronuclei at oral doses of 300 mg/kg at 48 hours and 900 mg/kg 24 and 48 hours. The Division in conjunction with the Genetic Toxicology Committee concluded that the sponsor had adequately assessed concerns regarding the genotoxic potential of roflumilast since both in vitro and in vivo chromosome aberration studies were negative.

Genotoxicity testing was also performed on two metabolites of roflumilast: roflumilast –N-oxide and ADCP. The former tests negative in Ames test and micronucleus test in V79 cells

in vitro. The latter tests negatively in the *in vivo* mouse micronucleus test and DNA[^{32}P]-post labeling assay in rat tissues.

Carcinogenicity

Roflumilast is carcinogenic in hamsters but not mice. Given orally for 2 years in Syrian golden hamsters, roflumilast causes undifferentiated carcinomas in nasal cavity in females at 8 mg/kg/day and in both males and females at 16 mg/kg/day. The hamster nasal tumor, however, may not be relevant to humans due to difference in roflumilast metabolisms between animals and humans. The tumorigenicity of the drug in hamster is believed to be the effect of a rodent species-specific metabolite, amino-dichloropyriden (ADCP)-N-oxide. The human nasal cavity lacks an enzyme that leads to the formation of ACDP-N-oxide. No evidence in tumorigenicity was observed at oral doses up 18 mg/kg/day in males and 12 mg/kg/day in females in mice.

The carcinogenic potential of roflumilast was evaluated in hamsters and mice. Two 2-year oral studies were conducted in hamsters and one studies in mice. All studies used oral (gavage) route of administration. In the first hamster study, Syrian golden hamsters (60/sex/dose) were treated with 0.25, 1, 4 and 8 mg/kg/day of roflumilast for 103 weeks. Additional hamsters received vehicle only (4% methocel) or no treatment at all as controls. The high dose female hamsters showed statistically significant increases in the incidence of undifferentiated carcinoma in the nasal cavity (incidence: 4/60-HD and 0/60 in the remaining groups). The mortality was similar across groups in females (58 – 73%); the males showed a statistically significant trend in dose-related mortality ($P < 0.004$). The cumulative mortality in males at the end of the treatment was 22%, 18%, 18%, 20%, 33% and 35% for the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. There were no significant differences in body weight between the vehicle control and treatment groups in either sex. No remarkable findings were observed in the males of any roflumilast-treated groups.

The second hamster study consisted of a treated group (16 mg/kg/day, n = 60/sex) and a sham control group (30/sex). The roflumilast group showed non-statistically significant increases in the incidence of undifferentiated carcinoma in the nasal cavity in both sexes (incidence: 0/30 for cage control and 5/60 for roflumilast treatment in each sex). The Executive CAC concluded that only the nasal tumor was a treatment-related effect, based on the low incidence of the basal neoplasms.

Both studies also showed dose-related and statistically significant increases in the incidence of uterine leiomyomas ($P < 0.003 – 0.024$). The Executive CAC did not consider the finding a treatment-related effect, based on its background incidence and the lack of significant difference in the sum of uterine leiomyomas and sarcomas, as discussed later in the review.

In mice, no evidence of tumorogenicity was seen at oral roflumilast doses up to 18 and 12 mg/kg/day in males and females, respectively. B6C3F1 mice (50/sex/dose) were treated with roflumilast in 4% methocal via oral gavage for 103 weeks. The respective roflumilast doses were 0.5, 2.0, 6.0, and 18 mg/kg/day in males and 0.5, 2.0, 6.0, and 12 mg/kg/day in females. There were 2 control groups, a cage control and a vehicle control. No effect on mortality was observed in the females. The HD males showed a significant increase in mortality. The respective cumulative survival rate for the sham, vehicle, LD, MLD, MHD and HD groups at

the end of the treatment was 88%, 90%, 90%, 90%, 82% & 18% in males and 88%, 78%, 92%, 86%, 90% and 82% in females. The increase started in month 18 of treatment and remained until the end of the study. There was no significant increase in tumor incidence between treated and control groups.

Overall, the available data indicate that roflumilast is carcinogenic in hamsters, but not in mice. This conclusion is reflective of the Executive CAC's evaluation on May 10, 2005. The Committee considered the above studies have adequately evaluated the carcinogenic potential of roflumilast in the laboratory.

2.6.6.5 Carcinogenicity

Mice Study (No. PR97/2001)

Study Title: 2-Year Oral [Gavage] Carcinogenicity Study with B9302-107 in B6C3F1 Mice

Key findings: Mice treated with roflumilast orally at doses up to 18 and 12 mg/kg/day in males and females, respectively, for 2 years did not show any evidence of tumorigenicity. B6C3F1 mice (50/sex/dose) were treated with roflumilast in 4% methocal via oral gavage for 103 weeks. The respective roflumilast doses were 0 (cage control), 0 (vehicle), 0.5, 2.0, 6.0, and 18 mg/kg/day in males and 0.5, 2.0, 6.0, and 12 mg/kg/day in females. The HD males showed a significant increase in mortality from treatment month 18 and onward. The cumulative survival rate at the end of the treatment was 88%, 90%, 90%, 90%, 82% & 18% for the sham, vehicle, LD, MLD, MHD and HD groups, respectively. No effect on mortality (78 – 92%) was observed in the females. Both high-dose males and females showed statistically significant decreases in body weights.

A number of dose-related histopathologic changes were noted in the MHD and HD groups. The changes in adrenals, heart, kidneys, stomach and spleen were observed in the HD males but not females. The lesion in the reproductive organs included atrophy of seminal vesicles, oligospermia in epididymides, and smaller testis in the high dose males and atrophy of the uterus in the MHD & HD Females. The MTD was exceeded in the males and was achieved in the females. No statistically significant increases in the incidence of any tumors were observed in any treated groups. Roflumilast is considered non-carcinogenic in mice.

Adequacy of the carcinogenicity study and the appropriateness of the test model

The study has adequately tested the carcinogenic potential of roflumilast in mice. The selections of roflumilast doses and the animal model were appropriate and in compliance with the Executive CAC recommendations. The maximum tolerated dose (MTD) was either exceeded or achieved both on increases in mortality (males) and decreases in body weight (males and females). The high dose in males actually exceeded the MTD due to excessive mortality (82%). In the females, the MTD was achieved, based on the decrease in body weight (11% reduction in body weight at the end of dosing) and treatment-related changes in the uterus.

Evaluation of the tumor findings

Roflumilast at oral doses up to 18 mg/kg/day in males and 12 mg/kg/day in females for 2 years did not cause significant increases in the incidence of any tumors in mice. This study, designed per Exec. CAC recommendations, has adequately evaluated the carcinogenic potential of roflumilast in mice. No statistically significant increase in any tumors was observed in any treatment groups. Thus, roflumilast is non-carcinogenic in mice.

Comment: As indicated in the mortality portion of the Results section, the study report appears to be biased in interpreting the study results. The report states that "overall, there was no substance-related increase in the incidence of mortality" (v0l. 65.1, p 6). The report considers the 21 deaths (found dead) "spontaneous" (vol.56.1, p6). The prevalence of so called "spontaneous" deaths was 10%, 4%, 6%, 4%, 14%, and 42% for the sham, vehicle, LD, MLD, MHD and HD male groups, respectively. These statements of the report contradict a fundamental toxicology concept: dose-response relations of toxicity-related effects. They also raise questions about the objectivity of the laboratory and the sponsor in interpreting study results and concerns that this bias may affect the validity of the study in determining carcinogenic potential. The review assumes that the bias occurred only in the data interpretation and considers the study valid.

Study No: PR 97/2001
Date of Submission & Serial No.: 26-SEP-2003 (draft) & 09-JAN-04 (final), 254
Volume and Page Nos.: C65.1, page 1
Conducting Laboratory and Location:

Quality control and test substance mixture: (b) (4)

Measurement of Pharmacokinetic Samples: (b) (4) Altana Pharma AG, Department of Drug Metabolism and Pharmacokinetics, Byk-Gulden-Str. 2, D-78467 Konstanz, Germany.

Toxicokineitc Evaluation and Reporting: Altana Pharma AG, Department of Drug Metabolism and Pharmacokinetics, Byk-Gulden-Str. 2, D-78467 Konstanz, Germany.

Histological Processing: (b) (4)

Histological Evaluation and Reporting: (b) (4)

Date of study initiation: March 1, 1999.
Date of Study Completion: April 23, 2003.
Study Report Date: August 18, 2003; amended on November 26, 2003.
GLP compliance: Yes, signed GLP statement included.
QA reports: Yes, signed statement included.
Drug lot #, & % purity: B9302-107, Batch# 298569; purity 101.4%.

STUDY PROTOCOL DESIGN AND METHODS:

Study Type: traditional 2-year oral gavage study
Species/strain: Mice, B6C3F1 Crl: BR
Number/sex/group; age at start of study: 50/sex/dose, 7 weeks at study initiation.
Animal weight at start of exposure: mean of 21.6 (Range 18 – 25) and 18.3 g (range 16 – 22) for males and females, respectively.
Animal housing: Individual caging, 21±2.0°C, 12 hr light dark cycle.
Treatment Duration: 24 months
Feed and water: NAFAG feed ad libitum, tap water ad libitum.
Formulation/vehicle: Suspension of 4% methocel containing 2 – 3 drops of Med Antifoam C.
Drug stability/homogeneity: 10%, 0.01% and 0.005% B9032-107 in 4% methocel E15 was measured over a period of 7 days (what does this mean?).

DESIGN:

Mice (50/sex/group) were treated with low, mid and high doses of roflumilast for 103 weeks to evaluate the carcinogenic potential of the drug. The study included dual controls: a sham group and a vehicle-only group (50/sex/group). Additional mice (20/sex) were included in the roflumilast treated groups for toxicokinetics evaluations. Finally, additional (sentinel) mice were included to check the healthy status of the animals during the study. Table 2 shows the overall design of the 24-month oral carcinogenicity study of roflumilast in mice.

Table 2: Design of the 24-Month Oral Carcinogenicity of Roflumilast in Mice

Group	Treatment	Roflumilast (mg/kg/day)		No. of Mice /sex	
		Male	Female	Tox.[b]	PK [c]
1	Sham control	0	0	50	0
2	Vehicle control	0	0	50	0
3/9[a]	Roflumilast	0.5	0.5	50	20
4/10	Roflumilast	2.0	2.0	50	20
5/11	Roflumilast	6.0	6.0	50	20
6/12	Roflumilast	18.0	-	50 M	20 M
7/13	Roflumilast	-	12.0	50 F	20 F
8	Sentinel [d]	0	0	24	-

a. Numbers after the slash sign indicate the groups dedicated for TK studies.
b. Survivors were terminated at week treatment 104.
c. These mice were sacrificed on week 13 after the last blood sample was drawn. No histological examination was done.
d. These mice were included for health check reasons. Six, 6 and 12 mice/sex were bled on week 1 and months 12 and 24. Blood samples were analyzed to confirm the health conditions. Autopsies were conducted after blood collection. No histological examination was done.

DOSES:

Doses: 0.5, 2, 6, 12 (F only) and 18 (M only) mg/kg/day.
Basis of dose selection: The doses were selected based on a MTD from a 13-week oral dose range finding study in mice (doses of 0, 6, 12 & 18 mg/kg/d). Adrenal atrophy and

hyperplasia were seen in all treated F and in HD M. Nasal olfactory epithelial degeneration and olfactory cell necrosis were seen at the mid and high doses. Therefore, doses of 18 mg/kg in M and 12 mg/kg in F were considered the MTD.

Relation to clinical use: This is an oral gavage study and the clinical route is also oral tablet for treatment of asthma and COPD.

CAC concurrence: Yes. The Executive CAC reviewed the study protocol on February 9, 1999 and recommended doses of 18 mg/kg/d in males and 12 mg/kg/d in females based on increased incidence and severity of adrenal and nasal lesions in the 3-month study at these doses.

Restriction paradigm for dietary restriction studies: None
Route of administration: Oral gavage (via gastric cannula) at 10 ml/kg volume.
Frequency of drug administration: Once a day, 7 days a week.
Controls employed: This study has dual controls. The two control groups are cage control and vehicle control.
Interim sacrifices: No.

Satellite PK or special study group(s): 20/sex/dose for the treatment groups, sacrificed at 3 months.

Deviations from original study protocol: No notable deviations; individual animals were dosed incorrectly on a single study day, and no data were collected in a few cases (BW, food consumption, hematology). These deviations are not expected to have any impact on the study.

OBSERVATIONS AND FREQUENCIES:

Viability:	Daily. Autopsy of all animals found dead
Clinical signs:	Daily, immediately after dosing and twice daily (M-F) after month 15.
Body weights:	D1, Week 1-13 weekly, then once every 4 weeks, D729 and at autopsy
Food consumption:	D1, Week 1-13 weekly, then once every 4 weeks, D729 and at autopsy
Ophthalmology:	N/A
Hematology:	@ autopsy, except for animals found dead
Clinical chemistry:	N/A
Gross pathology:	@ autopsy
Histopathology:	Adrenal glands, aorta, brain, clitoral glands, epididymides, esophagus, eyes, extraorbital lacrimal glands, Harder's glands (Harderian glands?), heart, kidneys, large intestine – (cecum and colon), larynx, liver, lungs, mammary gland, mesenteric lymph nodes, muscles, nasal turbinates, ovaries (w/ oviducts), pancreas, preputial gland, pituitary gland, prostate gland, salivary glands (mandibular), sciatic nerve, seminal vesicles, skin, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, sternum, stomach, testes, thymus, thyroid gland (w/ parathyroid), tibia (marrow) w/ knee joint, tongue, trachea, urinary bladder, uterus (plus cervix), & vagina as well as any abnormalities.

Toxicokinetics: Day 1, month 3: groups 9-13, n=5 at 0 (first 5 animals), 1 (second 5 animals), 2 (third 5 animals), 3 (fourth 5 animals), 4 (first 5 animals), 6 (second 5 animals), 8 (third 5 animals) & 24 (fourth 5 animals) hr. Each mouse was bled twice on day 1 and month 3. Blood samples were drawn via tail vein or retro-orbital venous plexus.
Month 24: from groups 3-7 mice, n=5 at 0 (first 5 animals), 2 (second 5 animals), 8 (third 5 animals) & 24 (fourth 5 animals) hr. If less than 20 animals were surviving, then 3/timepoint were sampled.

Palpable Mass Observations: Palpation for tissue masses was performed on the toxicity portion mice once every week using a scheme with a numerical/alpha-numerical roster for the description of locations where the masses appeared.

Statistical methods:

Data:	Statistical Method:
Body weight and food consumption:	None. Descriptive only.
Mortality:	Kaplan-Meier survival curve and log ranking test, $\alpha = 0.05$; controls combined
Tumor data:	Peto's linear trend test (alpha = 0.005 and 0.025 for common and rare tumors, respectively

RESULTS:

Mortality:

A dose-related increase in the incidence of deaths was observed in the males, but not in the females. Figures 2 and 3 (next 2 pages) present the survival curve as a function of time in males and females, respectively. Table 3 (next page) summarizes the nature of deaths. The cause of the death was not identified.

In the males, the high dose group showed a statistically significant increase in mortality. The increase started from week 81 onward. The survival rate at week 103 was 88%, 90%, 90%, 90%, 82% and 18% for the sham control, vehicle control, LD, MLD, MHD and HD groups, respectively. Among the 41 HD mice that died prematurely during the study, 21 were found dead and 20 were sacrificed due to moribund conditions.

Comment: The study report states that "overall, there was no substance-related increase in the incidence of mortality" (vol. 65.1, p 6). The report also states the 21 HD males died "spontaneously" (vol.56.1, 6). These animals were in fact those found dead in the cage. The prevalence of so called "spontaneous deaths" was 10%, 4%, 6%, 4%, 14%, and 41% for the sham, vehicle, LD, MLD, MHD and HD male groups, respectively. These statements of the report contradict a fundamental toxicology concept: dose-response relationships of a toxicity-related effect. They also raise questions about the objectivity of the laboratory and the sponsor in interpreting study results.

Table 3. Number and Type of Deaths

Sex	Male						Female					
Roflumilast (mg/kg/day)	0	Veh	0.5	2.0	6.0	18	0	Veh	0.5	2.0	6.0	12
Accidental death	0	0	0	1	0	0	0	0	1	1	0	1

Unscheduled sacrifice	1	3	2	2	3	20	2	5	2	3	5	1
Found dead	5	2	3	2	7	21	5	0	6	3	6	2
Total	6	5	5	5	9	41	7	5	9	7	11	4
Terminal sacrifice	44	45	45	45	41	9	43	45	41	43	39	46
Survival rate (%)	88	90	90	90	82	18	86	90	82	88	78	92

No treatment-related mortality was observed in the satellite animals designated for pharmacokinetic studies.

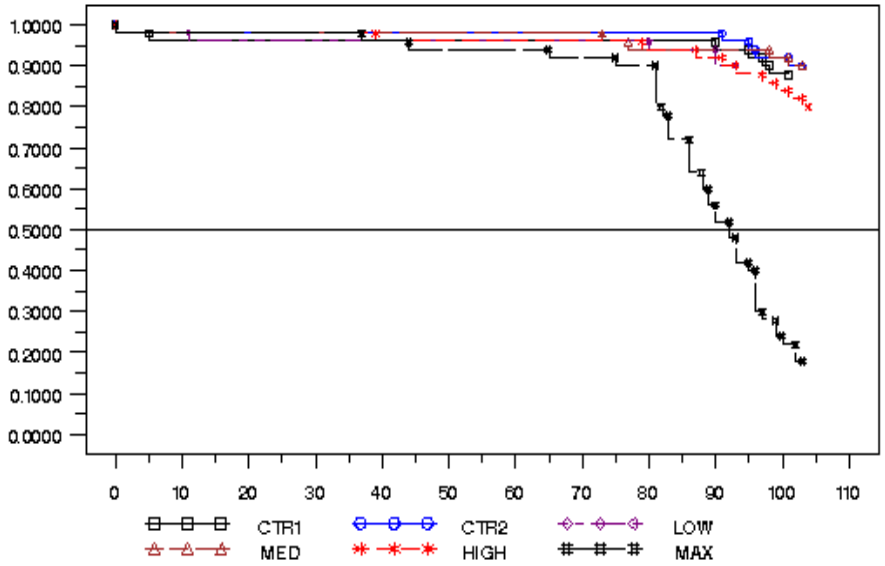

Figure 2: Kaplan-Meier survival curve of the 2-yr oral gavage carcinogenicity study of roflumilast in male mice.

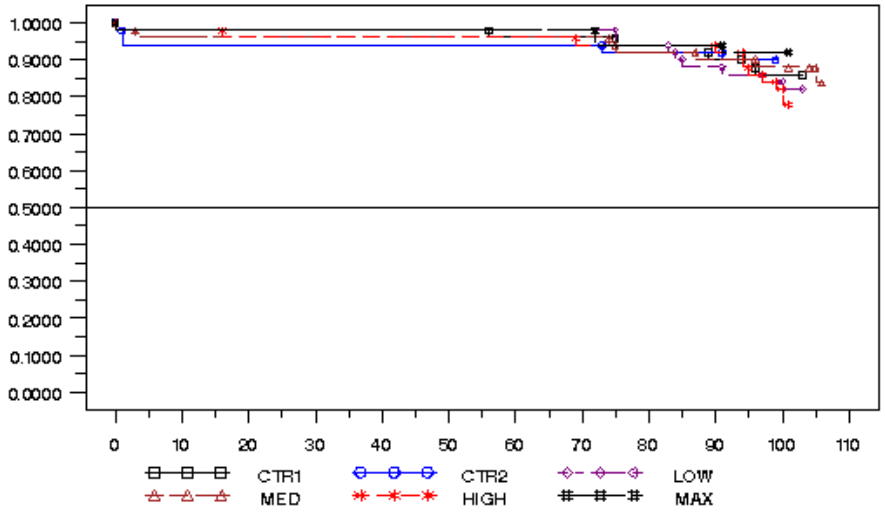

Figure 3: Kaplan-Meier survival curve of the 2-yr oral gavage carcinogenicity study of roflumilast in female mice.

Clinical signs: Numerous clinical signs were seen (Table 4). The incidence of some signs increased with dose. The most notable changes in males were penis prolapse, piloerection, decreased activity, and hypothermia. In both sexes, there was a dose dependent increase in white tip of tail, alopecia and miscellaneous signs (including cyanosis, altered respiratory rate, muscle spasms, etc). Males appeared to be more sensitive to Roflumilast than females.

Table 4. Clinical signs by instances/dose group

Sign	Sex	control	vehicle	0.5	2.0	6.0	18/12
Hunched posture	M	2	2	7	4	14	72
	F	0	1	1	1	8	7
Tail – white tip	M	3	0	9	8	2	18
	F	0	0	0	4	4	6
Penis prolapse	M	3	7	3	6	16	50
piloerection	M	1	6	7	4	12	42
	F	1	0	2	1	6	3
Decreased activity	M	1	5	5	1	3	18
	F	1	1	0	2	7	3
hypothermia	M	1	3	5	2	5	17
	F	0	1	1	4	1	1
nodule	M	4	0	2	1	2	1
	F	1	7	7	4	0	3
Alopecia/thin hair	M	0	3	4	2	10	12
	F	4	6	12	13	18	4
misc	M	3	8	5	4	8	26
	F	3	4	7	6	7	14

Shaded cells indicate the appearance of the values being different from both controls (no statistical evaluation conducted).

Palpable masses: No treatment-related effects were observed.

Body weight: Both high-dose males and females showed statistically significant decreases in body weight, when compared to the vehicle control group. Table 5 presents the mean body weights for the main study groups on Day 1 and Weeks 13, 26, 51, 79 and 104 of the treatment. Statistically significant decreases were noted in HD M from 3 months on and in HD F from 12 months on. The magnitude of the decrease was 7-22% in males and 4-12% in females, respectively.

Table 5. Mean Body Weight (g)

Sex	**Male**						**Female**					
Roflumilast (mg/kg/d)	**0**	**Veh**	**0.5**	**2.0**	**6.0**	**18**	**0**	**Veh**	**0.5**	**2.0**	**6.0**	**12**
Day 1	22	21	21	22	21	21	18	19	19	18	18	18
N	50	50	50	50	50	50	50	50	50	50	50	50
Week 13	28	27	27	27	27	25*	24	23	24	23	24	24
N	49	50	49	50	50	49	50	49	50	49	50	50
Week 26	31	29	30	29	29	26*	27	25	25	25	25	24
N	43	44	41	43	42	44	42	39	42	42	43	42

Week 51	33	31	31	30	29	27*	28	26	26	26	26	25*
N	49	50	49	50	49	48	50	49	50	49	49	50
Week 79	34	32	32	30	29	25*	29	27	28	26	26	25*
N	48	50	49	48	49	46	48	47	49	47	48	49
Week 104	31	29	29	28	26	23*	28	27	27	26	25	24*
N	44	45	45	45	41	9	43	45	41	44	39	46

* indicates significantly different from vehicle control

Figures 4 and 5 provide graphical presentations of body weight data as a function of treatment duration.

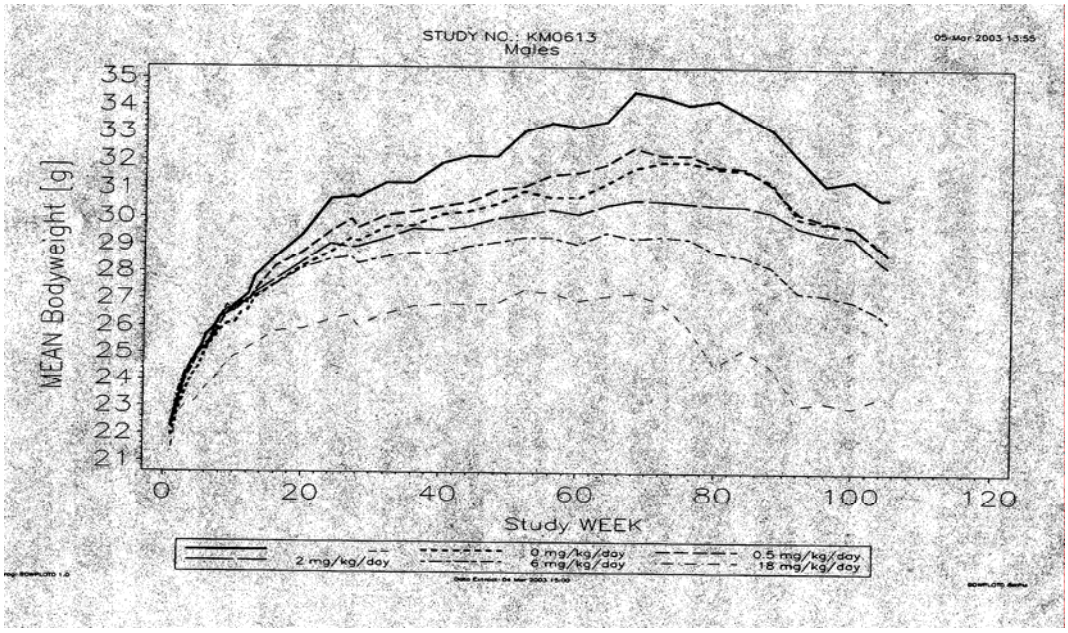

Figure 4:Body weight as a function of time in male mice.

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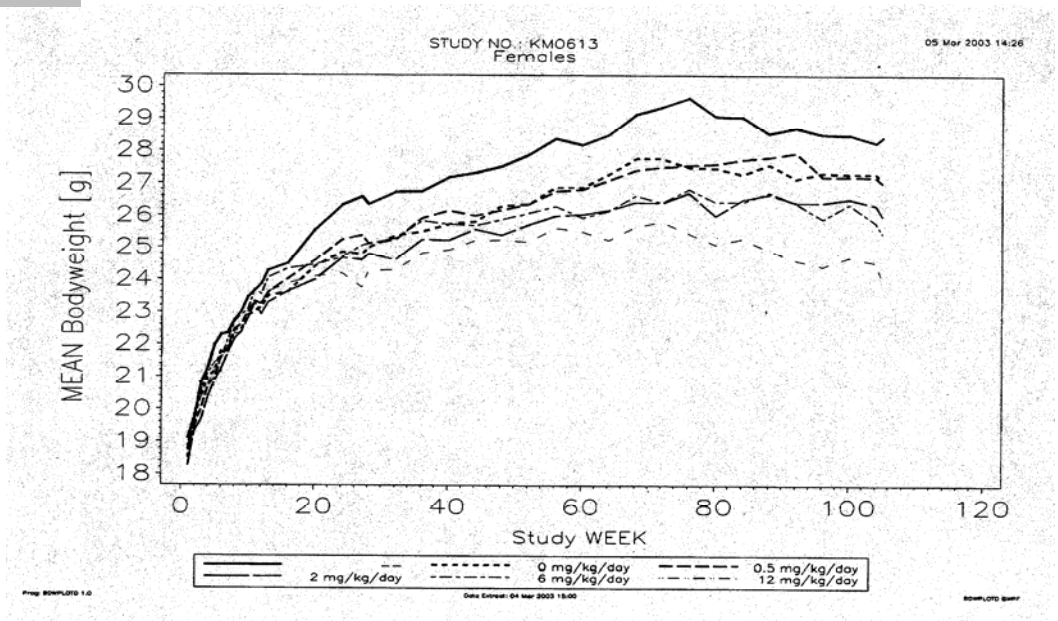


Figure 5: Body weight as a function of time in female mice.

Food consumption: No significant effect was observed (Table 6, below). Comparing to the vehicle control, the high dose mice showed increases in mean food consumption during the initial 2/3rd of the study and decreases in the last 1/3rd of the study. Magnitudes of the change were generally greater in males than females. However, none of the changes reached statistical significance.

Table 6: Mean Food Consumption (g)

Sex	Male						Female					
Roflumilast (mg/kg/d)	**0**	**Vehicle**	**0.5**	**2.0**	**6.0**	**18**	**0**	**Vehicle**	**0.5**	**2.0**	**6.0**	**12**
Week 1	**6**	**6**	**6**	**6**	**5**	**5**	**6**	**6**	**5**	**5**	**5**	**5**
N	50	50	50	50	50	50	50	50	50	50	50	50
Week 12-16	**7**	**6**	**6**	**7**	**7**	**8**	**6**	**6**	**6**	**6**	**7**	**7**
N	49	50	49	50	50	49	50	49	50	49	50	50
Week 23-27	**7**	**6**	**7**	**7**	**7**	**9**	**6**	**6**	**6**	**7**	**7**	**8**
N	43	44	41	43	42	44	42	39	42	42	43	42
Week 47-51	**6**	**6**	**6**	**6**	**7**	**7**	**6**	**6**	**6**	**6**	**6**	**7**
N	49	50	49	50	49	48	50	49	50	49	49	50
Week 67-71	**6**	**6**	**6**	**6**	**7**	**8**	**6**	**6**	**6**	**6**	**7**	**7**
N	48	50	49	48	49	46	48	47	49	47	48	49
Week 104	**6**	**6**	**6**	**6**	**6**	**5**	**7**	**7**	**7**	**7**	**7**	**6**
N	44	45	45	45	41	9	43	45	41	44	39	46

Hematology: Minor changes were observed in the HD group (Table 7, next page). The changes include small decreases in RBC (3-15%), hemoglobin counts, Hct (<5-9%), WBC counts (F:36 & M:57%), a statistical increase in segmented neutrophils (224% & 197%) and a statistically significant decrease in lymphocytes (56%, 27%). The MHD showed a small decrease in WBC counts (M:28% & F:21%).

Table 7. Hematology

Parameter	Sex	0	vehicle	0.5 mg/kg	2.0 mg/kg	6.0 mg/kg	M:18.0, F: 12.0
RBC Tera/l	M	8.32	8.8	8.28	8.05	7.84	7.50*
	F	8.6	8.58	8.41	8.22	8.27	8.24
Hb mmol/l	M	8.3	8.9	8.2	8.1	7.9	7.7*
	F	8.7	8.6	8.5	8.3	8.2	8.3*
MCV fl	M	48	47	48	49	49	51*
	F	48	48	48	48	48	48
Thrombocytes Giga/l	M	1457	1440	1507	1505	1525*	1604*
	F	937	981	1025	1099	1153*	1200*
WBC Giga/l	M	7.0	7.7	6.6	6.3	5.3	3.1*
	F	4.7	6.2	5.0	5.7	4.9	3.5*
Segmented neutron- /l	M	0.28	0.36	0.40	0.37	0.43	0.62*
	F	0.19	0.24	0.24	0.28	0.28	0.38*
Lymphocytes /l	M	0.68	0.6	0.58	0.59	0.53	0.36*
	F	0.76	0.72	0.71	0.68	0.68	0.58*

*indicates significantly different ($p<0.05$, Kruskal-Wallis) from cage control.

Gross pathology: The males showed dose-related changes in gross pathological examination, especially in the reproductive system (Table 8, below). Changes in the reproductive system include penis abnormalities (including prolapse, bloody penis, etc) and diminished testes, epididymides, prostate, seminal vesicles in the MHD and HD groups. Changes in other organs include skin exsiccosis, bloody/empty/ulcerated forestomach, glandular stomach, and empty intestines in HD males.

Table 8. Gross Pathology

Parameter	Sex	0	vehicle	0.5 mg/kg	2.0 mg/kg	6.0 mg/kg	M:18. 0F: 12.0
Autopsy BW (g)	M	30	27	27	27	25	22
	F	22	26	27	26	25	23
Diminished epididymides	M	1	2	2	1	2	13
Enlarged Harderian glands	M	0	1	0	4	5	5
	F	1	1	0	5	2	0
Empty intestines	M	0	0	0	0	2	4
	F	1	0	0	1	1	0
Penis abnormalities	M	4	5	6	6	11	18
Diminished prostate	M	1	4	2	2	6	17
Diminished seminal vescicles	M	1	3	2	1	7	16
Skin exsiccosis (dehydration due to	M	1	0	0	1	0	15
decrease fluid intake)	F	0	0	0	0	2	0
Diminished spleen	M	5	5	4	3	9	17
	F	0	0	1	0	2	2
Forestomach – bloody/empty/	M	2	1	1	2	2	13
ulcerated	F	3	0	2	3	3	2
Glandular stomach	M	2	2	2	2	9	14
bloody/empty/ulcerated/	F	2	2	2	5	4	3
hemmorrhage							
Diminished testes	M	3	3	5	3	8	20
Thymus-	M	19	15	16	17	33	45
involuted/diminished/missing	F	13	17	21	18	27	22

Shaded cells indicate values appearing different from controls (no statistical evaluation conducted).
N = 50 /group

Histopathology:

Non Neoplastic Lesions:

A number of non-neoplastic changes were observed in the MHD and HD groups in both sexes. Table 9 (below) summarizes the noticeable lesions. The high dose males showed distended heart (10/49), inflammation of posterior nasal cavity (8/10), lung congestion (19/50), glandular stomach erosion/ulcer (15/50), atrophy of the red pulp (23/50) and lymphoid depletion (19/50) in the spleen, pyelonephritis in the kidney (8/50), oligospermia in the epididymides, and small testes. A predominant majority of these lesions were observed in the prematurely-terminated mice. For example, abnormalities observed in the heart, lung and nasal cavity were exclusively found in the 41 pre-maturely terminated or dead males. The MHD males showed slight increases in the incidence of atrophy of the prostate gland, seminal vesicles, and lung congestion. The MHD and HD females showed decreases in ovary size (18/50–MHD, 31/50-HD), and uterine atrophy (22/50-MHD, 38/50-HD).

Table 9. Histopathology Findings

Parameter	Sex	0	vehi-cle	0.5 mg/kg	2.0 mg/kg	6.0 mg/kg	M:18.0 F: 12.0
Heart – distended chambers	M	1	0	2	0	2	10*
	F	0	0	2	0	0	1
Posterior nasal cavity - inflammation	M	2	4	2	0	2	8*
	F	0	0	0	0	2	0
Lung congestion	M	2	2	2	1	6	19*
	F	0	0	3	2	4	1
Glandular stomach – erosion/ulcer	M	0	1	2	0	5*	15*
	F	2	0	1	1	2	0
Epididymides - oligospermia	M	4	6	6	4	7	29*
Kidneys - Pyelonephritis	M	0	0	0	0	2	8*
Testes – diminished size	M	4	3	3	2	10	28*
Prostate gland - atrophy	M	2	4	3	2	10	31*
Seminal vescicles - atrophy	M	3	4	3	2	8	32*
Spleen	M	4	3	2	2	7	19*
Lymphoid depletion	F	0	0	0	0	2	2
Spleen	M	3	1	5	2	5	23*
Atrophy of red pulp	F	0	1	1	0	4	4
Ovaries – diminished in size	F	1	0	0	0	18*	31*
Uterus - atrophy	F	0	0	0	0	22*	38*

*indicates significantly different ($p<0.05$) from vehicle control.

Neoplastic Findings:

There was no statistically increased tumor incidence in male or female mice. For both sexes, the type, incidence and organ distribution of these neoplasms were not different between control and treated animals. Tables 10 and 11 (below) summarize noticeable findings in tumor incidence in males and females, respectively.

Table 10: Tumor incidence in male mice - Report on Test for Positive Linear Dose-Tumor Trends in Male Mice: Combined Control

Organ Name	Tumor Name	CTR1	CTR2	LOW	MED	MEDHI	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
PENIS	Granular cell tumor	0	0	0	0	0	1	0.0581	0.0003

Table 11: Tumor incidence in female mice - Report on Test for Positive Linear Dose-Tumor Trends in Female Mice: Combined Control

Organ Name	Tumor Name	CTR1	CTR 2	LO W	MED	MED HI	HIGH	P-Value (Exact Method)	P-Value (Asymptoti c Method)
SPINAL CORD	Fibrosarcoma	0	0	0	0	1	0	0.3345	0.3187
LUNG	Alveolar/bronchiolar adenoma	3	4	3	4	0	2	0.9048	0.9033
LUNG	Alveolar/bronchiolar	1	1	1	0	0	1	0.5731	0.5836

Organ Name	Tumor Name	CTR1	CTR 2	LO W	MED	MED HI	HIGH	P-Value (Exact Method)	P-Value (Asymptotic Method)
	carcinoma								
LUNG	Metastasis of sarcoma	1	1	0	0	0	0	1.0000	0.8816
STOMACH	Adenocarcinoma	1	0	0	0	0	0	1.0000	0.8176
SMALL INTESTINE	Adenoma	0	0	0	1	1	0	0.4029	0.4674
LIVER	Hepatocellular adenoma	2	1	2	0	3	2	0.2715	0.2856
LIVER	Hepatocellular carcinoma	2	2	2	1	3	1	0.6238	0.6474
LIVER	Hemangiosarcoma	0	0	0	1	0	0	0.5000	0.6707
OVARIES	Yolk sac carcinoma	0	0	1	0	0	0	0.6679	0.7866
OVARIES	Cystadenoma	0	1	0	1	0	1	0.3271	0.3433
OVARIES	Benign teratoma	0	0	0	0	1	0	0.3295	0.3240
UTERUS	Adenoma	0	1	0	0	0	0	1.0000	0.8128
UTERUS	Stromal polyp	1	0	1	0	0	0	0.8836	0.8641
UTERUS	Leiomyoma	0	0	0	0	1	0	0.3268	0.3204
PITUITARY GL	Adenoma of pars distalis	4	6	6	3	0	1	0.9977	0.9941
PITUITARY GL	Pituicytoma	0	0	0	0	1	0	0.3254	0.3167
THYROID GL	Follicular cell adenoma	3	1	2	1	0	1	0.8517	0.8582
THYROID GL	Follicular cell carcinoma	0	0	0	0	1	0	0.3307	0.3251
ADRENAL GL	A-cell adenoma	0	0	1	0	0	0	0.6589	0.7820
ADRENAL GL	Medullary tumor: ganglioneuro	1	0	0	0	0	0	1.0000	0.8136
ADRENAL GL	Medullary tumor: pheochromocyt	0	1	0	0	0	0	1.0000	0.8136
HEMOLYMPH. SYS	Histiocytic sarcoma	3	2	0	0	1	1	0.6959	0.7205
HEMOLYMPH. SYS	Malignant lymphoma	12	4	6	3	14	3	0.7200	0.7294
HEMOLYMPH. SYS	Malignant fibrous histiocytoma	0	0	0	0	1	0	0.3358	0.3244
SPLEEN	Hemangioma	1	1	1	0	0	0	0.9603	0.9103
SPLEEN	Hemangiosarcoma	1	0	0	0	0	0	1.0000	0.8121
PAROTID GL	Hemangioma	1	0	0	0	0	0	1.0000	0.8136
MANDIBULAR GL	Myoepithelioma	0	1	0	0	0	0	1.0000	0.8142
HARDERIAN GL	Adenoma	1	7	1	6	2	2	0.8233	0.8303
MAMMARY GL	Adenocarcinoma	1	0	0	2	1	0	0.6538	0.6963
SKIN/SUBCUTIS	Fibrosarcoma	0	0	1	0	0	1	0.2070	0.2178
SKIN/SUBCUTIS	Sarcoma (not otherwise specifi	0	0	1	0	0	0	0.6641	0.7852
FEMUR	Hemangioma	1	0	0	0	0	0	1.0000	0.8144

Toxicokinetics:

Plasma concentrations of roflumilast and roflumilast N-oxide rose in an approximately dose-dependent fashion. Plasma levels of roflumilast and its three metabolites were reported in Study Report No. 110/2002 (Vol. 65.5, page TK1). Plasma drug levels were determined on treatment day 1 and months 3 and 24. Samples on day 1 and month 3 were collected at pre-dosing and hours 1, 2, 3, 4, 6, 8 and 24 post-dosing (n = 5/sex/time point). They were analyzed with a validated HPLC with post column photochemical derivatization and fluorescence detection. Samples at month 24 (d722) were collected from animals of groups 3-7 at pre-dose and hours 2, 8 and 24 post dose. Roflumilast and roflumilast-N-oxide samples of month 24 and plasma levels of metabolites ADCP and ADCP-N-oxide on treatment day 1 and month 3 and 24 were assayed with LC/MS/MS. The detection limits were 0.5 and 1.0 ng/ml for ADCP and ADCP-N-oxide, respectively. No LOQs were provided for roflumilast and roflumilast-N-oxide but the sponsor states that the LOQ are the same as those described in the methods for study RR103/2001.

Plasma concentrations of roflumilast and roflumilast N-oxide rose in an approximately dose-dependent fashion. In study month 24, a greater than proportional increase in exposure to roflumilast N-oxide was seen in M. For the ADCP, on day 1 and month 3, the proportionate increase in AUC levels was lower than expected from the increase in roflumilast dose. The systemic exposure to ADCP-N-oxide increased approximately proportionally to the roflumilast dose. Table 12 (next page) summarizes the results of plasma roflumilast concentrations. For administered doses of 0.5, 2.0 & 6.0 mg/kg, systemic exposure to roflumilast, roflumilast-N-oxide, ADCP and ADCP-N-oxide appeared to be constant for the duration of the study.

Table 12: Plasma Concentration of Roflumilast in a 24-Month Oral Carcinogenicity Study in Mice

Dose (mg/kg)	Time point	**Roflumilast**		**Roflumilast N-oxide**		**ADCP**		**ADCP N-oxide**	
		$AUC_{(0-24h)}$ µg/l.h	**Cmax µg/l**	$AUC_{(0-24h)}$ µg/l.h	Cmax µg/l	**$AUC_{(0-24h)}$ µg/l.h**	**Cmax µg/l**	$AUC_{(0-24h)}$ µg/l.h	Cmax µg/l
0.5	D1	**28.9***	**5.29**	53.5*	15.2	**n.c.**	**n.c.**	39.4*	7.84
	M3	**23.9**	**4.68**	60.9	20.27	**n.c.**	**n.c.**	44.8	10.08
	M24	**n.a.**	**n.c.**	78.9	14.64	**n.c.**	**n.c.**	n.c.	6.50
2.0	D1	**88.5***	**18.62**	188.3*	41.03	**15.6***	**1.36**	146.6*	24.45
	M3	**73.2**	**15.21**	221.9	54.60	**15.6**	**1.69**	153.3	27.74
	M24	**71.5**	**7.25**	244.4	19.18	**n.c.**	**n.c.**	150.6	15.37
6.0	D1	**272.8***	**49.96**	579.1*	104.52	**24.0***	**3.93**	327.6*	53.13
	M3	**n.c.**	**36.27**	726.8	121.87	**18.0**	**3.08**	233.5	36.30
	M24	**231.4**	**20.13**	831.2	62.21	**25.5**	**2.53**	278.4	28.43
	Mean	**2252.1**	**35.5**	712.4	96.2	**22.5**	**3.2**	279.8	39.3
12.0 [2]	D1	**637.3***	**153.66**	1283.4*	278.4	**29.3***	**4.35**	446.2*	55.03
	M3	**521.0**	**113.79**	1579.7	261.8	**70.5**	**7.37**	842.5	98.13
	M24	**663.0**	**48.98**	2144.9	133.88	**134.0**	**13.13**	1042.0	85.67
18.0 [2]	D1	**617.0***	**144.48**	971.4*	213.11	**46.5***	**7.05**	573.3*	81.20

M3	**877.7**	**124.69**	2245.8	252.35	**142.8**	**13.61**	1302.5	110.37
M24[1]	961.1	154.14	3736.1	570.93	n.a.	7.56	n.a.	92.07

ADCP: 4-Amino-3,5-dichloro-pyridine;
[1]: n=3; *: $AUC_{(0\text{-}24h)}$ was calculated for study day 1 since extrapolation to infinity would require an elimination t1/2 which cannot be calculated for individual animals for each time.; n.a.: not ascertainable; n.c.: not calculated; D1: Study day 1, M3: Study month 3, M24: Study month 24.
2. n = 5/time point.

Mice had greater exposures to the N-oxide metabolites compared to the parent compound. Exposures were highest to roflumilast-N-oxide, then to ADCP-N-oxide followed by roflumilast and finally ADCP. Exposures to Roflumilast-N-oxide were approximately twice those to roflumilast; exposures to ADCP-N-oxide were usually slightly greater than those to roflumilast; exposures to ADCP were several fold lower than those to roflumilast.

Interpretation and Evaluation of Mouse Study

Roflumilast at oral doses up to 18 and 12 mg/kg/day in males and females, respectively, for 103 weeks did not cause significant increases in the incidence of any tumors in mice. B6C3F1 mice (50/sex/dose) were treated with dose of 0.5, 2.0, 6.0 and 12(F) or 18(M) mg/kg/d of roflumilast via oral gavage for 2 years. Additional mice (50/sex/group) received sham or vehicle (4% methocel) and servef as control. A statistically significant trend in dose-related mortality was observed in the males ($P < 0.0000$), but not in the females. The high dose group showed a statistically significant decrease in body weight. Clinical signs changes in males were penis prolapse, piloerection, decreased activity, and hypothermia. Males in the 6 and 18 mg/kg/d groups had penis abnormalities, diminished prostate, seminal vesicles and spleen and thymic changes.

Non-neoplastic lesions with roflumilast were seen in the heart, posterior nasal cavity, lung, glandular stomach, kidneys, spleen, and reproductive organs. The abnormality in the heart was extended chambers in the high dose males. The lesion in the nasal cavity was inflammation in the high dose males. The lesion in the lung was congestion in the high dose males. Lesions in the stomach were erosion and ulceration of the glandular region in the MHD and HD males. The lesion in the kidney was pyelonephritis in the HD males. The lesion in the spleen was atrophy of the red pulp in the HD males. The lesion in the reproductive organs included atrophy of seminal vesicles, oligospermia in epididymides, and smaller testis in the high dose males and atrophy of the uterus in the MHD & HD Females. The incidence of uterus atrophy was 0%, 0%. 0%, 0%, 54% and 76% for the sham, vehicle, LD, MLD, MHD and HD groups, respectively. The roflumilast treated groups did not show any statistically significant increases in the incidence of tumors.

This study adequately evaluated the carcinogenic potential of roflumilast in mice. The Executive CAC concurred with the study protocol and dose selection. The species, doses tested and duration of treatment were appropriate. The maximum tolerated dose (MTD) was either exceeded or achieved. In the males, the HD exceeded the MTD due to excessive mortality (82%). In the females, the MTD was achieved, based on the decrease in body weight and treatment-related changes in the uterus. The HD group showed an 11% reduction in body weight at the end of dosing. The MHD and HD groups showed 54 – 76% prevalence of uterus atrophy that was absent in the controls and lower dose groups. No statistically

significant increase in any tumors was observed in any treatment groups. Roflumilast is considered non-carcinogenic in mice under the conditions of the assay.

Hamster Studies

Study 7/2002: Up to 8 mg/kg/day of roflumilast for 2 years

Study Title: 24-Month Oral [Gavage] Carcinogenicity Study with B9302-107 [Roflumilast] in Syrian Hamster (up to 8 mg/kg/day)

Key findings:

Female hamsters treated with 8 mg/kg/day roflumilast (PO) for 2 years showed statistically significant increases in the incidence of carcinoma (undifferentiated) in the nasal cavity and leiomyomas in the uterus. Syrian golden hamsters (60/sex/dose) were treated via oral gavage with 0.25, 1, 4 and 8 mg/kg/day of roflumilast for 2 years. Additional hamsters received vehicle only (4% methocel) or no treatment at all as controls. The males showed a statistically significant trend in dose-related mortality ($P < 0.004$). The cumulative mortality in males at the end of the treatment was 22%, 18%, 18%, 20%, 33% and 35% for the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. The mortality was similar across groups in females and greater than that in males. There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The high dose females showed increases in the incidence of undifferentiated carcinomas in the nasal cavity and leiomyomas in the uterus. The respective incidence in the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups was 0, 0, 0, 0, 0 and 4 for the nasal carcinoma and 3, 2, 2, 4, 4 and 9 for the uterine leiomyomas. The Executive CAC concluded that the nasal tumor is a treatment-related effect while the uterine is not. The evaluation of the uterine tumor will be discussed later in the document. No remarkable findings were observed in the males of any roflumilast-treated groups.

Adequacy of the study and appropriateness of the test model

The study appears to have reached or exceeded the maximum tolerated dose (MTD) in the males, based on the dose related increases in mortality rate. The cumulative mortality rates in the 4 and 8 mg/kg/day group males (33% - 35%) were 60 – 75% higher than that (18% – 22% in the vehicle or cage controls in the males. Data, however, have not demonstrated that the MTD was also achieved in females. The cumulative mortality was similar among females and ranged from 61.7% (Mid high dose) and 73% (vehicle control and high dose). Although the cumulative mortality rate for the cage control is a slightly lower (58%), no statistical significance was achieved. Such data raise questions as to whether the MTD of roflumilast was achieved in the females, although the high dose of 8 mg/kg in females is the dose agreed upon by the Executive CAC previously based upon the dose-ranging study.

Female hamsters treated with 8 mg/kg/day roflumilast (PO) for 2 years showed statistically significant increases in the incidence of carcinoma (undifferentiated) in the nasal cavity and incidence of leiomyomas in the uterus. Syrian golden hamsters (60/sex/dose) were treated via oral gavage with 0.25, 1, 4 and 8 mg/kg/day of roflumilast for 2 years. Additional hamsters received vehicle only (4% methocel) or no treatment at all as controls. The males, but not the

females, showed a statistically significant trend in dose-related mortality ($P < 0.004$). The cumulative mortality at the end of the treatment in males was 22%, 18%, 18%, 20%, 33% and 35% for the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. The mortality was similar across groups in females (58 – 75%). There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The high dose females showed increases in the incidence of undifferentiated carcinomas in the nasal cavity and leiomyomas in the uterus. The respective incidence was 0, 0, 0, 0, 0 and 4 for the nasal carcinoma and 3, 2, 2, 4, 4 and 9 for the uterine leiomyomas in the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. No remarkable findings were observed in the males of any roflumilast-treated groups.

Interpretation and evaluation of the study is further discussed in the Overall Interpretation and Evaluation Section of the review.

STUDY IDENTIFICATION

Study No:	PR 7/2002
Study Protocol number:	(b) (4)
Date of Submission & Serial No.:	26-SEP-2003, 235
Volume and Page Nos.:	C59.1, page 61
Conducting Laboratory and Location:	(b) (4) Statistics non-neoplastic lesions: ALTANA Pharma, Institute of Pathology and Toxicology, Hamburg, Germany
Date of study initiation:	March 23, 1999
Date of Study Completion:	April 29, 2003
Study Report Date:	August 29, 2003
GLP compliance:	Yes, signed GLP statement included.
QA reports:	Yes, signed GLP statement included.
Drug lot #, & % purity:	Batch# 298569; purity 101.4%

STUDY PROTOCOL DESIGN AND METHODS

Study Type:	Traditional 2-year oral gavage study
Species/strain:	Hamster (Syrian Golden, Han:AURA)
Number/sex/group; age at start of study:	60 sex/dose, approximately 6 weeks
Animal weight at start of exposure:	Means of 76.5 g and 71.8 g for males and females
Animal housing:	Individually
Treatment Duration:	24 months
Feed and water:	Ad libitum
Formulation/vehicle:	Suspension of 4% methocel containing 10 - 15 drops of Med Antiform C/1000 ml.

Drug stability/homogeneity:	Concentrations of B9302-107 in prepared suspensions were analyzed between 22-APR-1999 to 03-AUG-2001. B9302-107 concentrations ranged between 103.4 – 103.7% of the target concentrations.

DESIGN

Hamsters (60/sex/dose) were given via oral gavage 0.25, 1, 4 or 8 mg/kg/day of roflumilast for 2 years. Additional groups received either vehicle only or no treatment (sham) at all to serve as controls. Surviving hamsters were sacrificed at the end of the treatment to examine the incidence of tumors. Table 13 (next page) shows the overall design of the study.

Table 13. Design of Study #7/2002

Group	Treatment	Roflumilast (mg/kg/day)	Hamster# /sex/dose	Dose volume (ml/kg)
A1/2	Cage control	0	60	-
B3/4	Roflumilast	0.25	60	5
C5/6	Roflumilast	1.0	60	5
D7/8	Roflumilast	4.0	60	5
E9/10	Roflumilast	8	60	5
F11/12	Vehicle control	0	60	5
G12/13	Sentinel *	-	30	-

* For monitoring health conditions of the hamsters in the living environment. These hamsters did not undergo the microscopic and histological evaluations.

DOSES:

Doses: 0, 0, 0.25, 1, 4 and 8 mg/kg/day.

Basis of dose selection: The report states that dose selection was based on the maximum tolerated dose (MTD) identified in a 3-month dose ranging study (Study# 252/98) in which the 8 mg/kg/day dose caused decreases of 10 and 21% in mean body weight gains in the male and females, respectively. However, the Executive CAC considered the high dose in males below the MTD and recommended raising the high dose to 16 mg/kg/day in males (Ref,: meeting minutes dated February 9, 1999). The sponsor conducted another 2-year study to address this comment. That study is reviewed later in the document.

Relation to clinical use: The route of administration of the study is identical to that of the intended clinical use (oral). The hamsters received roflumilast doses that were multiples of the human doses. Roflumilast doses in hamsters were 0.25, 1, 4 and 8 mg/kg/day. The highest human dose of roflumilast in clinical trials is 500 µg/day in COPD patients (10 µg/kg/day. On a mg/kg/day basis, the hamster doses are 25, 100, 400 and 800 times the maximum human dose, respectively. Based on systemic exposure, the roflumilast AUCs in two high-dose groups were 83 and 210 times that in humans (0.36 µg.h/ml at the maximum dose).

CAC concurrence: The Executive CAC concurred with the dose selection in the females but not the males. The Executive CAC reviewed the study protocol on February 9, 1999 and recommended raising the high dose in males to 16 mg/kg/day. Deliberation of the decision making process can be found in Exec. CAC minutes dated February 9, 1999 and Dr. Timothy McGovern's reviews dated February 2, 1999 and May 29, 2001.

Restriction paradigm for dietary restriction studies: None.
Route of administration: Oral gavage (via gastric cannula) at 5 ml/kg volume.
Frequency of drug administration: once a day, 7 days a week,
Controls employed: This study contains a dual control: one sham control and other the vehicle control.
Interim sacrifices: No.

Satellite PK or special study group(s): No additional hamsters were used.

Deviations from original study protocol: Survival was analyzed with Peto's test. No other significant deviations occurred.

OBSERVATIONS AND FREQUENCIES:

Viability:	Daily
Clinical signs:	Daily
Body weights:	Weekly
Food consumption:	Not evaluated
Ophthalmology:	Not evaluated
Hematology:	Prior to sacrifice
Clinical chemistry:	Not evaluated
Organ weights:	Necropsy
Gross pathology:	Necropsy
Histopathology:	A complete list of tissues
Final sacrifice:	Weeks 104 - 107
Toxicokinetics:	Weeks 13 and 104 at hrs 0, 1, 2, 3, 4, 6, 8 and 24 if possible.

Palpable Mass Observations: weekly.

Statistical methods:

Data:	Statistical Method:
Body weight:	ANOVA and t-test
Hematology:	ANOVA and t-test
Mortality:	Kaplan-Meier survival curve and log ranking test, $\alpha = 0.05$
Histology:	Fisher's exact
Tumor data:	Peto's linear trend test (alpha = 0.005 and 0.025 for common and rare tumors, respectively

RESULTS:

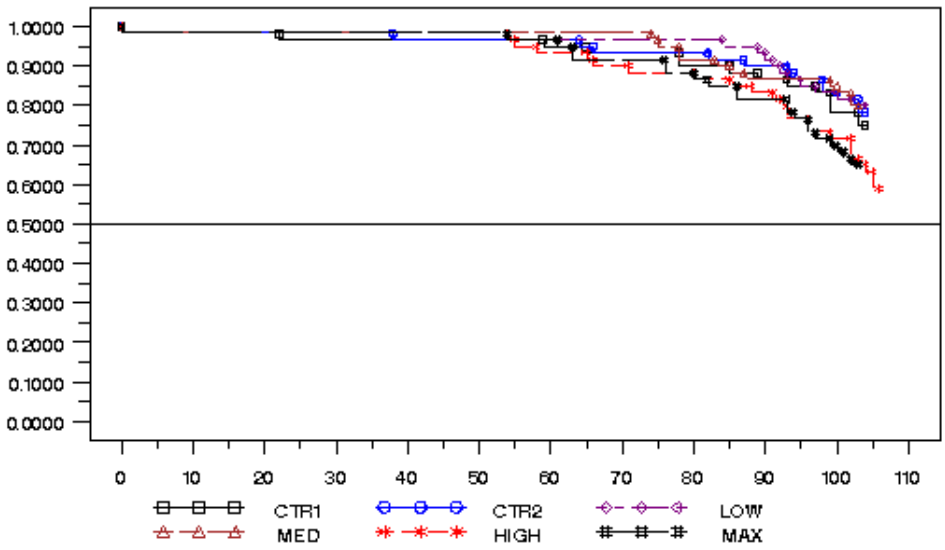
Mortality: The male hamsters showed a statistically significant, dose-related mortality ($p < 0.004$). The females did not show any dose-related mortality over the course of the study, although the mortality rate was increased slightly during the week 53-78 period in the highest dose group. The mortality rate in the females, however, was higher than the males. The respective cumulative mortality rate at the time of terminal sacrifice for the cage control, vehicle control, LD, LMD, LHD and HD groups was 21.7, 18.3, 18.3, 20, 33.3 and 35% in the males and was 58.3, 73.3, 68.3, 71.7, 61.7 and 73.3% in the females. Table 14 (next page) summarizes the mortality data of the study as a function of time. Figures 6 and 7 present the survival curve as a function of time. No tumor-related deaths were observed. A major cause for moribundity and mortality in females was left atrial thrombosis which is spontaneous in nature (up to 53%). Cause of death in males was not identified.

Table 14. Accumulative Mortality - Study 7/2002 [a]

Sex	Male						Female					
Roflumilast (mg/kg/day)	C1 [b]	C2 [c]	0.25	1	4	8	C1	C2	0.25	1	4	8
Weeks 0 - 52	1.7	1.7	0	0	0	0	0	0	5.0	0	3.3	3.3
53 - 78	6.7	5.0	1.7	5.0	10.0	8.3	10.0	16.7	11.7	15.0	18.3	25.0
79 - 91	11.7	8.3	8.3	11.7	16.7	15.0	23.3	40.0	26.7	33.3	35.0	48.3
92 - 103	21.7	18.3	18.3	20.0	33.3	35.0	58.3	73.3	68.3	71.7	61.7	73.3

a. Source: Tables 2 and 8 in Dr. Ted Guo's statistical review.[1]
b. Cage (sham) control.
c. Vehicle control.

Sufficient numbers of hamsters survived the treatment. Each group has at least 16 hamsters surviving to the scheduled sacrifice. Table 15 (page 30) presents the number of hamsters that survived the treatment and were available for the terminal sacrifice. Thirty-nine to forty-seven males per group were available for the terminal sacrifice. Only 16 to 25 females were available for the terminal sacrifice.



[1] The mortality rate in Dr. Guo's analysis differs slightly from that in the study report. Dr. Ted Guo analyzed the electronic data provided by sponsor most recently (Serial number 265, submitted on February 18, 2004). The following table presents the mortality rate in by the study report (vol. 59.1, page 83).

Accumulative Mortality (%) Presented in the Study Report

Sex	Male						Female					
Roflumilast (mg/kg/day)	C1[a]	C2[b]	0.25	1	4	8	C1	C2	0.25	1	4	8
Day 360	2	2	0	0	0	0	0	0	5	0	3	3
Day 540	5	5	2	3	10	8	8	15	12	15	17	25
Day 660	13	12	12	12	20	22	28	47	40	43	40	57
Day 748	25	22	20	20	38	35	58	73	75	73	73	65

Figure 6: Kaplan-Meier survival curve of the male hamsters – Study 7/2002.

CTR1, CTR2, LOW, MED, HIGH and MAX represents the cage control, vehicle control, 0.25, 1, 4 and 8 mg/kg/day of roflumilast, respectively (Source: Figure 3 in Dr. Ted Guo's statistical review).

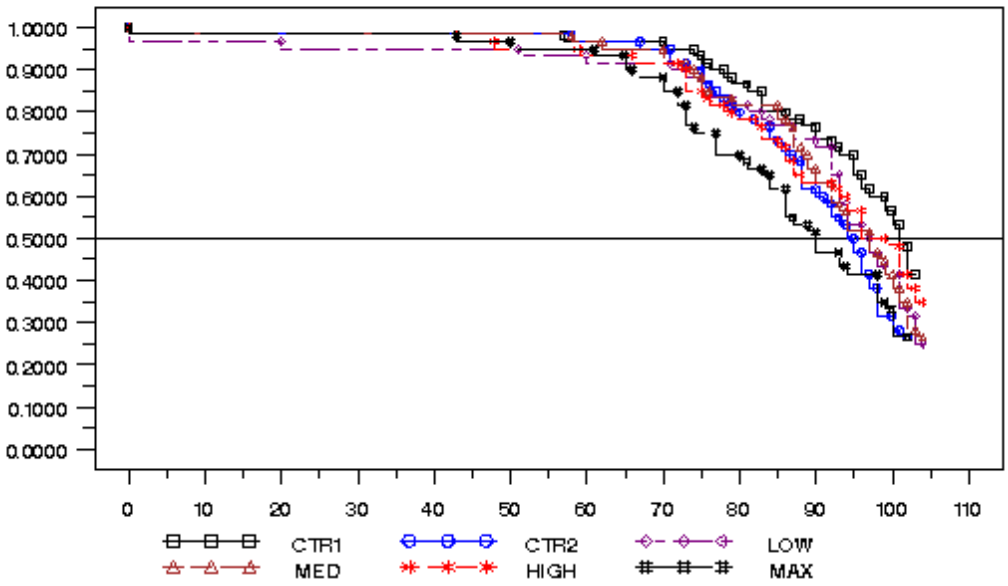


Figure 7. Kaplan-Meier survival curve of the 2-yr oral carcinogenicity study of roflumilast in female hamsters.

CTR1, CTR2, LOW, MED, HIGH and MAX represents the cage control, vehicle control, 0.25, 1, 4 and 8 mg/kg/day of roflumilast, respectively (Source: Figure 6 in Dr. Ted Guo's Statistical review).

Table 15. Number of Hamsters that Survived the Treatment (Study 7/2002) [a]

		Roflumilast (mg/kg/day)				
Sex	Sham	Vehicle	0.25	1	4	8
Male	47	49	49	48	40	39
Female	25	16	19	17	23	16

a. Source: statistical analysis by Dr. Ted Guo.

Clinical signs: The treated females showed a non-dose-related increase in the incidence of distended abdomen. Table 16 presents the prevalence of this finding in hamsters alive on week 104. The distended abdomen was attributed to ascite subsequent to cardiac thrombosis.

Table 16. Percentage of Hamsters with Distended Abdomen at Week 104 (Study 7/2002)

	Roflumilast (mg/kg/day) [a]					
Sex	Sham	Vehicle	0.25	1	4	8
Male	6	0	4	0	0	7
Female	4	6	18	17	27	18

a. Source: Study report, submission Vol. 59.1, page 84.

Palpable masses: No treatment-related effects were observed. Table 17 presents the percentage of surviving hamsters with palpable mass or nodule at Week 104. No remarkable findings were reported in the pre-terminally sacrificed animals.

Table 17. Percentage of Hamsters with Palpable Mass (Week 104) –Study 7/2002 [a]

	Roflumilast (mg/kg/day)					
Sex	Sham	Vehicle	0.25	1	4	8
Male	0	2	4	0	0	2
Female	0	6	0	11	0	12

a. Source: Study report, submission Vol. 59.1, page 84.

Body weight:

No remarkable effect was observed. Table 18 (next page) presents the absolute mean body weight at the major milestones of the study (treatment months 0, 12, 18, 24 and 24.2). The males showed a temporary statistically significant and dose-related decrease in body weight (from cage control) during the first 4 months of treatment. Statistical analyses from the vehicle control were not performed (or not given), but a similar trend appears to exist. The females, on the other hand, showed an increase in body weight, but the increase was small or comparable to the vehicle control.

Table 18. Mean Body Weight - Study 7/2002 [a]

Sex	Male						Female					
Roflumilast (mg/kg/day)	Cage	Veh	0.25	1	4	8	Cage	Veh	0.25	1	4	8
Day 0 (g)	77.0	76.0	76.0	76.5	76.3	76.1	71.8	71.9	71.6	72.0	71.6	71.8
Day 49 (g)	106	105	104	104	102*	101*	95.2	100*	98.6	99.9	99.2	99.1
Day 364 (g)	128	132	130	132	128	124	118	127*	124*	126*	124*	123*
N	60	60	60	60	60	60	60	60	57	60	58	58
Day 546 (g)	134	138	136	137	133	127*	126	134*	130	134*	131	127
N	56	57	59	58	54	55	55	50	53	51	50	46
Day 728 (g)	131	132	135	135	128	127	125	128	127	130	129	123
N	47	47	48	48	40	47	25	16	16	17	22	16
Day 742 (g)	131	134	133	132	133	126	119	126	125	120	131	126
N	13	17	15	14	12	13	9	6	5	5	5	7

a. Extracted from Table 9 of the report (Vol 59.2/page 212 – 221).
b. *, $P < 0.01$ (from cage control). Statistical analysis from the vehicle control was not done.

The report conducted analysis of body weight gain throughout the study. Compared to the cage control, the high dose males showed a 15.6% decrease in body weight gain while the females showed a slight increase (10.1%) in body weight gain The report stated that such a

degree of decrease in body weight gain in males justified that the dose of interest is the maximum tolerated dose (Vol. 59.1, page 86). This statement is not correct because the vehicle control and the HD groups have comparable body weight and weight gain.

Food consumption: Not evaluated.

Ophthalmology: No treatment-related effects were observed, although the clinical signs section included occasional observations of opacity, lacrimation and microphthalmus.

Hematology: No remarkable changes were observed. The high dose males showed slight but statistically significant increases in white blood cell and lymphocyte counts (approximately 30%) over the controls. Such a magnitude of increase is not toxicologically significant.

Gross pathology: No remarkable findings. Findings of note are summarized in Table 19 below.

Table 19. Noticeable Gross Pathological Findings (%) – Study 7/2002

Sex	Male [a]						Female					
Roflumilast (mg/kg/day)	C	V	0.25	1	4	8	C	V	0.25	1	4	8
Heart enlargement	12	10	12	5	13	3	32	42	47	30	23	15*
Nodules, forestomach	10	20	15	20	18	23	12	18	17	30*	32*	18
Kidney, altered surface	18	12	8	14	17	17	32	32	33	22	13*	17
Adrenals, enlargement	42	32	22*	37	30	18*	17	23	12	15	15	15
Testes, ↓ size	22	38*	15	12	12	13						

a. Constructed from section 7.4.1 of the study report (vol. 59.1, pages 86 - 88).
*, $P < 0.01$ (from cage control). Statistical analysis from the vehicle control was not done.

Histopathology:

Non-neoplastic Changes: No remarkable findings were observed. Table 20 (below) presents noticeable non-neoplastic histological changes in the study. Increases in liver lesions were observed in males receiving ≥ 1 mg/kg/day roflumilast. The lesions included acinar cell atrophy, mononuclear cell infiltration and hepatocyte hyperplasia. However, a dose-response relationship of these lesions was not clear; the incidence of lesions was similar between the 1, 4 and 8 mg/kg/day groups. No treatment-related effect was observed in the females.

Table 20. Noticeable Non-neoplastic Changes (%) –Study 7/2002

Organ/Lesions	Male						Female					
	Roflumilast (mg/kg/day)						Roflumilast (mg/kg/day)					
	C	V	0.25	1	4	8	C	V	0.25	1	4	8
Nasal cavity/ hyperplasia, Bowman's gland, olfactory epith.	12	5	4	9	12	12	11	9	4	14	8	6
Liver/ infiltrated by tumor cells	0	0	0	0	2	0	0	0	2	5	0	5
Acinar cell atrophy	18	23	8	35	28	33	37	30	25	37	32	35
Mononuclear cell infiltration	2	7	3	13	12	13	7	8	2	8	7	5
Hepatocyte metaplasia	0	7	5	12	13	13	0	0	2	8	3	3

Neoplastic Findings: Trend analyses of tumor incidence and roflumilast treatment revealed a statistically significant increase in nasal, uterus and larynx tumors. Table 21 presents the incidence of the tumors and p-values in statistical analysis. The statistical analysis was done against the cage control, the vehicle control, and the pooled cage and vehicle controls. The review uses the results against the vehicle under advice from the Executive CAC.

Table 21. Noticeable Tumor Incidence –Study 7/2002[a]

Organs	Tumor	Sex	Roflumilast (mg/kg/day)						P-Value
			Cage	Veh.	0.25	1	4	8	
Larynx	Neuroendocrine tumor [B],	M	0	0	0	0	0	2	0.0201
Nasal & para-nasal cavities	Carcinoma [M], undifferentiated	F	0	0	0	0	0	4	0.0006*
Uterus	Leiomyosarcoma [M]	F	2	1	0	3	4	2	NS
	Leiomyoma (TA) [B]	F	3	2	2	4	4	9	0.0022*
	Total		5	3	2	7	8	11	0.0224[c]

a. n = 60/dose.
b. Against the cage control.
*. Statistically significant from the vehicle control (source: Dr. Ted Guo's statistical review, p 17)

Nasal cavity: The high dose females showed a statistically significant increase in the incidence of undifferentiated carcinoma (P = 0.0006). The tumor incidence was 4/60 in the high dose females and 0/60 for the remaining groups.

Uterus: Dose-related increase in the incidence of uterus leiomyomas was observed in the females. Both leiomyomas and leiomyosarcomas were observed in the uterus. The former is a benign tumor while the latter is malignant. Only the incidence of leiomyomas was statistically significant (p = 0.0022). The combined incidence of leiomyomas and leiomyosarcomas are considered not significant.

Larynx: The high dose males showed a slight but statistically significant increase in the incidence of neuroendocrine tumors (2/60-HD males vs. 0/60 for the remaining groups). No definitive conclusions can be drawn from this finding because of the lack of the observation in the females.

Total tumor burdens: No treatment-related effect was observed. There was no difference in tumor burdens between the controls and the roflumilast treated groups in either sex. Table 22 presents the tumor burdens in males and females.

Table 22. Tumor Burden (incidence) – Study 7/2002

Sex	Male [a]						Female					
Roflumilast (mg/kg/day)	C	V	0.25	1	4	8	C	V	0.25	1	4	8
# hamster w/ tumors	56	55	50	56	54	49	52	56	55	59	53	52
# hamster w/ benign tumors	47	50	45	54	45	42	47	50	52	54	45	49
# hamster w/ malignant T.	25	19	19	12	23	16	28	29	29	37	34	27
# Tumors - Total	102	94	93	99	93	77	135	147	138	153	128	126
# Tumors - benign	73	74	72	85	69	59	95	112	103	109	90	90
# Tumors - maglignant	29	20	21	14	24	18	40	35	35	44	38	36
# Tumor - metastasizing	3	3	1	4	4	8	7	5	9	7	7	6

a. n = 60 /sex/dose (Source: study report Vol. 59.1, page 90)

Toxicokinetics:

Plasma levels of roflumilast and its three metabolites were reported (Study Report No. 72/2002, Vol. 59.9 – 10, pages 59.9/27 – 59.10/279). Plasma drug levels were determined at pre-dosing and hours 1, 2, 3, 4, 6, 8 and 24 post-dosing on treatment day 1 and month 3 and 24 (n = 2/sex/time point).[2] Day 1 and month 3 samples were analyzed with isocratic HPLC with post column photochemical derivatization and fluorescence detection. Samples of Month 24 and all cage and vehicle controls were assayed with LC/MS/MS. The limit of quantitation was not reported.

Table 23 (below) summarizes the AUCs of these compounds. All compounds were detectable in the MHD and HD groups, but the levels of roflumilast, ADCP and ADCP-N-oxide were below the limit of quantitation in the LD and MLD groups. Roflumilast levels were lowest among the four compounds. Roflumilast levels were approximately 1/40th, 1/3rd, and 1/10th of that of Roflumilast-N-oxide, ACDP and ACDP-N-oxide, respectively.

Table 23. Mean Plasma AUCs of Roflumilast and its metabolites – Study 7/2002

Roflumilast oral dose (mg/kg/day)	Plasma AUCs (µg.h/L)			
	Roflumilast	Roflumilast-N-Oxide	ACDP	ADCP-N-Oxide
0.25	-	88.8	-	-
1	-	255.2	-	-
4	30.4	1,203.9	100.9	345.8
8	69.0	3,073.3	222.8	821

a, NA = not ascertained due to the level below the low limit of detection, ND = not determined. (Source: vol. 59.9, p 29).

There was no significant difference in plasma levels of roflumilast and its metabolites between sexes. Table 24 (next page) presents the mean plasma AUCs of Roflumilast and its metabolites in male and female hamsters. These numbers are computed to facilitate labeling review should it become necessary.

[2] The study report incorrectly identifies n as 6 – 7 per time point. According to the study design, "Blood samples (approximately 0.3 ml) were collected from … 8 animals [each group] … at the following time point: Pre-dose, 1, 2, 3, 4, 6, 8 and 24 hours post dose. Blood sample were drawn … according the following schedule: The first two animals of each group and subset were used for pre-dose and 4 hour value. The second two animals of each group and subset were used for 1 and 6 hour value. The third two animals of each group and subset were used for 2 and 8 hour value. The last two animals of each group and subset were used for the 3 hour and 24 hour value (Vol. 59.9, p40)." Thus, the n per time point should be two rather than 8 that the study report states.

Table 24. Mean Plasma AUCs of Roflumilast and its metabolites in Males and Females – Study 7/2002

Roflumilast oral dose (mg/kg/day)	Treatment Time	Plasma AUCs (µg.h/L)							
		Male				Female			
		Roflumilast	Roflumilast-N-Oxide	ACDP	ADCP-N-Oxide	Roflumilast	Roflumilast-N-Oxide	ACDP	ADCP-N-Oxide
0.25	Day 1	NA	97.9	ND	ND	NA	91.6	ND	ND
	Month 3	NA	114.1	ND	ND	NA	115.7	ND	ND
	Month 24	NA	55.2	ND	ND	NA	58.6	ND	ND
	Average		89.1				88.6		
1	Day 1	NA	240.5	ND	ND	NA	247.5	ND	ND
	Month 3	NA	307.9	ND	ND	NA	328.2	ND	ND
	Month 24	NA	204.0	ND	ND	NA	203.6	ND	ND
	Average		250.8				259.7		
4	Day 1	16.3	964.0	72.3	378.0	NA	1,005.9	62.2	361.7
	Month 3	37.2	1,427	101.6	372.8	32.8	1,687.1	105.5	384.7
	Month 24	40.0	1,233.7	120.0	837.7	NA	937.2	145.1	764.5
	Average	31.2	1,208.2	98.0	529.5	32.8	1,210.1	104.3	503.6
8	Day 1	31.8	2,045	127.6	656.3	34.7	2,397.2	125.6	666.9
	Month 3	142.6	3,659.5	248.6	573.7	91.9	4,821.3	262.0	966.3
	Month 24	52.8	2,720.8	270.6	1,139.4	66.4	2,893.3	303.4	1,960.7
	Average	75.7	2,808.4	215.6	789.8	64.3	3,370.6	230.3	1,198.0

a, NA = not ascertained due to the level below the low limit of detection, ND = not determined. (Source: vol. 59.9, p 41 - 60).

Evaluation and Interpretation of Study 7/2202

Female hamsters treated with 8 mg/kg/day roflumilast (PO) for 2 years showed statistically significant increases in the incidence of carcinoma (undifferentiated) in the nasal cavity and incidence of leiomyomas in the uterus. Syrian golden hamsters (60/sex/dose) were treated via oral gavage with 0.25, 1, 4 and 8 mg/kg/day of roflumilast for 2 years. Additional hamsters received vehicle only (4% methocel) or no treatment at all as controls. The males, but not the females, showed a statistically significant trend in dose-related mortality ($P < 0.004$). The cumulative mortality at the end of the treatment in males was 22%, 18%, 18%, 20%, 33% and 35% for the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. The mortality was similar across groups in females (58 – 75%). There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The high dose females showed increases in the incidence of undifferentiated carcinomas in the nasal cavity and leiomyomas in the uterus. The respective incidence was 0, 0, 0, 0, 0 and 4 for the nasal carcinoma and 3, 2, 2, 4, 4 and 9 for the uterine leiomyomas in the cage control, vehicle control, and 0.25, 1, 4 and 8 mg/kg/day of roflumilast groups, respectively. The Executive CAC concluded that the nasal tumor is a treatment-related effect while the uterine is not. No remarkable findings were observed in the males of any roflumilast-treated groups.

The study appears to have reached or exceeded the maximum tolerated dose (MTD) in the males, based on the dose related increases in mortality rate. The cumulative mortality rates in

the 4 and 8 mg/kg/day group males (33% - 35%) were 60 – 75% higher than that (18% – 22% in the vehicle or cage controls in the males. Data, however, have not demonstrated that the MTD was also achieved in females. The cumulative mortality was similar among females and ranged from 61.7% (Mid high dose) and 73% (vehicle control and high dose). Although the cumulative mortality rate for the cage control is a slightly lower (58%), no statistical significance was achieved. Such data raise questions as to whether the MTD of roflumilast was achieved in the females, although the high dose of 8 mg/kg in females is the dose agreed upon by the Executive CAC previously based upon the dose-ranging study.

Interpretation and evaluation of the study is further discussed in the Overall Interpretation and Evaluation Section of the review.

Study 233/2003: 16 mg/kg/day of Roflumilast for 2 Years

Study Title: Complementary 24-Month Oral [Gavage] Carcinogenicity Study with B9302-107 [Roflumilast] in Syrian Golden Hamster (Control cage and 16 mg/kg/day)

Key findings: Hamsters (60/sex) treated with 16 mg/kg/day of roflumilast for 24 months showed non-statistically significant increases in the incidence of tumors in the nasal and para-nasal cavity in both sexes and uterine leiomyomas in females. The respective incidence of all tumors in the nasal cavity for the control and treatment groups was 0/30 and 7/60 in males and 0/30and 8/60 in females. The nasal tumors included carcinoma, adenocarcinoma and adenoma. Undifferentiated Carcinomas accounted for most tumor incidence (5/60 in each sex). The incidence of leiomyomas in the uterus was 2/30 and 10/60 for the cage control and roflumilast-treated groups. The study lacks appropriate control - vehicle group. The sample size of the cage control group is also small. The lack of proper control and the small sample size raises question about the validity of the study.

The 16 mg/kg/day dose of roflumilast seemed to have exceeded the maximum tolerated dose in hamsters, based on increased mortality in the treated group. The respective cumulative mortality at week 78 for the control and treated groups was 0% and 16.7% in males and 10% and 40% in females. The difference in mortality persisted throughout the study in the males but disappeared in females soon thereafter. The respective cumulative mortality at week 104 for the control and treated groups was 23% and 52% in males and 93% and 87% in females.

STUDY IDENTIFICATION
Study No: 233/2003
Study Protocol number: (b) (4)
Date of Submission & Serial No.: 28-SEP-2003, 235
Volume and Page Nos.: C59.11, page 1
Conducting Laboratory and Location:
(b) (4)

Statistics non-neoplastic lesions: ALTANA Pharma, Institute of Pathology and Toxicology, Hamburg, Germany.
Date of study initiation: August 27, 2000.
Date of Study Completion: June 23, 2003.
Study Report Date: August 29, 2003
GLP compliance: Yes, signed GLP statement included.
QA reports: Yes, signed GLP statement included.
Drug lot #, & % purity: Batch# 298569; purity 101.4%.

STUDY PROTOCOL DESIGN AND METHODS

Study Type: traditional 2-year oral gavage study
Species/strain: Hamster (Syrian Golden, Han:AURA)
Number/sex/group; age at start of study: 30/sex and 60/sex for the cage control and treatment groups, respectively; additional 44 hamsters were used to monitor health condition of the hamsters during the study. The hamsters were approximately 6 weeks at the start of the study.
Animal weight at start of exposure: means of 90.4 – 91.2 g and 82.3 – 83.3 g and for males and females, respectively.
Animal housing: Individually.
Treatment Duration: 24 months
Feed and water: ad libitum.
Formulation/vehicle: Suspension of 4% methocel containing 10 - 15 drops of Med Antiform C/1000 ml.
Drug stability/homogeneity: Good. Concentrations of roflumilast in prepared suspensions were analyzed. Roflumilast concentrations ranged between 103.4 – 103.7% of the target concentrations.

DESIGN

Syrian golden hamsters (60/sex) were given via oral gavage 16 mg/kg/day of roflumilast for 2 years. Additional 30 hamsters per sex did not receive any treatment to serve as cage control. Surviving hamsters were sacrificed at the end of the treatment. All hamsters were examined for tumor formation. Table 25 (below) shows the overall design of the study.

Table 25. Design of Study 233/2003

Group	Treatment	Roflumilast (mg/kg/day)	Hamster# /sex/dose	Dose volume (ml/kg)
H15/16	Cage control	0	30	-
I17/18	Roflumilast	16	60	5
K19/20	Sentinel [a]	-	22	-

a. For monitoring health conditions of the hamsters in the living environment. These animals were sacrificed at week 13. No histologic evaluation was performed.

DOSES:

Doses: 0 and 16 mg/kg/day.

Basis of dose selection: This is the Executive CAC's recommendation for the top roflumilast dose in males. The sponsor had previously initiated a study (Study #7/2002) at a top roflumilast dose of 8 mg/kg/day. The Executive CAC determined the 8 mg/kg/day dose to be too low in the males on February 9, 1999. The sponsor subsequently conducted the current study to complement Study 7/2002 and to comply with the CAC's recommendation. The executive CAC considered 8 mg/kg/day roflumilast in the females acceptable.

Relation to clinical use: The oral route of administration of the study is the same as the intended clinical route of administration.

CAC concurrence: The Executive CAC has concurred with the dose selection for the males on February 9, 1999.

Restriction paradigm for dietary restriction studies: None.
Route of administration: Oral gavage (via gastric cannula) at 5 ml/kg volume.
Frequency of drug administration: once a day, 7 days a week
Controls employed: A cage control.
Interim sacrifices: No.
Satellite PK or special study group(s): No additional hamsters were used.

Deviations from original study protocol: No significant deviations occurred.

OBSERVATIONS AND FREQUENCIES:

Viability:	Daily
Clinical signs:	Daily
Body weights:	Weekly
Health status:	Bacteriological investigation during the study and virology evaluation at month 3
Food consumption:	Not evaluated
Ophthalmology:	Not evaluated
Hematology:	Prior to sacrifice
Clinical chemistry:	Not evaluated
Organ weights:	Necropsy
Gross pathology:	Necropsy
Histopathology:	A complete list of tissues.
Final sacrifice:	Week 105
Toxicokinetics:	Weeks 1, 13 and 104 at hrs 0, 1, 2, 3, 4, 6, 8 and 24 if possible.

Palpable Mass Observations: Not evaluated.

Statistical methods:

Data:	Statistical Method:
Body weight:	ANOVA and t-test
Hematology:	ANOVA and t-test
Mortality:	Kaplan-Meier survival curve and log ranking test, $\alpha = 0.05$
Histology:	Fisher's exact
Tumor data:	Peto's linear trend test (alpha = 0.005 and 0.025 for common and

rare tumors, respectively

RESULTS:

Dose: 0 and 16 mg/kg/day. The drug concentration of roflumilast in the diet was investigated 10 times during the study. Mean roflumilast concentrations was 104% of the target.

Mortality: The treated males showed a statistically significant increase in mortality ($p < 0.005$). The cumulative mortality rate at the time of terminal sacrifice was 23 and 52% for the cage control and treated groups, respectively. The females did not show a significant difference in mortality (93% - treatment vs. control - 87%) at the end of the study although mortality rates were increased through week 91. Table 26 summarizes the mortality data of the study as a function of time. Figures 8 and 9 present the survival curve as a function of time. No tumor-related deaths were observed. Left atrial thrombosis was a major cause of death. The respective prevalence of the left atrial thrombosis in the cage control and the roflumilast-treatment group was 40% and 10% in males and 60% and 15% in females.

Table 26. Accumulative Mortality (%) – Study 233/2003 [a]

Treatment	Male				Female			
	0 - 52	53 - 78	79 - 91	92 - 104	0 - 52	53 - 78	79 - 91	92 - 104
Control	0	0	3.3	23.3	3.3	10.0	50.0	93.3
Roflumilast	11.7	16.7	23.3	51.7	25.0	41.7	61.7	86.7

a. Source: Dr. Ted Guo's review: Tables 14 and 17.

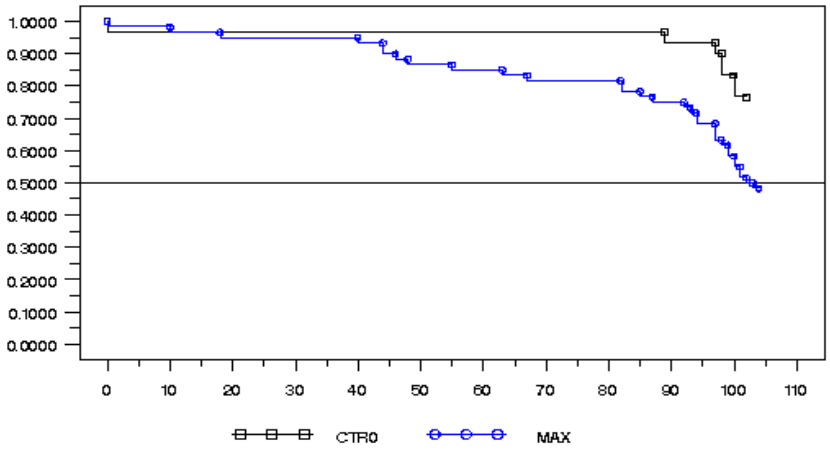


Figure 8. Kaplan-Meier survival curve of the 2-yr dietary carcinogenicity study of roflumilast in male hamsters.

CTR0, and MAX represent the cage control and 16 mg/kg/day of roflumilast, respectively.

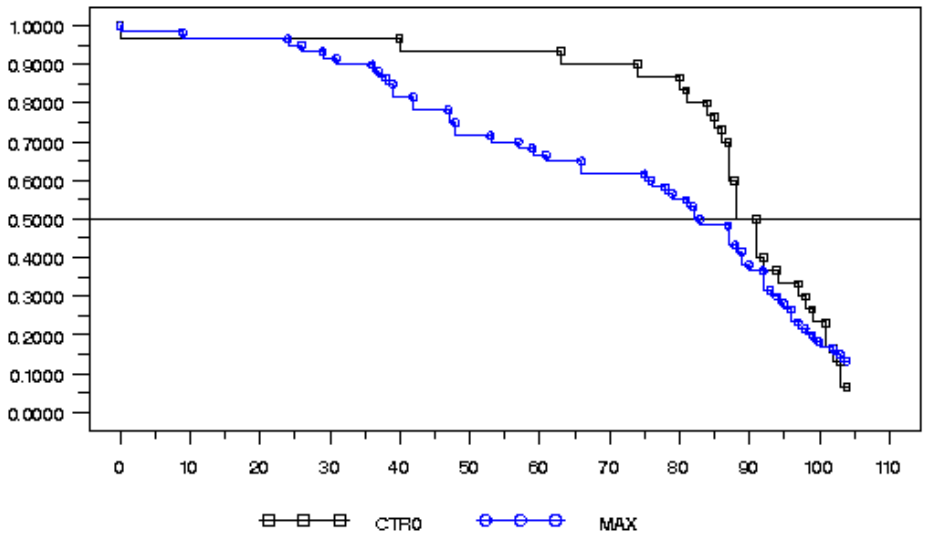


Figure 9. Kaplan-Meier survival curve of the 2-yr dietary carcinogenicity study of roflumilast in female hamsters.

CTR0 and MAX represent the cage control, and 16 mg/kg/day of roflumilast, respectively.

Clinical signs: No remarkable findings were observed.

Body weight: The treated hamsters showed statistically significant decreases in body weight starting from Week 7 of the treatment ($p < 0.05$). Table 27 (below) presents the body weight at the several milestones of the study. The decrease in body weight was 14% and 23% on day 727 for the male and female, respectively.

Table 27. Mean Body Weight

Sex	Male [a]				Female			
Treatment	Cage control		Roflumilast		Cage control		Roflumilast	
Parameter	Grams	N	Grams	N	Grams	N	Grams	N
Day -1	91.2	30	90.4	60	82.3	30	83.3	60
Day 48	111.3	30	109.2	60	98.3	30	102.1*	60
Day 363	139.8	30	125.5*	53	126.8	29	122.4*	45
Day 545	139.8	30	127.2*	50	132.6	27	126.4*	35
Day 727	142.0	23	122.1*	30	149.5	4	115.1*	9

a. Source: Vol. 59.12, pages 3 – 12.

Health Status: No remarkable findings.

Hematology: The treated male hamsters showed statistically significant decreases in red blood cell numbers, hemoglobin concentration and hematocrit; and increases in platelet and lymphocyte numbers (Table 28, below). A number of changes were observed in the females, but these changes may not be toxicologically significant, given the small sample size (N = 2 for the vehicle control) and small magnitude of the changes.

Table 28. Noticeable Hematologic Findings – Study 233/2003

Sex	Male [a]				Female			
Treatment	Cage control (N = 22)		Roflumilast (N = 27)		Cage control (N = 2)		Roflumilast (N = 8)	
Parameter	Mean	SD	Mean	SD	Mean	SD	Mean	SD
RBC (Tril./L)	7.79	1.10	7.07**	0.58	6.97	0.26	6.88	0.56
Hb (mmol/L)	9.5	1.1	8.6**	0.6	9.0	0.4	8.2	0.6
HCT (%)	45.0	5.8	40.6**	3.0	41.9	1.6	39.5	2.9
MCV (fl)	57.8	1.9	57.5	2.4	60.1	0.1	57.5*	2.2
MCH (fmol)	1.22	0.04	1.22	0.03	1.29	0.00	1.19*	0.04
MCHC (mmol)	21.2	0.5	21.2	0.5	21.4	0.0	20.8*	0.3
Platelet (G/L)	762	179	908**	136	719	27	1034	221
Lymph (G/L)	2.81	0.78	3.53**	0.80	2.76	0.94	4.04	1.18

a. Source: Vol. 59.12, pages 13 – 14.

Gross pathology: No remarkable findings were observed.

Non-neoplastic changes: The treated hamsters showed basal cell hyperplasia of the olfactory epithelium in the Bowman's gland of the nasal and para-nasal cavities. The respective incidence of the lesion in the control and treated groups was 5/30 and 22/60 in the males and 2/30 and 23/60 in the females. The respective percentage of animals with the lesion in the control and treatment groups was 16.7% and 36.7% in the male and 13.3% and 38.3% in the females.

Neoplastic changes: The treated hamsters showed non-statistically significant increases in the incidence of tumors in the nasal cavity, forestomach, and uterus, when compared to the concurrent cage control. Dr. Ted Guo performed the statistical analysis in September 9, 2004 (see review, attached). Table 29 (next page) presents the notable tumor findings of the study. The findings are evaluated further with considerations to the results of the other completed study in the same strain of animals. This evaluation can be found in the Interpretation and Evaluation of Hamster Studies section.

In the nasal cavity, both sexes of the treated hamsters showed increases in the incidence of carcinoma, adenocarcinoma and adenoma. The total incidence of nasal tumors was 0/60 and 15/120 in the control and treated groups. The most significant increase was the incidence of undifferentiated carcinoma (5 for each sex of the treated animals).

In the uterus, the treated hamsters showed an increase in the incidence of leiomyoma (2/30-C vs. 10/60-T) but a decrease in the incidence of leiomyosarcoma (5/30-C vs. 2/60-T). The incidence of all uterus tumors between control and treated hamsters was comparable (7/30 vs. 12/60).

Table 29. Notable Tumor Incidence - Study 233/2003

Sex	Male		Female	
Treatment	Control (N = 30)	Roflumilast (N = 60)	Control (N = 30)	Roflumilast (N = 60)
Nasal and para-nasal cavity				
Carcinoma, [M] undifferentiated	0	5	0	5
Adenocarcinoma [M], of Bowman's gland	0	1	0	1
Adenoma [B], of Steno gland(s)	0	1	0	2
Total	0	7	0	8
Forestomach/ carcinoma [M], squamous cell	0	0	1	0
Papilloma (TA) [B], squamous cell	6	12	1	7
Uterus/ Leiomyosarcoma (TA), [M]			5	2
Leiomyoma (TA), [B]			2	10

In the forestomach, the roflumilast treated female hamsters showed an increase in the incidence of squamous papilloma of the squamous cell (1/30-C vs. 7-12/60-T). This tumor, however, appears to be spontaneous in nature and is not considered treatment-related. The incidence of the tumor in the treated group is similar to the background level of Study 7/2002: 6/60 – 7/60 in male controls and 5/60 – 9/60 in female controls. Tumor incidences in the treated groups were as high as 11/60 and 13/60. Such incidences were not found to be statistically different from the concurrent control. The tumor incidence in the forestomach of the treated hamsters in the current study is similar to that of Study 7/2202. Consequently, this increase is not considered to be of importance. No noticeable increases in other tumors were observed. Table 30 (below) presents the tumor burden of the study and Appendix 3 presents the overall tumor prevalence.

Table 30. Tumor Burden – Study 233/2003

Sex	Male [a]		Female	
Lesion	Control (N = 30)	Roflumilast (N = 60)	Control (N = 30)	Roflumilast (N = 60)
# hamster w/ tumors	24	44	29	37
# hamster w/ single tumors	10	24	11	14
# hamster w/ multiple tumors	14	20	18	35
# hamster w/ benign tumors	22	35	22	36
# hamster w/ malignant tumors	8	21	17	18
# hamster w/ metastasizing tumors	0	4	4	4
# Tumors - Total	41	65	63	85
# Tumors - benign	31	42	42	58
# Tumors - malignant	10	23	21	27
# Tumor - metastasizing	0	4	4	4
% hamster w/ tumors	80	73	97	62
% hamster w/ single tumors	33	40	37	23
% hamster w/ multiple tumors	47	33	60	38
% hamster w/ benign tumors	73	58	73	58
% hamster w/ malignant tumors	27	35	57	30
% hamster w/ metastasizing tumors	0	7	13	7

a. source: vol. 59.12, p 31.

Toxicokinetics: Plasma concentrations of roflumilast and roflumilast-N-oxide were determined on day 1 and week 12 with LC/MS/MS. Blood samples were collected from 12 hamsters at 0, 1, 2, 3, 4, 6, 8 and 24 hours post dosing. Sex of the samples was not identified. Table 31 summarizes the AUC and Cmax for roflumilast and roflumilast-N-oxide. The plasma concentrations of both compounds increased significantly at week 12 when compared to day 1. Roflumilast AUC at week 12 was 7 times that on day 1. Roflumilast-N-oxide AUC was approximately 3 times that on day 1. The report was silent on the reason for the difference. Note that such a difference was not observed in the initial study.

Table 31. Plasma concentrations of Roflumilast and Roflumilast–N-oxide – Study 233/2003

Time	AUC (µg.h/L) (n = 12)		Cmax (µg/L)	
	Roflum	R-Oxide	Roflum	R-Oxide
Day 1	75.4	4,243	4.34	404.1
Week 12	519.8	11,336	37.9	1,043.2
Mean	397.6	7,789.5	21.1	723.7

Evaluation and Interpretation of Study 233/2003

Results of the study showed that hamsters (60/sex) treated with 16 mg/kg/day of roflumilast for 24 months had non-statistically significant increases in tumor incidence in the nasal and para-nasal cavity in both sexes and uterine leiomyomas in females. The tumors in the nasal cavity included carcinoma, adenocarcinoma and adenoma. Undifferentiated carcinomas accounted for most of the tumor incidence (0/30 in conrol and 5/60 in treated group in each sex). The incidence of leiomyomas in the uterus was 2/30 and 10/60 for the cage control and roflumilast-treated groups.

Causal effects of tumors in the nasal cavity and uterus cannot be readily ascertained due to the lack statistical significant difference in tumor incidences between the groups and inherent flaws of the study. Nor can the study be regarded as a complete and independent study because of flaws in study design: it lacks a vehicle control group; the sample size of the cage control is rather small; too few females survived the study to yield a meaningful statistical analysis - only 2 and 8 hamsters for the cage control and treated groups were available for terminal sacrifice. The Interpretation and Evaluation of the Hamster Studies section (later) provides a detailed discussion of the study results.

INTERPRETATION AND EVALUATION OF HAMSTER STUDIES

Roflumilast at oral doses of 8 and 16 mg/kg/day for 2 years in hamsters causes nasal tumors in both males and females. The carcinogenic potential of roflumilast in Syrian hamsters was evaluated in two 2-year traditional studies (Studies 7/2002 and 233/2003). The two studies

complement each other (Table 29, below) to comply with the Executive CAC's recommendation on the selection of the top roflumilast doses.[3]

Table 32. Overall Design of Roflumilast Carcinogenicity Studies in Hamsters

Study No.	Group Code	Treatment	Roflumilast (mg/kg/day)	Hamster# /sex/dose	Dose volume (ml/kg)
7/2002	G12/13	Sentinel	-	30	-
	A1/2a	Cage control	0	60	-
	F11/12	Vehicle control	0	60	5
	B3/4	Roflumilast	0.25	60	5
	C5/6	Roflumilast	1.0	60	5
	D7/8	Roflumilast	4.0	60	5
	E9/10	Roflumilast	8	60	5
233/2003	I17/18	Roflumilast	16	60	5
	H15/16	Cage control	0	30	-
	K19/20	Sentinel	-	22	-

a. Numbers before and after the slash represent the male and female subgroups, respectively.

Study 7/2002 was initiated at roflumilast doses of 0.25, 1, 4 and 8 mg/kg/day in both sexes in March 1999. Such a dose selection in males did not comply with the executive CAC recommendation of 16 mg/kg/day. Seventeen months into Study 7/2002, the sponsor initiated Study 233/2003 at a roflumilast dose of 16 mg/kg/day in both males and female hamsters in August 2000. Study 233/2003, however, is an abbreviated study that consisted of only one roflumilast group (60/sex) and a cage control group (30 hamsters/sex). Study 233/2003 was conducted in an attempt to satisfy the Agency's recommendation for the top roflumilast dose in males but it also complicated the data interpretation due to its study design and timing. A retrospective view is that Study 233/2003 might not be needed because the first study (Study 7/2002) had achieved the maximum tolerated dose.

Conclusions about the tumorigenic potential of roflumilast vary depending on whether the above two studies are analyzed separately or considered in combination. Tumors of interest of these studies are the nasal and uterine tumors. In the nasal tumor case, separate analyses of the individual studies showed that roflumilast is carcinogenic in female hamsters only, while consideration of the studies together showed strong evidence of tumorigenic effect of the drug in both sexes. In the uterine tumor case, both studies showed statistically significant and dose-related increases in the incidence uterine leiomyomas. However, the combined incidence of uterine leiomyomas and leiosarcomas was not increased. Table 31 (below) presents the prevalence of nasal tumors in both studies. Table 32 (page 50) summarizes the uterine tumor data.

The review team sought advice from the Executive CAC regarding appropriateness of pooling the data for analysis and interpretation. The committee recommended against such a practice

[3] The Executive CAC recommended the top roflumilast dose to be 16 and 8 mg/kg/day in males and females, respectively. The committee's recommendation was based on changes in body weight gain in a 13-week oral toxicity study in the same strain of hamsters (see CAC minutes dated February 9, 1999). Roflumilast in the dose ranging study was 4, 8 and 16 mg/kg/day. The low, mid and high dose groups showed respective decreases in body weight gains by 0%, 10% and 20% in males and 15%, 21% and 34% in females, compared to the vehicle control.

citing possible confounding factors in such a practice. Dr. Ted Guo (the statistical reviewer) objected to the Committee's recommendation (source: Email message from Dr. Guo on May 11, 2005). The following analyses are done in compliance with the CAC recommendations. Each study is treated as an independent study. Information from other studies is considered on its scientific merits.

Treatment Related Findings: ***Nasal tumors***

Oral roflumilast treatment for 2 years causes the undifferentiated nasal carcinomas in hamsters at doses of ≥ 8 mg/kg/day, but not at ≤ 4 mg/kg/day. Table 33 (below) presents the prevalence of notable nasal tumors in hamster studies. Study 7/2002 showed that females treated with 8 mg/kg/day roflumilast had a statistically significant increase in the incidence of undifferentiated carcinomas in nasal cavity (p = 0.0006). Study 233/2003 revealed statistically non-significant increases in the incidence of all tumors and undifferentiated carcinomas in the nasal cavity. The total incidence of nasal tumors (carcinoma, adenocarcinoma and adenoma) in the cage control and roflumilast treated groups was 0/30 and 7/60 in males and 0/30 and 8/60 in females. Undifferentiated carcinomas accounted for the majority of the tumors (5 each per sex). Statistical analysis against the cage control did not reveal any positive trend in tumor incidence, although a lower incidence (4/60) had been found to be positive in the original study (Study Report 7/2002). The results were presented to the Executive CAC. The Committee determined that the nasal tumor is a treatment-related effect although the second study did not reach statistical significance. The conclusion was based on the rarity of nasal tumor in the hamsters, the dose-response relation in the current studies, and the statistical significance of study 7/2002.

Table 33. Percentage of Hamsters with Nasal Tumors

Tumor	Sex	Study No.	Control		Roflumilast (mg/kg/day) [a]					P-Value (Exact)
			Cage	Veh.	0.25	1	4	8	16	
Carcinoma [M], undifferentiated	M	7/2002	1.7	0	0	0	0	0	-	NS
		233/2003	0	-	-	-	-	-	8.3	0.0587 [c]
	F	7/2002	0	0	0	0	0	6.7	-	.0006 [b]
		233/2003	0	-	-	-	-	-	8.3	.081 [c]
Adenocarcinoma, Bowman's gland [M]	M	233/2003	0	-	-	-	-	-	1.7	NS
	F	233/2003	0	-	-	-	-	-	1.7	NS
Adenoma, Bowman's gland [B]	M	233/2003	0	-	-	-	-	-	1.7	NS
	F	233/2003	0	-	-	-	-	-	3.3	NS

a. n = 60 hamsters/sex except for the cage control for Study B (233/2003) which consists of 30 hamsters/sex.
b. Against vehicle control (Asymptomatic method).
c. Against cage control (exact method).

TREATMENT-UNRELATED FINDINGS

Other tumors which increase statistically significantly or non-significantly are incidental and not treatment-related. These tumors include uterine leiomyomas and tumors in the larynx, forestomach, mammary gland and uterus. The following section discusses the interpretation of the tumor findings.

Uterine tumors

Female hamsters treated with 8 and 16 mg/kg/day of roflumilast orally for 2 years showed statistically significant and dose-related increases in the incidence of uterine leiomyomas. The increase in the combined incidence of leiomyomas and leiosarcomas, however, did not reach statistical significance. Table 34 (below) presents the prevalence the uterine tumors in these studies. Figure 10 (next page) presents the time-course of leiomyoma detection of the studies. Leiomyomas also occurred at an earlier time than the controls and lower dose groups. The data suggest that the induction of leiomyomas may be a roflumilast treatment-related effect.

Table 34. Percentage of Female Hamsters with Uterine Tumors

Tumor	Study No.	Control		Roflumilast (mg/kg/day) [a]					P-Value (Exact)
		Cage	Vehicle	0.25	1	4	8	16	
Leiomyoma [B]	7/2002	5	3.3	3.3	6.7	6.7	15	-	0.0030
	233/2003	6.7						16.7	0.0242
	Mean	5.6	3.3	3.3	6.7	6.7	15	16.7	0.0000 [b]
Leiomyosarcoma [M]	7/2002	3.3	1.7	0	5.0	6.7	3.3	-	NS
	233/2003	16.7	-	-	-	-	-	3.3	NS
	Mean	7.7	1.7	0	5.0	6.7	3.3	3.3	NS
Total	Mean	13.3	5.0	3.3	11.7	13.3	18.3	20.0	0.0000

a. n = 60/sex/dose except for the cage control in Study 233/2003 which has 30 hamsters/sex.
b. P = 0.0000 indicates that the p value is smaller than 0.0001.

The sponsor argued that there was no treatment-related effect in regard to uterine tumors in hamsters. Rationales for the argument were presented in a report entitled "Assessment of the Tumorigenic Potential of Roflumilast (vol. 59.1, pages 1-28). The rationales are:

1. The spontaneous rate of leiomyomas in hamsters is similar to that of background in hamsters.
2. There is no evidence of shortened tumor latency or increased incidence of malignant tumors with dose.
3. There is no increase in the combined incidence (prevalence) of uterine leiomyomas and leiomyosarcomas.
4. Repeat-dose toxicity studies do not reveal pre-neoplastic lesions in hamsters.
5. There is no evidence of increased incidence of smooth muscle cells at other locations in the hamster carcinogenicity studies.
6. No compound-related uterine neoplasia is seen in the mouse carcinogenicity study.

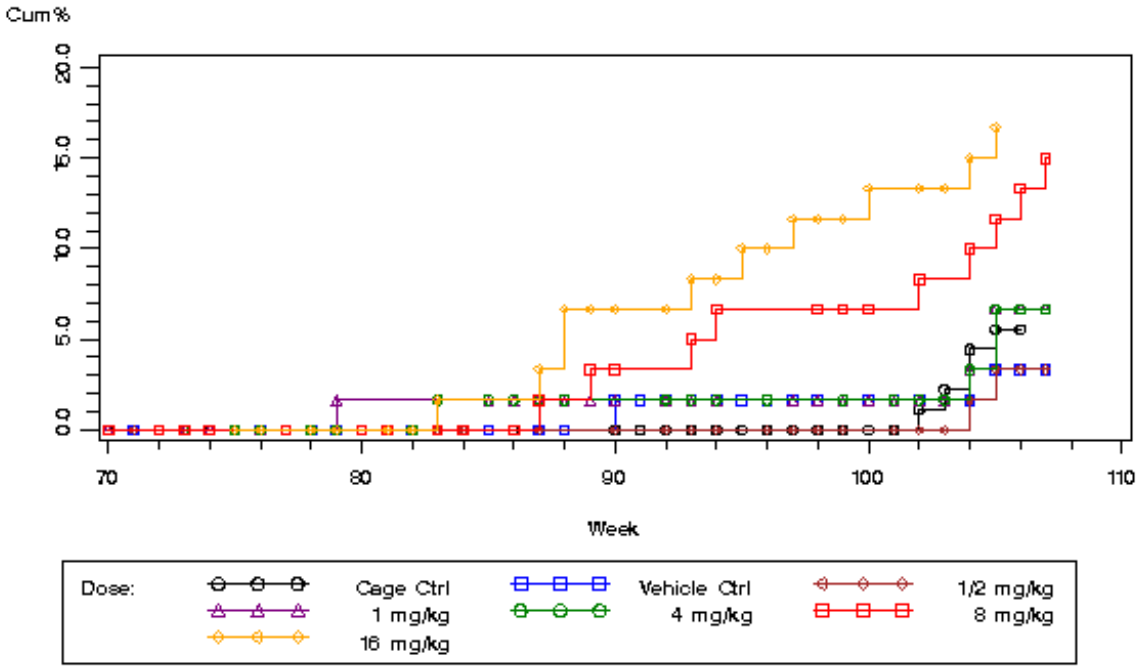


Figure 10. Time-course of uterine leiomyomas in the 2-yr oral gavage carcinogenicity studies of roflumilast in female hamsters – Studies 7/2002 and 233/2003 combined.

N = 60/dose except for the cage control that has 90 hamsters. The cage control is pooled data of Studies 7/2002 and 233/2003. (source: Figure 19 in Dr. Ted Guo's review)

Evidence supporting the background tumor rate includes studies by Sher et al. (1982)[4], Pour et al. (1976)[5], Kirkman (1972)[6] and Kamino et al. (2001)[7]. The prevalence of leiomyomas in these references is generally low (< 3%), with exception of Kamino (2001) who reported a rate of approximately 10%. Kamino et al. observed tumor developments over a natural life span of 184 female hamsters. Ninety three hamsters (about 51%) died during the period of weeks 66 – 104. Nine of them (about 10%) showed uterine leiomyomas. The prevalence of uterine leiomyomas was 0 - 15% (Table 35, below).

Table 35. Spontaneous Rate of Leiomyomas in Syrian Hamsters (Han:AURA)

Time (weeks)	≤ 52	53-66	66-78	79-91	92-104	105-117	≥ 118	Total
# Hamsters died	9	7	18	21	54	53	22	184
# with Leiomyomas	0	0	0	1	8	8	1	18
% with leiomyomas	0	0	0	5	15	15	8	10

The life span of hamsters in the Kamino study, however, differs somewhat from the current study. Hamsters in the current study have a considerably shorter life span. Approximately

[4] Sher, SP. Tumors in control hamsters, rats and mice: literature tabulation, Crit Revi. Toxicol. 1862;10(1) 49-79.
[5] Pour, P et al. Spontaneous tumors and common diseases in two clolonies of Syrian hamsters. III Urogenical system and endocrine glands. J National Cancer Inst. 1976;25:949-961.
[6] Kirkman H Hormone-related tumors in Syrian Hamsters Progr Exp Tumors Res 1972;16:201-240.
[7] Kamino K et al., Spectrum and age-related incidences of spontaneous tumors in a colony of Han:AURA hamsters. Exp Toxic Pathol, 2001;52:539-544.

50% of hamsters in the Kamino study survived beyond 104 weeks. Less than 13% of hamsters in study 7/2003 survived to Week 104. Also, the roflumilast studies have larger sample sizes. The sum of female hamsters in the controls and low, mid-low and mid doses is 330, a number 3.3 times that of the Kamino study. The current study has a prevalence rate of 5.6% uterine leiomyomas in female hamsters. This rate is closer to the rate of other references than the Kamino study. The Executive CAC considers the argument of background incidence reasonable.

Data from the roflumilast studies appear to contradict the sponsor's argument of a lack of shortening of the tumor latency period of leiomyomas. Figure 8 (previous page) shows that roflumilast treatment at 8 and 16 mg/kg/day shifted the tumor prevalence curve to the left. The 16 mg/kg/day group has the largest increase in tumor rate and earliest time of occurrence. The 8 mg/kg/day group follows the trend but to a lesser degree. No significant changes are noticed at roflumilast doses of 4 mg/kg/day or less. The Executive CAC determined that it was not appropriate to conduct analysis based on pooled data.

The sponsor also argues that uterine leiomyomas are not a treatment-related effect because there is no evidence of dose-related incidence of leiomyosarcomas or the combined incidence of leiomyomas and leiomyosarcomas (Table 34, previous page). Leiomyoma is a benign tumor while leiomyosarcoma is a malignant tumor. Some drugs such as beta adrenergic agonist are known to cause leiomyomas without subsequent transformation to malignant leiomyosarcomas. The effect of roflumilast on hamster uterine is, however, unknown.

Overall, the executive CAC considered the sponsor's argument acceptable, given the lack of increase in the incidence of leiomyosarcomas. The review will not address the remaining arguments.

Other Tumors

Squamous Papillomas in Forestomach

Study 233/2003 showed a non-statistically significant increase versus cage controls in the incidence of squamous papillomas in the forestomach in the 16 mg/kg/day females (P = 0.077). The incidence of squamous papillomas was 1/30-control and 7/60-roflumilast. Such a prevalence of the tumor in the female is similar to that in the males (6/30 and 12/60 for the control and roflumilast-treated groups, respectively) of the same study. It is also comparable to that in females in Study 7/2002 (range: 5/60 – 13/60). Table 36 presents the tumor prevalence in these two studies. The increase in the forestomach tumor in female hamsters in Study 233/2003 is not considered a roflumilast treatment-related effect.

Table 36. Percentage of Hamsters with Forestomach Tumors

Tumor	Sex	Study No.	Roflumilast (mg/kg/day) [a]						
			Cage	Veh.	0.25	1	4	8	16
Carcinoma [M], squamous cell	F	7/2002	0	0	0	0	0	1.7	-
		233/2003	3.3	-	-	-	-	-	0
		Mean	1.1	0	0	0	0	1.7	0
Papilloma [B] squamous cell	M	7/2002	11.7	10	15	18.3	18.3	18.3	-
		233/2003	20	-	-	-	-	-	20
		Mean	14.4	10	15	18.3	18.3	18.3	19.2
	F	7/2002	8.3	15	15	16.7	21.7	10	-
		233/2003	3.3	-	-	-	-	-	11.7
		Mean	6.7	15	15	16.7	21.7	10	11.7

a. n = 60 hamsters/sex except for the cage control for Study B (233/2003) which consists of 30 hamsters/sex.
b. Including a myxomatous fibrosarcoma [M] in a female hamster in Study 7/2002.

Neuroendocrine Adenomas in Larynx

Male hamsters receiving 8 mg/kg/day of roflumilast in Study 7/2002 showed a statistically significant increase in the incidence of benign neuroendocrine tumor in larynx (P = 0.021). The prevalence of the tumor in the group, however, is comparable to that of the cage control females in Study 233/2003. Also, neither sex in the 16 mg/kg/day group in Study 233/2003 showed such a tumor. Table 37 (below) presents the prevalence of the tumor in both sexes of two studies. The lack of a dose-response indicates that the neuroendocrine tumor observed in the larynx of the 8 mg/kg/day males is not a treatment-related effect.

Table 37. Percentage of Hamsters with Neuroendocrine Tumors in Larynx

Tumor	Sex	Study No. [b]	Roflumilast (mg/kg/day) [a]						
			Cage	Veh.	0.25	1	4	8	16
Malignant	M	7/2002	0	1.7	0	0	0	1.7	-
		233/2003	0	-	-	-	-	-	0
		Mean	0	1.7	0	0	0	1.7	0
Benign	M	7/2002	0	0	0	0	0	3.3	-
		233/2003	0	-	-	-	-	-	0
		Mean	0	0	0	0	0	3.3	0
	F	7/2002	0	0	1.7	1.7	0	1.7	-
		233/2003	3.3	-	-	-	-	-	1.7
		Mean	2.2	0	1.7	1.7	0	1.7	1.7

a. n = 60 hamsters/sex except for the cage control for Study B (233/2003) which consists of 30 hamsters/sex.

Mammary Gland Adenocarcinoma and Uterine Adenosquamous Carcinoma

Dr. Ted Guo's statistical analysis also identifies positive trends in adenocarcinoma in the mammary glands and adenosquamous carcinoma in the uterus in female hamsters. Table 38 presents the incidence of these tumors. The prevalence of these tumors in the MHD and HD groups are not remarkably different from that of the control groups. These tumors are not considered to be treatment-related or toxicologically significant from the nonclinical viewpoint.

Table 38. Other Noticeable Tumors (Incidence) in Female Hamsters

		Roflumilast (mg/kg/day)						
		Cage	Veh.	0.25	1	4	8	16
N		90	60	60	60	60	60	60
Mammary gland	Adenocarcinoma [M]	0	1	1	0	0	1	2
Uterus	Adenosquamous Carcinoma [M],	0	0	0	0	0	1	1

Overall, the undifferentiated nasal carcinomas associated with roflumilast treated in hamsters are considered a treatment-related effect. Other tumors are considered incidental and not treatment-related.

Achievement of the MTD

The dose selection of roflumilast in hamsters appears to have achieved or exceeded the MTD. This conclusion is based on the mortality data and decreases in body weight. Dose-related increases in mortality and decreases in body weight were observed in both males and females.

a. Mortality

Dose-related increases in mortality were observed in both males and females. In males, the cumulative mortality at the terminal sacrifice time (Day 728 – 742) was 22%, 18%, 18%, 20%, 33%, 35% and 50% for the cage control, vehicle control and 0.25, 1, 4, 8 and 16 mg/kg/day of roflumilast, respectively.[8] Mortality data indicate that the 16 mg/kg/day dose has exceeded the MTD in males. One could argue that even the 8 and 4 mg/kg/day achieved or exceeded the MTD. The evidence for the increased mortality in the 4 and 8 mg/kg/day dose, however, was not apparent to the sponsor, nor to the Agency, until week 80 (approximately October 20, 2000) or later when the 16 mg/kg/day treatment had already been started.[9] If one agrees that 4 and 8 mg/kg/day groups both have achieved the MTD, there would have been no need to conduct the second study (Study 23/2003).

In females, dose-related increases in mortality existed although only a portion of Study 7/2002 showed such a trend. The cumulative mortality at 18 months was 9%, 15%, 12%, 15%, 17%, 25% and 40% for the cage control, vehicle control and 0.25, 1, 4, 8 and 16 mg/kg/day of roflumilast, respectively. This trend was no longer apparent by the end of the study; the cumulative mortality was similar across groups (in percentage): 68% (mean of 58 and 93% for Studies 7/2002 and 233/2003, respectively), 73%, 75%, 73%, 65%, 73% and 87% for the cage control, vehicle control and 0.25, 1, 4, 8 and 16 mg/kg/day of roflumilast, respectively). The lack of dose-response in survival rate in the final sacrifice in females suggests that high mortality may be spontaneous in nature in females. However, a close examination of the mortality data, especially mortality from week 45 and onward showed a clear dose-response relationship. There was a statistically significant increase in the mortality in the 16-mg/kg/day group.

[8] Note the mortality rate for the cage control and the 16 mg/kg/day groups differ slightly from the review of individual study reports. The mortality for the cage control was 22% and 23% for studies 7/2002 and 233/2003, respectively. The morality in the 16 mg/kg/day group was listed as 52%. The difference apparently was attributed to the data pooling for statistical analysis and cut-off time for the sacrifice in the date pooling.

[9] The treatment of Study 7/2002 was started on April 6, 1999. Study 233/2003 was initiated on August 27, 2000 and the treatment was started on September 27th, 2000.

Body weight

Both males and females treated with 16 mg/kg/day of roflumilast also showed statistically significant decreases in body weight compared with the cage control ($P < 0.05$). The reduction on day 727 was 14% and 23% in males and females, respectively. There was no significant difference in body weight between the controls and 8 mg/kg/day hamsters.

The high mortality and body weight reduction in hamsters in both sexes receiving 16 mg/kg/day roflumilast indicates that this dose has exceeded the maximum tolerated dose. The 8 mg/kg/day dose also appears to have achieved or exceeded the MTD. Overall, the studies have achieved or exceeded the MTD of roflumilast in rats.

Relevance of Hamster Tumor Findings to Humans

The nasal tumors associated with roflumilast treatment may not be relevant to humans. This conclusion is based on the difference in roflumilast metabolism between rodents (including hamsters) and humans. Hamsters treated with 8 and 16 mg/kg/day of showed statistically significant, dose-related increases in undifferentiated carcinomas in the nasal cavity. The sponsor concluded that these tumors are not relevant to humans. The Executive CAC concurred with the sponsor in this regard. The following two documents summarize the sponsor rationales.

1. Assessment of the Tumorigenic Potential of Roflumilast (B9302-107). Report Date of September 18, 2003 by Gerd Bode et al. of Altana, vol. 59.1, pages 1 – 33.
2. Summary Report/Overview/Assessment: Roflumilast: Causal Relationship between Olfactory Metabolism and Olfactory Toxicity in Rodents. Report No. 159/2002. vol. 59.1, pages 34 – 60.

Rationales supporting the conclusion that nasal tumors in hamsters are not relevant to humans include: 1) roflumilast metabolic pathways differ between rodents and humans, 2) physiology and biochemistry of nasal epithelium in rodents and humans differ, 3) tumorigenicity of roflumilast is attributed to ADCP-N-oxide that is negligible in humans and 4) hamsters possess much higher plasma AUCs of roflumilast and its metabolites than humans. The following section briefly describes each rationale.

1) Roflumilast metabolic pathways in animals and humans.

The report extends the metabolic pathways of roflumilast from one compartment (plasma) to three tissue compartments: plasma, nasal epithelium and urine. The report indicates that roflumilast pathways vary with the tissue compartments (Figure 11, next page). In plasma, roflumilast is metabolized to roflumilast-N-oxide (M07), B9302-077, and ADCP by enzymes CYP1A2 and CYP3A4. In rodent nasal epithelial cells, CYP2G1 further converts ADCP ADCP-N-oxide. ADCP-N-oxide is further converted to an unstable epoxide intermediate by the same enzyme. The epoxide intermediate will react with -SH groups of glutathione or proteins to form protein adducts. Human nasal tissues lack the enzyme that converts ADCP to ADCP-N-oxide.

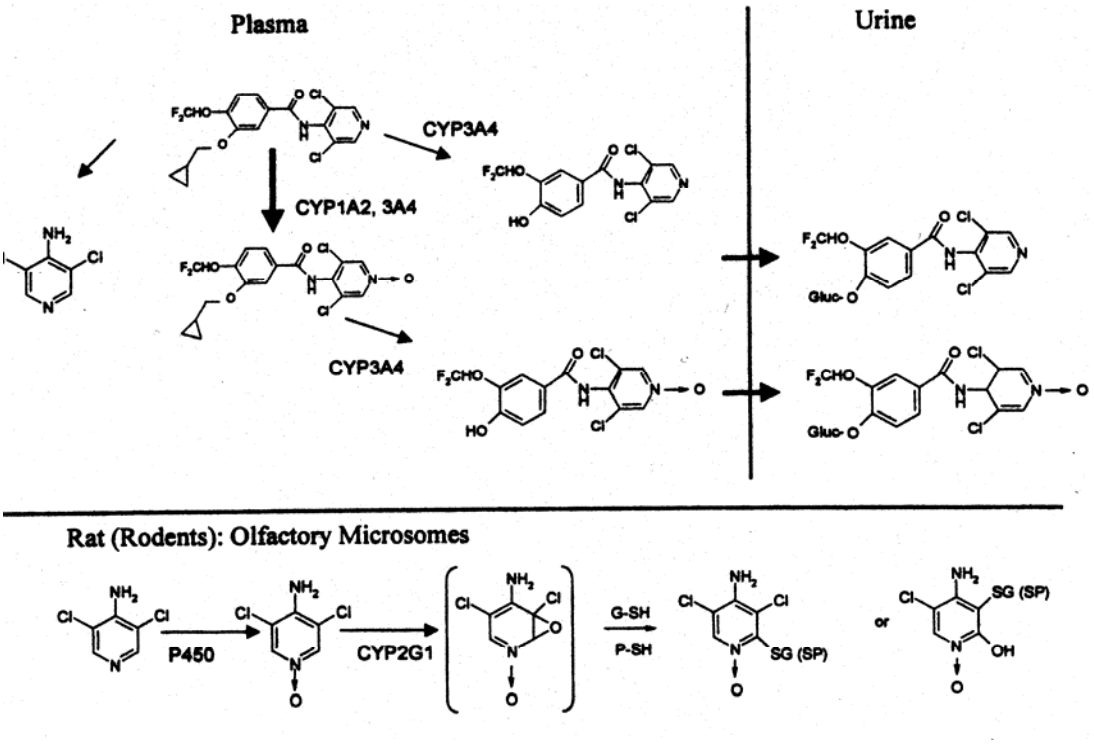


Figure 11. **Hypothesized metabolic pathways of roflumilast.**

Compounds in the plasma compartment are roflumilast (top), roflumilast-N-oxide (or M07, Middle), B9302-077 (top right), and ADCP (left) by CYP1A2 and CYP3A4.

2) Physiology and biochemistry of nasal epithelium in animals and humans

Nasal epithelium of rodents, monkeys and humans metabolizes ADCP, a metabolite of roflumilast, differently. The rodent nasal epithelium contains CYP2A3 that converts ADCP to ADCP-N-oxide. The latter is further converted by CPY2G1 to a reactive intermediate species, epoxide (See item 4, below, for additional discussion). Epithelial cells of monkeys and humans do not produce ADCP-N-oxide because of the lack of counterpart enzymes. The capacity of olfactory and lower respiratory tract epithelia (and liver) of species of interest in converting ADCP to ADCP-N-Oxide was studied *in vitro*. Microsomes from above tissues were incubated with 100 ng/ml of ^{14}C-ADCP for 2 hours. The rate of ADCP disappearance and ADCP-N-oxide formation was determined. The production of ADCP-N-oxide varies with species and tissues. Table 39 (below) summarizes the results. The results show that the mouse, rat and hamster tissues metabolize ADCP rapidly, while the human tissue metabolizes slowest. A negligible amount of ADCP-N-oxide is formed in human tissues. The human liver, which has the highest capacity of ADCP-N-oxide formation, transforms only 2% of ADCP into ADCP-N-oxide. The data demonstrate that the rodents produce significantly more ADCP-N-oxide than humans. Rats also concentrate the ADCP in the nasal region (Study Report No. 159/2002, vol. 59.1, p 46). Finally, significant lesions observed in the early toxicity studies can eventually lead to tumor formation.

Table 39. Metabolism of ADCP and Formation of ADCP-N-oxide by Microsomes

Microsome	% ADCP catabolized [a]	% ADCP-N-oxide formed

source	Olfactory	Respiratory	Liver	Olfactory	Respiratory	Liver
Rat	98	75	9	92	72	4
Mouse	82	93	92	78	88	88
Hamster	96	77	94	89	77	93
Dog	98	98	4	92	95	1
Monkey	30	10	71	1	< LLOQ [b]	15
Human	12	10	43	< LLOQ	< LLOQ	2

a. Source: vol. 59.1, page 49.
b. LLOQ, the low limit of quantitation = 0.5 pmoles ADCP equivalents equating to a rate of metabolism of 0.5 pmoles/hr/mg protein.

3) Tumorigenicity of ADCP-N-oxide

The report does not provide any direct evidence of ADCP-N-oxide carcinogenicity in hamsters. Carcinogenicity of ADCP-N-oxide is based on indirect evidence that includes:

1) Neoplastic and pre-neoplastic lesions of the nasal cavity occur only in species that produce ADCP-N-oxide, a reactive intermediate. Rodents (i.e., mice, rats and hamsters) possess enzyme CYP2G1 that convert ADCP to ADCP-N-oxide. Human (also dogs and monkeys) nasal cavity does not produce such a compound due to the lack of similar enzymes. *(Comment: The mouse carcinogenicity data of roflumilast does not fit into this hypothesis. Roflumilast does not induce nasal tumors although severe nasal lesions were observed after repeat-dose treatment with roflumilast).*

2) Binding to nasal tissue is detected in rats receiving ADCP-moiety labeled roflumilast, but not the benzyl-moiety labeled. Neither did the binding occur in humans when given the benzyl-moiety labeled roflumilast. *(Comment: the binding studied is in rats, not hamsters.)*

3) The rats uptake roflumilast readily. The ratio of nasal tissue to plasma roflumilast concentrations in rats is approximately 5. The ratio is about 1 in mice and hamsters. Note the ratio in humans is less than approximately 0.5.

4) ADCP-N-oxide is likely responsible for the nasal toxicity of roflumilast. Rodent CYP2G1 converts ADCP-N-oxide to an epoxide intermediate. The latter reacts with the -SH group of the protein and forms protein adducts. Nasal toxicity of roflumilast can be modified by compounds that change the intracellular glutathione concentration. Phorone depletes intracellular glutathione concentration. Co-administration of phorone with 5 mg/kg roflumilast or 0.5 mg/kg ADCP enhances the nasal toxicity of these compounds. Metyrapone inhibits cytochrome P450 activity. Co-administration of metyrapone prevents or alleviates the nasal lesion of roflumiast (100 mg/kg) or ADCP (6 mg/kg) from occurring. Similar observations were made with dichlobenil and ADCP derived from piclamilast.

5) Piclamilast, another PDE4 inhibitor, exhibits similar nasal toxicity profile. The nasal toxicity of piclamilast is also modified by dichlobenil and piclamilast-derived ADCP in similar fashion.

4. Plasma AUCs comparison of roflumilast and metabolites in animals and humans.

The apparent difference in the roflumilast metabolic pathways across species results in significant differences in the plasma levels of roflumilast and its metabolites in animals and humans. The most noticeable difference is the level of ADCP-N-oxide. Table 36 (below) summarizes plasma levels of roflumilast and its metabolites in animals and humans. Significant amounts of the metabolite are present in the rodent plasma, but not in the plasma of non-rodents such as dogs, monkeys and humans. Roflumilast-N-oxide concentration is the highest in all species but dogs. The level of ADCP is below the low limit of quantitation (i.e., 5.5, 1 and 3 μg/L in dogs, monkeys and humans, respectively). ADCP-N-oxide is not detectable in dogs, monkeys, or humans.

Table 40. Plasma Levels of Roflumilast and its Metabolites in Animals and Humans

Species	Rat	Hamster	Mouse	Dog	Monkey	Human
Roflumilast (mg/kg/day)	1.5	8	12	13	0.5	0.01
Sampling time (week)	4	13	26	13/52	13/42	1
	Plasma AUCs $_{0\text{-}24}$ (μg.h/l)					
Roflumilast	32.4	88.9	689.7	1056	792.0	32.9
Roflumilast-N-oxide	927.5	2781.6	2196.7	74.8	2384.9	351.4
ADCP	499.5	186.8	65.7	54.8	47.4	4.14
ADCP-N-oxide	470.5	873.1	503.5	<LLOQ[a]	<LLOQ	<LLOQ
Report No.	31/2001 44/2001 42/2002	147/99	197/2001	4/2001	108/2002	128E/97 177/98
NOAEL (mg/kg/day)	0.8	4	4	2.0	0.5	> 0.01

Source: Vol. 59.1, page 44.
a. LLOQ = low limit of quantitation: 5.5, 1 and 3 μg/L in dogs, monkeys and humans, respectively.

The above justifications appear adequate to explain the mechanism for nasal tumors in hamsters. They also appear to support a lack of relevancy of hamster nasal tumors to humans.

Conclusion:

Roflumilast is carcinogenic in hamsters. Male and female hamsters receiving 8 and 16 mg/kg/day of roflumilast orally for 24 months show increases in the incidence of nasal tumors.

APPENDCES

Appendix 1. Minutes of February 9, 1999 Executive CAC Meeting

Executive CAC,
February 9, 1999

Committee: Joseph DeGeorge, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-900, Alternate Member
Glenna Fitzgerald, Ph.D., HFD-120, Alternate Member
Joseph Sun, Ph.D., HFD-570, Team leader
Timothy McGovern, Ph.D., HFD-570, Presenting Reviewer
Adele Seifried, Project Manager

Author of Draft: Timothy McGovern

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

IND #: Pre-IND
Drug Name: Roflumilast (BY217)
Sponsor: Byk Gulden (represented by Altana)

Roflumilast is a PDE IV inhibitor, and is intended for the treatment of asthma. The sponsor sought Agency concurrence with their proposal to use the hamster rather than the rat for carcinogenicity testing due to the unique metabolic profile of Roflumilast and nasal cavity toxicity found in the rat. In addition, the sponsor sought concurrence for dose selection in hamster and mouse studies. The anticipated clinical dose is 0.5 mg per day. Roflumilast was negative in the Ames Assay, an in vitro human lymphocyte chromosome aberration assay, an HPRT test with V79 cells, and an in vitro micronucleus test with V79 cells, but was positive in the in vivo micronucleus test in mice. The metabolite, B9202-045 (dichloroaminopyridine, DCAP), tested negatively in the in vivo mouse micronucleus test.

Selection of hamster for carcinogenicity testing.

The sponsor proposed using the hamster in place of the rat for carcinogenicity testing with Roflumilast based upon comparative metabolic profiling in which significantly greater serum levels of the metabolite DCAP were detected in the rat versus the hamster, mouse or humans. The Agency concurs with the sponsor on the selection of the hamster based on the greater similarity of the metabolic profile of Roflumilast in hamsters to humans.

Dose selection for hamster carcinogenicity studies.

The sponsor proposed doses of 0.25, 1 and 4 mg/kg/day by oral gavage. The doses for the proposed 18-month study were selected based upon determination of the MTD from a 3-month oral gavage study in hamsters (doses of 0, 4, 8, and 16 mg/kg). Body weight gain was

reduced by 10 and 20% in mid- and high-dose males, respectively, and 15, 21 and 34% in low-, mid- and high-dose females, respectively. Only the effect in high-dose females was statistically significant. Nasal olfactory epithelium disorganization was observed at the mid- and high doses, but lesion severity was not convincing. Also, increased incidence and severity of male reproductive organ effects were noted at all treatment doses. However, the findings used by the sponsor to select the MTD (male reproductive organ effects at all doses and reduced body weight gain at the mid-dose in females) were not considered by the Committee to adversely influence animal survival. Doses of 16 mg/kg in males and 8 mg/kg in females were considered the MTD since body weight gain was significantly decreased at the high dose of 16 mg/kg in females in the 3-month study. The sponsor is also encouraged to include dual control groups unless sufficient contemporary historical data are available. In addition, the carcinogenicity study in hamsters should be extended to 24 months rather than the proposed 18 months. Since it is currently unclear as to what percentage reduction in body weight gain is detrimental to hamster survival, the high doses should be reduced in the event of severe toxicity. The sponsor should contact the Agency if significant mortality occurs in either the control(s) or in the treated groups.

Dose selection for mouse carcinogenicity studies.

The sponsor proposed doses of 0.5, 1.7 and 6 mg/kg/day by oral gavage. The doses for the 24-month study were selected based upon determination of the MTD from a 3-month oral gavage study in mice (doses of 0, 6, 12 and 18 mg/kg/day). Adrenal atrophy and hyperemia were noted at all doses in females and at doses of ≥ 12 mg/kg in males. In addition, nasal olfactory epithelial degeneration and olfactory cell necrosis were noted at the mid- and high-doses. However, the MTD selected by the sponsor, based upon histological findings in the adrenal gland and nasal cavity, was not considered by the Committee to adversely influence animal survival. Rather, doses of 18 mg/kg in males and 12 mg/kg in females were considered the MTD due to the increased incidence and severity of the adrenal and nasal lesions in the 3-month study.

Executive CAC Recommendations and Conclusions:

Species Selection:

1. The Agency concurs with the sponsor's proposal to use the hamster for carcinogenicity testing rather than the rat.

Hamster Study:

1. Doses of 16, 8 and 4 mg/kg in males and 8, 3, and 1 mg/kg in females are recommended based upon reduced body weight gain. This recommendation is made on the condition that studies summarized in the sponsor's carcinogenicity protocol package of 12/30/1998 are submitted and are found to be in agreement with the submitted studies.

2. The sponsor is encouraged to include an additional control group other than vehicle control unless sufficient contemporary historical data are available.

3. Study duration should be 24 months rather than the proposed 18 months. The sponsor should contact the Agency in the event of significant mortality.

Mouse Study:

1. Doses of 18, 6 and 2 mg/kg in males and 12, 6, and 2 mg/kg in females are recommended based upon adrenal atrophy and nasal degeneration. This recommendation is made on the condition that studies summarized in the sponsor's carcinogenicity protocol package of 12/30/1998 are submitted and are found to be in agreement with the submitted studies.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:/
/Division File HFD-570, Pre-IND Roflumilast
/HFD-570/McGovern/Sun/Hilfiker
/ASeifried, HFD-024

Appendix 2: Tumor Incidence in Hamsters (Study No. 7/2002)

Organ/Tumor	Male						Female					
	Roflumilast (mg/kg/day)						Roflumilast (mg/kg/day)					
	C	V	0.25	1	4	8	C	V	0.25	1	4	8
Pituitary/ Adenocarcinoma [M]	0	0	0	0	0	0	0	0	1	2	0	0
Adenocarcinoma [B]	1	0	2	0	0	1	6	6	4	7	3	3
Adenoma [B]	0	0	0	0	0	0	0	0	1	0	0	0
Eye/ Carcinoma [M], undif.	0	0	0	0	1	0	0	0	0	0	0	0
Harderian gland/ adenoma [B]	3	2	1	4	0	0	2	4	3	2	2	0
Lacriminal gland/ adenoma [M]	0	0	0	0	0	0	1	0	0	0	0	0
Nose and nasal cavity												
Carcinoma, [M] undifferentiated	1	0	0	0	0	0	0	0	0	0	0	4
Adenoma [B], of Steno gland(s)	0	0	0	0	0	1	0	0	0	0	0	0
Larynx/ neuroendocrine tumor [M]	0	1	0	0	0	1	0	0	0	0	0	0
neuroendocrine tumor [B]	0	0	0	0	0	2	0	0	0	0	0	0
Trachea/ neuroendocrine T [B]	0	0	0	0	0	0	0	0	0	1	0	0
Thyroid/ carcinoma [M], follicle C.	0	0	0	0	0	0	0	0	1	0	1	0
Adenoma [B]/ follicle cell	0	1	0	0	0	0	2	3	0	0	0	1
Carcinoma [M]/ C-cells	0	0	0	0	0	0	0	2	0	2	0	1
Adenoma [B]/ C-cell	1	0	3	2	0	3	2	0	5	2	1	5
Parathyroid/ adenoma [B]	1	0	1	1	1	2	5	9	2	6	6	8
Heart /schannoma [B], endocardi.	0	0	0	0	0	0	0	0	0	0	1	0
Thoracic cavity/ # examined	12	8	6	6	12	5	18	26	32	25	11	9
Osteosarcoma [M]	0	0	0	0	0	0	0	0	0	2	0	0
Fibrosarcoma [M]	0	0	0	0	0	0	0	0	0	1	0	0
Sarcoma [M], NOS	0	0	0	0	1	0	0	0	0	0	0	0
Salivary gland/ Adenocarcinoma, [M], squamous cell	0	0	0	0	0	0	0	0	0	1	0	0
Schannoma [M]	0	0	0	0	0	0	0	0	0	0	0	1
Forestomach/ carcinoma [M] squamous cell	0	0	0	0	0	0	0	0	0	0	0	1
Papilloma (TA) [B], squamous	7	6	9	11	11	11	5	9	9	10	13	6
Fibrosarcoma, [M]	0	0	0	0	0	0	0	0	0	0	0	1
Glandular stomach, adenocarc. [M]	0	0	0	0	0	0	0	0	1	0	0	0
Adenoma [B]	1	0	0	0	0	0	0	0	0	0	0	0
Liver/ carcinoma [M], heptatocul.	0	0	0	0	0	0	1	0	0	0	0	0
Adenoma [B], hepatocellular	0	0	1	0	0	0	1	0	0	0	0	1
Cholangiocarcinoma [M]	0	0	1	0	0	0	0	1	0	0	0	0
Cholangioma [B]	0	0	2	0	0	0	1	0	0	0	1	1
Hemangiosarcoma [M]	2	0	0	0	0	0	2	1	1	1	0	2
Hemangioma [B]	0	0	0	0	0	1	1	0	1	0	0	0
Gall bladder/ adenoma [M]	0	0	1	0	0	0	0	0	0	0	1	0
Pancreas/ carcinoma [B], islet cell	1	0	0	0	1	0	0	0	0	0	0	1
adenoma [M], islet cell	2	3	0	4	2	3	2	2	2	1	2	1
Mesantery/ fibrous histocytoma [M]	1	0	0	0	0	0	0	0	0	0	0	0
Mesentery lymph node/leiosarcoma	0	0	0	0	0	0	1	0	0	0	0	0
Cecum/ Adenoma [B]	0	0	0	0	0	0	0	0	0	1	0	0
Colon/ hemangioma [B]	0	0	0	0	1	0	0	0	0	0	0	0
Rectum/ granuler cell tumor [B]	0	0	0	0	1	0	0	0	0	0	0	0
Kidney/ nephroblastoma [M]	0	0	0	0	2	0	0	0	0	0	0	0
Carcinoma, renal tunule []	0	0	0	0	0	0	0	0	0	0	1	1
Adenoma, renal tubule [B]	0	0	0	0	0	0	1	0	0	0	0	1
Sarcoma/ NOS	0	0	0	0	0	0	0	0	1	0	0	0

Organ/Tumor	Male						Female					
	Roflumilast (mg/kg/day)						Roflumilast (mg/kg/day)					
	C	V	0.25	1	4	8	C	V	0.25	1	4	8
Adrenals/ Carcinoma (A) [M], cort.	13	12	10	7	10	8	11	8	7	8	13	4
Adenoma [B], cortical	35	42	35	44	35	27	27	39	32	40	29	30
Adenoma [B], subcapsular cell	22	19	16	19	13	9	1	1	1	0	0	1
Pheochromacytoma [B]	0	0	0	1	2	0	0	0	0	2	1	0
Epididymides/ leiomyosarcoma [B]	0	0	0	0	0	1						
Prostate/ schnnoma [B]	0	0	0	1	0	0						
Ovaries/ Tumor [M], granulosa cell							0	0	0	1	0	1
Tumor [B], granulosa cell							8	6	6	6	5	3
Tumor [M], granulosa theca cell							0	1	1	0	0	0
Tumor [M], granulosa theca cell							1	2	1	2	2	2
Thecoma [M]							2	1	1	0	1	1
Thecoma [B]							0	1	0	0	0	0
Tumor [B], sex cord stromal, mix							0	0	0	0	1	0
Uterus/ adenocarcinoma (TA), [M]							10	7	11	10	7	6
Carcinoma. [M], adenosquamous							0	0	0	0	0	1
Adenoma [B]							2	4	5	4	2	2
Polyps [B], endometrial stromal							7	7	9	4	2	5
Polyps [B], glandular							0	1	0	0	0	1
Leiomyosarcoma (TA), [M]							2	1	0	3	4	2
Leiomyoma (TA), [B]							3	2	2	4	4	9
Hemangiosarcoma [M]							0	0	0	1	1	0
Schwannoma [B]							1	0	0	0	0	0
Tumor granular cell [B]							0	0	0	1	0	0
Vagina/ carcinoma [M], squamous							1	1	2	2	0	0
Papilloma (TA) [B], squamous							16	13	16	13	13	10
Mammary G./ adenocarcinoma [M]	0	0	0	0	0	0	0	1	0	0	0	1
Skin/ carcinoma [M], squamous	1	0	0	0	0	0	0	0	0	0	0	0
Papilloma [B], squamous cell	0	0	0	1	0	0	0	0	0	0	0	0
Melanoma [M]	1	0	0	1	0	0	0	0	0	0	0	0
Fibrous histocytoma [M]	0	0	0	0	1	0	0	1	0	1	0	0
Fibrosarcoma [M]	0	0	0	0	0	0	0	1	0	1	0	0
Hemangiosarcoma [M]	0	0	0	0	0	0	0	1	0	1	0	0
Schwannoma [M]	0	0	0	0	0	0	1	0	0	1	1	0
Sarcoma [M], NOS	0	0	0	0	0	1	0	0	0	0	0	1
Bones/ Numnbers examined	1	4	1	2	-	4	1	2	2	1	1	1
Osteosarcoma [M]	0	1	1	0	-	1	0	1	0	0	0	0
Chondroma [B]	0	0	0	1	0	0	0	0	0	0	0	0
Vetebrea/ Osteosarcoma [M]	0	0	0	0	0	0	0	0	0	0	0	1
Femur/ Osteosarcoma [M]	0	0	0	0	0	0	0	0	1	0	1	0
Skeletal muscle/ rhabdomyocarc.	0	0	0	0	1	0	0	0	0	0	1	0
Spleen/ Hemangiosarcoma [M]	2	0	0	2	1	1	2	2	4	2	4	1
Hemangioma [B]	0	1	0	0	0	0	0	0	2	0	0	1
Hepatop./lymphoret.-tissue												
Lymphoma [M]	6	5	8	1	4	2	6	5	1	4	3	4
Sarcoma [M], histocytic	0	0	0	0	0	0	0	0	1	1	0	0
CNS/ schwannoma [B]										1/1		

a. n = 60/group unless specified.

Appendix 3. Tumor Incidence in Hamsters Study 233/2003

Sex	Male		Female	
Treatment	Control (N = 30)	Roflumilast (N = 60)	Control (N = 30)	Roflumilast (N = 60)
Pituitary/ Adenocarcinoma [B]	0	0	4	1
Harderian gland/ adenoma [B]	3	2	1	2
Nasal and para-nasal cavity				
Carcinoma, [M] undifferentiated	0	5	0	5
Adenocarcinoma [M], of Bowman's gland	0	1	0	1
Adenoma [B], of Steno gland(s)	0	1	0	2
Larynx/ neuroendocrine tumor [B]	0	0	1	1
Thyroid/ Adenoma [B], follicle C.	0	0	0	1
Adenoma [B]/ C-cell	1	4	0	1
Parathyroid/ adenoma [B]	1	0	2	1
Thoracic cavity/ Sarcoma [M], NOS	0/9	0/9	1/16	0/9
Oral cavity/ hemangiosarcoma [M]	0/5	1/5	-	0/2
Teeth/ hemangiosarcoma [M]	0/8	0/11	1/12	0/3
Abdominal cavity/ mesothelioma [M]	1/2	0/9	0/13	1/9
Neurofibrosarcoma [M]	0/2	1/9	0/13	0/9
Forestomach/ carcinoma [M], squamous cell	0	0	1	0
Papilloma (TA) [B], squamous cell	6	12	1	7
Liver/ Hemangiosarcoma [M]	0	1	0	1
Hemangioma [B]	0	0	0	1
Cholangioma [B]	0	0	0	2
Pancreas/ adenoma [B], islet cell	2	0	1	1
Cecum/ Adenocarcinoma [M]	0	1	0	0
Leiomyosarcoma [M]	1	0	0	0
Colon/ hemangioma [B]	1	0	0	0
Adrenals/ Carcinoma (A) [M], cortical	8	10	2	1
Adenoma (TA) [B], cortical	11	23	16	14
Adenoma [B], subcapsular cell	6	0	1	0
Prostate/ hemangiosarcoma [M]	0	1		
Ovaries/ Tumor [M], granulosa cell			5	2
Tumor [B], granulosa theca cell			2	2
Uterus/ adenocarcinoma (TA), [M]			4	6
Carcinoma. [M], adenosquamous			0	1
Adenoma [B]			1	2
Polyps [B], endometrial stromal			1	1
Leiomyosarcoma (TA), [M]			5	2
Leiomyoma (TA), [B]			2	10
Vagina/ carcinoma [M], squamous			1	0
Papilloma (TA) [B], squamous			4	7
Mammary G./ adenocarcinoma [M]	0	0	0	2
Skin/ Schwannoma [M]	0	0	0	2
Liposarcoma [M]	0	1	0	0
Bones/ osteosarcoma [M]	-	1/4	-	1/2
Sarcoma [M], NOS	-	0/4	-	1/2
Spleen/ Hemangiosarcoma [M]	0	0	0	1
Hepatop./lymphoret.-tissue, Lymphoma [M]	1	0	5	2
Sarcoma [M], histocytic	0	0	1	0

Appendix 4. Minutes of the May 10, 2005, Executive CAC Meeting

EXECUTIVE CAC

Date of Meeting: May 10, 2005

Committee: Abigail Jacobs, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Adebayo Laniyonu, Ph.D., HFD-160, Alternate Member
Timothy McGovern, Ph.D., HFD-570, Team Leader
Luqi Pei, Ph.D., HFD-570, Presenting Reviewer

Author of Draft: Luqi Pei, Ph.D.

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

IND #: 57,883
Drug Name: Roflumilast
Sponsor: Pfizer and Altana

Background:

Roflumilast is a phosphodiesterase IV (PDE_4) inhibitor being developed as a therapy for asthma and chronic obstructive pulmonary disease (COPD). Roflumilast tested positive in an *in vivo* mouse micronucleus test, but negative in the following assays: Ames test for bacterial gene mutation, *in vitro* chromosome aberration assay in human lymphocytes, *in vitro* HPRT test with V79 cells, an *in vitro* micronucleus test with V79 cells, DNA adduct formation assay in rat nasal mucosa, liver and testes, and *in vivo* mouse bone marrow chromosome aberration assay. In the *in vivo* mouse micronucleus test, roflumilast induced micronuclei at an oral dose of 300 mg/kg at 48 hours and 900 mg/kg 24 and 48 hours.

Genotoxicity testing was also performed on two metabolites of roflumilast: roflumilast –N-oxide and ADCP. The former tested negative in Ames test and micronucleus test in V79 cells *in vitro*. The latter tested negative in *in vivo* mouse micronucleus test and DNA[^{32}P]-post labeling assay in rat tissues.

Mouse Carcinogenicity Study:

B6C3F1 mice (50/sex/dose) were treated with roflumilast by oral gavage for 103 weeks. The respective roflumilast doses were 0.5, 2.0, 6.0 and 18 mg/kg/day in males and 0.5, 2.0, 6.0 and 12 mg/kg/day in females. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males showed a statistically significant and dose-related trend in increased mortality ($P < 0.0000$). The high dose females showed a statistically significant decrease in body weight relative to the vehicle or cage control ($P < 0.05$). Roflumilast-treated mice did not show statistically significant increases in the incidence of any tumors.

Hamster Carcinogenicity Studies

The carcinogenic potential of roflumilast was evaluated in two 2-year bioassays in Syrian golden hamsters. In one study, 60 hamsters/sex/dose were treated with 0.5, 1, 4 and 8 mg/kg/day of roflumilast by oral gavage for 103 weeks. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males, but not the females, showed a statistically significant trend in increased mortality ($P < 0.004$). There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The females showed a statistically significant trend for an increased incidence of undifferentiated carcinomas in the nasal cavity. The incidences for the nasal carcinomas were 4/60 in the high dose group and 0/60 for the remaining groups. No remarkable neoplastic findings were observed in the males of any roflumilast-treated groups.

In a second study, 60 hamsters per sex were treated with 16 mg/kg/day of roflumilast for 103 weeks. An additional 30 hamsters per sex that did not receive any treatment served as cage controls. Both roflumilast-treated males and females showed significant increases in mortality. The roflumilast treated groups showed numerical increases in the incidence of undifferentiated carcinomas in the nasal cavity (incidence: 0/30 – cage control and 5/60 – roflumilast in each sex), which were not statistically significant by pair-wise comparisons. However, the background incidence of nasal neoplasms is very low (0.03% or 1/2649)

Executive CAC Recommendations and Conclusions:

Mouse Study:

- The committee concurred that the mouse study was adequate.
- The committee concurred that the study was negative for drug–induced tumors.

Hamster Studies:

- The Committee accepted the studies as adequate in evaluating the carcinogenicity potential of roflumilast in hamsters.
- The Committee concurred that the studies were positive for nasal carcinomas in females at 8 mg/kg/day and in both males and females at 16 mg/kg/day.
- The Committee concurred that based on rarity, the nasal neoplasms appear to be drug related. The nasal neoplasms may be due to a rodent-specific metabolite and thus may not be relevant to humans The finding, however, should be noted in the labeling.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, HFD-570
/T. McGovern, Team leader, HFD-570
/L. Pei, Reviewer, HFD-570

/L. Jafari, CSO/PM, HFD-570
/A. Seifried, HFD-024

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/s/

Abby Jacobs
5/18/05 07:30:26 AM

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Luqi Pei
6/27/2007 09:27:32 AM
PHARMACOLOGIST

Timothy McGovern
6/27/2007 09:39:09 AM
PHARMACOLOGIST
I concur.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22522	ORIG-1	FOREST RESEARCH INSTITUTE	DAXAS(ROFLUMILAST 500 MCG TABLETS

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/s/

LUQI PEI
01/22/2010

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22522	ORIG-1	FOREST RESEARCH INSTITUTE	DAXAS(ROFLUMILAST 500 MCG TABLETS

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/s/

LUQI PEI
01/25/2010

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22522	ORIG-1	FOREST RESEARCH INSTITUTE	DAXAS(ROFLUMILAST 500 MCG TABLETS

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/s/

LUQI PEI
03/19/2010

MOLLY E SHEA
03/19/2010

Appendix 5

Memorandum by Dr. Luqi Pei
Completed on January 25, 2010
In NDA 22-522

MEMORANDUM

TO: NDA 22-522 and IND 57,883 Files

FROM: Marcie Wood Ph.D., and Luqi Pei, Ph.D.

DATE: January 25, 2010

RE: Re-evaluation of the Relevance of Roflumilast-Treatment-Related Hamster Tumor Data to Humans

This memo documents the deliberation of the Center's Executive Carcinogenicity Assessment Committee (ECAC) decision to revise its interpretation of the roflumilast tumorigenicity data to humans. On January 19, 2010, the ECAC issued an addendum to the 10-MAY-2005 meeting minutes. The addendum states: "… that currently available data show that ADCP N-oxide does not appear to be a rodent-specific metabolite and the statement in the 10-MAY-2005 meeting minutes that [T]he nasal neoplasms may be due to a rodent-specific metabolite and thus may not be relevant to humans' is no longer accurate." The addendum (attached) was issued upon reviewing the currently available data submitted in the NDA 22-522 submission on July 15, 2009 as discussed below.

Background Information

Roflumilast causes dose-related and statistically significant increases in the incidence of nasal tumors in hamsters, but not in mice, in 2-year oral carcinogenicity studies. The Executive CAC reviewed the study reports and concluded that the tumor finding was irrelevant to humans on May 10, 2005. The conclusion was based on 2 observations: 1) the tumorigenicity of roflumilast was attributed to one of its metabolites, ADCP N-oxide; and 2) hamsters produced the compound but humans did not. The carcinogenicity data are discussed briefly later in the document. The 10-MAY-2005 ECAC meeting minutes states:

> "The Committee concurred that the studies were positive for nasal carcinomas in females at 8 mg/kg/day and in both males and females at 16 mg/kg/day. The Committee concurred that based on rarity, the nasal neoplasms appear to be drug related. The nasal neoplasms may be due to a rodent-specific metabolite and thus may not be relevant to humans. The finding, however, should be noted in the labeling."

Roflumilast NDA application (#22-522) is currently in house for review. Additional data provided in the original submission of NDA 22-522 showed that humans also produce ADCP N-oxide as observed in urine. The primary reasons for the discrepancies in ADCP N-oxide levels in humans were the improved analytical method for plasma samples (from 3 ng/ml to 1 ng/ml) and new urine drug level data that were unavailable previously.

Roflumilast Carcinogenicity Data

Roflumilast at daily doses of 8 and 16 mg/kg/day for 2 years caused statistically significant increases in the incidence of nasal tumors in hamsters, but not in mice at doses up to 18 mg/kg/day. Briefly, two 2-year oral carcinogenicity studies were completed in Syrian golden hamsters. In the first study, 60 hamsters/sex/dose were treated with 0.5, 1, 4 and 8 mg/kg/day of roflumilast by oral gavage for 103 weeks. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males, but not the females, showed a statistically significant trend in increased mortality ($P < 0.004$). There were no significant differences in body weight between the vehicle control and treatment groups in either sex. The females showed a statistically significant trend for an increased incidence of undifferentiated carcinomas in the nasal cavity. The incidences for the nasal carcinomas were 4/60 in the high dose group and 0/60 for the remaining groups. No remarkable neoplastic findings were observed in the males of any roflumilast-treated groups.

Table 1 Tumor Prevalence in the 2-yr Carcinogenicity Study in Hamsters

Tumor	Sex	Study No.	Tumor Prevalence (%) [a]							P-Value (Exact)
			Control		Roflumilast (mg/kg/day)					
			Cage	Veh.	0.25	1	4	8	16	
Carcinoma, undifferentiated	M	7/2002	1.7	0	0	0	0	0	-	NS
		233/2003	0	-	-	-	-	-	8.3	0.0587 [c]
	F	7/2002	0	0	0	0	0	6.7	-	.0006 [b]
		233/2003	0	-	-	-	-	-	8.3	.081 [c]
Adenocarcinoma, Bowman's gland	M	233/2003	0	-	-	-	-	-	1.7	NS
	F	233/2003	0	-	-	-	-	-	1.7	NS
Adenoma, Bowman's gland	M	233/2003	0	-	-	-	-	-	1.7	NS
	F	233/2003	0	-	-	-	-	-	3.3	NS
Total	M	7/2002	0.8	0	0	0	0	3.3	-	-
	F	233/2003	0	-	-	-	-	-	12.5	-

a. Extracted from Table 2.6.6 – 24 of the submission. n = 50 and 30/sex/group for studies 7/2002 and 233/2003, respectively.
b. Against vehicle control (asymptotic method)
c. Against cage control (exact method)

In a second study, 60 hamsters per sex were treated with 16 mg/kg/day of roflumilast for 103 weeks. An additional 30 hamsters per sex that did not receive any treatment served as cage controls. Both roflumilast-treated males and females showed significant increases in mortality. The roflumilast treated groups showed numerical, but statistically non-significant increases in the incidence of undifferentiated carcinomas in the nasal cavity (incidence: 0/30 – cage control and 5/60 – roflumilast in each sex). However, the background incidence of nasal tumors is very low (0.03% or 1/2649). Based on the totality of the data from the two studies, the ECAC concluded that roflumilast was carcinogenic in hamsters at doses of 8 and 16 mg/kg/day.

In mice, B6C3F1 mice (50/sex/dose) were treated with roflumilast by oral gavage for 103 weeks. The respective roflumilast doses were 0.5, 2.0, 6.0 and 18 mg/kg/day in males and 0.5, 2.0, 6.0 and 12 mg/kg/day in females. Each sex also included a vehicle control (0.4% methocel) and a cage control group for reference. The males showed a statistically

significant and dose-related trend in increased mortality ($P < 0.0000$). The high dose females showed a statistically significant decrease in body weight relative to the vehicle or cage control ($P < 0.05$). Roflumilast-treated mice did not show statistically significant increases in the incidence of any tumors.

Role of ADCP N-oxide in Roflumilast Carcinogenicity

It was concluded that ADCP-N-oxide was exclusively responsible for the carcinogenicity of roflumilast in hamsters. This conclusion was based on indirect evidence that includes the following:

1) Neoplastic and pre-neoplastic lesions of the nasal cavity occur only in species that produce ADCP-N-oxide, a reactive intermediate. Rodents (i.e., mice, rats and hamsters) possess enzyme CYP2G1 that convert ADCP to ADCP-N-oxide. The human (also dog and monkey) nasal cavity does not produce such a compound due to the lack of similar enzymes.

2) Binding to nasal tissue is detected in rats receiving ADCP-moiety labeled roflumilast, but not the benzyl-moiety labeled compound. Binding also did not occur in humans given the benzyl-moiety labeled roflumilast.

3) Rats uptake roflumilast readily. The ratio of nasal tissue to plasma roflumilast concentrations in rats is approximately 5. The ratio is about 1 in mice and hamsters. Note the ratio in humans is less than approximately 0.5.

4) ADCP-N-oxide is responsible for the nasal toxicity of roflumilast. Rodent CYP2G1 converts ADCP-N-oxide to an epoxide intermediate. The latter reacts with the -SH group of the protein and forms protein adducts. Nasal toxicity of roflumilast can be modified by compounds that change the intracellular glutathione concentration. Phorone depletes intracellular glutathione concentration, and co-administration of phorone with 5 mg/kg roflumilast or 0.5 mg/kg ADCP enhances the nasal toxicity of these compounds. Metyrapone inhibits cytochrome P450 activity, and co-administration of metyrapone prevents or alleviates the nasal lesions of roflumiast (100 mg/kg) or ADCP (6 mg/kg) from occurring. Similar observations were made with dichlobenil and ADCP derived from piclamilast.

5) Piclamilast, another PDE4 inhibitor, exhibits a similar nasal toxicity profile. The nasal toxicity of piclamilast is also modified by dichlobenil and piclamilast-derived ADCP in similar fashion.

ADCP N-oxide Levels in Humans

ADCP N-oxide was observed in human plasma and urine. These data were not available at the time of the carcinogenicity study assessments. The metabolic pathways of ADCP N-oxide in humans are not clear at present time. Regardless, the data provided in the NDA submission showed the presence of ADCP N-oxide in both plasma and urine in humans. Particularly, ADCP N-oxide is detected in plasma in approximately 10% (2/19) healthy subjects. Table 2 showed pharmacokinetic profile of ADCP N-oxide in the two individuals. It is noted that the

Cmax of the compound in the two subjects were similar (~1.3 ng/ml) and were only slightly above the lower detection limit of 1 ng/ml. The mean AUC was approximately 20 ng.h/ml.

Table 2:

ADCP and ADCP N-oxide: Summary of pharmacokinetic characteristics in two Study Subjects (No. 11 and No. 15) after multiple once-daily oral doses of 500 µg roflumilast (Study Day 12)

Pharmacokinetic Characteristic	Subject #11		Subject #15	
	ADCP	ADCP N-oxide	ADCP	ADCP N-oxide
$AUC_{(0\text{-}24h)}$ [µg/l x h]	18.0	13.9	6.3	24.2
C_{max} [µg/l]	0.94	1.31	0.64	1.28
t_{max} [h]	0.00	6.0	1.00	24.00
$t_{1/2}$ [h]	n.a.	n.a.	n.a.	n.a.

Table 3 presents urine ADCP N-oxide levels in humans. The compound accounted for approximately 15% of the total radio-activity. Since the urine is the major route (~70%) of excretion of roflumilast after oral administration, data suggest that ADCP N-oxide contributes to significant systemic exposure (~10.5%). These results, however, were from a radio-labeled study in which ADCP N-oxide was detected in the urine from pooled samples.

Table 3 Metabolic Profile of Roflumilast in the Urine

	Percentage (%) of Recovered Roflumilast Doses [a]					
	Mouse	Rat	Hamster	Dog	Monkey	Human
Roflumilast				**3.2**	**5.3**	
M03 & M1 [c]	10.1	1.3	6.5		30.6	31.1
M05, M02 & M08	45.9	10.9	32.3	9.6	26.7	18.3
M04* & M04	3.3				6.8	16.4
Roflumilast N-oxide	**1.6**				**11.0**	**1.3**
ADCP N-oxide	**15.0**	**37.7**	**11.8**			**14.9**
ADCP		**19.9**				
N-methyl-ADCP				60.6		

The blank boxes indicate data not available.

The observation that humans apparently produce ADCP N-oxide contradicts the previous conclusion that humans do not produce ADCP N-oxide (Note 1, page 5). A close look reveals no real discrepancies between the previous and current reviews. Primary reasons for such a conclusion were an improved analytical method for ADCP N-oxide levels in plasma samples in humans and urine drug level data that were unavailable previously. The lower limit of quantitation was 3 and 1 ng/ml in 2005 (or earlier) and new data, respectively.

Relevance of Hamster Carcinogenicity Data to Humans

The currently available data show that the previous conclusion regarding the relevance of hamster tumorigenicity data of roflumilast to humans is no longer accurate. It was previously concluded that roflumilast tumorigenicity was not relevant to humans because humans were not believed to produce ADCP N-oxide, an epigenetic carcinogen of roflumilast metabolite.

Rationale for ADCP N-oxide tumorigenicity was discussed earlier in Section entitled "Role of ADCP N-oxide in Roflumilast Carcinogenicity". Briefly, nasal tumorigenicity in hamsters is attributed to the formation of rodent-specific, SH-reactive metabolite: ADCP-N-oxide epoxide**.** CYP2G1 catalyzes the formation of ADCP N-oxide from ADCP. The enzyme further transforms ADCP N-oxide into ADCP-N-oxide epoxide. Human tissues do not express the CYP2G1 or its counterpart and thus do not produce ADCP-N-oxide. Consequently, no epoxide is produced because the lack of appropriate substrate.

The currently available data, however, do not support the argument that humans do not produce ADCP N-oxide. The nonclinical and clinical pharmacology disciplines held a meeting on January 8, 2010 to discuss whether humans produce ADCP N-oxide when taking roflumilast orally. The discussion was prompted by the observation that ADCP N-oxide was not only detected in the human plasma, but also in urine as discussed earlier. It was concluded that humans do produce ADCP N-oxide. Presence of ADCP N-oxide in humans suggests that the tumor finding may be relevant to humans.

Conclusion

The presence of ADCP N-oxide, an epigenetic carcinogen of roflumilast metabolite, in both hamsters and humans shows that the previous conclusion that ADCP N-oxide is a rodent-specific metabolite is no longer accurate. The ECAC discussed the available data on January 19, 2010 and issued an addendum to revise the May 10, 2005 meeting minutes.

Notes:

1. **Plasma Levels of Roflumilast and its Metabolites in Animals and Humans (2005 data)**

Species	Rat	Hamster	Mouse	Dog	Monkey	Human
Roflumilast (mg/kg/day)	1.5	8	12	13	0.5	0.01
Sampling time (week)	4	13	26	13/52	13/42	1
	Plasma AUCs $_{0\text{-}24}$ (µg h/l)					
Roflumilast	32.4	88.9	689.7	1056	792.0	32.9
Roflumilast-N-oxide	927.5	2781.6	2196.7	74.8	2384.9	351.4
ADCP	499.5	186.8	65.7	54.8	47.4	4.14
ADCP-N-oxide	470.5	873.1	503.5	<LLOQ[a]	<LLOQ	<LLOQ
Report No.	31/2001 44/2001 42/2002	147/99	197/2001	4/2001	108/2002	128E/97 177/98
NOAEL (mg/kg/day)	0.8	4	4	2.0	0.5	> 0.01

a. LLOQ = low limit of quantitation: 5.5, 1 and 3 µg/L in dogs, monkeys and humans, respectively.

Executive CAC
Date of Meeting: January 19, 2010

Committee: David Jackson-Kram, Ph.D., ONDIO, Member
Abigail Jacobs, Ph.D., ONDIO, Member
Paul Brown, Ph.D., ONDIO, Member
Luqi Pei, Ph.D., Acting Supervisor
Marcie Wood, Ph.D., Presenting Reviewer

Author of Draft: Marcie Wood and Luqi Pei

The following information reflects a brief summary of the Committee discussion and its recommendations.

IND and NDA#: IND 57,883 and NDA 22-522
Drug Name: Roflumilast
Sponsor: Nycomed

Addendum to the Minutes of May 10, 2005 Executive CAC Meeting

Background:

The current available data submitted in NDA 22-522 show that both humans and hamsters produce ADCP N-oxide, a tumorigenic metabolite of roflumilast. This finding apparently contradicts the previous finding that only rodents including hamsters produced ADCP N-oxide in vivo. The presence of the tumorigenic compound in humans raises questions about the accuracy of the Committee's 10-MAY-2005 conclusion that the nasal tumors in hamsters were irrelevant to humans.

In two 2-year oral carcinogenicity studies, roflumilast caused dose-related increases in the tumor incidence in the nasal cavity in hamsters. The Committee reviewed the study reports on May 10, 2005 and concluded that the tumors were attributed to one of the roflumilast metabolites, ADCP N-oxide. Hamsters, especially tissues in their nasal cavity, produced high levels of ADCP N-oxide while humans did not appear to product any detectable amount of it. The Committee concluded that the hamster tumor finding was irrelevant to humans. The minutes of the May 10, 2005 Committee meeting state:

> "The Committee concurred that based on rarity, the nasal neoplasms appear to be drug related. The nasal neoplasms may be due to a rodent-specific metabolite and thus may not be relevant to humans. The finding, however, should be noted in the labeling."

Additional data provided in the roflumilast NDA submitted on July 15, 2009, however, showed that humans also produced ADCP N-oxide. Specifically, ADCP N-oxide accounted for 15% of the total radioactivity in the urine. Seventy percent of orally administered roflumilast dose is excreted by urine. Some patients (approximately 10%)

also show detectable amount of ADCP N-oxide in plasma. The presence of ADCP N-oxide contradicts the previous observation that humans did not produce ADCP N-oxide.

The more recent detection of ADCP N-oxide levels in humans was apparently attributed to the improved analytic method and the urine data that were previously unavailable. The improved analytical method in blood sample analysis resulted in a lower detection limit. Specifically, the detection limit was 3 and 1 ng/ml in previous and current submissions, respectively. The human plasma ADCP N-oxide level was approximately ≤1.31 ng/ml.

Executive CAC Recommendations and Conclusions:

Hamster Studies:

- The Committee concurred that currently available data show that ADCP N-oxide does not appear to be a rodent-specific metabolite and the statement in the 10-MAY-2005 meeting minutes that "[T]he nasal neoplasms may be due to a rodent-specific metabolite and thus may not be relevant to humans" is no longer accurate.
- The other recommendations and conclusions in the minutes of May 10, 2005 are still appropriate.

David Jackson-Kram, Ph.D., ONDIO,
Chair, Executive CAC

2.6 PHARMACOLOGY / TOXICOLOGY REVIEW

NDA Pharmacology Fileability Check List

Reviewer: Luqi Pei, Ph.D.

NDA No: 22-522
Drug Name: Daxas (Roflumilast) tablets
Date of submission: July 29, 2009 (stamp date)
Date of 45-day file-ability meeting: September 4, 2009
Information to the Sponsor: No
Date of check list: September 8, 2009

(1) On its face, is the pharmacology/toxicology section of the NDA organized in a manner to allow substantive review? Yes.

(2) On its face, is the pharmacology/toxicology section of the NDA legible for review? Yes.

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

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

(4) If the formulation to be marketed is different from the formulation used in the toxicology studies, are repeating or bridging the studies necessary? No.

If no, state why not: No bridging toxicity studies are necessary although there is a difference in formulations between the nonclinical and clinical programs. Clinical formulation will be 500-mg roflumilast oral tablets. Nonclinical toxicity studies are done with oral suspensions via oral gavage (or gastric tubes). These studies have achieved sufficient systemic drug exposures and identified the target organs of toxicity in animals. Also, there are no novel excipients in the clinical formulation.

If yes, has the applicant made an appropriate effort to repeat the studies using the ‘to

be marketed' product, to bridge the studies or to explain why such repetition or bridging should not be required?

(5) Are the proposed preclinical labeling sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) appropriate (including human dose multiples expressed in either mg/m^2 or comparative systemic exposure levels) and in accordance with 201.57?

No. Dose ratios between animals and humans in preclinical sections (carcinogenesis, mutagenesis and impairment of fertility, pregnancy category and overdosage) are not given, or units are not expressed. The proposed label format, however, generally follows the new product labeling rule. The dose ratio issue can be addressed during labeling review.

(6) Has the applicant submitted all special studies/data requested by the Division prior to the submission including but not limited to pre-NDA discussion? Yes.

(7) On its face, does the route of administration used in the pivotal toxicity studies appear to be the same as the intended clinical route? Yes.

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

(8) Has the applicant submitted a statement(s) that all of the toxicity studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations? Yes.

(9) Has the applicant submitted any studies or data to address any impurity or extractable issues (if any)? Yes, there were few studies address drug impurities.

(10) Are there any outstanding preclinical issues? Not identified yet.

If yes: Identify below.

(11) From a preclinical perspective, is this NDA fileable? Yes

If no, state below why it is not.

(12) Should any additional information/data be requested? No additional information is needed at present. The minutes of the Pre-NDA of April 16, 2008 meeting indicates that the available nonclinical data constitute a complete package for a NDA submission. The adequacy of the nonclinical data in support of approval of the drug will be a review issue.

NDA Planning Timeline

NDA No.: 22-522 (Daxas tablets)

Date of planning timeline: September 8, 2009

PDUFA Due Date: May 17, 2010

Projected review completion date: March 14, 2010

	Milestone Dates
Pharmacology and ADME	February 1, 2010
Toxicology	February 10, 2010
General toxicity studies	N/A
Carcinogenicity studies and mutagenicity studies	February 1, 2010
a. Statistical consult request for CA studies	N/A
b. Submission of CA studies for CAC concurrence	N/A
Reproductive studies	November 30, 2009
Special studies and others	February 28, 2010
Labeling	February 15, 2010

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____

Concurrence Yes ___ No ___

cc:

NDA 22-522 Review Team Timelines

Filing/Planning Mtg:	September 4, 2009
Filing Date:	September 15, 2009
74 Day Letter:	September 29, 2009
MCR Mtg:	December 14, 2009
Full Labeling Mtg:	February 15, 2010
WU Mtg:	March 13, 2010
Primary Review Due:	March 24, 2010
Secondary Review Due:	March 31, 2010
Labeling T-con:	April 6, 2010
CDTL Memo:	April 7, 2010
Division Director Memo:	April 14, 2010
Action Package Readiness:	April 16, 2010
Action Package to ODE II IO:	April 26, 2010
PDUFA Date:	May 17, 2010

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

LUQI PEI
09/08/2009

JEAN Q WU
09/08/2009