CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
22-134

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22134
Supporting document/s:	none
Applicant's letter date:	09/28/2009
CDER stamp date:	09/29/2009
Product:	(b) (4) ophthalmic solution
Indication:	Prevention of itching associated with allergic conjunctivitis
Applicant:	Vistakon Pharmaceuticals, LLC.
Review Division:	Division of Anti-Infectives and Ophthalmology Products
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Template Version: December 7, 2009

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Approvable from a Pharmacology/Toxicology Perspective

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

The tables below summarize the highest doses which did not lead to impaired fertility in the rat Segment I study or impaired litter parameters or fetal harm in the rat and rabbit Segment II studies.

Safety Factor Assessment Based on Human Equivalent Dose Comparisons for the Segment I and II Study NOAEL Values

Study Type/ Study Number	Species	NOAEL (mg/kg/day)	HED (mg/kg/day) [c]	Safety Margin (based on human clinical dose)[d]
Segment I/ N122467/1	Rat	20 [a]	3.2	1317
Segment II/ N122419/1	Rat	40 [b]	6.4	2634
Segment II/ N122418/2	Rabbit	80	25.6	10535

[a] The NOAEL value for female fertility

[b] The NOAEL value for female reproduction and teratogenicity, however, over half of the mothers died in this group.

[c] Normalization based on body surface area. Conversion factors of 0.16 for rats and 0.32 for rabbits.

[d] Clinical human daily dose = 0.25% R89674/eye/day administered to both eyes for seven days. Assuming bilateral doses of 34 µl of 0.25% R89674 per day = 170 µg/day or for a 70 kg human, 2.43 µg/kg/day

Safety Factor Assessment Based on Plasma Exposure Comparisons

Study Type/ Study Number	Species	NOAEL (mg/kg/day)	R90692 AUC (μg x h/ml)	Safety Margin (based on human AUC)[d]
Segment I/ N122467/1	Rat	20 [a]	Not available	NA
Segment II/ N122419/1	Rat	40 [b]	2.44 [c]	230
Segment II/ N122418/2	Rabbit	80	93.6	8819

[a] The NOAEL value for rat female fertility

[b] The NOAEL value for rat female reproduction and teratogenicity, however, over half of the mothers died in this group.

[c] $AUC_{1\text{-}8h}$ for R90692 for the 20 mg/kg/day dose group. Exposure data for the NOAEL dose (40 mg/kg/day) was not available.

[d] AUC in human: For R90692: $AUC_{0\text{-}last}$ = 10.613 ng x hr/ml for 0.25% R89674/eye/day administered to both eyes.

It is recommended that under the 8.1 Pregnancy section, information supporting a Pregnancy category B be reworded to include the NOAEL mg/kg/day doses in the rat and rabbit reproduction studies which did not result in maternal mortality, impaired litter parameters, or harm to the fetus.

Based on the plasma exposure comparisons above, Section 8.1 Pregnancy should be rewritten as follows:

Pregnancy Category B. Reproduction studies performed in rats and rabbits revealed no evidence of impaired female reproduction or harm to the fetus due to alcaftadine. Oral doses in rats and rabbits of 20 and 80 mg/kg/day, respectively, produced plasma exposure levels 230 and 8800 times the plasma exposure at the recommended human ocular dose. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Similarly, the second paragraph of Section 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility should be rewritten as follows based on the exposure comparison from the Segment II rat study:

Alcaftadine was found to have no effect on fertility of male and female rats at oral doses up to 20 mg/kg/day (230 times the plasma exposure at the recommended human ocular dose).

1.2 Brief Discussion of Nonclinical Findings

A. Brief overview of nonclinical findings.

- R89674 (alcaftadine, R089674) is a histamine H_1, H_2, and H_4 antagonist intended for use as a topical ophthalmic treatment for allergic conjunctivitis. In pharmacology studies, R89674 was shown to bind histamine H_1 and H_2 receptors with nM affinity, maintain conjunctival epithelial integrity, prevent vascular leak, and inhibit early and late phase allergic inflammation.
- In pharmacokinetic studies, topical ocular administration of [^{14}C]R89674 resulted in preferential distribution to aqueous humor, iris-ciliary body, cornea, and eyelids. Plasma radioactivity levels were much lower and below the limit of quantification within four hours after dose administration. Over 60% of the radioactivity was excreted in urine. In a 6-month repeated-ocular dose toxicology study in rabbits, R89674 was quickly metabolized to its active carboxylic acid metabolite, R90692. R89674 is primarily metabolized by liver cytosolic enzymes. R90692 plasma C_{max} and AUC_{0-t} levels increased in a roughly dose-dependent manner and were relatively constant throughout the dosing period suggesting little or no accumulation of R90692. Plasma $t_{1/2}$ values for R90692 ranged from approximately 1-3 hours. Human plasma proteins bound approximately 40% and 63% of R89674 and R90692 respectively. R90692 was the only major metabolite in human cultured hepatocytes, and R89674 was primarily metabolized by liver cytosolic enzymes rather than CYP-450 enzymes. Neither R89674 nor R90692 greatly inhibited the major CYP-450 isozymes.
- Repeated topical ocular administration of R89674 in 14-day and 6-month studies in rabbits did not cause significant systemic or ocular toxicity. The NOEL value for both ocular and systemic toxicity in the 6-month study was considered to be 0.5% TID. After 6-months of dosing, the high dose produced plasma R90692 C_{Max} values of 53.9 (male) and 67.9 (female) ng/ml and $AUC_{(0-t)}$ values of 49.3 (male) and 72.1 (female) ng x hr/ml.
- In a 6-month oral repeated-dose study in rats, slight to moderate toxicity occurred at R89674 doses of ≥ 20 mg/kg/day. The liver appeared to be the target organ for toxicity. Liver weights were increased in male and female rats and female liver histopathology included hepatic atrophy and diffuse hyperplasia of oval cells and bile ducts. The NOAEL for this study was considered to be 5 mg/kg/day and R90692 AUC_{0-t} values associated with this dose were 0.627 (male) and 0.493 (female) μg x h/ml.
- In a 6-month oral repeated-dose study in dogs, a few toxicological effects occurred in the high-dose group (40 mg/kg/day) including transient salivation, rough haircoat, increased incidences of focal alopecia, decreased body weights and body weight gains, shortened PQ interval, and a slight increase in systolic blood pressure. Some hematological and serum chemistry values were also changed and the NOAEL for this study was considered to be 10 mg/kg/day which was associated with a R90692 $AUC_{0-\infty}$ value of 29.6 μg x h/ml.
- R89674 was negative for mutagenicity and did not increase chromosome aberrations in a full panel of genetic toxicology assays including the Ames test, a

chromosome aberration assay, a thymidine kinase mutation assay, and two mouse micronucleous assays. R90692 also did not increase mutations in an Ames test. A waiver for carcinogenicity evaluation was obtained.

- In a Segment I reproductive toxicity study in rats, R89674 did not cause adverse fertility effects at doses of ≤ 60 mg/kg/day in males and ≤ 20 mg/kg/day in females. In Segment II studies in rats and rabbits, female fertility was not impaired, no teratogenic effects occurred, and maternal lethality did not occur at doses ≤ 20 mg/kg/day in rats or doses ≤ 80 mg/kg/day in rabbits. In a Segment III study in rats, the NOAEL for maternal toxicity was considered to be 20 mg/kg/day, but the reproductive NOEL was considered to be 30 mg/kg/day as no adverse effects on F_0 reproduction occurred. The NOAEL for offspring viability and growth was 5 mg/kg/day.
- Several organic impurities were qualified in valid Ames' tests and *in vitro* chromosomal aberration tests with uniformly negative results for mutagenicity or chromosomal damage. In addition, test substances enriched for two specific impurities were tested in 14-day repeated-dose ocular toxicology studies in rabbits, and the impurities did not produce significant ocular or systemic toxicity.
- In both the 14-day and 6-month repeated-dose ocular studies in rabbits, no ocular or systemic toxicity occurred, and the NOEL values were the highest administered doses of 0.5% R89674/eye TID. When the 0.5% TID ocular NOEL doses in rabbits are normalized to human vitreal volume or conjunctival surface area, they provide 28- and 7- fold safety margins compared to the proposed human clinical daily ocular dose of 0.25% R89674.
- In the 6-month rat and dog oral repeated-dose toxicology studies where some R89674-related changes in hematological and clinical chemistry did occur as well as histopathology of the liver in rat, the exposure levels associated with the NOAEL doses were much higher than those measured clinically. The R90692 AUC values corresponding to the NOAEL doses in the 6-month rat and dog studies provide margins of exposure of 40 and 22 for male and female rats respectively and 2789 for dogs relative to the clinical R90692 $AUC_{0\text{-last}}$ of 10.613 ng x h/ml in the clinical pharmacokinetic protocol # 05-003-09. Similarly, the human equivalent dose (HED) values based on surface area normalization of the rat and dog NOAEL values provide safety margins of 329 and 2222 for the rat and dog NOAELs respectively.

2 Drug Information

2.1 Drug

2.1.2 Generic Name

Alcaftadine

2.1.3 Code Name

R89674, R089674

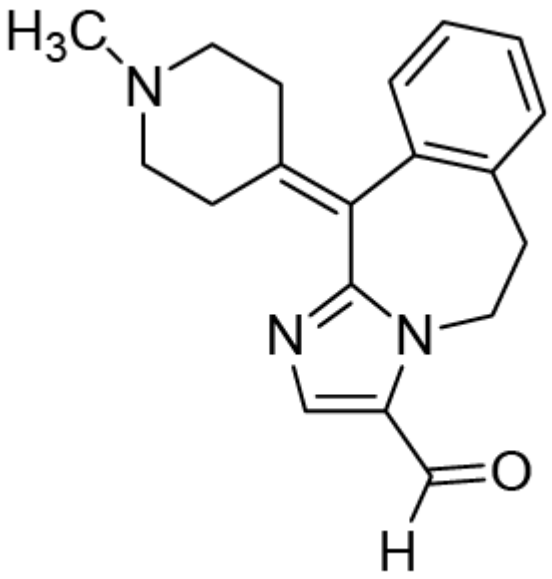
2.1.4 Chemical Name

6,11-dihydro-11-(1-methyl-4-piperidinylidene)-5*H*-imidazo[2,1-b] [3] benzazepine-3-carboxaldehyde (CAS Index name)
4-(1-methyl-piperidine-4-ylidene)-9,10-dihydro-4*H*-3,10a-diaza-benzo[*f*]-azulene-1-carbaldehyde (Beilstein)

2.1.5 Molecular Formula/Molecular Weight

$C_{19}H_{21}N_3O$/ 307.39 Daltons

2.1.6 Structure



2.1.7 Pharmacologic class

Histamine H_1, H_2, and H_4 receptor antagonist

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

IND 66884

2.3 Clinical Formulation

2.3.1 Drug Formulation

The proposed drug product formulation for (b) (4)™ is shown below in Table 1.

Table 1: (b) (4)™ Ophthalmic Solution 0.25% Drug Formulation

Components	Reference to Quality Standard	Function	Concentration mg/mL
Alcaftadine	In-House	Active Ingredient	2.5
Sodium Phosphate Monobasic Monohydrate	USP	(b) (4)	
Edetate Disodium, Dihydrate	USP		
Benzalkonium Chloride (50% Solution) [1]	NF		
Sodium Chloride	USP		
Sodium Hydroxide (1N Solution) [2]	NF	pH Adjustment	pH Adjustment Target 7.0
Hydrochloric Acid (1N Solution) [2]			
Purified Water	USP	(b) (4)	

[1] Equivalent to 0.05 mg/mL Benzalkonium Chloride.

[2] If needed, 1N NaOH solution and/or 1N HCl solution may be added to adjust the pH to 7.0.

2.3.2 Comments on Novel Excipients

The excipients shown in Table 1 are of USP or NF grade, compendial, and not considered novel for ophthalmic formulations.

2.3.3 Comments on Impurities/Degradants of Concern

There are six specified related substances including starting material, intermediates, and process impurities in the final synthesis process. Residual solvents that are monitored include (b) (4). In addition, (b) (4) content are also monitored. The specification limits for impurities are shown in Table 2 below. The specified limits for the residual solvents were all below those recommended in the ICH guidance "Guidance for Industry Q3C Impurities: Residual Solvents." Several of the organic impurities were qualified in repeated-dose ocular toxicology studies and genetic toxicology studies.

Table 2: R89674 Specifications

Test	Specification
	(b) (4)

* Release specification only.

2.4 Proposed Clinical Population and Dosing Regimen

(b) (4) ™ Ophthalmic Solution is intended to be administered topically to each eye at a concentration of 0.25% once daily for the prevention of itching associated with allergic conjunctivitis.

2.5 Regulatory Background

This is the initial NDA application for this product.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacodynamics

1. Receptor profiling of pheniramine maleate, ketotifen fumarate, and R89674 anti-allergic compounds – *in vitro* pharmacology Cerep study R89674. Study No.: 1510.
2. *In vitro* receptor binding profile of alcaftadine (R089674). Study No.: JNJ-224016-AAA.
3. Histamine H_4 receptor activity of R89674 and R90672. Study No.: DD06639.
4. Evaluation of Janssen anti-allergics R83546, R89674, R129160 in a murine model of allergic conjunctivitis. Study No.: 03-011-02D.
5. Evaluation of Janssen anti-allergics (R129160, norberastine citrate, and R89674). Study No.: 03-011-02B.
6. Evaluation of mast cell stabilization capacity of R89674 relative to olopatadine. Study No.: 05-011-05A.
7. Degranulation assay of RBL-CCR1 cells to test the effect of R90692 metabolite and R89674 drug product on mast cell degranulation. Study No.:07-003-01.
8. An evaluation of R089674 for ability to maintain the integrity of conjunctival epithelium measured through assessment of conjunctival epithelial tight junction markers. Study No.: 05-011-05C.
9. An evaluation of R89674 ophthalmic solution for ability to prevent vascular leak and cellular infiltrates using a murine model of allergic conjunctivitis. Study No.: 05-011-05B.
10. A comparison of R89674 0.25% and patanol (olopatadine 0.1%) for ability to preserve the integrity of the conjunctival epithelium in a murine model of allergic conjunctivitis. Study No.: 07-003-091.

Secondary Pharmacodynamics

1. Antihistamine acivity of R89674 in the compound 48/80 and histamine-induced lethality tests in rats and guinea pigs, respectively. Study No: N111636/1
2. Anti-allergic profile of R089674 in a model of acute- and late-phase allergic conjunctivitis in the guinea-pig. Study No.: N111642/1.
3. *In vivo* antiallergic activity of R87314 and R89674 in the Ascaris allergy test in dogs: a comparison with noberastine, loratadine, and terfenadine. Study No.: N109248/1.
4. Binding of test compound to melanin (compound R89674-AAA). Study No.: AAL00003-04-982.

Safety Pharmacology

1. Effects of R089674 on EEG power spectral activity in waking rats. Study No.: N111699/1.
2. Effects of R089674 on cardiovascular and behavioral parameters in instrumented, awake dogs: dose 0.63 mg/kg orally. Study No.: N111653.
3. Effects of R089674 on cardiovascular and behavioral parameters in instrumented, awake dogs: single oral dose of 2.5 mg/kg. Study No.: N125298/1.

Pharmacokinetics/Toxicokinetics

Absorption

1. A pilot study on the absorption and plasma levels of R089674 and of its active metabolite R090692 in the male Wistar rat and the D.H. guinea-pig after single oral administration of R089674 at 1.0 mg/kg. Study No.: N111588/1.
2. A pilot study on the absorption, plasma kinetics and tissue distribution of R089674 and its active metabolite R090692 in the male Wistar after single oral administration of R089674 at 2.0 mg/kg. Study No.: N115975/2.
3. Pharmacokinetics and absolute bioavailability of R089674 and R090692 in male Beagle dogs after a single oral and intravenous administration of a R089674 or R090692 solution at 2.5 mg/kg. Study No.: N111677.
4. Plasma kinetics of R089674 and metabolically formed R090692 after single intravenous (5 mg/kg) administration and absorption and tissue distribution of R089674 and metabolically formed R090692 after single oral (5 mg/kg) administration of ^{14}C-R089674 in the male SPF Wistar rat. Study No.: N125250.
5. Pharmacokinetics and absolute bioavailability of R089674 and R090692 in male Beagle dogs after a single oral and intravenous administration of a R089674 or R090692 solution at 2.5 mg/kg. Study No.: N125342/1.

Distribution

1. The plasma protein binding and the distribution of R089674 and its carboxylic acid metabolite (R090692) in blood. Study No.: N125358/1.
2. Mass balance and ocular tissue distribution of [^{14}C]R089674 following ocular administration to male New Zealand White rabbits. Study No.: AAL00004.

Metabolism

1. The metabolism and excretion of R089674 in the male and female SPF Wistar rat after single oral administration of 5 mg ^{14}C-R089674/kg. Study No.: N125294/1.
2. The *in vitro* metabolism of R089674 and R090692 in hepatocytes and liver subcellular fractions of male and female, adult and neonatal mice, male and female rats, female rabbit, male dog, and human. Study No.: N126519/1.

3. An *in vitro* study on the metabolism of ^{14}C-R089674 in human liver microsomal fractions and on the human microsomal cytochrome P-450 form(s) involved in the metabolism of R089674. Study No.: N125328/1.

Pharmacokinetic Drug Interactions

1. Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes after six months dosing of R089674 in an aqueous solution in male and female SPF Wistar rats. Study No.: N125478/1.
2. *In vitro* determination of the drug-drug interaction potential of R089674 in human liver microsomes: effects of possible co-medication of the metabolism of R089674. Study No.: N125331/1.

Toxicology

Single-Dose Toxicology

1. Single-dose oral toxicity study with R089674 in SPF Albino Swiss mice (CD1). Study No.: N125347/1.
2. The acute oral toxicity of the anti-allergic compound R89674 in mice. Study No.: N111662/1.
3. Single-dose intravenous toxicity study with R089674 in SPF Albino Swiss mice (CD1). Study No.: N125346/1.
4. The acute intravenous toxicity of the anti-allergic compound R89674 in mice. Study No.: N111661/1.
5. Single-dose oral toxicity study with R089674 in SPF Wistar (Hannover) rats. Study No.: N125345/1.
6. The acute oral toxicity of the anti-allergic compound of R89674 in rats. Study No.: N111659/1.
7. The acute intravenous toxicity of the anti-allergic compound R89674 in rat. Study No.: N111658/1.
8. Single-dose intravenous toxicity study with R089674 in SPF Wistar (Hannover) rats. Study No.: N125344/1.
9. Evaluation of Janssen anti-allergics R83546, R89674, R129160 for irritation potential in the rabbit. Study No.: 03-011-02A.

Repeat-Dose Toxicology

1. A 14-day repeat-dose ocular toxicity study in rabbits for R89674 and impurities. Study No.: 05-6067-G1.
2. 14-day evaluation of the ocular toxicity of an ophthalmic solution containing (b) (4) and (b) (4) following multiple topical instillations in the eyes of New Zealand white rabbits. Study No.: P1006064.
3. 14-day repeat-dose ocular study in rabbits. Study No.: 04-1959-G1.
4. A 6-month repeat-dose ocular toxicity study in rabbits. Study No.: 05-2456-G1.
5. One-week oral toxicity study in rabbits. Study No.: N122462/1.

6. One-month toxicity study in SPF Wistar Rats. Study No.: N111689/1.
7. Toxicokinetics of R089674 and its metabolite R090692 in the SPF Wistar rat in a one-month subchronic oral pilot study on R089674 at 2.5, 10, and 40 mg/kg/day. Study No.: N111676.
8. One–month toxicity study in Beagle dogs. Study No.: N111690/1.
9. Toxicokinetics of R089674 and its metabolite R090692 in the Beagle dog in a one-month subchronic oral pilot toxicity study on R089674 at 1.25, 5, and 20 mg/kg/day. Study No.: N111678.
10. Six-month repeated-dose oral toxicity study with R089674 in SPF Wistar rats. Study No.: N122461/2.
11. Toxicokinetics of R089674 and its metabolite R090692 in SPF Wistar rats in a 6-month oral toxicity study with aqueous solutions of R089674 at 5, 20, and 80 (40/60) mg/kg/day. Study No.: N122430.
12. Six-month repeated-dose oral toxicity study with R089674 in Beagle dogs. Study No.: N122463/2.
13. Toxicokinetics and tissue distribution of R089674 and its metabolite R090692 in Beagle dogs in a 6-month oral toxicity study with aqueous solutions of R089674 at 2.5, 10, and 40 mg/kg/day. Study No.: N122428.

Genetic Toxicology

1. *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. Study No.: 05-5302-G2.
2. R89674 spiked with (b) (4) and (b) (4) bacterial mutation test. Study No.: 961392.
3. R89674 spiked with (b) (4) and (b) (4) chromosome aberration test. Study No.: 961393.
4. R089674-experiment No. 3553 *Salmonella typhimurium* reverse mutation assay. Study No.: N122201.
5. R090692-experiment No. 4011 *Salmonella typhimurium* reverse gene mutation test. Study No.: N122464/2.
6. Chromosomal aberration assay in CHO cells – OECD. Study No.: 05-5302-G1.
7. R089674 experiment No. 3552 micronucleus test in mice short study. Study No.: N113173.
8. R089674 experiment No. 4013 micronucleus test in mice. Study No.: N122466.
9. Toxicokinetics of R089674 and its metabolite R090692 in SPF Albino Swiss mice after single oral administration of aqueous formulations of R089674 at 2.5, 10, and 40 mg/kg in the micronucleus test. Study No.: N125295.
10. R089674: mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the (b) (4) technique. Study No.: N125233.

Reproductive and Developmental Toxicity

1. R089674 Experiment No. 3971 male and female fertility study in Wistar rats (Segment I). Study No.: N122467.

2. R089674 Experiment No. 3969 study for effects on embryo-fetal development in SPF Wistar rats (Segment II). Study No.: N122419.
3. Toxicokinetics of R089674 and its metabolite R090692 in pregnant SPF Wistar rats in and oral segment II reproduction toxicity study with aqueous solutions of R089674 at 5, 20, and 80 mg/kg/day. Study No.: N122427.
4. Study for effects in Embryo-foetal development in albino rabbits (Segment II). Study No.: N122418.
5. Toxicokinetics of R089674 and its metabolite R090692 in the albino rabbit in an oral segment II reproduction toxicity study with aqueous solutions of R089674 at 10, 40, and 80 mg/kg/day. Study No.: N122426.
6. Oral gavage development and prenatal/postnatal reproduction toxicity study of R89674 in rats, including a postnatal behavior/functional evaluation. Study No.: AAL00006.

4 Pharmacology

4.1 Primary Pharmacology

Mechanism of action

The mechanism of action of R89674 was elucidated in receptor binding studies summarized below in Table 3.

Table 3: Primary Pharmacodynamics-Mechanism of Action

Study No./ Study Type/Study Design	Assay Type	Results
R&D Technical Report # 1510/ Receptor binding/ Competitive binding experiments to determine the relative affinity of R89674, pheniramine maleate, and ketotifen fumarate for H_1 and H_2 histamine receptors, α_1 and α_{2A} adrenergic receptors, and M_1 and M_2 muscarinic receptors.	*In vitro*/ Recombinant human receptors except for the α1 adrenergic receptor which was from rat brain	**R89674 K_i values**: H_1 histamine: 3.1 nM H_2 histamine: 58 nM α_1 adrenergic: > 1 μM α_2 adrenergic: > 1 μM M_1 muscarinic: 71 nM M_2 muscarinic: 670 nM
JNJ-224016-AAA/ *In vitro* binding potential/ Competitive binding experiments to determine the affinity of R089674 for a total of 40 receptors	*In vitro*/ Animal (rat, mouse, guinea pig) membrane preparations or cell lines transfected with human receptors	1. R089674 bound to the human H_1 receptor with a K_i of 1.1 nM. 2. R089674 affinity was 100 fold higher and 500 fold higher for rat muscarinic receptors and for some serotinergic and adrenergic receptors respectively. 3. R089674 K_is were > 10 μM for other receptors, transporters, and ion channel binding sites including the hERG channel.
DD06639/ Receptor binding/ Competitive binding of R89674 and R90692 to the human H_4 histamine receptor	*In vitro*/ SK-N-MC cells stably transfected with histamine H_4 receptor	1. R89674 was an antagonist of the human H_4 receptor with a K_i value of 2.9 μM. 2. The K_i value for R90692 for the H_4 receptor was > 100 μM.

Drug activity related to proposed indication

The pharmacodynamic activity of R89674 in relation to its proposed indication was examined in antiallergic models in mice and rabbits, mast cell stabilization assays, assays measuring conjunctival epithelial integrity, vascular leak assays, and in assays measuring early- and late- phase inflammation. These studies are summarized below in Table 4.

Table 4: Primary Pharmacodynamics-Drug Activity

Study No./ Study type/ Experimental Design	Species(Strain)/ Route and Dose/ N/Sex/Group	Results
03-011-02D/ Antiallergic activity: Ragweed model of ocular allergic responses/ R89674, R129160, citrate, noberastine, olopatadine, or ketotifen were topically administered to mouse eyes 15 minutes before ocular ragweed challenge.	Mice (SWR/J)/ Ocular doses of 0.0625% R89674/ 6 male mice/group	1. R89674 (0.0625%) inhibited ragweed-induced squinting and face washing, conjunctival hyperemia, chemosis, lid edema, and tearing in mice. 2. Of all the tested agents, R89674 was most effective in inhibiting each of the allergic ocular responses.
03-011-02B/ Antiallergic activity: 48/80 model of ocular histaminic responses/ R129160, noberastine citrate, olopatadine, or ketotifen were instilled into rabbit eyes 15 minutes or three hours before 48/80 challenge to both eyes.	Rabbit (NZW)/ Ocular doses of 0.01, 025, and 0.05% R89674/ 6 male rabbits/group	1. R89674 (0.05%) demonstrated superiority to other agents in its effect on total redness (onset and duration), chemosis (onset only), and lid swelling (onset and duration).
05-011-05A Mast cell stabilization/ Mast cells undergoing degranulation following IgE binding were treated with R89674 or olopatadine.	*In vitro*/ Fresh human conjuntival tissues/0.5 mg/ml R89674 and 0.2 mg/ml olopatadine	1. Unlike olopatadine, R89674 at a concentration of 0.5 mg/ml (0.05%) was ineffective in mast cell stabilization.
07-003-01/ Mast cell stabilization/ Mast cells undergoing degranulation following IgE binding were treated with R89674 or R90692.	*In vitro*/ Cultured RBL-CCR1 cells/ R89674 (0.083-0.0083%) and R90692 (0.083-0.00083%)	1. R89674 and R90692 at concentrations as low as 0.00083% produced a significant degree of mast cell stabilization in this test system.
05-011-05C/ Ragweed-induced degradation of conjunctival epithelial tight junctions/ Ocular instillations of R89674 one and two hours before ragweed challenge into both eyes of sensitized mice.	Mice (strain not reported)/ R89674 (5 μl/eye x2) concentratons not reported/ Numbers per group not reported	1. R089674 significantly inhibited ragweed-induced qualitative changes to the conjunctival epithelium tight junction proteins, ZO-1 and E-cadherin. 2. These results indicate that R89674 was able to maintain the integrity of conjunctival epithelium tight junctions.
05-011-05B/ Ragweed-induced ocular vascular leakage and late-phase inflammation/ Ocular instillations of R89674 one and two hours before ragweed challenge into both eyes of sensitized mice.	Mice (strain not reported)/ R89674 (12.5 μg; 5 μl/eye; x 2)/ 10-12 mice per group	1. R89674 significantly inhibited ragweed-induced vascular leak. 2. R89674 did not inhibit ragweed-induced late-phase ocular eosinophil or neutrophil recruitment.
07-003-091/ Ragweed-induced late phase ocular inflammation and degradation of conjunctival epithelial tight junctions/ Ocular instillations of R89674 or olopatadine one and two hours before ragweed challenge into both eyes of sensitized mice.	Mice (BALB/c)/ R89674 (0.025%, 10 μl per eye, x 2)/ 16 mice per group	1. R89674 significantly inhibited degradation of the epithelial cell gap junction proteins, ZO-1 and E-cadherin, while olopatadine did not. 2. Treatment with R89674 significantly inhibited eosinophil recruitment to the conjunctiva while treatment with olopatadine did not.

4.2 Secondary Pharmacology

Secondary pharmacodynamic studies evaluating the histamine-blocking activities of R89674 have been conducted in rats, guinea pigs, and dogs. In addition, an *in vitro* melanin binding study was conducted. The secondary pharmacology studies are summarized below in Table 5.

Table 5: Secondary Pharmacology

Study No./ Study type/ Study Design	Species(Strain)/ Route and Dose/ N/Sex/Group	Results
N111636/1/ Antihistamine activity/ 48/80 and histamine-induced lethality. R89674 was administered one hour before lethal doses of 48/80 (rats) or histamine (guinea pigs).	Rats (Wistar) and Guinea pigs (Dunkin-Hartly-Purbright)/ R89764 doses ranging from 0.00063 to 10 mg/kg administered PO or SC/ 5 animals per group for both species.	1. R89674 potently inhibited histamine-mediated 48/80-induced lethality in rats with peak effect doses of 0.014 and 0.56 mg/kg after SC and PO administration respectively. The onset of action was rapid (< 1 hr), and the duration of action was approximately ≥ 8 hours for both routes. 2. R89674 also potently inhibited histamine-induced lethality in guinea pigs with a peak oral dose of 0.016 mg/kg. The onset of action was rapid (< 1 hr) and the duration of action exceeded 24 hours.
N111642/1/ Antiallergic activity/ Guinea pig model of allergic conjunctivitis. Ocular instillations of R089674, loratadine, ketotifen, terfenadine, astemizole, and oxatomide were administered 24 and one hour before ocular challenge.	Guinea pigs (Dunkin-Hartley)/ Oral doses of 0.005, 0.01, 0.05, 0.1, 0.5, and 1 mg/kg R089674/ 6 guinea pigs per group.	1. R89674 inhibited both early and late-phase reactions in the 0.1 to 1 mg/kg dose range. 2. The antihistamine potency profile was as follows for the tested compounds: levocabastine > R089674 > ketotifen, astemizole, cetirizine, loratadine > oxatomide, terfenadine. 3. The potency to inhibit the acute effects of allergen challenge was as follows: R089674 > levocabastine > oxatomide, ketotifen, terfenadine > astemizole, cetirizine, loratadine. 4. The anti-inflammatory potency was as follows: dexamethasone > R089674 > terfenadine. None of the other compounds inhibited eosinophilic recruitment.
N109248/1/ Antiallergic activity/ Ascaris-induced skin reactions in dogs. R89674, R87314, loratadine, noberastine, and terfenadine were administered 1, 4, 20 and 72 hours before Ascaris skin challenge	Dog (strain was not reported)/ Oral administration of a range of concentrations/ N/group was not reported.	1. The potency of all the compounds was greatest 4 hours after administration. 2. R89674 and R87318 (a structurally related compound) demonstrated the lowest ED_{50}s at this timepoint, but the oral activity of R89674 declined more rapidly than for R87318 or the other compounds.
AAL00003-04-982/ melanin binding activity/ R89674.	Synthetic melanin/ 0-30 μM R89674, 0-30 μM chloroquine (positive control).	1. R89674 (0-30 μM) did not exhibit affinity for melanin relative to chloroquine which bound melanin with a K_d of 2.8 μM.

4.3 Safety Pharmacology

R89674 safety pharmacology studies were conducted to examine central nervous activity in rats and cardiovascular and behavioral activity in dogs.

Study title: Effects of R089674 on EEG power spectral activity in waking rats.

Study no.:	N111699/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Neuropsychopharmacology, Janssen Research Foundation, B-2340 Beerse, Belgium
Date of study initiation:	Not reported. Report is dated May 1995
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R089674, lot and purity were not reported

Methods

The central nervous system activity of R089674 was evaluated by power spectral analysis of the electroencephalogram (EEG) in conscious male Wistar rats. A series of 10 minute EEG recordings were performed at 1, 2, 4, and 6 hours following oral administration of R089674 (0.16, 0.63, and 2.5 mg/kg).

Results

Animals treated with 0.16 and 0.63 mg/kg R089674 demonstrated only marginal modifications of the EEG power activity in all the frequency bands. The 2.5 mg/kg dose significantly increased the EEG power in the 9.8 to 32.0 Hz frequency range two hours after treatment. Six hours after treatment the 2.5 mg/kg treated rats demonstrated increased EEG power in the 1.2 to 4.6 Hz and 7.0 to 9.6 Hz frequency range. The EEG activity changes are suggestive of a sedative effect.

Study title: Effects of R089674 on cardiovascular and behavioural parameters in instrumented, awake dogs: dose 0.63 mg/kg orally.

Study no.:	N 111653/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Cardiovascular and Pulmonary Pharmacology. Janssen Research Foundation, B-2340 Beerse, Belgium
Date of study initiation:	The initiation date was not reported. The study report is dated April 1995.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R089674, lot # GDIE0174070 1, the

purity was not reported.

Methods

The possible cardiovascular and behavioral effects of R089674 (0.63 mg/kg, single oral dose) were investigated in surgically instrumented male Beagle dogs.

Results

No behavioral changes were observed as a result of R089674 treatment.

Relative to the solvent group, R089674 did not produce any consistent effects on the following cardiovascular parameters monitored for two hours after dosing: heart rate, diastolic and systolic blood pressure, pressure rate product, cardiac relaxation, LV dp/dt max, cardiac output, stroke volume, total systemic resistance, coronary artery blood flow, and ECG characteristics. A slight but significant decrease (median peak value of -7%) in LV dp/dt max/p was observed at 0.25 and 0.5 hours following dosing.

Study title: Effects of R089674 on cardiovascular and behavioural parameters in instrumented, awake dogs: single oral dose of 2.5 mg/kg

Study no.:	N125298/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Cardiovascular and Pulmonary Pharmacology. Janssen Research Foundation, B-2340 Beerse, Belgium
Date of study initiation:	The initiation date was not reported. The study report is dated May 1997.
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R089674, batch # LVDE-0087-010-1, the purity was not reported.

Methods

The possible cardiovascular and behavioral effects of R089674 (2.5 mg/kg, single oral dose) were investigated in surgically instrumented male Beagle dogs. Animals were dosed then monitored at regular intervals (usually 5 minutes) and observed for the next two hours.

Results

No changes in behavior were noted in 5/8 dogs in the solvent treated group, and 4/7 dogs in the R089674-treated group. In the R089674-treated group, three dogs urinated during the 2 hour, postdose observation period, and two of these dogs appeared slightly

agitated. In the solvent control group, vomiting was observed in two dogs and short periods of extrasystoles were observed in one dog.

Relative to the solvent group, R089674 did not produce any consistent effects on the following cardiovascular parameters monitored for two hours after dosing: heart rate, diastolic and systolic blood pressure, pressure-rate product, cardiac relaxation, LV dp/dt max, LV dp/dt max/p, relaxation time constant (τ), stroke volume, total systemic resistance, and ECG-interval durations (PQ, QRS, QT, QTc Bazett and QTc Janssen). R089674 tended to increase cardiac output and significant differences (median peak effect of +23%) with the solvent control animals were apparent at 15 minutes and 45 minutes following administration.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Methods of Analysis

R89674 and R90672 in biological samples were measured using a high performance liquid chromatography-ultra violet (HPLC-UV; detection wavelengths of 270 and 290 nm) method. In studies using ^{14}C labeled R89674, liquid scintillation counting (LSC) and HPLC with radioactive detection were employed. Plasma protein binding was assessed with equilibrium dialysis.

Absorption

In addition to toxicokinetic analyses associated with the repeated-dose toxicology studies, several pharmacokinetic studies in rats, and dogs were conducted to examine R89674 absorption. A pilot study was performed to assess the absorption and plasma levels of R89674 and R90692 in male rats and guinea pigs following single oral dosing of R89674 at 1.0 mg/kg (Study No. N111588/1). An additional pilot study (N115975/2) assessed the absorption, plasma kinetics, and tissue distribution of R89674 and the metabolite R90692 in male rats after a single oral R89674 dose of 2 mg/kg. The plasma pharmacokinetics of R89674 were assessed following a single intravenous dose (5 mg/kg) or a single oral dose (5 mg/kg) of ^{14}C-R89674 in male SPF Wistar rats (Study No. N125250/1). The pharmacokinetics and absolute bioavailability of R89674 and R90672 were assessed in male Beagle dogs following single oral and intravenous doses of R89674 or R90692 at a dose of 2.5 mg/kg (Study No. N125342/1). Also the plasma pharmacokinetics of R89674 and two metabolites, the acid metabolite R90692, and the secondary alcohol metabolite, R87314, were assessed in a pilot study in three Beagle dogs after a single oral dose (0.63 mg) of each of these compounds (Study No. N111677/1).

Table 6: Pilot Absorption Studies

Study No. and Title	Results
Study No. N111588/1: A pilot study on the absorption and plasma levels of R089674 and of its active metabolite, R090692, in male Wistar rats and D.H. guinea pigs after a single oral administration of R089674 at 1.0 mg/kg.	1. Plasma levels of unchanged R089674 were below the limit of quantification at all time points in both rats and guinea pigs. 2. The maximum plasma concentration of the metabolite, R090692, was much higher in both species. 3. In rats, the plasma R090692 T_{max} (h), C_{max} (ng/ml), $T_{1/2}$ (h), and AUC_{0-3h} (ng x h/ml) were: 0.33, 83, 1.4, and 114 respectively. 4. In guinea pigs, the plasma R090692 T_{max} (h), C_{max} (ng/ml), $T_{1/2}$ (h), and AUC_{0-3h} (ng x h/ml) were: 0.33, 235, 0.8, and 196 respectively.
Study No. N115975/2: A pilot study on the absorption, plasma kinetics and tissue distribution of R089674 and its active metabolite, R090692, in male Wistar rats after a single oral administration of R089674 at 2.0 mg/kg.	1. Plasma levels of unchanged R089674 were below the limit of quantification at all time points. 2. Maximum plasma concentrations for the carboxylic acid metabolite, R090692 were much higher. 3. The plasma R090692 T_{max} (h), C_{max} (ng/ml), $T_{1/2}$ (h), and AUC_{0-3h} (ng x h/ml) were: 0.33, 110, 2.1, and 194 respectively. 4. Maximum metabolically formed R090692 was observed in the liver and kidney 20 minutes after gavage (the same as plasma) and peak levels in liver and kidney were 29 and 5 times those in plasma respectively. 5. Brain levels of R090692 peaked at one hour and peak brain concentrations were approximately 26 fold lower than peak plasma concentrations.
Study No. N111677: A pilot study on the plasma kinetics of R089674, R090692 and R087314 in the Beagle dog after a single oral dose of each of these compounds at 0.63 mg/kg	1. R089674 and R087314 were rapidly metabolized to R090692. 2. The best plasma exposure for the acid metabolite, R090692, was obtained following R089674 oral administration rather than oral administration of R090692 itself. 3. Following administration of R089674, the plasma T_{max} (h), C_{max} (ng/ml), $T_{1/2}$ (h), and $AUC_{0-\infty}$ (ng x h/ml) for R090692 were: 1.0, 353, 1.8, and 1324 respectively. 4. Following administration of R090692, the plasma T_{max} (h), C_{max} (ng/ml), and AUC_{0-3h} (ng x h/ml) for R090692 were: 1.7, 186, and 921 respectively. 5. Following administration of R087314, the plasma T_{max} (h), C_{max} (ng/ml), and AUC_{0-3h} (ng x h/ml) for R090692 were: 2.7, 182, and 957 respectively. 6. Plasma concentrations of R087314, when measured, were below detection limits after single oral doses of R089674 or R090692.

Study title: Plasma kinetics of R089674 and metabolically formed R090692 after a single intravenous (5 mg/kg) administration and absorption and tissue distribution of R089674 and metabolically formed R090692 after a single oral (5 mg/kg) administration of ^{14}C-R089674 in the male SPF Wistar rat.

Study no.:	N125250/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Janssen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B-2340 Beerse, Belgium
Date of study initiation:	February 17, 1997
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	^{14}C-R089674, Batch # 1303, specific activity of 590 MBq/mmole, radiochemical purity of 99.2%.

Methods

^{14}C-R089674 was administered either by single intravenous (ten groups of four rats) or by single oral (eight groups of four rats) administration to male SPF Wistar rats at 5 mg/kg. The intravenously dosed animals were sacrificed at 7, 15, and 30 minutes and 1, 2, 4, 8, 24, 48, and 96 hours after dosing. The orally dosed animals were sacrificed 20 minutes and 1, 2, 4, 8, 24, 48, and 96 hours after dosing. All animals were sampled for blood (processed to plasma), and samples of the following tissues were also collected from the orally dose animals: brain, pituitary gland, eyes, lacrimal glands, lymph nodes, salivary glands, thyroid, thymus, heart, lung, liver, kidney, adrenal glands, pancreas, spleen, esophagus, stomach, small and large intestine, tesicle, seminal vesicle, epididymus and prostate, urine bladder, muscle, skin, peri-renal and subcutaneous fat, brown fat, bone marrow, bone, and trachea. Total radioactivity was determined with scintillation counting.

Results

After intravenous administration of 5 mg/kg ^{14}C-R089674, plasma concentrations of unchanged R089674 declined with a $t_{1/2}$ of approximately 0.1 hours, and were below the limit of quantification one hour post-dose. The volume of distribution of R089674 was estimated at 6.8 L/kg which is much greater than the blood volume for rats (50-70 ml/kg) suggesting substantial tissue distribution. The total plasma clearance was estimated as 35.7 L/kg indicating extrahepatic and extrarenal clearance.

The results indicate that R089674 was rapidly metabolized to its carboxylic acid metabolite, R090692. The $AUC_{0-\infty}$ of R089674 was only 7% of that of the total radioactivity, and minutes after intravenous administration of ^{14}C-R089674, plasma concentrations of R090692 were much higher than those of R089674. The $AUC_{0-\infty}$ of

R090692 was 92% of that of the total radioactivity, and R090692 demonstrated a $t_{1/2}$ of approximately one hour. The R089674 and R090692 pharmacokinetic parameters for intravenous and oral administration are summarized in Table 7.

After oral administration of ^{14}C-R089674, plasma concentrations of R089674 were below the limit of detection at all sampling time-points. The oral bioavailability was estimated to be <7%. Maximum plasma levels of R090692 were observed at 20 minutes after dosing and plasma $t_{1/2}$ for R090692 was approximately 2 hours. The $AUC_{0-\infty}$ for R090692 was 79% of that of the total radioactivity.

Table 7: Pharmacokinetic Parameters of Unchanged R089674 (UD) and R090692 in Plasma Following Intravenous and Oral Administration of ^{14}C-R089674

Parameter (units)	*UD*	*R090692*
Intravenous		
β (h^{-1})	5.65	-[1)]
$t_{1/2,\beta}$ (h)	0.123	0.932
Vc (l/kg)	6.83	-
Vd_{ss} (l/kg)	6.83	-
Cl (l/h/kg)	35.7	-
$AUC_{0-\infty}$ (µg.h/ml)	0.140	1.92
T_{max} (h)	-	0.25
C_{max} (µg/ml)	-	2.43 ± 0.33
Oral		
T_{max} (h)	-	0.33
C_{max} (µg/ml)	< 0.010	0.194 ± 0.049
$t_{1/2,\beta}$ (h)	-	1.99
$AUC_{0-\infty}$ (µg.h/ml)	< 0.01	0.477
F_{abs} (%)	< 7	-

1) could not be calculated

Approximately 80% of the dose was recovered in the gastrointestinal contents up to eight hours after oral administration in agreement with the low oral bioavailability for R089672. In most of the examined tissues, maximum concentrations of the total radioactivity were observed within the first hour of oral administration indicating a rapid distribution of R089674 and R090692. The highest tissue concentrations of radioactivity were found in the small and large intestines and in the urinary bladder. Radioactive exposure levels (AUC_{0-8h}) were 22-25 times higher in liver and stomach tissue and 5-12

times higher in the esophagus, trachea, prostate, kidney, and seminal vesicles compared to plasma. However, radioactive exposures in other tissues including eyes and skin were similar to those of plasma. The lowest radioactive concentrations were found in subcutaneous fat, testicle, and brain. Radioactive concentrations rapidly declined in all the examined tissues and no irregular tissue retention or accumulation was observed. The relative tissue exposures following oral administration of ^{14}C-R089674 are summarized in Table 8.

Table 8: Tissue Radioactive Exposures Following Oral Administration of ^{14}C-R089674.

Time (h) / Tissue	$AUC_{0\text{-}8\ h}$ of TR (µg-eq.h/ml or g)	$AUC_{0\text{-}8\ h}$-ratio tissue/plasma
Plasma	0.588 (0.474[1])	(1.00)
Brain	-	≤ 0.2[2]
Pituitary gland	-	≤ 1[2]
Eyeballs	-	≤ 0.5[2]
Lacrimal gland	0.428	0.73
Lymph nodes	0.690[1]	1.46[1]
Salivary gland	-	0.69[2]
Thyroid	-	1.63[2]
Thymus	-	0.60[2]
Heart	-	0.49[2]
Lung	1.09	1.86
Liver	12.8	21.9
Kidney	3.28	5.59
Adrenal gland	-	≤ 5[2]
Pancreas	1.56[1]	3.29[1]
Spleen	0.616[1]	1.30[1]
Oesophagus	7.28	12.4
Stomach tissue	14.9	25.3
Small int. tissue	92.5	157
Large int. tissue	42.0	71.4
Testicle	-	0.22[2]
Seminal vesicle	2.90	4.93
Epididymis	-	0.61[2]
Prostate	3.90	6.64
Urine-bladder	63.7	108
Muscle	0.347[1]	0.73[1]
Skin	0.433[1]	0.91[1]
Perirenal fat	-	3.05[2]
Subcutaneous fat	-	0.24[2]
Brown fat (hib. gl.)	0.403	0.69
Bone marrow	-	1.18[2]
Bone	0.370	0.63
Trachea	3.97	6.76

1) $AUC_{0\text{-}4\ h}$-value or their ratio

2) $C_{1\ h}$-ratio

Study title: Pharmacokinetics and absolute bioavailability of R089674 and R090692 in male Beagle dogs after a single oral and intravenous administration of a R089674 or R090692 solution at 2.5 mg/kg.

Study no.:	N125342/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Janssen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B2340 Beerse, Belgium
Date of study initiation:	November 28, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Lot # ZR089674PFA061, purity was not reported. R090692 (actually R123869: R090692 containing one equivalent of crystal water), lot # JWEE_0002_059_1, purity was not reported.

Methods

Four male beagle dogs were orally and intravenously dosed with 2.5 mg/kg R089674 or R090692 according to the dosing schedule shown below in Table 9.

Table 9: Experimental Dosing Schedule for Study No. N125342/1

	Group I		Group II	
Dog No.	12565	12785	12892	12897
Phase I	R089674	R089674	R090692	R090692
	Route: IV	Route: PO	Route: IV	Route: PO
Phase II	R089674	R089674	R090692	R090692
	Route: PO	Route: IV	Route: PO	Route: IV
Phase III	R090692	R090692	R089674	R089674
	Route: IV	Route: PO	Route: IV	Route: PO
Phase IV	R090692	R090692	R089674	R089674
	Route: PO	Route: IV	Route: PO	Route: IV

Blood samples processed to plasma were obtained predose and at 0.125 (IV only), 0.25 (IV only), 0.5, 1, 3, 6, 8, 10 and 24 hours after each dose administration. Plasma samples were analyzed for R089674 and/or R090692 with an HPLC method.

Results

Plasma concentrations of R089674 following intravenous administration of R089674 decreased rapidly and were at or below the quantification limit 6 hours after dosing. The

relatively large volume of distribution (average of 4.6 L/kg) indicated extravascular distribution. The plasma $t_{1/2}$ was short with a mean value of 0.918 hours. Plasma concentrations of the active metabolite, R090692, following intravenous administration of R089674 exceeded those of the parent compound 0.5 hours after dosing. The mean C_{max} concentration for R090692 was 0.970 μg/ml at one hour after dosing, and the plasma $t_{1/2}$ for R090692 was approximately 4 hours.

After oral administration of R089674, plasma concentrations of R089674 were low and below the quantification limit 6 hours after dosing. The mean C_{max} was 0.040 mg/ml, and the T_{max} occurred variably from 0.5 to 3 hours after dosing. Absolute bioavailability of R089674 was a relatively low value of approximately 15%. Plasma concentrations of R090692 were higher than those of R089674 3 hours after R089674 oral dosing and maximum plasma concentrations of 1.13 mg/ml were variably achieved from 0.5 to 3 hours after dosing. The individual and mean plasma concentrations of R089674 and R090692 following intravenous and oral dosing of R089674 are shown in Table 10 and Table 11 respectively.

Table 10: Individual and Mean Plasma Concentrations of R089674 in Beagle Dogs After Single Intravenous and Oral Administrations of 2.5 mg/kg R089674

Intravenous administration						
Time post-dose		12565	12785	12892	12897	mean ± S.D.
0		NQ	NQ	NQ	NQ	NQ
0.125		(b) (4)				0.611 ± 0.213
0.25						0.545 ± 0.129
0.5						0.354 ± 0.075
1						0.210 ± 0.045
3						0.037 ± 0.008
6		NQ[a]	0.015	NQ	NQ	NQ[b]
8		NQ	NQ	NQ	NQ	NQ
10		NQ	NQ	NQ	NQ	NQ
24		NQ	NQ	NQ	NQ	NQ
V	l/kg	5.12	5.37	4.14	3.82	4.62 ± 0.76
k_{10}	h^{-1}	0.932	0.521	0.921	0.821	0.799 ± 0.192
Cl	l/h/kg	4.80	2.80	3.82	3.13	3.64 ± 0.88
$t_{1/2}$	h	0.744	1.330	0.753	0.844	0.918 ± 0.279
AUC_{0-t}[c]	µg.h/ml	0.492	0.865	0.618	0.745	0.680 ± 0.161
$AUC_{0-\infty}$	µg.h/ml	0.521	0.894	0.655	0.798	0.717 ± 0.163
Oral administration						
Time post-dose		12565	12785	12892	12897	mean ± S.D.
0		NQ	NQ	NQ	NQ	NQ
0.5		(b) (4)				0.029 ± 0.025
1						0.029 ± 0.013
3						0.025 ± 0.010
6		NQ	NQ	NQ	NQ	NQ
8		NQ	NQ	NQ	NQ	NQ
10		NQ	NQ	NQ	NQ	NQ
24		NQ	NQ	NQ	NQ	NQ
C_{max}	µg/ml	0.032	0.036	0.065	0.028	0.040 ± 0.017
T_{max}	h	1	3	0.5	3	1.9 ± 1.3
$AUC_{0-3\ h}$	µg.h/ml	0.072	0.070	0.104	0.053	0.075 ± 0.021
F_{abs}	%	17.9	15.5	18.2	10.9	15.6 ± 3.37

[a] NQ: < 0.010 µg/ml.
[b] median.
[c] AUC_{0-t}: AUC to the timepoint of the last measurable plasma concentration (t = 3 or 6 h).

Table 11: Individual and Mean Plasma Concentrations of R090692 in Beagle Dogs After Single Intravenous and Oral Administrations of 2.5 mg/kg R089674

Intravenous administration					
Time post-dose	12565	12785	12892	12897	mean ± S.D.
0	NQ	NQ	NQ	NQ	NQ
0.125	(b) (4)				0.342 ± 0.295
0.25					0.463 ± 0.070
0.5					0.773 ± 0.098
1					0.970 ± 0.106
3					0.573 ± 0.087
6					0.257 ± 0.098
8					0.150 ± 0.059
10					0.090 ± 0.033
24					0.011[a]
C_{max} µg/ml	0.836	0.953	1.09	0.999	0.970 ± 0.106
T_{max} h	1	1	1	1	1
$t_{1/2}$ h	2.23	4.65	4.72	4.89	4.12 ± 1.27
$AUC_{0-\infty}$ µg.h/ml	3.58	6.14	4.83	4.69	4.81 ± 1.05
Oral administration					
Time post-dose	12565	12785	12892	12897	mean ± S.D.
0	NQ	NQ	NQ	NQ	NQ
0.5	(b) (4)				0.639 ± 0.709
1					0.844 ± 0.509
3					0.846 ± 0.290
6					0.355 ± 0.169
8					0.198 ± 0.115
10					0.115 ± 0.060
24	NQ	0.012	NQ	NQ	NQ[a]
C_{max} µg/ml	0.787	1.25	1.69	0.807	1.13 ± 0.43
T_{max} h	1	3	0.5	3	1.9
$t_{1/2}$ h	2.69	3.45	3.27	2.46	2.97 ± 0.47
$AUC_{0-\infty}$ µg.h/ml	4.90	8.03	5.22	3.97	5.53 ± 1.75

[a] median.

The plasma concentrations of R090692 following intravenous administration of R090692 demonstrated a biphasic decline. The volume of distribution of the central compartment was an average of 0.312 L/kg which is approximately 3 times larger than the blood volume of dogs (70 to 110 ml/kg) suggesting some degree of extravascular distribution, but much lower than that of R089674 following IV administration of

R089674. Clearance of R090692 was also much lower at 0.332 L/h/kg and the $t_{1/2}$ was 2.1 hours.

After oral dosing of R090692, a C_{max} value of 0.625 mg/ml was attained between 1 and 3 hours after dosing, and the absolute bioavailability was 40.9%. The pharmacokinetic parameters for plasma R090692 following intravenous and oral administration of R090692 are summarized below in Table 12.

Table 12: Individual and Mean Pharmacokinetic Parameters of R090692 in Beagle Dogs after Single Intravenous and Oral Administrations of 2.5 mg/kg R090692

Intravenous administration						
Parameter		#12565	#12785	#12892	#12897	Mean ± SD
A	µg/ml	6.52	6.53	5.49	7.22	6.44 ± 0.72
B	µg/ml	1.31	2.41	1.34	1.68	1.68 ± 0.51
α	h^{-1}	2.40	2.32	2.20	3.32	2.56 ± 0.51
β	h^{-1}	0.286	0.351	0.314	0.349	0.325 ± 0.031
$t_{1/2\beta}$	h	2.42	1.98	2.21	1.99	2.12 ± 0.21
V_c	l/kg	0.320	0.280	0.366	0.281	0.312 ± 0.041
Cl	l/h/kg	0.343	0.258	0.370	0.358	0.332 ± 0.051
$V_{d\beta}$	l/kg	1.20	0.735	1.18	1.03	1.04 ± 0.21
$AUC_{0\text{-}10\ h}$	µg.h/ml	7.69	10.4	7.21	7.35	8.16 ± 1.50
$AUC_{0\text{-}\infty}$	µg.h/ml	7.94	10.7	7.42	7.52	8.40 ± 1.55
Oral administration						
Parameter		#12565	#12785	#12892	#12897	Mean ± SD
C_{max}	µg/ml	0.648	0.598	0.837	0.418	0.625 ± 0.172
T_{max}	h	3	1	1	3	2.00 ± 1.15
$t_{1/2\beta}$	h	2.74	3.33	2.00	6.11	3.55 ± 1.79
$AUC_{0\text{-}24\ h}$	µg.h/ml	3.37	2.83	3.36	2.97	3.13 ± 0.275
$AUC_{0\text{-}\infty}$	µg.h/ml	3.64	3.15	3.47	3.13	3.35 ± 0.25
F_{abs}	%	45.8	29.4	46.8	41.6	40.9 ± 7.99

Distribution

The absorption and tissue distribution of R89674 and R90692 were examined following a single intravenous or oral dose of ^{14}C-R89674 (a dose of 5 mg/kg for both routes) in male SPF Wistar rats (Study No. N125250/1). A similar pilot study was conducted in rats using a single 2 mg/kg oral dose of R89674 (Study No. N115975/2). Study No. N125342/1 assessed the pharmacokinetics and absolute bioavailability of R89674 and

R90692 in male beagle dogs after single 2.5 mg/kg oral and intravenous doses of both compounds. The tissue distribution of repeated-doses of R89674 was examined as part of the toxicokinetic analysis from the 6-month oral toxicology study in dogs (Study No. for the toxiciokinetic analysis: N122428/3).

The plasma protein binding of R89674 and R90692 were examined in plasma samples from male adult humans, male Beagle dogs, male and female rats, female rabbits, and male Swiss mice (Study No. N125358/1).

The ocular tissue distribution of ^{14}C-R89674 was examined in male rabbits following a single dose of 0.15 ml/eye with sampling periods of 0.5, 1, 2, 4, 6, 8, and 168 h (Study No. AAL00004). Distribution of ^{14}C-R89674 was measured in aqueous humor, iris-ciliary body, conjunctiva, lens, optic nerve, retinal/choroid, sclera, cornea, and eyelids.

Study title: The plasma protein binding and the distribution of R089674 and its carboxylic acid metabolite (R090692) in blood.

Study no.:	N125358/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Pharmacokinetics, Janssen Research Foundation, Turnhoutseweg 30, B-2340 Beerse, Belgium
Date of study initiation:	March 20, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	1. ^{3}H-R089674, batch 1318, radiochemical purity of 98.1%; batch 1330, radiochemical purity of 97.9%; specific activity of 27.7 mCi/mg. 2. Unlabeled R089674, batch ZR089674PFA061, purity not identified. 3. ^{3}H-R089674, batch 1320, radiochemical purity of 99.0%; batch 1331, radiochemical purity of ≥ 98.0%; specific activity of 974 mCi/mg. 4. R090692.H20, lot name JWEE-0002-059; purity not identified.

Methods

The plasma protein binding of R089674 and of R090692 was determined in individual human plasma samples. Human plasma from five males was combined with 5 ng ^{3}H-R089674/ml or with 20 ng ^{3}H-R090692/ml. The protein binding of R089674 and R090692 were also determined in individual or pooled plasma samples from animals. Dog and rabbit plasma, pooled rat plasma, and pooled mouse plasma were combined

with 100 ng/ml of ^{3}H-R089674 or with 1000 ng/ml of ^{3}H-R090692. Dog plasma was also fortified with 5 ng/ml of ^{3}H-R089674 or with 20 ng/ml of ^{3}H-R090692.

The binding of ^{3}H-R089674 and ^{3}H-R090692, at 10 and 50 ng/ml respectively, to various concentrations of human serum albumin (≈ 0.3 to 6 g/100 ml) and human α1-acid glycoprotein (≈ 0.02 to 0.2 g/100 ml) were studied. After addition of the radioactive compounds, duplicate samples were subject to equilibrium dialysis for the plasma protein and human protein binding experiments. Radioactive levels were measured in duplicate samples of plasma or protein incubation solutions before dialysis and in the contents of the plasma, protein, and buffer compartments of the dialysis cells.

Blank whole blood obtained from the same human subjects and animals (mice, rats, rabbits, and dogs) as in the plasma protein binding experiments was combined with the same concentrations of ^{3}H-R089674 and ^{3}H-R090692 as that used in the plasma protein binding experiments. After incubation, plasma was obtained from the blood solutions and radioactivity was measured in triplicate plasma samples.

Results

Both ^{3}H-R089674 and ^{3}H-R090692 were stable during equilibrium dialysis against human serum albumin for four hours at 37°C. ^{3}H-R090692 in plasma and blood were shown to be stable under experimental conditions; however, there were species and matrix differences in the stability of ^{3}H-R089674. In human, dog, and mouse plasma incubated for four hours, and in human and dog blood incubated for 30 minutes, R089674 underwent a small degree of degradation (2.4 to 12.9% for plasma and 7.3 and 15.3% for dog and human blood respectively). In rat blood and plasma and mouse blood there was substantial conversion (≈ 55% for plasma and 27.9-47.2% for blood) to metabolites. In rabbit plasma and blood, ^{3}H-R089674 was almost completely converted to metabolites. The metabolic conversion products were R090692 in blood and plasma and an alcohol metabolite, R087314, in blood. Because of the substantial instability of R089674 in rat and rabbit plasma and blood, and mouse blood, the plasma blood distribution of R089674 could not be determined in any of these species, and plasma protein binding could not be determined in rat and rabbit plasma. The plasma protein binding and blood distribution of R090692 was determined for all species.

While R089674 bound plasma proteins to a low to moderate degree in all species where measurements were possible, the degree of binding to R090692 varied between the other species and humans. The plasma protein binding of R089674 averaged 41%, 52% and 40% in dog, mouse and human plasma respectively. The plasma protein binding of R090692 averaged 12-14% in rats and mice, 20% in dogs, 28% in rabbits, and 63% in humans. Higher concentrations of R089674 and R090692 were sometimes incubated with plasma from dogs and mice (5 and 100 ng/ml ^{3}H-R089674 and 20 and 1000 ng/ml ^{3}H-R090692 for dogs and 100 ng/ml ^{3}H-R089674 and 1000 ng/ml ^{3}H-R090692 for mice) compared to the concentrations incubated with human plasma (5 ng/ml ^{3}H-R089674 and 20 ng/ml ^{3}H-R090692). However, concentration effects did not appear to influence binding, because in dog plasma, plasma protein binding did not

change for R089674 and R090692 incubated at 5 and 100 ng/ml or 20 and 1000 ng/ml respectively.

Approximately 27% and 10% of the tested concentrations of ^{3}H-R089674 bound human serum albumin (HSA) and α1-acid glycoprotein (α1-AGP) respectively, while 72% of ^{3}H-R090692 bound to HSA with negligible binding to α1-AGP.

Blood distribution was very similar for human and dog blood. In human and dog blood, the blood to plasma concentration ratio for R089674 was approximately 1.4. The largest fraction of R089674 distributed to blood cells (62-66%) and approximately 15% bound to plasma proteins, and 20-23% remained unbound in plasma water. The blood to plasma concentration ratio of R090692 was approximately 0.63 in human blood and 0.75-0.89 in animal blood. In human blood, the largest fraction of R090692 was bound to plasma proteins (≈ 52%), and approximately 16% bound to red blood cells with 32% remaining unbound in plasma water. In the blood of dogs, rats, mice and rabbits, the largest fraction of R090692 remained unbound in plasma water (49-62%) with 7-23% bound to plasma proteins, and 20-39% bound to blood cells.

Study title: Mass balance and ocular tissue distribution of [^{14}C]R089674 following ocular administration to male New Zealand White rabbits.

Study no.:	AAL00004
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 7, 2005
GLP compliance:	Yes, except Sponsor did not supply stability data or compliance information for test article or vehicle characterization.
QA statement:	Yes
Drug, lot #, and % purity:	[^{14}C]R089674, Batch # 1890, radiochemical purity of 98.34%

Methods

New Zealand White Rabbits were administered 0.15 mg/eye (~7.7 μCi/eye) R89674 to both eyes. Urine and feces were collected at various intervals until 168 hours after dosing. Groups of animals were euthanized at 0.5, 1, 2, 4, 6, 8, and 168 hours after dosing, and blood samples for analysis were collected just prior to euthanasia and processed to plasma. After euthanasia, selected ocular tissues (conjunctiva, upper and lower eyelids, aqueous humor, optic nerve, cornea, lens, iris-ciliary body, vitreous humor, retina with choroid, sclera) were collected from each animal, and radioactivity was measured.

Results

Mean concentrations of radioactivity in the left eye and right eye at each sampling time-point were generally similar, with concentrations declining from early maximal values. Up to and including the 8 hour postdose sampling time, the highest mean concentrations of radioactivity were observed in the aqueous humor, iris-ciliary body, cornea, and eyelids. In general, mean C_{max} values were observed at the first sampling time point following dosing (0.5 hour); however, three ocular tissues (right and left aqueous humor and left optic nerve) had C_{max} values observed at 1 hour postdose, and 8 ocular tissues (conjunctiva, upper and lower eyelids, aqueous humor, optic nerve, cornea, lens, iris-ciliary body, vitreous humor, retina with choroid, sclera) had C_{max} values observed from 2-6 hours postdose. The percentage of radioacitivity measured in ocular tissues at each sampling time-point is noted in Table 13 below.

Table 13: Mean (n = 3) Percentages of Radioactivity in Ocular Tissues at 0.5, 1, 2, 4, 6, 8, and 168 Hours After a Single Ocular (0.15 mg/eye; both eyes) Administration of [^{14}C]R089674 to Male New Zealand White Rabbits.

	Percent of Radioactive Dose															
	0.5 h				1 h				2 h				4 h			
	Eye, Left		Eye, Right		Eye, Left		Eye, Right		Eye, Left		Eye, Right		Eye, Left		Eye, Right	
Matrix	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aqueous Humor	0.47	0.08	0.31	0.20	0.60	0.17	0.42	0.05	0.38	0.35	0.30	0.36	0.39	0.09	0.45	0.18
Upper Eyelid	1.49	0.78	1.27	0.47	1.05	0.75	0.86	0.71	2.15	0.48	0.32	0.31	0.81	0.58	0.50	0.65
Lower Eyelid	1.62	0.54	1.55	1.38	0.72	0.30	1.15	1.06	5.45	2.68	1.61	2.46	1.19	1.22	1.02	1.56
Conjunctiva	0.55	0.27	0.34	0.20	0.19	0.11	0.18	0.09	0.11	0.03	0.05	0.05	0.08	0.03	0.05	0.01
Cornea	1.94	0.56	2.11	0.84	1.46	0.33	1.55	0.70	0.98	0.03	0.99	0.40	0.57	0.10	0.62	0.18
Iris-ciliary Body	0.19	0.15	0.11	0.03	0.15	0.05	0.13	0.04	0.19	0.11	0.18	0.04	0.13	0.03	0.10	0.10
Lens	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01
Optic Nerve	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Retina/Choroid	0.03	0.01	0.04	0.02	0.03	0.01	0.03	0.02	0.02	0.02	0.04	0.02	0.01	0.01	0.07	0.06
Sclera	0.65	0.20	0.44	0.17	0.42	0.24	0.37	0.04	0.17	0.01	0.20	0.03	0.12	0.04	0.11	0.03
Vitreous Humor	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.18	0.29	0.02	0.01	0.01	0.01	0.01	0.00

	Percent of Radioactive Dose											
	6 h				8 h				168 h			
	Eye, Left		Eye, Right		Eye, Left		Eye, Right		Eye, Left		Eye, Right	
Matrix	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aqueous Humor	0.15	0.02	0.16	0.03	0.10	0.04	0.09	0.02	0.00	0.00	0.00	0.00
Upper Eyelid	0.54	0.29	0.72	0.72	0.83	0.59	1.01	0.71	0.11	0.08	0.10	0.11
Lower Eyelid	0.74	0.29	0.63	0.58	0.81	0.18	0.59	0.45	0.14	0.05	0.15	0.12
Conjunctiva	0.05	0.05	0.04	0.04	0.02	0.02	0.02	0.01	0.00	0.00	0.00	0.00
Cornea	0.27	0.04	0.27	0.08	0.19	0.02	0.19	0.06	0.00	0.00	0.00	0.00
Iris-ciliary Body	0.08	0.01	0.06	0.02	0.07	0.01	0.04	0.00	0.00	0.00	0.00	0.00
Lens	0.01	0.01	0.02	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00
Optic Nerve	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Retina/Choroid	0.00	0.00	0.03	0.01	0.00	0.00	0.02	0.01	0.00	0.00	0.00	0.00
Sclera	0.07	0.01	0.08	0.02	0.04	0.01	0.05	0.01	0.00	0.00	0.00	0.00
Vitreous Humor	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

Mean plasma concentrations of radioactivity were < 0.1 μg-eq/g at all sampling times. The maximal mean concentration was observed at 0.5 h postdose. Concentrations then declined from maximal concentrations and were measurable through 4 hours postdose. The percentage of radioactive dose in plasma is summarized in Table 14.

Table 14: Mean (n=3) Percentages of the Radioactive Dose in Plasma After a Single Ocular (0.15 mg/eye; both eyes) Administration of [^{14}C]R089674 to Male New Zealand White Rabbits.

Time (h)	Percent of Radioactive Dose	
	Mean	SD
0.5[a]	3.52	0.16
1[b]	1.87	0.35
2[c]	0.37	0.02
4[d]	0.09	0.15
6[e]	0.00	0.00
8[f]	0.00	0.00
168[g]	0.00	0.00

a Samples collected from Animal Nos. 1, 2, and 3.
b Samples collected from Animal Nos. 4, 5, and 6.
c Samples collected from Animal Nos. 7, 8, and 9.
d Samples collected from Animal Nos. 10, 11, and 12.
e Samples collected from Animal Nos. 13, 14, and 15.
f Samples collected from Animal Nos. 16, 17, and 18.
g Samples collected from Animal Nos. 19, 20, and 21.

[^{14}C]R089674-derived radioactivity was eliminated almost exclusively in urine, with mean totals of 62.26% and 18.71% recovered in urine and feces respectively, through 168 hours postdose. The percentage of radioactivity recovered in urine and feces is summarized in Table 15.

Table 15: Mean (n=3) Recoveries of Radioactivity in Urine and Feces for Each Collection Interval After a Single Ocular (0.15 mg/eye; both eyes) Administration of ^{14}C-R089674 to Male New Zealand White Rabbits.

	Percent of Radioactive Dose			
	Urine		Feces	
Time (h)	Mean	SD	Mean	SD
0-4	12.15	21.05	0.70	1.00
4-8	0.00	0.00	0.77	0.63
8-24	34.66	21.68	9.72	9.55
24-48	10.76	8.60	4.77	4.27
48-72	3.39	1.90	1.27	0.67
72-96	0.46	0.47	0.83	0.62
96-120	0.43	0.57	0.34	0.29
120-144	0.31	0.27	0.16	0.28
144-168	0.11	0.11	0.15	0.27

Metabolism

The *in vivo* metabolism of R89674 was assessed as part of the absorption studies, N125250/1, N11588/1, N115975/2, N125342/1, and N111677/1 reviewed above. In addition, the *in vivo* metabolism of R89674 and metabolites in male and female rats were evaluated after a single oral dose of 5 mg/kg ^{14}C-R89674 (Study No. N125294/1). *In vitro,* the metabolism of R89674 and R90692 was characterized in hepatocytes and liver subcellular fractions from mice, rats, rabbits, dogs, and humans (Study No. N126519/1). Also, the metabolism of ^{14}C-R89674 in human liver microsomal fractions and the CYP-450 isozymes involved in the metabolism of R89674 were evaluated (Study No. N125328/1).

Study title: The metabolism and excretion of R089674 in the male and female SPF Wistar rat after a single oral administration of 5 mg ^{14}C-R089674

Study no.:	N125294/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Janssen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B-2340 Beerse, Belgium
Date of study initiation:	February 17, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	^{14}C-R089674, batch # 1303, radiochemical purity of 99.0%, specific

activity of 16 mCi/mmole.

Key Findings: After a single oral dose of 5 mg of ^{14}C-R089674/kg in male and female rats, the radioactivity was completely excreted within 96 hours of dosing, and the primary route of excretion was via feces (87-92%) followed by urine (11-15%). The major metabolite was the carboxylic acid metabolite, R090692, in feces, urine, and plasma.

Methods

Five male and five female SPF Wistar rats were dosed by oral gavage with 1.0 ml of ^{14}C-R089674 formulation per 100 g body weight to provide a dose of 5 mg/kg. Urine and feces were collected from these rats. In addition, four groups of four female rats were dosed in the same manner for later collection of plasma samples. Urine was collected at 0-4h, 4-8 h, 8-24 h, 24-48 h, 48-72 h, and 72-96 h after dosing, and feces was collected every 24 hours for four days. Blood was collected and processed to plasma at 1, 4, 8, and 24 hours. Total radioactivity was measured in the urine and feces, as well as the major metabolites using radio-HPLC. Plasma samples were analyzed for total radioactivity, R089674 and R090692 content, and the metabolic pattern of R089674.

Results

Radioactivity was excreted rapidly and predominantly in the feces, and excretion routes and rates were comparable between male and female rats. Approximately 93-96% of the administered radioactivity was excreted within 24 hours after dosing with 79-86% excreted in the feces and 10-15% excreted in the urine. From 24-48 hours after dosing, 6-8% of the dose was excreted in feces and 0.2-0.3% in urine, and from 0-96 hours after dosing, 87-92% of the dose was recovered in feces, 11-15% in urine and 0.1-0.4% in cage washings. Excretion was complete 96 hours after dosing with recovery of approximately 100% of the dose.

Several R089674 metabolites were identified as shown in the metabolic scheme in Figure 1 below. The primary metabolite was R090692 in urine, feces, and plasma samples as shown in the mass balance tables (Table 16 and Table 17) below. R090692 accounted for 45-72% of all metabolites. Other metabolites represented 1-6% of the administered dose. Only a small fraction (≤ 0.6%) of R089674 was excreted unchanged. There were no major gender-related differences in the nature and relative amounts of the metabolites in each sample fraction.

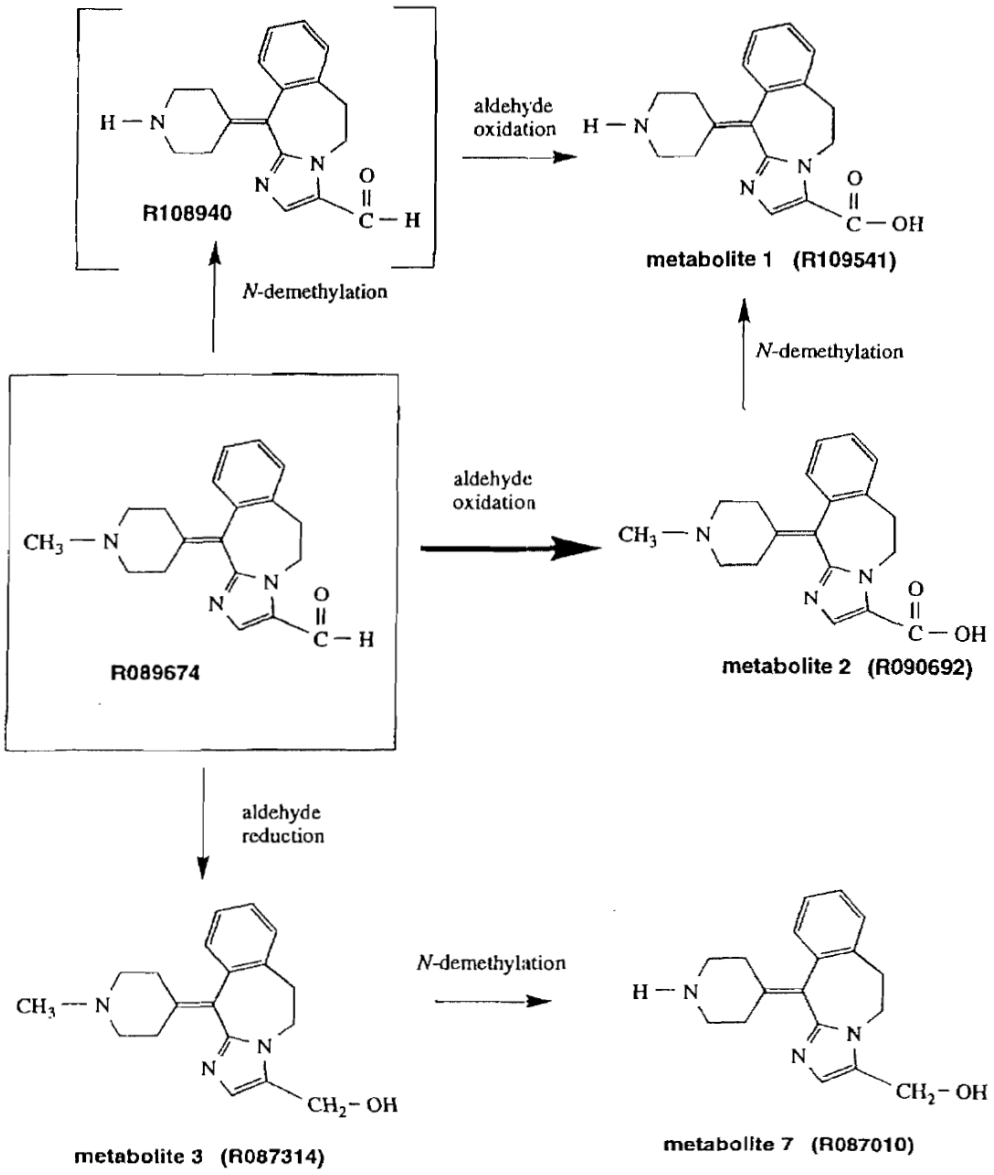


Figure 1: Metabolic Scheme for R089674

Table 16: Mass Balance of R089674 (UD) and Its Major Metabolite in the Pooled Plasma Samples. Data is expressed as the percentage of radioactivity with the concentration in ng-eq/ml in parentheses. Metabolite #2 is as shown in the metabolic scheme.

	males			
Metabolite or UD	0.33-hour (224.6)[a)]	1-hour (200.1)	2-hour (107.7)	4-hour (49.4)
2	75.3 (169.2)	66.8 (133.7)	51.4 (55.4)	56.2 (27.8)
UD	<2.7 (<6.0)	<3.4 (<6.8)	<6.5 (<7.0)	<21.6 (<10.7)

	females		
Metabolite or UD	1-hour (262.5)	4-hour (33.1)	8-hour (24.2)
2	77.6 (203.7)	53.7 (17.76)	52.2 (12.63)
UD	<1.5 (<4.0)	<15.0 (<5.0)	<14.9 (<3.6)

a) total radioactivity concentration in ng-eq./ml.

Table 17: Mass Balance of Unchanged R089674 (UD) and its Major Metabolites in Urine and Feces Collected in the First 48 Hours. Data is expressed as the percentage of dose. Metabolite numbers are as shown in the metabolic scheme with metabolites 8-12 unidentified.

Metabolite or UD	male rats			female rats		
	urine (10.5 %)[a]	faeces (57.1 %)	sum (67.6 %)	urine (14.8 %)	faeces (57.8 %)	sum (72.6 %)
8	- [b]	0.9	0.9	-	1.3	1.3
1	-	2.1	2.1	-	1.5	1.5
2	9.5	39.3	48.8	12.0	33.2	45.2
9	-	2.3	2.3	-	4.7	4.7
10	-	5.0	5.0	-	5.8	5.8
3	-	2.4	2.4	-	3.7	3.7
11	-	3.0	3.0	-	3.2	3.2
12	-	0.9	0.9	-	1.8	1.8
UD	<0.05	<0.2	<0.05	<0.06	0.6	0.6
Sum	9.5	55.9	65.4	12.0	55.8	67.8

[a] total radioactivity (as % of the administered dose) in urine and faeces.
[b] below the limit of quantification.

Study title: The *in-vitro* metabolism of R089674 and R090692 in hepatocytes and liver subcellular fractions of male and female, adult and neonatal mice, male and female rats, female rabbit, male dog, and human.

Study no.:	N126519/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Jannsen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B-2340 Beerse, Belgium.
Date of study initiation:	January 28, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	1. ^{14}C-R089674, Batch #1298, radiochemical purity of ≥ 99%. 2. ^{14}C-R090692, Batch #1301, radiochemical purity of ≥ 99%.

Methods

Hepatocytes and liver subcellular fractions from male and female mice, male and female rats, female rabbits, male dogs, and humans were incubated with ^{14}C-R089674 and ^{14}C-R090692, and the metabolic profile for each compound in each species was

determined. Hepatocyte cultures and subcellular fractions were characterized for CYP-450 content and metabolism of two substrates (7-ethoxycoumarin and scoparone).

Results

The CYP-450 content, and biotransformation of 7-ethoxycoumarin and scoparone were comparable to previously reported values and activities in all species.

The metabolism of R089674 was pronounced in incubates of 12000 x g supernatant fractions for all species except dog. In male rat and female rabbit microsomes, metabolism was extensive, intermediate metabolism occurred in the microsomes of female mice and male rabbits, and the least metabolism occurred in the microsomes of male mice, dogs, and humans. Pronounced metabolism was also observed in hepatocyte incubations for each species except humans. The metabolism of R089674 as a whole was least extensive in human hepatocytes with unchanged drug accounting for 38% with human hepatocytes in suspension culture (120 minute incubation) and 76% in human hepatocytes in primary culture (43 hour incubation). The results are summarized in Table 18.

The metabolic profiles for each species are shown below in Figure 2. The main metabolite for all species was R090692 both in incubations with subcellular fractions and incubations with hepatocytes. Metabolite 6 was another major metabolite in hepatocyte incubations of male and female mice and rats. Minor metabolites included Metabolite 1 in male and female, adult and neonatal mice, male and female rats, rabbits and dogs; Metabolite 3 in male and female neonatal mice, female rats, dog and humans; Metabolite 4 in male and female mice, male and female rats, rabbits and dog; and Metabolite 5 in male mice, male and female rat, dog and human. Biotransformation of ^{14}C-R090692 did not occur in every species including humans, and ^{14}C-R090692 was metabolized to Metabolite 6 only in hepatocytes from male and female mice and rats, and male neonatal mice. Of the test species used in the toxicology studies, the metabolic profile of dogs and female rats contained all the metabolites present in the metabolic profile of humans.

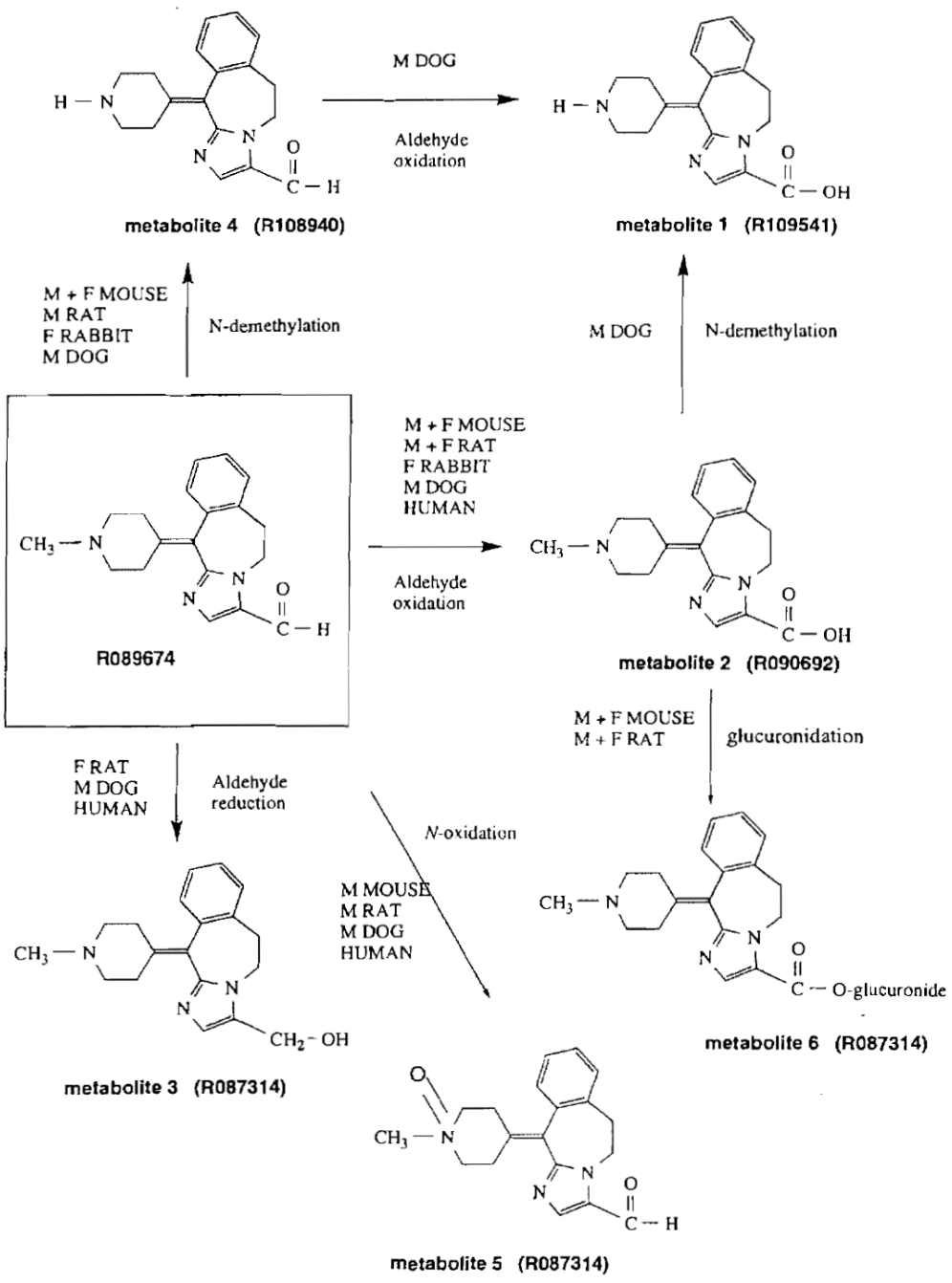


Figure 2: *In Vitro* Metabolic Pathways for R089674 in the Liver of Male and Female Mouse, and Male and Female Rat, Rabbit, Dog, and Human.

Table 18: Mass Balance of R089674 and Major Metabolites In Human Hepatocyte Cultures and Liver Subcellular Fractions.

	^{14}C-R089674					
	suspension culture		primary cell culture		12000 g SN	microsomes
	60 min	120 min	20 h	43 h	120 min	120 min
Metabolite						
2	41.5	47.0	3.0	3.3	95.3	12.1
3	5.8	10.3	9.8	14.8	2.2	-
5	2.2	2.2	- (1)	1.9	-	-
PD (R089674)	45.7	38.0	79.8	75.9	-	81.4
SUM	95.2	97.5	92.6	95.9	97.5	93.5

(1) not detected (detection limit: 200 dpm) or radioactivity < 1% of the injected sample radioactivity.

Study title: An *in vitro* study on the metabolism of ^{14}C-R089674 in human liver microsomal fractions and on the human microsomal cytochrome P-450 forms involved in the metabolism of R089674.

Study no.: N125328/1
Study report location: Electronic transmission
Conducting laboratory and location: Jannsen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B-2340 Beerse, Belgium.
Date of study initiation: February 18, 1997
GLP compliance: No
QA statement: No
Drug, lot #, and % purity:
1. ^{14}C-R089674, Batch # 1297, specific activity of 590 MBq/nmol, radiochemical purity of ≥ 98% (determined with HPLC).
2. Unlabelled R089674, Batch # ZR089674PFA061, purity was not reported.
3. Unlabelled R123869 (R090692 containing 1 equivalent of crystal water), Batch # JWEE-002-059-1, purity was not reported.

Methods

Ten batches of human liver microsomes obtained from kidney transplant donors or patients undergoing partial lobectomy were used for this study. The microsomal batches

were previously characterized for CYP-450 content and activity for the following CYP-450 isozymes: CYPA1,2; CYP2A6; CYP2C19; CYP2C8,9,10; CYP2D6; CYP3A4; and CYP4A. The metabolism of ^{14}C-R89674 was analyzed in all ten batches of human liver microsomes according to an established protocol. The amount of unchanged ^{14}C-R89674 and its metabolites was determined by radio-HPLC.

Diagnostic CYP-450 inhibitors were used to determine which CYP-450 isozmes were involved in R089674 metabolism. The diagnostic inhibitors with their target CYP-450 isozymes shown in parentheses were as follows: 7,8,-benzoflavone (CYP1A2), furafylline (CYP1A2), mephenytoin (CYP2C19), gestodene (CYP3A4), phenacetin (CYP1A2), tobutamide (CYP2C8,9), phenytoin (CYP2C), sulphaphenazole (CYP2C10), coumarin (CYP2A6), aniline (CYP2E1), p-nitrophenol (CYP2E1), quinidine (CY2D6) and troleandomycin (CYP3A4). In the inhibitor experiments, one batch of human liver microsomes was incubated with ^{14}C-R89674 in the presence of each different inhibitor according to established protocols. The amount of unchanged ^{14}C-R89674 and its metabolites was determined by radio-HPLC.

Also the metabolism of specific CYP-450 substrates was examined in a single batch of human liver microsomes in the absence and presence of different concentrations of R089674 (0, 1, 10, and 100 ng/ml) and R090672 (0, 50, 200, and 1000 ng/ml) according to established protocols. The following CYP-450 substrates (with target isozyme in parentheses) were used: tobutamide (CYP2C8,9), chlorzoxazone (CYP2E1), cyclosporine-A (CYP3A4), caffeine (CYP1A2), Coumarin (CYP2A6), lauric acid (CYP4A), testosterone (CYP3A4), and debrisoquine (CYP2D6).

Results

The metabolism of R08674 was comparable in the different batches of human liver microsomes. The main *in-vitro* metabolite was the carboxylic acid metabolite, R090692; however, R090692 only accounted for 4.25 ± 2.24% of the added radioactivity. These results indicate that hepatic microsomes metabolize R089674 to R090692 much less than what is seen *in vivo*, and suggest that CYP-450 enzymes are probably not a major metabolic system involved in the metabolism of R089674.

Experiments with diagnostic inhibitors indicated that approximately 50% inhibition of the overall microsomal metabolism of R089674 and of the formation of R090672 was observed after incubation of the microsomes with mephenytoin (inhibitor of CYP2C19) troleandomycin and gestodene (both inhibitors of CYP3A4). Furafylline and 7,8-benzoflavone, both inhibitors of CYP1A2 inhibited the overall metabolism of R089674 by a maximum of 27%; however, phenacetin, also an inhibitor of CYP1A2, did not affect the overall R089674 metabolism. Inhibitors of CYP2A6, CYP2D6, and CYP2E1 also did not affect the overall metabolism of R089674.

In the experiments examining the effects of R089674 or R090692 on the metabolism of specific CYP-450 substrates, no substantial inhibition of the metabolism of any of the substrates was observed with the highest concentrations of R089674 (100 ng/ml) or

R090692 (1000 ng/ml). The results suggest that CYP-450 enzymes are not a major pathway for R089674 metabolism, and that significant drug-drug interactions mediated by inhibition of CYP-450 enzymes are not expected for R089674 or R090692.

Excretion

The excretion of R89674 following a single oral dose in male and female rats was evaluated as part of Study No. N125294/1 reviewed above in the Metabolism section. The excretion of R89674 following topical ocular administration in rabbits was evaluated as part of Study No. AAL00004 reviewed above.

Pharmacokinetic Drug Interactions

The potential for drug-drug interactions for R89674 was examined in human liver microsomes in Study No. N125328/1 reviewed above. The induction and inhibition of hepatic drug metabolizing enzymes was also assessed in liver microsomes (Study No. N125478/1) prepared from male and female rats dosed in the six-month oral toxicity study (Study No. N122461/2). The R89674 potential for drug-drug interactions was further studied in conjunction with co-medications in human liver microsomes and supernatant fractions (Study No. N125331/1).

Study title: Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes after six months dosing of R089674 in an aqueous solution in male and female SPF Wistar rats.

Study no.:	N125478/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Janssen Research Foundation, Department of Pharmacokinetics and Department of Toxicology, Turnhoutseweg 30, B-2340 Beerse, Belgium.
Date of study initiation:	November 8, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, batch No. ZR089674PFA011, purity was not reported.

Methods

Parts of the livers of male and female SPF Wistar rats from Study No. 3900, a 6-month oral toxicology study, were used for this study. Microsomes were prepared from the liver pieces of each rat, and the microsomal protein, and CYP-450 content were determined for each sample. In addition, the activity of specific CYP-450 isozymes was assessed for each sample. The enzyme activity assays were validated for their ability to measure enzyme induction by demonstrating increased enzymatic activity for liver microsomes prepared from rats treated with known CYP-450 inducers (phenobarbital, β-

naphtoflavone, dexamethasone, ethanol, and clofibrate) compared to the enzymatic activity of liver microsomes prepared from non-induced rats.

Results

In male rats, oral dosing with R089674 at any of the three dose levels (5, 20, and 80/40/60 mg/kg/day) for 6 months did not increase the relative liver weight, microsomal protein content, or CYP-450 content compared to control animals. Microsomes from male rats treated with the medium dose of R089674 (20 mg/kg/day) had reduced (60% of the control group value) 7-ethoxyresorufine-*O*-deethylase activity, and microsomes from rats treated with the lowest R089674 dose of 5 mg/kg/day were slightly induced (117% of control group values) for lauric acid hydroxylase activity.

In female rats, significant elevation of the relative liver weight (130% of the control group) was observed in liver microsomes from rats treated with the medium dose (20 mg/kg/day) of R089674, and CYP-450 content was decreased at the 80/40/60 mg/kg/day R089674 high-dose group (75% of the control group) in liver microsomes. None of the enzymatic activities were induced; however, in liver microsomes from female rats treated with the high dose, the aniline hydroxylase, ethylmorphine-*N*-demethylase, and 7-ethoxyresorufine-*0*-deethylase activities were inhibited 48%, 24%, and 62% respectively compared to the control group.

As the observed effects occurred at R090692 plasma levels greatly exceeding clinical plasma levels, the potential for R089674-mediated drug interactions is expected to be minimal.

Study title: *In vitro* determination of the drug-drug interaction potential of R089674 in human liver microsomes: effects of possible co-medication on the metabolism of R089674.

Study no.:	N125331/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Janssen Research Foundation, Department of Pharmacokinetics, Turnhoutseweg 30, B-2340 Beerse, Belgium.
Date of study initiation:	March 3, 1997
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	^{14}C-R089674, Batch 1297, specific activity of 590 MBq/mmol, and radiochemical purity of 98%.

Methods

^{14}C-R089674 was incubated with human liver microsomes or human liver 12,000 x g supernatant fractions in the absence and presence of drugs known to be metabolized

by different CYP-450 isozymes. The overall metabolism of R089674 and the formation of the major metabolite R090692 were measured. Drugs which were examined for their possible inhibitory effects on R089674 biotransformation included: adrenaline, beclomethasone, budesonide, codeine, diphenhydramine, erthyromycin, ketotifen, loratadine, salbutamol, terfenadine, carboxyterfenadine, and theophylline.

Results

The metabolism of R089674 was slow in liver microsomes and only low amounts (6.33 ± 0.35% of the injected radioactivity) of the major metabolite, R090692 was formed. In contrast incubation of ^{14}C-R089674 with human liver 12,000 x g supernatant resulted in a rapid and complete metabolism of R089674 to R090692, indicting that the metabolism of R089674 was almost completely independent of metabolism by CYP-450 enzymes.

The overall metabolism of R089674 and the formation of R090692 in human liver microsomes or in human liver 12,000 x g supernatant was not inhibited after incubation with adrenaline, beclomethasone, budesonide, codeine, diphenhydramine, salbutamol, terfenadine, carboxyterfenadine, and theophylline. R089674 metabolism in the 12,000 x g supernatant fraction of human liver was slightly inhibited after incubation with ketotifen, loratidine, and erythromycin (between 5 and 57% inhibition) at concentrations 5-8 times the therapeutic plasma level. These same three compounds substantially inhibited R089674 metabolism in human liver microsomes. However, the clinical importance of this finding is limited by the understanding that R089674 is not metabolized to a great extent in human liver microsomes by CYP-450 enzymes but is primarily metabolized by cytosolic human liver enzymes.

5.2 Toxicokinetics

Toxicokinetic results are reported in the reviews of the toxicology studies where they were performed.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose intravenous and oral toxicity studies were performed in mice and rats, and a single-dose ocular study was performed in New Zealand White rabbits. The results are summarized in Table 19, Table 20, and

Table **21**.

Table 19: Single-Dose Toxicology Studies in Mice

Study No.	Species/ Strain	Test Article/ Route/ Dose	Number/ Gender/ Group	Results
N125347/1	CD-1 Mice	R89674/ Oral gavage/ 0, 30, 60 mg/kg	5 males 5 females	1. Male (100%) and female (40%) mortality was observed at the 60 mg/kg dose. 2. Tremors and sedation were noted at 30 and 60 mg/kg. 3. Paralysis was observed at 60 mg/kg. 4. Surviving mice fully recovered after two (30 mg/kg) or three (60 mg/kg) hours.
N111662/1	CD-1 Mice	R89674/ Oral gavage/ 0, 40, 80, 160, 640 mg/kg	2 males 2 females	1. Mortality, clonic convulsions, loss of righting reflex, dyspnea, tremors, tachypnea, spasms, sedation, and death were induced at ≥ 80 mg/kg. 2. All the surviving animals recovered fully within one hour, and no pathological effects were found. 3. The lowest lethal dose was 80 mg/kg.
N125346/1	CD-1 Mice	R89674/ Intravenous/ 0, 10 and 30 mg/kg	5 males 5 females	1. No adverse effects occurred in the 10 mg/kg group. 2. All mice died immediately after dosing in the 30 mg/kg dose group.
N111661/1	Swiss Albino Mice	R89674/ Intravenous/ 0, 10, 20 and 40 mg/kg	2 males 2 females	1. R89674 acutely induced 100% mortality at doses of 40 mg/kg preceded by clonic convulsions, loss of righting reflex, tremors, and dyspnea. 2. R89674 also induced ataxia in male and female mice and tachypnea in female mice which did not lead to death at a dose of 20 mg/kg.

Table 20: Single-Dose Toxicology Studies in Rats

Study No.	Species/ Strain	Test Article/ Route/ Dose	Number/ Gender/ Group	Results
N125345/1	Wistar Rat	R89674/ Oral gavage/ 0, 160, 320, 640 mg/kg	5 males 5 females	1. Tremors and sedation were noted in both genders at all dose levels. 2. Male rats dosed at 640 mg/kg resulted in 80% drug-related mortality.
N111659/1	Wistar Rat	R89674/ Oral gavage/ 0, 160, 320, 640 mg/kg	2 males 2-5 females	1. At doses of ≥ 320 mg/kg, rats experienced mortality (females only), spasms, tremors, dyspnea, and behavioral depressant effects (hypotonia, prostration, ataxia, sedation, hypothermia, palpebral ptosis), piloerection and wet urogenital region. 2. The NOAEL was considered to be 160 mg/kg.
N111658/1	Wistar Rat	R89674/ Intravenous/ 0, 40 mg/kg	2 males 2 females	1. R89674-related convulsions were noted immediately after dosing. 2. All rats were fully recovered 30 minutes after dosing, and no mortality occurred.
N125344/1	Wistar Rat	R89674/ Intravenous/ 0, 40 mg/kg	5 males 5 females	1. Toxic effects included loss of righting reflex, spasms, tremors, dyspnea, and muscular hypotonia. 2. Two out of five male rats died with the 40 mg/kg dose. No mortality occurred in female rats. 3. All surviving rats fully recovered 1 hour after dosing.

Table 21: Single-Dose Toxicology Studies in Rabbits

Study No.	Species/ Strain	Test Article/ Route/ Dose	Number/ Gender/ Group	Results
03-011-02A	Rabbits/ NZW	R89674/ Topical ocular/ 0.0625%	6 males	1. Ocular administration of R89674 (0.0625%) did not cause eye irritation.

6.2 Repeat-Dose Toxicity

R89674 was tested in two ocular repeated-dose studies of 14-day (Study No. 04-1959-G1) and 6-months (Study No. 05-2456-G1) duration in rabbits. In addition, two organic impurities of the R89674 drug substance were examined in 14-day repeated-dose

ocular studies (Study Nos. 05-6067-G1 and P1006064) in rabbits, and these studies are summarized below in Table 22.

Table 22: Ocular Repeated-Dose Toxicology Studies

Study No./ Study type/ GLP Status	Species/ Test Article/ Route/Dose	Results
05-6067-G1/ 14-day ocular repeated-dose study in rabbits/ GLP compliant	New Zealand White rabbits/ R89674 and (b) (4) Topical ocular/ 40 µl TID of 0.05% solutions to the left eye.	1. No test article-related ocular toxicity (determined by slit-lamp, fluorescein staining, fundoscopy, tonometry, and histopathology) was observed. 2. No test article-related changes in hematology, clinical chemistry, coagulation parameters, or urinalysis were noted.
P1006064/ 14-day ocular repeated-dose study in rabbits/ GLP compliant	New Zealand White rabbits/ (b) (4) Topical ocular/ 40 µl TID to the right eye of a 0.003% solution.	1. No test article–related mortality or weight loss or ocular toxicity (determined by gross observation, slit-lamp examinations and histopathology) was observed. 2. Test article-treated animals demonstrated significantly higher blood levels of RBCs, total protein, albumin, calcium, and globulin compared to vehicle control animals; however, the Sponsor did not consider these slight changes to be biologically significant.

Study title: 14 day repeat dose ocular toxicity study in rabbits-OECD

Study no.:	04-1959-G1
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/21/2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Lot # 2, purity was not identified.

Key Study Findings

R089674, administered by the topical ocular route at doses as high as 0.5% did not cause significant ocular or systemic toxicity.

Methods

Doses:	0, 0.05%, 0.10%, 0.25%, or 0.5% R089674 to the left eye.
Frequency of dosing:	TID
Route of administration:	Topical ocular
Dose volume:	40 µl
Formulation/Vehicle:	The (b) (4) study report does not indicate the vehicle formulation
Species/Strain:	albino rabbits (oryctolagus cuniculus)
Number/Sex/Group:	5 males and 5 females per group

Age:	At least 11 weeks old
Weight:	2.36 to 3.05 kg
Satellite groups:	none
Unique study design:	Four groups of ten rabbits were administered either vehicle, 0.05%, 0.10%, 0.25%, or 0.5% R089674 three times per day via topical ocular dosing to the left eye for 14 days. The right eye remained untreated and served as the control. Animals were sacrificed on Day 14. The study design is summarized in Table 23.
Deviation from study protocol:	1. Rabbits were not assessed for vomiting as indicated in the protocol as rabbits are not able to vomit. 2. Testes and epididymus were weighed together instead of separately as indicated in the protocol.

Table 23: Study Design for Study No.: 04-1959-G1

Group	Sex		Dose	Dosing	Dose
	Males	Females	Concentration (w/v)	Schedule	Volume
Low	5	5	0.05%	t.i.d. OS	40 µl
Mid A	5	5	0.10%	t.i.d. OS	40 µl
Mid B	5	5	0.25%	t.i.d. OS	40 µl
High	5	5	0.50%	t.i.d. OS	40 µl
Control	5	5	N/A	t.i.d. OS	40 µl

t.i.d OS = three times a day in the left eye

Observations and Results

Mortality

One animal died during the study due to head trauma, but the death was not considered R089674-related.

Clinical Signs

Clinical observations were performed on each animal prior to the start of dosing and daily thereafter. Clinical observations included but were not limited to changes in the skin, fur, eyes, and mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern. Particular attention was directed to observations of central nervous system signs (seizures, tremors, salivation) and diarrhea.

No adverse clinical signs were noted in any of the animals during the course of the study.

Body Weights

Body weights were recorded prior to dosing, weekly, and at the termination of the study.

Aside from a few animals which lost minimal weight, all the test animals gained weight over the course of the experiment, and biologically significant differences between the groups were not noted.

Feed Consumption: Not measured

Ophthalmoscopy

Opthalmic examinations were performed prior to dosing, on Days 1, 7, and 14 and just prior to sacrifice (Day 15). Examinations were performed after the second daily dose. Examinations included slit-lamp biomicroscopy, fluorescein staining, fundoscopy, and tonometry. If fluoroscein staining was noted in any animal on Day 1, slit-lamp exams were performed every 24 hours until resolution.

No significant R089674-related ophthalmology findings were noted during the course of the study. Occasional and sporadic fluoroscein staining was noted but was not related to dose group. High-dose animals (0.5% R089674) were observed to tightly close the treated eye immediately after dosing; however, this reaction was not associated with any visible signs of adverse reaction.

ECG: Not measured

Hematology: Not measured

Clinical Chemistry: Not measured

Urinalysis: Not measured

Gross Pathology

Gross pathology assessments were performed immediately upon euthanasia and included examination of the external surface of the body, all orifices, and the cranial, thoracic, and abdominal cavities, and their contents.

No R089674-related abnormalities were noted at necropsy.

Organ Weights

The following organs were weighed upon necropsy: liver, kidneys, adrenal glands, testes with epididymides, uterus, ovaries, thymus, spleen, brain (cerebrum, cerebellum, pons/medulla), and heart.

Organ weight analysis did not reveal any findings considered to be R089674-related. The relative weight of testes/epididymes was significantly higher in 0.25% dose group males compared to 0.10% dose group males; however, differences were not apparent in other groups, and the difference was not considered biologically relevant.

Histopathology

Adequate Battery

An extensive battery of tissues was fixed and retained for future analysis. However, only the ocular tissues of the control and high-dose group and any gross lesions from all groups were examined histologically. The sections of the eye included the eyelids, conjunctiva, cornea, iris, retina, and optic nerve of each specimen. If pathology was seen in the high-dose group, then histopathology was performed on samples from the lower dose groups as well.

Peer Review: No

Histological Findings

Histological assessment of the ocular tissues from the control and high-dose groups did not reveal any histopathological changes related to R089674. Subacute inflammation was noted in the palpebral and bulbar conjunctiva in all animals but this was considered to be normal histology of the conjunctiva and not R089674-related.

Special Evaluation: No special evaluations were performed

Toxicokinetics: Toxicokinetics were not performed.

Stability and Homogeneity

The stability of the dose solutions was not assessed, but dose verification analysis was performed. The 0.5 mg/ml dose solution was 92.3% of the target level, and the other doses (1.0, 2.5, and 5.0 mg/ml) were within 99% of the target level.

Study title: A 6-month repeat dose ocular toxicity study in rabbits

Study no.:	05-2456-G1
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	4/29/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R89674 Ophthalmic Solution, Batch # PD05076 (2.5 mg) purity of 99.91%; Batch # PD05079 (5 mg), Purity of 99.86%

Key Study Findings

No overt clinical signs of R89674-related ocular or systemic toxicity occurred through Day 182 of the study at topical ocular R89674 doses of ≤ 0.5% administered TID. Also no changes in ophthalmology, clinical chemistry, hematology, coagulation, or hematology parameters were associated with R89674 administration. The NOEL was considered to be 0.5% (TID).

Methods

Doses:	0.25% TID, 0.5% BID, 0.5% TID
Frequency of dosing:	BID or TID dosing
Route of administration:	Topical ocular
Dose volume:	40 μl/dose
Formulation/Vehicle:	Vehicle is referred to as R89674 vehicle which is not explicitly described in the report, but appears to be a buffered aqueous solution which contains 0.093 mg/ml benzalkonium chloride.
Species/Strain:	New Zealand White rabbits (*Oryctolagus cuniculus*)
Number/Sex/Group:	See Table 24 below
Age:	At least 11 weeks old at the start of the study
Weight:	2.02 to 2.82 kg at the start of the study
Satellite groups:	Recovery Study Animals in Groups 1 and 4 were dosed for 6 months, then sacrificed three months later on Day 273.
Unique study design:	Animals received topical ocular doses of 40 μl of the test substance or vehicle into the left eye of the animal. The other eye remained untreated and served as an untreated control. Two different cohorts of animals in Groups 1-4 were sacrificed on Day 92 (6/sex/group) and on Day 183 (6/sex/group). The dosing groups and

regimens are summarized in Table 24 (Sponsor's table) below.

Deviation from study protocol: None of the following deviations were thought to affect the overall integrity of the study.

1. Three animals were replaced up to 28 days after the initiation of dosing following unscheduled deaths instead of within the first three days only as specified in the protocol.
2. The relative humidity in the animal rooms was slightly outside the specified range of 30–70% on one day of the study.
3. One animal was not weighed at the one-month timepoint as specified in the protocol.
4. The reticulocyte fraction was not measured as part of the hematological assessment for blood collected at the 3-month timepoint.
5. For one animal, the final ocular assessment was not within 72 hours of the final dose of R89674, but occurred within one week of the last dose.
6. Animals in Groups 2 and 4 were administered test substances 5 minutes after instead of 5 minutes before the four-hour PK blood draw on Days 91 and 182.

Table 24: Study Design for Study No.: 05-2456-G1

Dose Group	Time Point (Months)	Sex		Dose Concentration (w/v)	Dosing Schedule	Dose Volume	Sacrifice Day
		Male	Female				
1	6	6	6	N/A	t.i.d. OS*	40 µL	183
2	6	6	6	0.25%	t.i.d. OS	40 µL	183
3	6	6	6	0.5%	b.i.d. OS	40 µL	183
4	6	6	6	0.5%	t.i.d. OS	40 µL	183
1	3	6	6	N/A	t.i.d. OS	40 µL	92
2	3	6	6	0.25%	t.i.d. OS	40 µL	92
3	3	6	6	0.5%	b.i.d. OS	40 µL	92
4	3	6	6	0.5%	t.i.d. OS	40 µL	92
1	8	3	3	N/A	t.i.d. OS**	40 µL	273
4	8	3	3	0.5%	t.i.d. OS**	40 µL	273

* b.i.d. OS = 2 times per day left eye; target times = 8:00 and 16:00, ± 30 minutes
* t.i.d. OS = 3 times per day left eye; target times = 8:00, 12:00, and 16:00, ± 30 minutes
** These groups were dosed for 6 months and followed by a 2 month recovery period.

Observations and Results

Mortality

Animals were observed once daily for morbidity and mortality as part of the clinical observations.

Clinical Signs

Clinical observations were performed on each animal prior to the start of dosing and daily thereafter. Clinical observations included but were not limited to changes in food consumption, changes in the skin, fur, eyes, mucous membranes, respiratory system, gastrointestinal system, circulatory system, autonomic and central nervous system, somatomotor activity, and behavioral pattern.

Generally, there were no adverse R89694-related clinical signs. Over the first month of the study, there were five unscheduled deaths of study animals, but these deaths were associated with enteric infections or trauma during dosing or blood collection procedures.

Body Weights

Body weights of all animals were recorded prior to dosing and then monthly. Moribund and found dead animals were weighed at the time of sacrifice or detection respectively.

Some statistically significant differences were noted between male rabbits from Groups 2 and 3 after four, five, and six months of treatment; however, Group 3 males receiving the higher cumulative dose of R89674 demonstrated the higher body weights. One month after the cessation of dosing, at 7 months, the three remaining males from the highest dose group (Group 4; 0.5% R89674 TID) demonstrated significantly lower body weights (13.4%) compared to the three remaining control male animals. A non-significant reduction in body weight (12.1%) was also noted for Group 4 males relative to the control males at 8 months. These differences, however, were not considered by the Sponsor to be biologically significant because dose-related trends were not noted, and because similar differences did not occur among the female animals.

Food Consumption

Food consumption was monitored qualitatively as part of the daily clinical assessment, and quantitatively by measuring food consumption for one week out of each month.

No differences were noted for food consumption among the dose groups at any time-point.

Ophthalmoscopy

Ophthalmic examinations using the McDonald-Shadduck Scoring System were performed on both eyes of each animal prior to dosing on Day 1 and on approximately Day 28, then monthly, and within 72 hours of the last dose administration. Examinations were performed after the first daily dose. Examinations included: slit-lamp biomicroscopy, fluoroscein staining, fundoscopy, and tonometry. Tonometry was performed using a (b) (4) one day after each ophthalmic examination.

There were no significant ophthalmic findings in any of the treated eyes, and there were no differences among the treatment groups. Daily observations of the animals did not reveal an irritating effect of the test substance. Ocular findings that were noted during the study included one animal with a red eye, and two instances each of constricted pupils and milky discharge. However, these findings were very infrequent, not persistent, and did not appear to be related to treatment.

ECG: Not performed

Hematology

Blood was collected for hematology and clinical chemistry assessment after fasting overnight on the day of sacrifice for each animal. A standard panel of hematology parameters were assessed.

Among the 6-month male groups, Group 4 (0.5% TID), demonstrated a significantly higher total white blood cell count compared to control animals and Group 2 (0.25% TID). Also Group 3 from the 6-month females, demonstrated a significantly lower percent neutrophils than the control group and Group 4. All of the averages for all hematology parameters, however, fell within normal ranges, did not demonstrate dose-dependent changes, or did not occur in both genders, and thus were not considered biologically significant.

Coagulation Studies

A serum fraction of the blood collected at sacrifice was assessed for prothrombin time and partial thromboplastin time.

Group 2 demonstrated a significantly higher prothrombin time than both Group 3 and Group 4 among 3-month females. Also Group 3 demonstrated a significantly lower activated partial thromboplastin than Group 4 among 6-month females. For both parameters, however, all the group averages were within normal ranges, and the changes were not considered biologically meaningful.

Clinical Chemistry

The serum fraction of the blood was prepared from blood collected on the sacrifice day for each animal and a standard panel of clinical chemistry parameters was assessed.

Group 2 demonstrated significantly more creatinine than the control group among 3-month males and Group 4 demonstrated significantly higher serum glucose than the control group among 6-month females. For both parameters, however, the average for each group was within normal ranges, and the effects were not considered biologically meaningful.

Urinalysis

Urine was collected from each animal at sacrifice by cystocentesis. Urine was assessed for appearance, specific gravity, protein, and glucose.

No R89674-related urinalysis effects were noted.

Gross Pathology

Sacrificed animals were immediately examined for gross pathology. The external surface of the body, all orifices, cranial thoracic and abdominal cavities, and their contents were examined for each animal.

No R89674-related gross pathology effects were noted.

Organ Weights

Organ weights were obtained for the following organs: Liver, kidneys, adrenal glands, testes (male), vesicular gland (male), prostate (male), ovaries (female), uterus (females), thymus, lungs (with mainstem bronchi), spleen, brain (cerebrum, cerebellum, pons/medulla), pituitary gland, heart, thyroid (with parathyroid), and submandibular gland.

A statistically significant difference was noted for relative organ weight of the mandibular gland between Group 3 and control females; however, all averages were within historical ranges. Also a significant difference was noted for relative prostate weight among 6-month males, but pair-wise statistical comparison did not identify differences between groups. None of the organ weight changes were considered biologically meaningful because the differences did not occur in both genders and were not dose-dependent.

Histopathology

An extensive battery of tissues was collected from each experimental animal; however, only ocular tissues from Groups 1 and 4 for both the three- and six-month sacrifice time

points were prepared for histopathology. The eye tissues prepared for examination included: the eyelids (with palpebral conjunctiva), bulbar conjunctiva, cornea, iris, retina, and optic nerve.

Adequate Battery: Yes for eyes; however, other tissues were not examined for histopathology

Peer Review: No

Histological Findings

Mild inflammatory infiltration and lymphoid aggregates were noted in the palpebral and bulbar conjunctival tissues of both the control group (Group 1) and the high-dose group (Group 4). However, these effects were not considered R89674-related, and were considered normal and not adverse.

Special Evaluation

No special evaluations were conducted.

Toxicokinetics

Baseline blood was drawn from the marginal ear vein of 6 males and 6 females prior to treatment. On Days 1, 28, 91, and 182, blood was collected from 6 males and 6 females from each of the four dose groups. Samples were collected pre-dose and then at 5 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, and 8 h after administration of the first daily dose of R89674. Blood was processed to plasma and frozen before toxicokinetic analysis.

Neither R89674 nor its metabolite R90692 were detected in the plasma from Group 1 control animals. In the groups administered R89674, plasma levels of R89674 were detectable 5 minutes after administration (1.38, 1.10, and 2.32 ng/ml for Groups 2, 3, and 4 respectively after the first administration) but subsequently fell below the lower limit of quantification. However, R90674, the primary R89674 metabolite, was detectable at the five minute sampling timepoint and subsequent timepoints for all doses. At all sampling timepoints (Days 1, 28, 91, and 182) R90692 C_{max} and AUC_{0-t} values were relatively constant, suggesting little or no accumulation of R90692 occurred with any of the dose regimens. The R90692 C_{max} concentrations resulting from topical ocular administration of 0.25% R89674 TID was approximately 60% of that resulting from administration of 0.5% R89674 TID at all sampling timepoints. The plasma $t_{1/2}$ values for R90692 ranged from approximately 1 to 3 hours. The pharmacokinetic parameters of R90692 in plasma are summarized in the Table 25 (Sponsor's table) below.

Table 25: Pharmacokinetics of R90692 in Plasma.

Dose Group	Group 2 (0.25% t.i.d.)							
Study Day	1		28		91		182	
Sex	M	F	M	F	M	F	M	F
Tmax (min.)	25.8 [10.21]	30.0 [0.0]	25.8 [10.21]	30.0 [0.0]	17.5 [13.7]	21.7 [12.9]	21.7 [12.9]	25.8 [10.2]
Cmax (ng/mL)	63.5 [25.0]	64.4 [22.5]	33.4 [16.9]	49.0 [13.7]	40.7 [26.3]	36.5 [17.9]	33.8 [11.9]	27.4 [10.6]
AUC (0-t) (ng-min/mL)*	3933.5 [1452.7]	4011.5 [964.5]	2282.6 [1038.3]	3133.7 [815.4]	2305.3 [1434.7]	2234.4 [1095.2]	2032.8 [692.0]	2167.5 [916.9]
T1/2 (min.)*	60.6 [16.8]	63.3 [14.4]	55.7 [3.46]	32.7 [49.5]	74.3 [58.1]	61.9 [15.6]	72.5 [25.3]	77.2 [71.4]

[Standard Deviation]
*Calculated based on plasma levels during first dosing interval

Dose Group	Group 3 (0.5% b.i.d.)							
Study Day	1		28		91		182	
Sex	M	F	M	F	M	F	M	F
Tmax (min.)	25.8 [10.2]	26.7 [20.4]	21.7 [12.9]	30.3 [0.0]	17.5 [13.7]	25.8 [10.2]	30.0 [0.0]	25.8 [10.2]
Cmax (ng/mL)	107.3 [42.4]	74.1 [43.8]	63.3 [30.0]	78.7 [31.1]	77.9 [28.9]	75.0 [45.1]	32.9 [25.4]	74.2 [30.3]
AUC (0-t) (ng-min/mL)*	7242.7 [3081.7]	5632.0 [3791.9]	4636.0 [2646.4]	5680.0 [2447.4]	4778.2 [2665.1]	5489.2 [3149.0]	2785.7 [1944.4]	5987.0 [2722.5]
T1/2 (min.)*	118.6 [26.2]	125.1 [54.6]	111.6 [24.9]	106.4 [27.4]	107.3 [51.2]	198.5 [117.0]	126.2 [80.0]	20.7 [271.7]

[Standard Deviation]
*Calculated based on plasma levels during first dosing interval

Dose Group	Group 4 (0.5% t.i.d.)							
Study Day	1		28		91		182	
Sex	M	F	M	F	M	F	M	F
Tmax (min.)	30.0 [0.0]	25.8 [10.2]	30.0 [0.0]	25.0 [12.3]	15.0 [13.7]	30.0 [0.0]	21.7 [12.9]	25.8 [10.2]
Cmax (ng/mL)	114.8 [43.8]	109.6 [48.5]	82.7 [27.5]	60.9 [34.0]	55.8 [16.3]	45.7 [19.4]	53.9 [47.4]	67.9 [23.7]
AUC (0-t) (ng-min/mL)*	8989.7 [3786.4]	6609.3 [3114.5]	5733.0 [1986.6]	3883.5 [2130.1]	3238.1 [996.8]	3148.8 [1373.8]	2959.4 [2531.3]	4325.7 [1162.7]
T1/2 (min.)*	55.6 [26.6]	143.6 [240.5]	60.4 [22.3]	111.2 [89.0]	62.2 [7.8]	27.4 [56.9]	78.9 [26.9]	56.9 [23.3]

[Standard Deviation]
*Calculated based on plasma levels during first dosing interval

Stability and Homogeneity

Stability and homogeneity of the R89674 test solutions was not reported for this study.

Oral Repeated-Dose Studies

Systemic toxicity patterns were examined in a one-week, repeated-dose oral study in rabbits (Study No. N122462/1) and in one- and six-month oral repeated-dose studies in both rats (Study Nos. N111689/1 and N122461/2) and dogs (Study Nos. N111690/1 and N122463/2). The rabbit one-week study and the rat and dog one-month studies are summarized below in Table 26.

Table 26: Nonpivotal Oral Repeated-Dose Toxicology Studies.

Study No./ Study type/ GLP Status	Species/ Test Article/ Route/Dose	Results
N122462/1/ One-week oral repeated-dose study in rabbits/ Non-GLP	Female Cunistar albino rabbits/ R089674/ Oral gavage/ 0, 1, 10, 40, 80 mg/kg/day	1. Doses of R089674 of ≤ 80 mg/kg did not produce toxicologically relevant effects on mortality, clinical observations, hematological values, and gross pathology. 2. R089674 administered at 80 mg/kg resulted in slightly decreased weight gain and food consumption as well as decreased serum sodium, and inorganic phosphate and increased spleen weight. Organs were not examined histopathologically. 3. The NOAEL was considered to be 40 mg/kg/day
N111689/1/ One-month oral repeated-dose study in rats/ Non-GLP	Male and female Wistar rats/ R089674/ oral gavage/ 0, 2.5, 10, and 40 mg/kg/day	1. Doses of R089674 of ≤ 40 mg/kg did not produce toxicologically relevant effects on mortality, clinical observations, ophthalmology examinations, body weight gain, or serum chemistry. 2. R089674 administered at 40 mg/kg/day increased urinary volume, increased spleen weight and decreased thyroid weight in male rats, and increased liver weight in female rats; however, no coincident histopathological effects were observed. 3. The mean C_{max} concentrations of R090692 were 71, 227, and 1343 ng/ml in males and 95, 415, and 3310 ng/ml in females for the 2.5, 10, and 40 mg/kg/day doses respectively. 4. The AUC_{0-8h} values for R090692 were 687 (male) and 760 (female) and 2854 (male) and 5894 (female) ng x h/ml for the 10 and 40 mg/kg/day doses respectively. 5. The NOAEL was considered to be 10 mg/kg/day
N111690/1/ One-month oral repeated-dose study in dogs/ Non-GLP	Male and female Beagle dogs/ R089674/ oral gavage/ 0, 1.25, 5, and 20 mg/kg/day	1. Doses of R089674 of ≤ 20 mg/kg did not produce consistent or toxicologically relevant effects on any measured parameter including mortality, clinical observations, ophthalmology examinations, body weight gain, food consumption, ECG, blood pressure, hematology, serum chemistry, or urinalysis. 2. R089674 was rapidly metabolized to its major metabolite R090692 with $t_{1/2}$ values ranging from 0.73 to 1.89 hours. 3. The mean C_{max} values of R089674 were, 33, 392, and 1337 ng/ml and the mean $AUC_{(0-24h)}$ values were 49, 475, and 2670 ng x h/ml for the 1.25, 5, and 20 mg/kg/day dose groups respectively. 4. The mean C_{max} values of the major metabolite, R090692 were, 960, 4887, and 9659 ng/ml and the mean $AUC_{(0-24h)}$ values were 3070, 18671, and 55385 ng x h/ml for the 1.25, 5, and 20 mg/kg/day dose groups respectively. 5. The NOAEL was considered to be 20 mg/kg/day

Study title: Six-month repeated dose oral toxicity study (Exp. No. 3900) with R089674 in SPF Wistar rats.

Study no.:	N122461/2
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	May 6, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, lot # ZR089674PFA011, conversion factor of 1.

Key Study Findings

Slight to moderate toxicity occurred in rats orally administered ≥ 20 mg/kg R089674 for 6 months. The NOAEL dose was considered to be 5 mg/kg/day. Toxicity was most pronounced in the high-dose group orally administered 80/40/60 mg/kg/day. In the high-dose group, male rats demonstrated transiently increased blood urea nitrogen, slight increases in serum glucose, triglycerides, and slight decreases in total bilirubin and aspartate aminotransferase, as well as a marginal increase in liver weight. Toxicity in the high-dose group was more pronounced in females, and this toxicity correlated with higher R090692 exposure levels. Several hematological parameters were altered (slight decreases in hematocrit, hemoglobin, and red blood cells, slight increases in white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and thrombocytes) as well as slight to moderate alterations in several serum values (decreases in total protein, albumin, and creatinine, and increases in potassium, aspartate aminotransferase, cholesterol, inorganic phosphate, phospholipids, total bilirubin, alkaline phosphatase, and alanine aminotransferase). In the high-dose females, liver weight was increased with histopathological changes in the form of hepatic atrophy and diffuse hyperplasia of oval cells and bile ducts.

Methods

Doses:	0, 5, 20, and 80/40/60 mg/kg/day. The 80/40/60 group received 80 mg/kg/day on Days 0-8; no dosing on Days 9-12; 40 mg/kg/day on Days 13-90; and 60 mg/kg/day on Days 91-180.
Frequency of dosing:	Once per day
Route of administration:	Oral gavage
Dose volume:	0.8 ml per 100 g body weight/day
Formulation/Vehicle:	The vehicle contained HCl and demineralized water (pH 4.0 $\pm$ 0.1).
Species/Strain:	SPF Wistar rats
Number/Sex/Group:	20 males and 20 females per group
Age:	Approximately 4 weeks old at delivery

Weight:	Not indicated in report
Satellite groups:	None
Unique study design:	Rats were dosed by oral gavage once per day in 4 dosage groups of 40 animals (20 males and 20 females) each at doses of 0 (vehicle), 5, 20, and 80/40/60 mg/kg/day. The study design is summarized in Table 27 below.
Deviation from study protocol:	1. Due to early mortality, both males and females in the 80/40/60 group received 80 mg/kg/day on Days 0-8; no dosing on Days 9-12; 40 mg/kg/day on Days 13-90; and 60 mg/kg/day on Days 91-180.

Table 27: Study Design for Study No. N122461/2.

Group	Dose (mg/kg/day)	Dose Volume (µl/100g Body Weight/Day)	Number per Group	
			Males	Females
Vehicle Control	0	800	20	20
Low	5		20	20
Medium	20		20	20
High	80/40/60*		20	20

* The 80/40/60 group received 80 mg/kg/day on Days 0-8; no dosing on Days 9-12; 40 mg/kg/day on Days 13-90; and 60 mg/kg/day on Days 91-180.

Observations and Results

Mortality

One male from the R089674 low-dose group and 6 females from the high-dose group died during the study. The male demonstrated extensive lung congestion probably resulting from improper gavage procedure. Four female rats in the high-dose group were found dead on Day 9 after eight days of dosing with 80 mg/kg/day of R089674. One other female rat in the high-dose group died on Day 14. The death of these five female rats was considered to be R89674-related. One other high-dose female died on Day 118 during dosing and this death was considered to be a dosing accident. Subsequent to the high dose female deaths on Day 9, dosing was discontinued for four days then resumed at a lower dose of 40 mg/kg/day for approximately two and one half months. As no clinical signs occurred in the rats dosed with 40 mg/kg/day for this prolonged period, the dose was raised to 60 mg/kg/day on Day 91 of the study.

Clinical Signs

All rats were observed at least once a day for signs of waning health, abnormal behavior, unusual appearance, occurrence of untoward clinical effects, manifestations of toxic and pharmacological responses, morbidity, and mortality.

No relevant differences in the incidences of clinical signs were noted for male and female rats in the R089674 low- and medium-dose groups and male rats in the high-dose group compared to the vehicle control group. Relevant clinical signs consisted of abdominal masses and/or yellow vaginal discharge in several females from the high-dose group. All of the relevant clinical signs were noted within a few days of dosing with 80 mg/kg/day R089674, and the signs subsided and did not recur upon initiation of dosing with 40 mg/kg/day.

Body Weights

Individual body weights were obtained on the first day of the dosing period, at weekly intervals during the dosing period, and at the end of the dosing period.

Male rats in the low-, medium-, and high-dose groups and females in the low-dose group did not experience any changes in body weight or body weight gain relative to vehicle control animals. Female rats in the medium-dose group demonstrated significantly increased body weight in Weeks 4-12 and in Week 14, and a significant increase in body weight gain from Weeks 2-14 and in Weeks 16 and 20. High-dose females demonstrated significant increases in body weight in Weeks 4 and 5, Weeks 7-16, Weeks 18-22, and in Week 24, and significant increases in body weight gain from Week 3 until the end of the study.

Food Consumption

Weekly food consumption was quantified for each rat.

No differences in weekly food consumption or total food consumption were noted for low- and high-dose males and for low-dose females compared to the vehicle control animals. Medium-dose males demonstrated significantly increased weekly food consumption in Weeks 4, 6, 21, and 22, but no differences in total food consumption. Medium-dose females demonstrated increased weekly food consumption in Weeks 4 and 5, but no difference in total food consumption, and high-dose females demonstrated significantly increased weekly food consumption from Week 3 until the end of the study and increased total food consumption.

Ophthalmoscopy

An ophthalmology examination (conjunctiva, sclera, cornea, iris, lens, and fundus) was performed on the first day of the study (before dosing) and in Week 25 for the first 10 animals of each sex in the control and high-dose groups.

No ophthalmology findings were apparent in any of the study rats before the initiation of dosing. At week 25 of the dosing period only three ophthalmology findings were observed, fundus hyperreactivity in a male control rat, and localized corneal opacities in both eyes of one female in the high-dose group. The findings were not considered to be R089674 related.

ECG: Not assessed

Hematology

Blood was collected for hematology analysis after approximately 3 months of dosing and near the end of the dosing period for each group. A standard panel of hematology parameters was examined.

Hematology values did not demonstrate R089674-related changes in low-, medium-, and high-dose male rats or in low-dose female rats. In medium-dose females, hematocrit, hemoglobin, and red blood cells were slightly and transiently decreased, a slight increase in the number of thrombocytes was noted after three months of dosing, and slight increases in thrombocytes and eosinophils were observed after 6 months. In high-dose females, slight decreases in hematocrit, hemoglobin, and red blood cells and slight increases in the number of white blood cells, thrombocytes, neutrophils, lymphocytes, and monocytes were observed after 3 and 6 months. Eosinophil numbers were also slightly higher in high-dose females after 6 months. Although limited to females and often of small magnitudes and limited toxicological concern, many of the hematological changes noted above occurred at both 3 and 6 months, were dose dependent, and appeared to be R089674 related. The three- and six-month hematology values are summarized in Table 28 and Table 29 respectively.

Table 28: Mean Hematology Values Recorded in Weeks 13 and 14.

Parameter	Dosage group (mg/kg)							
	Males				Females			
	Control	Low:5	Medium:20	High:80/40/60	Control	Low:5	Medium:20	High:80/40/60
HCT: Haematocrit %	44.5	44.7	45.1	44.6	42.7	42.1	41.5 *	41.5 *
HGB: Haemoglobin g/dl	15.4	15.5	15.7	15.5	15.2	15.1	14.7 **	14.8 **
RBC: R.B.C. 10E6/mm3	8.93	9.01	9.20 **	8.97	8.22	8.05	7.99 *	7.86 **
WBC: W.B.C. 1000/mm3	11.3	11.0	10.6	11.5	8.5	8.4	9.6	11.8 ***
THR: Thrombocytes 1000/mm3	996	977	989	915 *	931	966	1078 **	1106 **
NRC: Normoblasts/100 W.B.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MCV: Mean cell volume fl	49.8	49.7	49.1	49.8	52.0	52.4	52.0	52.8
MCH: Mean cell haemogl. pg	17.3	17.3	17.0	17.3	18.5	18.7	18.4	18.8
MHC: Mean cell h. conc.g/dl	34.6	34.7	34.7	34.7	35.7	35.8	35.5	35.7
ABSOLUTE DIFFERENTIAL COUNT x 1000/mm3								
NEU: Neutrophils	1.14	1.18	1.10	1.32	1.05	1.09	1.15	1.26 *
EOS: Eosinophils	0.19	0.23	0.22	0.21	0.15	0.15	0.17	0.17
BAS: Basophils	0.04	0.03	0.03	0.04	0.02	0.02	0.02	0.03 *
LYC: Lymphocytes	9.70	9.41	9.02	9.74	7.08	6.88	7.97	10.02 ***
MOC: Monocytes	0.23	0.19	0.18 *	0.21	0.23	0.24	0.26	0.31 **

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

Table 29: Mean Hematology Values Recorded in Weeks 25 to 26.

Parameter	Dosage group (mg/kg) Males Control	Low:5	Medium:20	High:80/40/60	Females Control	Low:5	Medium:20	High:80/40/60
HCT: Haematocrit %	44.2	44.2	44.0	44.5	43.1	42.8	42.8	41.5 *
HGB: Haemoglobin g/dl	15.2	15.3	15.2	15.4	14.7	14.7	14.6	14.0 ***
RBC: R.B.C. 10E6/mm3	8.81	8.94	8.94	8.85	7.94	7.78	7.85	7.61 *
WBC: W.B.C. 1000/mm3	10.6	10.9	10.1	11.1	6.9	7.0	8.0 *	10.3 ***
THR: Thrombocytes 1000/mm3	1053	1097	1108	987	966	986	1036 *	1155 **
NRC: Normoblasts/100 W.B.C.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MCV: Mean cell volume fl	50.2	49.5	49.3	50.2	54.3	55.0	54.5	54.6
MCH: Mean cell haemogl. pg	17.3	17.1	17.0	17.4	18.5	18.9 *	18.6	18.4
MHC: Mean cell h. conc.g/dl	34.4	34.6	34.6	34.6	34.1	34.4	34.1	33.8
ABSOLUTE DIFFERENTIAL COUNT x 1000/mm3								
NEU: Neutrophils	1.24	1.46	1.47	1.60	0.89	0.99	1.21 *	1.79 ***
EOS: Eosinophils	0.19	0.17	0.18	0.20	0.15	0.15	0.19 *	0.21 *
BAS: Basophils	0.04	0.03	0.03 *	0.04	0.02	0.02 *	0.02	0.02
LYC: Lymphocytes	8.82	8.92	8.17	9.01	5.58	5.56	6.25	7.88 ***
MOC: Monocytes	0.27	0.29	0.24	0.27	0.26	0.25	0.32	0.43 **

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

Clinical Chemistry

Blood was collected and processed to serum for clinical chemistry analysis after approximately 3 months of dosing and near the end of the dosing period for each group. A standard panel of serum chemistry parameters was examined.

Low-dose male and female rats did not demonstrate changes in any serum parameters. Medium-dose males exhibited a slight and transient increase in blood urea nitrogen after 3 months but not after 6 months. In medium-dose female rats, total protein was slightly decreased after three and six months of dosing and serum potassium was slightly increased after six months. In the high-dose group males, blood urea nitrogen was transiently increased after three months of dosing, glucose was increased and total bilirubin was decreased after three and six months, and triglycerides were increased and aspartate aminotransferase was decreased after six months of dosing. In the high-dose females, total serum protein, albumin, and creatinine were decreased and total bilirubin was increased after three and six months of dosing. Cholesterol was decreased at three months, but increased after six months. Potassium, inorganic phosphate, phospholipids, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels were increased after six months of dosing. Many of the serum

chemistry changes appeared to be dose dependent and R089674 related, but as with the hematological changes, the effects were most pronounced in females. The serum chemistry changes which occurred were of limited toxicological relevance as they were not always consistent between males and females, and generally small in magnitude. The three- and six- month mean serum chemistry values are summarized in Table 30 and Table 31 respectively.

Table 30: Mean Serum Chemistry Values Recorded in Weeks 13 to 14.

		Dosage group (mg/kg)							
		Males				Females			
Parameter		Control	Low:5	Medium:20	High:80/40/60	Control	Low:5	Medium:20	High:80/40/60
SOD: Sodium	mmol/l	145	145	145	145	145	145	145	145
POT: Potassium	mmol/l	5.4	5.3	5.2 *	5.3	5.0	5.1	5.3 *	5.2
CHL: Chloride	mmol/l	101	101 *	101 *	102 *	101	102	101	102 **
CAL: Calcium	mg/dl	10.8	10.7	10.8	10.9	11.3	11.1	11.3	11.2
INP: Inorg. phosphate	mg/dl	7.3	7.1	7.2	7.2	6.7	6.6	7.0	7.3
TOP: Total protein	g/dl	6.7	6.8	6.9	6.8	7.4	7.3	7.0 *	6.5 ***
ALB: Albumin	g/dl	3.8	3.9	3.9 *	3.9	4.5	4.4	4.3	4.0 ***
GLU: Glucose	mg/dl	122	125	127	130 **	120	120	119	118
CHO: Cholesterol	mg/dl	69	75	70	72	89	93	84	77 ***
TGL: Triglycerides	mg/dl	177	209	216	217	187	197	240	203
PLP: Phospholipids	mg/dl	153	163	156	164	204	208	212	211
BUN: Blood urea nitr.	mg/dl	17.1	17.8	19.4 **	19.0 **	16.5	17.9 *	16.3	16.7
CRS: Creatinine	mg/dl	0.53	0.53	0.55 *	0.53	0.57	0.62	0.55	0.50 ***
BIL: Total Bilirubin	mg/dl	0.06	0.05	0.05	0.05 *	0.06	0.08 *	0.08 *	0.09 **
ALP: Alkal. phosphatase	U/l	220	209	224	215	134	139	132	164
AST: Aspartate aminotr.	U/l	112	111	106	103	107	117	110	118
ALT: Alanine aminotran.	U/l	65	67	60	61	67	65	57 *	65

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

Table 31: Mean Serum Chemistry Values Recorded in Weeks 25 to 26.

	Dosage group (mg/kg)							
	Males				Females			
Parameter	Control	Low:5	Medium:20	High:80/40/60	Control	Low:5	Medium:20	High:80/40/60
SOD: Sodium mmol/l	143	143	143	143	140	140	140	139
POT: Potassium mmol/l	5.5	5.4	5.3	5.5	4.4	4.6	4.8 **	4.9 ***
CHL: Chloride mmol/l	101	101	101	101	99	99	99	99
CAL: Calcium mg/dl	10.9	10.9	11.1	11.1 *	11.6	11.6	11.7	11.9
INP: Inorg. phosphate mg/dl	6.7	6.5	6.7	6.8	6.4	6.3	6.7	7.5 ***
TOP: Total protein g/dl	7.0	7.2	7.3 **	7.1	8.0	7.9	7.6 **	7.3 **
ALB: Albumin g/dl	3.8	3.9 *	3.9 **	3.8	4.7	4.6	4.5	4.0 ***
GLU: Glucose mg/dl	127	134 *	135 *	139 ***	119	120	117	112
CHO: Cholesterol mg/dl	88	92	95	92	111	114	109	158 ***
TGL: Triglycerides mg/dl	190	228	263 *	280 **	291	356	350	286
PLP: Phospholipids mg/dl	170	181	188	189 *	273	290	292	371 ***
BUN: Blood urea nitr. mg/dl	16.2	16.4	16.3	16.2	18.4	19.2	18.7	19.7
CRS: Creatinine mg/dl	0.55	0.55	0.55	0.55	0.58	0.59	0.60	0.57 *
BIL: Total Bilirubin mg/dl	0.07	0.06	0.06	0.05 **	0.09	0.08	0.09	0.14 ***
ALP: Alkal. phosphatase U/l	164	167	164	167	103	110	105	123 *
AST: Aspartate aminotr. U/l	110	102	92	82 ***	86	93	90	106 **
ALT: Alanine aminotran. U/l	54	57	54	49	58	63	60	69 **

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

Urinalysis

Urine was collected for urinalysis after approximately 3 months of dosing and near the end of the dosing period for each group. Urine was assessed for volume, pH, proteins, glucose, occult blood, ketones, bilirubin, urobilinogen, specific gravity, and sediment.

There were no dose-dependent changes in urinary parameters in low-, medium-, and high-dose male rats or in low- and medium-dose female rats. In the high-dose females, a slight increase in urinary volume was recorded after three and six months of dosing. The urinalysis parameters measured after three and six months are summarized in Table 32 and Table 33 respectively.

Table 32: Urinalysis Mean Values After Approximately Three Months of Dosing.

Parameter	Dosage group (mg/kg)							
	Males				Females			
	Control	Low:5	Medium:20	High:80/40/60	Control	Low:5	Medium:20	High:80/40/60
SGR: Specific gravity	1.041	1.040	1.045	1.043	1.045	1.048	1.043	1.040
ACI: pH	6.7	6.9 **	6.8	6.8	5.9	6.2	6.0	5.7
VOL: Volume ml / 16 h	5.9	5.9	6.4	5.9	3.3	3.5	3.5	5.7 **
score								
PRO: Proteins 0-4	0.85	1.00	0.85	0.90	0.40	0.60	0.30	0.33
GLU: Glucose 0-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
KET: Ketones 0-4	0.90	1.00	1.10	0.85	0.70	0.75	0.65	0.73
UBS: Urobilinogen-score 0-4	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00
BIU: Bilirubin 0-3	0.05	0.00	0.00	0.00	0.05	0.15	0.10	0.07
BLD: Occult blood 0-4	0.50	0.26	2.00 ***	1.85 ***	0.00	0.10	0.05	0.07
SEDIMENT: score								
RBU: Red blood cells 0-3	0.05	0.00	0.15	0.10	0.00	0.00	0.05	0.00
WBU: White blood cells 0-3	0.00	0.00	0.00	0.00	0.35	0.30	0.20	0.13
BAC: Bacteria 0-3	1.05	1.11	0.95	0.70 *	1.05	0.65	0.80	0.47
SPR: Sperms 0-3	1.50	0.89 *	0.65 **	1.05	0.00	0.00	0.00	0.00
MCN: Mucin 0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FGI: Fungi 0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FAT: Fat droplets 0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

score: 0 = negative, 1 = low, 2 = moderate, 3 = high, 4 = very high

Table 33: Urinalysis Mean Values After Approximately Six Months of Dosing.

		Dosage group (mg/kg)							
		Males				Females			
Parameter		Control	Low:5	Medium:20	High:80/40/60	Control	Low:5	Medium:20	High:80/40/60
EPITHELIAL CELLS:	score								
SQM: Squamous	0-3	0.30	0.11	0.45	0.40	0.35	0.35	0.40	0.67
CYL: Cylindrical	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
REN: Renal	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
CASTS:	score								
HYL: Hyaline	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GRN: Granular	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.07
BLO: Blood	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PUS: Pus	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EPI: Epithelial	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
WAX: Waxy	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CRYSTALS:	score								
TPH: Triphosphate	0-3	1.45	1.53	1.90	1.25	0.55	0.65	0.30	0.20
COX: Calcium oxalate	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AMP: Amorphous	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DCP: Dicaphosphate	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
URA: Uric acid	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CAC: Calcium carbonate	0-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

score: 0 = negative, 1 = low, 2 = moderate, 3 = high, 4 = very high

Gross Pathology

Gross pathology, described as a “full necropsy” was assessed for each animal as soon as possible after sacrifice.

Most of the gross pathology changes were only occasionally observed, and their incidences were considered normal. In males, none of the gross pathology findings were considered toxicologically relevant. In high-dose female rats toxicologically relevant findings were observed in the female rats which died following the eight days of 80 mg/kg/day dosing. These findings included: changes in liver (hardened and/or yellow), pancreas (pale), thoracic and peritoneal cavities (fluid retention), urinary bladder (yellow), and contents of the small and large intestine (watery and hemorrhagic). In surviving high dose female rats after six months of dosing, gross

pathology changes were limited to the liver and included swollen liver (4/14 rats) pale liver (4/14 rats), more pronounced lobulation (2/14 rats), stippled liver (2/14 rats) and brown focus (1/14 rats)

Organ Weights

The following organs were weighed as soon as possible after necropsy: adrenals, brain, gonads (testes or ovaries), heart, kidneys, liver, lungs, pancreas, spleen, thymus, and thyroids. Organ weight relative to body weight (relative organ weight) was also determined.

Organ weights were not altered in low-dose male and female rats. In medium-dose rats, liver weight was marginally increased in males (8.3%), but more substantially increased in females (18.4%) relative to the vehicle control group. In the high-dose males the liver weight was also marginally increased (7.4%), and moderate liver weight increases (53.5%), and slight increases in spleen (19.0%) and pancreas (12.4%) weights were noted in high-dose females.

Histopathology

Adequate Battery: The extensive battery of tissues noted in the Histopathology Inventory Table (Table 40) were examined for histopathology for all groups in this study.

Peer Review: No

Histological Findings

Marked differences in histopathology findings were apparent between males and females. Males and female rats in the low-dose group demonstrated findings comparable to those of vehicle control rats. In some males in the medium- and high-dose groups, there were minor, non-significant liver findings including clear hepatocytes (glycogen storage, cell swelling), cellular vacuolation (lipid-like) and foci of cellular alteration (mainly clear cells).

In the surviving medium- and high-dose females, significant, dose-related liver alterations of slight to pronounced severity were observed. This liver histopathology primarily followed the same pattern as that demonstrated to a slight, insignificant degree in the medium- and high-dose males, but with increased incidence and severity. High-dose females also demonstrated cellular irregularities and bile duct proliferation within the periportal area, and one female was afflicted with a small hepatocellular adenoma.

In the five female high-dose rats which died within the second week of the study, there was pronounced liver atrophy, and a pronounced diffuse hyperplasia of oval cells and bile ducts. These rats also displayed marked lymphoid depletion and cellular necrosis within the lymph nodes, spleen, and thymus which was considered to be secondary to the liver changes.

Special Evaluation

Liver samples of four rats (2 male and 2 female) were processed for electron microscopy examination.

In one of the high dose female rats, electron macroscopic examination of the liver revealed a slight increase in glycogen and a slight increase in fat droplets within the hepatocellular cytoplasm.

Also possible induction and/or inhibition of hepatic drug metabolizing enzymes was examined using rats from this study and the results of this analysis are reported in a separate report, Study No. N125478/1.

Toxicokinetics

Toxicokinetics were performed for Main Study rats as well as a satellite group of rats (4 males and 4 females for all R089674 dose groups). The toxicokinetic results are reported in a separate report (No. N122430/2) and summarized below. Toxicokinetic analysis was performed with samples from animals in the high-dose group receiving 80 mg/kg/day on Day 9 of dosing before reducing the dose for this group. Samples were also obtained one hour after dosing on Days 1, 7, 14, 28, 58, 121, and 149 and composite sampling was performed 1, 2, 4, 8, and 24 hours after dosing on Days 90 and 182.

R089674 plasma levels were low and generally below the limit of detection one or two hours after dosing. On Days 90 and 182, the plasma C_{max} and $AUC_{0\text{-}24h}$ values of the much higher R090692 concentrations increased approximately dose-proportionally for the male rats and in a greater than dose-proportional manner for female rats. Female rats demonstrated substantially higher C_{max} (10.5 μg/ml) and $AUC_{0\text{-}t}$ (34.8 μg x hr/ml) values compared to male rats (3.51 μg/ml and 7.93 μg x hr/ml respectively) in the high-dose group on Day 182. Also R090692 plasma concentrations in the low (5 mg/kg/day) and medium (20 mg/kg/day) dose groups were higher on Day 182 compared to Day 90 suggesting some degree of accumulation. The plasma concentrations and pharmacokinetic parameters for the Day 90 and Day 182 samples are summarized below in Table 34.

Table 34: The Mean (n = 2) Plasma Concentrations (µg/ml) and Pharmacokinetic Parameters of R090692 on Days 90 and 182.

	Males						Females					
	5 mg/kg		*20 mg/kg*		*40 mg/kg*	*60 mg/kg*	*5 mg/kg*		*20 mg/kg*		*40 mg/kg*	*60 mg/kg*
Time	day 90	day 182	day 90	day 182	day 90	day 182	day 90	day 182	day 90	day 182	day 90	day 182
1	0.224	0.349	0.684	1.55	1.98	3.51	0.193	0.214	1.30	1.81	3.34	10.5
2	0.115	0.145[a]	0.298	0.556	0.598	1.44	0.087	0.152	0.362	0.697	1.28	3.38
4	0.026	0.061	0.147	0.163	0.243	0.628	NQ	0.051	0.305	0.382	0.632	2.08
8	NQ[b]	NQ[a]	0.038	0.031	0.071	0.184	NQ	NQ	0.049	0.119	0.632	1.29
24	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	0.141	NQ	0.033	0.030
C_{max}	0.224	0.349	0.684	1.55	1.98	3.51	0.193	0.214	1.30	1.81	3.34	10.5
T_{max}	1	1	1	1	1	1	1	1	1	1	1	1
AUC_{0-t}[c]	0.421	0.627	1.66	2.92	3.76	7.93	0.236	0.493	2.94	4.25	13.7	34.8

[a] n = 1.
[b] NQ: not quantifiable (< 0.025 µg/ml).
[c] AUC_{0-t}: up to the last measurable concentration.

Stability and Homogeneity

The stability of the R089674 dosing solutions was confirmed up to one month after preparation and shown to fall between 96-100% for all solutions. The concentration and stability values are summarized in Table 35 below.

Table 35: Concentration and Stability of R089674 Formulations

				Nominal concentration of R089674 (in mg/ml)		
Date of		SP-UV result	CA	0.625	2.5	10
Preparation	Analysis			Concentration found in % of nominal concentration		
May 7, 1996	May 13,1996	96-249	15509	99.4	98.4	99.9
	May 23,1996	96-275	15509	100.2	98.8	100.6
	May 30,1996	96-290	15509	98.4	99.6	100.3
	June 10,1996	96-316	15509	99.0	99.2	100.0

Reviewer Comment: *It should be noted that the appendixes containing individual animal data were not supplied with the electronic report for this study.*

Study title: Six-month repeated-dose oral toxicity study (Exp. No. 3965) with R089674 in Beagle dogs.

Study no.:	N122463/2
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	August 9, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Lot Name: ZR089674 PFA011, conversion factor = 1.0

Key Study Findings

A few, relatively minor, toxicological effects occurred in the high-dose group (40 mg/kg/day) including transient salivation, rough haircoat, increased incidences of focal alopecia, shortened PQ interval, and a slight increase in systolic blood pressure. Body weight and weight gain were also decreased in the high-dose group. In conjunction with this change, a number of organs were decreased in weight; however, the organ weight changes were generally not accompanied by histopathological changes. Some laboratory values were also affected in the high-dose group including decreased red blood cell volume, decreased serum calcium, cholesterol, and phosholipids, and increased serum blood urea nitrogen. The Sponsor considered the NOAEL to be 10 mg/kg/day.

Methods

Doses:	0, 2.5, 10, and 40 mg/kg/day
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Dose volume:	2 ml/kg
Formulation/Vehicle:	R089674 was dissolved in demineralized water and HCl
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/sex/group
Age:	6-8 months old at the start of dosing
Weight:	Group averages of 8.9 to 9.2 kg at the start of the experiment.
Satellite groups:	None; plasma samples from the first two Main Study male and female dogs in each group were analyzed for toxicokinetics.
Unique study design:	See study design table below
Deviation from study protocol:	1. On a few occasions, minimum and/or maximum temperatures slightly below or above the predefined minimum temperature of 17°C were recorded in the testing room.

Similarly, relative humidity values slightly above or below the predefined range were recorded.
2. The occasional temperature and humidity deviations were not considered to have had relevant effects on the conduct of the study.

Table 36: Study Design for Study No. N122463/2

Groups: Dose	Dose Volume	Male	Female
Control	2 ml/kg	4	4
Low: 2.5 mg/kg		4	4
Medium: 10 mg/kg		4	4
High: 40 mg/kg		4	4

Observations and Results

Mortality

Dogs were observed daily for mortality. No mortality was noted for any group.

Clinical Signs

All dogs were observed daily for signs of waning health, abnormal behavior, unusual appearance, and occurrence of untoward clinical effects.

At 40 mg/kg, all animals demonstrated slight transient salivation which stopped occurring in males after 7 weeks of dosing, and at later timepoints for female dogs. Dogs in the high dose group (40 mg/kg) also experienced slightly more rough coat, and skin irritation/alopecia than control dogs.

Body Weights

Body weight was recorded prior to the dosing period, at weekly intervals during the study, and at the end of the study.

Body weights were significantly decreased in weeks 25 (13%) and 26 (14%) in the high-dose group receiving 40 mg/kg.

Feed Consumption

Individual food consumption was measured at weekly intervals.

No significant R089674-related changes in food consumption were noted.

Ophthalmoscopy

An ophthalmological examination of the conjunctiva, sclera, cornea, anterior chamber, iris, lens, and fundus was performed on all dogs prior to the dosing period and toward the end of the dosing period. The eyes were examined with a slit-lamp biomicroscope and an indirect binocular ophthalmoscope after induction of mydriasis with atropine sulphate 1% eyedrops.

No R089674-related ophthalmology findings were observed after 6 months of dosing.

ECG

ECG and heart rate were carried out in lead II of the Einthoven Standard lead system. ECG and heart rate measurements were carried out prior to the dosing period, after approximately one to three months of dosing, and toward the end of the dosing period.

No R089674-induced arrhythmias were observed during the course of the study; however, some changes in ECG intervals were noted. While most of the changes were considered mild, or occurred predosing and therefore were not relevant, a significant shortening of the PQ interval at three and six months in the high-dose group (40 mg/kg) was considered R089674 related.

Hematology

Individual blood samples were obtained and hematology examinations were performed in all dogs twice before the start of the dosing period, after 2, 4, 8, 12, 16, and 20 weeks of dosing and toward the end of the study. A standard panel of hematology and coagulation parameters including partial thromboplastin time was examined.

Hematology parameters were generally not affected by R089674, and most of the changes that were seen were not considered relevant because they were only temporarily seen, were not dose related, or fell within the reference range of the historical control data. Dogs in the high-dose group (40 mg/kg), however, demonstrated significantly decreased mean cell volume at Weeks 4, 8, 12, 16, 20, and 26; and significantly decreased mean cell hemoglobin at Weeks 8, 12, and 26 which may be R089674-related.

Clinical Chemistry

Clinical chemistry samples were obtained and assessed according to the same schedule as the hematology examinations. A standard panel of serum analysis values was examined.

Most changes in clinical chemistry values were not considered relevant because they already existed before dosing was started, they were not dose-related, they were transient, and/or the values were within historical ranges. Some statistically significant

changes in the high-dose group (40 mg/kg) were considered R089674- related including: decreased calcium at Weeks 8, 12, 16, 20, and 26 (2.8-4.7%); decreased cholesterol at Weeks 8, 12, 16 and 20 (23.3-26.6%); decreased phospholipids at Weeks 8, 12, 16, and 20 (15.9-21.3%); and increased blood urea nitrogen (BUN) at Weeks 4, 8, 12, 16, and 20 (20-53%). BUN levels were also significantly increased in the R089674 medium-dose group at weeks 12, 16, and 20 (18-29%), although the values fell within the historical control range. The increased BUN was not accompanied by increased creatinine levels or histopathological changes in the kidney.

Urinalysis

Urine for urinalysis was collected from all dogs prior to the dosing period, after approximately one and three months of dosing, and toward the end of the study. Urine pH, proteins, glucose, ketones, urobilinogen, bilirubin, occult blood, specific gravity, and sediment were assessed.

Sporatic incidental changes in urinalysis values were noted; however, these changes were not considered R089674-related because the changes were small, not dose-related, transient, and/or the values fell within the historical range.

Gross Pathology

Following sacrifice at the end of the six-month dosing period, a full necropsy was performed on all animals, and all macroscopic changes were noted.

As noted in the clinical observations, the dogs in the high-dose group experienced a slightly higher incidence of alopecia. None of the other differences in gross pathology between the control group and any of the R089674 groups reached statistical significance, and most of the gross pathology observations were considered normal for Beagle dogs.

Organ Weights

Following necropsy, the organs listed in the Histopathology Inventory Table (Table 40) were weighed.

Several absolute or relative organ weight changes were noted in the high-dose group; however, some of these changes may have reflected the decreased body weight in this group. Also some values, although significantly different than those of the control group, fell within historical control range. Included in the significant changes in the high-dose group were: decreased absolute heart weight, decreased absolute pancreas weight, increased relative kidney weight, decreased absolute and relative thymus weights, decreased absolute thyroid weight, decreased absolute weight of the female gonads, increased relative weight of the hypophysis, and decreased relative prostate weight. The Sponsor considered only the decrease in thymus weight and prostate weight to be possibly R089674-related because these weights decreased slightly more than

proportionally to the decrease in body weight. However, the weight changes in these organs did not correlate with significant histopathology.

Histopathology

Adequate Battery: yes, as listed in the Histopathology Inventory Table (Table 40).

Peer Review: No

Histological Findings: The slight skin inflammation associated with alopecia in the high dose group (40 mg/kg), was comparable to that observed in the control group. Also a non-significant slightly more pronounced thymic involution in the high-dose group was considered by the Sponsor to be related to the reduced body weight gain in this group.

Special Evaluation

Indirect blood pressure measurements (systolic and diastolic pressure) were carried out simultaneously with the ECG measurements prior to the study, after approximately one and three months of dosing, and toward the end of the study.

Male and female dogs in the high-dose (40 mg/kg) group demonstrated significantly higher systolic blood pressure after six months of dosing; however, the systolic blood pressure for this group was not significantly different than that of the control group at the one- and three-month measurements.

Toxicokinetics

Toxicokinetics were performed and the results are reported in Study No. N122428/3. The results are summarized below. Blood samples for toxicokinetic analysis were obtained and processed to plasma on the Days 1 and 176 at 0.5, 1, 3, 6, 8, 19, and 24 hours after dose administration. Also on Days 2, 3, 7, 14, 28, 56, and 119, blood samples were obtained 24 hours after dosing. An additional blood sample was obtained on Day 28 at 0.5 hours after dose administration and blood samples were obtained on the last experimental day, Day 184, just before euthanasia and necropsy. In addition to blood collection, tissue samples were obtained from the same animals on Day 184 approximately 24 hours after the last dose. Samples were obtained from liver, lung, brain, heart, kidney, adrenal, pancreas, fat, and muscle. Plasma and tissue samples were analyzed for R089674 and R090692 with a validated HPLC method.

Plasma concentrations of R089674 attained similar peak levels after single and repeated dosing and decreased to levels below the level of quantification within 1-10 hours following dosing depending on the dose. C_{max} values for plasma R089674 increased in a more than dose-proportional manner (5-11 times). Plasma R090692 concentrations were much higher than those of R089674 and generally peak plasma levels were attained in the first hour following dosing. R089674 and R090692 did not

appear to accumulate and plasma concentrations were similar after single and repeated-dosing. Steady state plasma R090692 levels were attained on Day 3 of dosing. R090692 $AUC_{0-\infty}$ values increased in an approximately dose-proportional manner. Male and female dogs demonstrated similar plasma levels and pharmacokinetic parameters for both R089674 and R090692 in all the dose groups after single and repeated dosing. The plasma concentration and pharmacokinetic parameters of R090692 following single and repeated dosing of R089674 are summarized below in Table 37.

Table 37: Mean (n = 4) Plasma Concentrations and Pharmacokinetic Parameters of R090692 in Beagle Dogs Following Single (Day 1) and Repeated (Day 176) Dosing.

Time post-dose (h)	*2.5 mg/kg/day*		*10 mg/kg/day*		*40 mg/kg/day*	
	Single	Repeated	Single	Repeated	Single	Repeated
0	NQ[a]	0.018 ± 0.005	NQ	0.050 ± 0.014	NQ	0.379 ± 0.230
0.5	2.38 ± 0.26	1.68 ± 0.32	6.98 ± 0.85	7.19 ± 0.82	20.4 ± 5.9	21.2 ± 1.3
1	2.34 ± 0.24	2.06 ± 0.38	7.67 ± 0.31	7.38 ± 0.46	24.5 ± 3.4	22.3 ± 0.8
3	0.969 ± 0.155	0.864 ± 0.198	3.56 ± 0.21	3.36 ± 0.37	15.2 ± 0.9	11.4 ± 2.4
6	0.309 ± 0.059	0.301 ± 0.083	1.24 ± 0.16	1.14 ± 0.16	5.71 ± 0.92	4.56 ± 1.15
8	0.156 ± 0.037	0.176 ± 0.046	0.612 ± 0.080	0.635 ± 0.034	3.04 ± 0.61	2.66 ± 0.66
10	0.090 ± 0.027	0.117 ± 0.041	0.345 ± 0.074	0.376 ± 0.060	1.80 ± 0.39	1.67 ± 0.42
24	NQ[a]	0.016[b]	0.039 ± 0.016	0.051 ± 0.017	0.244 ± 0.153	0.377 ± 0.316
C_{max} (µg/ml)	2.41 ± 0.26	2.06 ± 0.38	7.67 ± 0.31	7.64 ± 0.34	24.5 ± 3.4	22.3 ± 0.8
R090692/R089674[c]	[24]	[39]	[11]	[11]	[7]	[6]
T_{max} (h)	0.5	1	1	0.75	1	1
$t_{½}$ (h)	2.88 ± 1.42	4.74 ± 1.56	4.42 ± 0.70	4.83 ± 0.47	4.74 ± 0.98	6.56 ± 3.13
$AUC_{0-24\ h}$ (µg.h/ml)	7.94 ± 1.20	7.60 ± 1.38	29.3 ± 2.1	28.7 ± 1.9	115 ± 17	99.7 ± 14
$AUC_{0-\infty}$ (µg.h/ml)	8.16 ± 1.22	-	29.6 ± 2.1	-	117 ± 16	-

[a] NQ: < 0.010 µg/ml.
[b] Median.
[c] Peak plasma concentrations ratio of R090692 over R089674.

Individual tissue concentrations of R89674 were not quantifiable at the 2.5 and 10 mg/kg/day dose levels, and only quantifiable in certain tissues at the 40 mg/kg/day dose. The highest tissue concentrations of R89674 were obtained in the adrenals followed by kidney and lung. Tissue concentrations of R90692 were several fold higher than those of R89674 and quantifiable in most examined tissues at all doses. The highest tissue concentrations of R90692 were found in kidney and liver followed by lung, fat, and heart. The lowest concentrations were found in muscle, adrenals, and brain. R90692 tissue to plasma concentration ratios decreased with increasing dose. The mean tissue and plasma concentrations of R90692 collected on the last day of dosing (Day 184) are shown below in Table 38.

Table 38: Mean (n = 4; ± SD) tissue and plasma concentrations (ng/g or ng/ml) of R090692 in Beagle Dogs.

	2.5 mg/kg/day	10 mg/kg/day	40 mg/kg/day
Kidney	145 ± 54	568 ± 212	3072 ± 1034
Liver	128[a]	407 ± 140	1622 ± 321
Pancreas	73[a]	228 ± 62	1375 ± 428
Lung	90[a]	247 ± 70	1256 ± 432
Fat	71[a]	269 ± 191	1093 ± 515
Heart	54[a]	194 ± 71	686 ± 162
Muscle	51[a]	151 ± 41	537 ± 128
Adrenal	< 222[a]	< 165[a]	428 ± 214
Brain	50[a]	76 ± 3	239 ± 40
Plasma	32 ± 7	148 ± 66	910 ± 504

[a] Median.

Stability and Homogeneity

The R089674 dosing solutions were shown to be stabile up to 27 days after preparation (Table 39).

Table 39: Stability of R089674 in Dosing Solutions

Date of		SP-UV		Nominal concentration of R089674 (in mg/ml)		
Preparation	Analysis	result	CA	1.25	5	20
August 13, 1996	August 14, 1996	96-401	15902	100.3	99.0	100.5
	August 26, 1996	96-411	15902	100.6	99.6	98.2
	September 2,1996	96-426	15902	100.2	99.4	98.4
	September 9, 1996	96-439	15902	99.8	99.4	99.6

Reviewer Comment: *It should be noted that the appendixes containing individual animal data were not supplied with the electronic report for this study. Also the supplied summary tables, with the exception of the plasma toxicokinetic tables, often did not delineate gender differences.*

Table 40: Histopathology Inventory Table

Study	N122461/2	N122463/2
Species	Rat	Dog
Adrenals	X	X, *
Aorta	X	X
Bone Marrow smear	X	
Bone (femur)	X	X
Brain	X	X, *
Cecum		
Cervix		
Colon	X	X
Duodenum	X	X
Epididymis	X	X
Esophagus	X	X
Extraorbital lacrimal gland	X	
Eye	X	X
External ear	X	
Fallopian tube		
Gall bladder		X
Gross lesions	X	X
Harderian gland		
Heart	X	X, *
Hypophysis		*
Ileum	X	X
Injection site		
Jejunum	X	X
Kidneys	X	X, *
Lachrymal gland		X
Larynx		
Liver	X	X, *
Lungs	X	X, *
Lymph nodes, bronchial		X
Lymph nodes mandibular		
Lymph nodes, mesenteric	X	X
Mammary Gland	X	X
Nasal cavity		
Nasal turbinates	X	
Optic nerves		X
Ovaries	X	X, *

Pancreas	X	X, *
Parathyroid	X	X
Peripheral nerve		X
Pharynx		
Pituitary	X	X
Prostate	X	X, *
Rectum		
Salivary gland	X	X
Sciatic nerve		
Seminal vesicles		
Skeletal muscle	X	X
Skin		X
Spinal cord	X	X
Spleen	X	X, *
Sternum		
Stomach	X	X
Testes	X	X, *
Thymus	X	X, *
Thyroid	X	X, *
Tongue		X
Trachea	X	X
Urinary bladder	X	X
Uterus	X	X
Vagina	X	X
Zymbal gland		

X, histopathology performed
*, organ weight obtained

7 Genetic Toxicology

The genetic toxicology of R89674 and its major metabolite, R90692, was assessed *in vitro* in an Ames test. Also, R089674 was examined in a thymidine kinase mutation assay, and R089674 as well as putative impurities ((b) (4) R89674 crude material and (b) (4)) were assessed in a chromosome aberration assay in mammalian cells. *In vivo*, R89674 was assessed in micronucleus assays in mice. In other genetic toxicology studies, the R89674 product impurities, (b) (4) as well as R89674 spiked with (b) (4) and (b) (4) were assessed in Ames tests, and R89674 spiked with (b) (4) and (b) (4) was assessed in an *in vitro* chromosome aberration study.

Table 41: Genetic Toxicology Studies with R89674-Impurities

Study No./ Study Type/ GLP status	Test Articles	Species/ Strains	Results
05-5302-G2/ Ames Assay/ GLP compliant	R89674, (b) (4) [redacted] R89674 crude material, (b) (4) [redacted]	TA98, TA100, TA1535, TA1537, WP2	The test articles at concentrations of 0.01, 0.1, 1, 10, 100, and 1000 μg/plate with and without metabolic activation did not significantly increase the frequency of revertants compared to the negative control group.
961392/ Ames Assay/ GLP compliant	R89674 spiked with (b) (4) [redacted]	TA1535 TA1537 TA98 TA100 WP2	The test article at concentrations of 1.58, 5.0, 15.8, 50, 158, and 500, 1581, and 5000 μg/plate with and without metabolic activation did not significantly increase the frequency of revertants compared to the negative control group.
961393/ *In Vitro* Chromosome Aberration Test/ GLP compliant	R89674 spiked with (b) (4) [redacted]	Human blood lymphocytes	The test article at concentrations of 198, 397, 775 and 1550 μg/ml did not induce chromosomal damage after four hours of incubation with and without S9 activation or after 24 hours without metabolic activation.

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

Study title: R089674-Experiment No. 3553: *Salmonella typhimurium* reverse mutation assay (Ames test).

Study no.:	Experiment No. 3553; Study No.: N122201
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	March 23, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # R089674PL1, purity was not reported

Key Study Findings

R089674 did not induce a significant increase in the number of revertant colonies beyond the solvent control incidence with or without metabolic activation.

Methods

Strains:	*Salmonella typhimurium* strains TA1538, TA98, TA1537, TA1535 and TA100
Concentrations in definitive study:	50, 100, 250, 500, 1000, 2500, and 5000 μg/plate with and without S9 activation.
Basis of concentration selection:	A range finding experiment with TA100 was conducted using R089674 concentrations of 25, 50, 100, 250, 500, 1000, 2000, 3000, 4000, and 5000 μg/plate in the presence and absence of S9 activation. Based on the results of this experiment, it was decided to test R089674 in the definitive study up to a concentration of 5000 μg/plate.
Negative control:	Vehicle (DMSO) controls with and without S9 activation.
Positive control:	2-nitrofluorene for TA1538 and TA98 without S9 activation; sodium azide for TA1535 and TA100 without S9 activation; 9-aminoacridine for TA1537 without S9 activation, and 2-aminoanthracene for all strains with S9 activation.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	48-hour incubation time, with automatic counting of the plates after incubation.

Study Validity

The following criteria for a valid study were met:

1. All of the test components passed sterility tests.
2. None of the test strains grew on histidine-free plates demonstating their histidine requirement.
3. None of the strains grew on the UV irradiated side of the plate demonstrating their uvr B mutation.
4. Growth of the strains were clearly inhibited by crystal violet demonstrating the rfa mutation.
5. The strains TA98 and TA100 showed resistance to ampicillin demonstrating the presence of R-factor plasmid pKM101.
6. The vehicle reversion counts fell within the historical range.
7. The positive control values fell within the historical range, and demonstrated ≥ 2 fold increase in the number of revertant colonies above the solvent control value

for the strains TA98 and TA100 and ≥ 3 fold increase above the solvent control value for the strains TA1535, TA1537, and TA1538.
8. The experiments were performed to the accepted limit of 5000 μg/plate.
9. Decreasing concentration levels from the highest acceptable concentration level provided an adequate number of data points.
10. R089674 was shown to be stable in solution for at least 5 days.

Results

At concentrations of 50, 100, 250, 500, 1000, 2500, and 5000 μg/plate in the definitive test, R089674 did not reveal a significant increase in the number of revertant colonies with or without S9 activation. A similar result was observed for the range-finding experiment. With most of the strains, visible thinning of the background lawn and/or pinpoint deletions indicating cytotoxicity were observed at concentrations of 2500 and/or 5000 μg R089674 per plate. Each of the appropriate positive controls produced a significant increase in the number of revertant colonies for each strain.

Study title: R090692-experiment No. 4011: Salmonella typhimurium reverse mutation test

Study no.:	N122464/2
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	September 30, 1996
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	R090692, Batch # PL1, purity was not identified

Key Study Findings

With and without S9 activation, and at concentrations of 50, 100, 250, 500, 1000, 2500, and 5000 μg/plate, R090692 did not reveal a significant increase in the number of revertant colonies in TA98 and TA100 test systems.

Methods

Strains:	*Salmonella typhimurium* strains TA98 and TA100
Concentrations in definitive study:	50, 100, 250, 500, 1000, 2500, and 5000 μg R090692 per plate
Basis of concentration selection:	Solubility and range-finding studies were not performed. Concentrations in the definitive study were chosen to cover a full range of concentrations beginning with the maximum required concentration of 5000 μg R090692 /plate and including six lower concentrations.
Negative control:	DMSO
Positive control:	Without S9 activation: Sodium azide for TA98 and 2-nitrofluorene for TA100. With S9 activation: 2-aminoanthracene for both bacterial strains.
Formulation/Vehicle:	R090692 was dissolved in DMSO
Incubation & sampling time:	Plates were incubated in the dark at 37°C for 48 hours.

Study Validity

1. The components of the test did not show contamination.
2. Neither strain was able to grow on histidine-free plates thus confirming their histidine requirement.
3. Neither strain was able to grow on the UV irradiated side of the plate, thus demonstrating their uvr B mutation.
4. The growth of both strains was clearly inhibited around the paper disc treated with crystal violet thus demonstrating the rfa mutation.
5. Both strains were resistant to ampicillin, thus demonstrating the presence of the R-factor plasmid pKM101.
6. The solvent (DMSO) reversion counts fell within historical values.
7. The positive controls demonstrated at least a 2-fold increase in the number of revertant colonies above the solvent control values, and positive control reversion counts fell within the historical range for both bacterial strains.
8. The maximum dose of R090692 required by ICH guidances, 5000 μg/plate, was used in the study.

Results

With and without S9 activation, none of the tested concentrations of R090692 revealed a significant increase in the number of revertant colonies for either bacterial strain.

7.2 *In Vitro* Chromosomal Aberration Assays in Mammalian Cells

Study title: Chromosomal Aberration Assay in CHO cells-OECD

Study no.:	05-5302-G1
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/27/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	1. R89674, batch # 05P0189, purity of 99.5% 2. (b) (4), batch # 882.100.2b, purity of 98.35% 3. R89674 (b) (4) batch # 882.100.3d, purity of 98.34% 4. R89674 crude material, batch # 05B0317, purity of 98.70% 5. (b) (4), batch # 882.100.5d, purity of 96.52%

Key Study Findings

None of the concentrations (10, 100, and 1000 μg/ml) of the five different test substances (R89674, (b) (4) R89674 crude material, and (b) (4)) caused significant increases in the number of chromosome aberrations with and without metabolic activation. The study design was not optimal for study validity in that the highest test substance concentration (1000 μg/ml) produced 50-60% cytotoxicity instead of the recommended 80% cytotoxicity for mammalian cell mutagenicity studies.

Methods

Cell line:	Chinese Hamster Ovary–K1(CHO-K1) cells
Concentrations in definitive study:	10, 100, and 1000 μg/ml
Basis of concentration selection:	The dose levels in the definitive study were selected based on the results of a range-finding assay. In the range-finding assay, a high degree of cytotoxicity occurred with the 5000 and 2500 μg/ml concentrations of all the test substances, and 1000 μg/ml concentrations produced 50-60% cytotoxicity.
Negative control:	Ham's F-12 medium
Positive control:	Without metabolic activation: 0.075 μg/ml mitomycin C (MMC). With metabolic activation: 35 μg/ml cyclophosphamide (CP)
Formulation/Vehicle:	Test substances were dissolved in Ham's F-12 medium
Incubation & sampling time:	Cells were incubated with test substances ± S9 activation for 3 hours then incubated with fresh media (sans test substances or S9) for 21 hours then washed and treated with colcemid for one hour prior to harvesting. A confirmatory assay utilizing a longer test substance exposure for 21 hours without S9 activation was also performed.

Study Validity

The high dose for the test substances was 1000 μg/ml which caused approximately 50% cytotoxicity. According to "Guideline for Industry: Specific Aspects of Genotoxicity Tests for Pharmaceuticals." the highest study concentration ideally should produce at least 80% cytotoxicity in mammalian cell mutation tests. Thus the highest concentration for the main test should have been higher, somewhere between 2500 μg/ml which produced 100% cytotoxicity and 1000 μg/ml which produced approximately 50% cytotoxicity.

Aberration frequencies for the negative and positive control conditions were within historical ranges. Also the induction of chromosome aberration by the positive control substances with and without metabolic activation was significant compared to the corresponding negative control groups.

Results

None of the concentrations (10, 100, and 1000 μg/ml) of the five different test substances (R89674, (b) (4) R89674 crude material,

and [redacted] (b) (4)) caused significant increases in the number of chromosome aberrations with and without metabolic activation. The confirmatory assay results were similar to those of the definitive study.

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

Study title: R089674-Experiment No.: 3552: Micronucleus test in mice short study.

Study no.:	Experiment No. 3552; Study No.: N113173
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	March 28, 1995
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # R089674PL1, purity was not reported

Key Study Findings

R089674 did not cause a significant increase in the number of micronucleated polychromatic erythrocytes. It was concluded that R089674 did not induce structural and/or numerical chromosome aberrations in erythrocytes of bone marrow under the experimental conditions.

Methods

Doses in definitive study:	10, 20, 40, and 80 mg/kg
Frequency of dosing:	Single dose
Route of administration:	Oral
Dose volume:	0.1 ml/10 g body weight
Formulation/Vehicle:	Vehicle: HCl (0.1N) + NaOH (0.1N) + demineralized water
Species/Strain:	Albino Swiss SPF mice-8 weeks of age at start
Number/Sex/Group:	3 male and 3 female mice/group.
Satellite groups:	none
Basis of dose selection:	The high dose (80 mg/kg) was shown to be the maximum tolerated dose.
Negative control:	Vehicle control
Positive control:	40 mg/kg cyclophosphamide (lot# 60949/1)

Study Validity

Bone marrow slides were prepared and examined by light microscopy. A total of 1000 polychromatic (PCE) and normochromatic (NCE) erythrocytes per mouse were counted

to determine the ratio of polychromatic to normochromatic erythrocytes. A total of 1000 PCE were counted per mouse and the number of micronucleated PCE was recorded. At the same time, the number of micronucleated NCE was recorded in the fields containing the 1000 PCE.

The study was considered valid because:

1. The positive control substance induced a statistically significant increase in the number of micronucleated PCEs.
2. The number of micronucleated PCEs in the vehicle control mice fell within the historical range.
3. The high dose employed in the study was the maximum tolerated dose (80 mg/kg), and three additional doses including one half (40 mg/kg), and one fourth (20 mg/kg) of the maximum tolerated dose were tested.

Results

Mortality: All of the male and female mice in the 10, 20, and 40 mg/kg R089674 groups survived until they were sacrificed at the end of the 48 hour test period. Two (out of three) male and two female mice in the 80 mg/kg group died within 24 hours of dosing, and the mortality was considered to be related to R089674 administration.

Clinical observations: No clinical signs resulted from administration of 10 or 20 mg/kg R089674, but administration of 40 and 80 mg/kg R089674 produced sedation.

Body weight: Body weight changes were comparable for all groups.

Red blood cell proliferation: The proportion of PCE to the total of PCE plus NCE was not changed in any of the R089674 treatment groups indicating red blood cell proliferation in bone marrow was not changed by R089674. Treatment with the positive control (40 mg/kg cyclophosphamide) produced a significant reduction ($p \leq 0.01$) in red cell proliferation in male and female mice relative to the vehicle control group.

Incidence of micronucleated PCE: The number of micronucleated PCE found for each of the vehicle control males and females fell within the historical range. The positive control, 40 mg/kg cyclophosphamide, produced a significant ($p \leq 0.01$) increase in the number of micronucleated PCE. None of the doses of R089674 (10, 20, 40, and 80 mg/kg) significantly increased the number of micronucleated PCE in male or female mice. Under all the test conditions, male and female mice experienced similar results.

Study title: R089674-Experiment No. 4013: Micronucleus test in mice

Study no.:	Experiment No. 4013; Study No. N122466
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	September 23, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # ZR089674PFA011, purity was not reported.

Key Study Findings

None of the doses of R089674 produced a biologically or statistically significant increase in the number of micronucleated polychromatic erythrocytes.

Methods

Doses in definitive study:	2.5 (low), 10 (medium) and 40 (high) mg/kg body weight
Frequency of dosing:	Single dose
Route of administration:	Oral gavage
Dose volume:	0.1 ml/10g body weight
Formulation/Vehicle:	Demineralized water + HCl 0.1N (adjustment to pH 4.0 $\pm$ 0.1)
Species/Strain:	Mouse/Albino Swiss, approximately 8 weeks old on the experiment start date.
Number/Sex/Group:	5 males and 5 females per group
Satellite groups:	Toxicokinetics: 6/sex for the low- and medium-dose groups, and 10/sex for the high-dose group.
Basis of dose selection:	The dose levels were selected based on the previous short micronucleus test (Exp. No. 3552)
Negative control:	Vehicle control
Positive control:	40 mg/kg cyclophosphamide
Sampling Times:	24 and 48 hours for the negative control and R089674 treatment groups and 48 hours for the positive control group.

Study Validity

The study employed a high dose of 40 mg/kg which was not the maximum tolerated dose, but probably was the highest dose which would not result in mortality within two days and thus was considered the highest acceptable dose. The Sponsor did not

employ half of this dose for the medium dose, but instead used a one quarter dose (10 mg/kg) and a much lower dose (2.5 mg/kg).

The study was valid for the following criteria:

1. The positive control substance induced a statistically significant increase in the number of micronucleated PCEs.
2. The number of micronucleated PCEs in the vehicle control mice fell within the historical range.

Bone marrow slides were prepared and examined by light microscopy. A total of 1000 polychromatic (PCE) and normochromatic (NCE) erythrocytes per mouse were counted to determine the ratio of PCE to NCE. A total of 2000 PCEs were counted per mouse and the number of micronucleated PCE was recorded. At the same time, the number of micronucleated NCE was recorded in the fields containing the 2000 PCE.

Results

Mortality: Two male mice in the 40 mg/kg R089674 high-dose group died before sacrifice time. All other male and female mice in all the R089674-dose groups survived until sacrifice at the end of the 24 and 48 hour experimental periods.

Clinical observations: No clinical signs were noted for any of the R089674 dose groups

Body weight: The high-dose R089674 group (40 mg/kg) experienced a significant weight loss ($p \leq 0.05$) after 24 hours compared to the vehicle control group. No other groups demonstrated significant weight changes at either the 24 or 48 hour time points.

Red blood cell proliferation: Males in the high-dose R089674 group receiving 40 mg/kg demonstrated a significant decrease in the proportion of PCEs to the total of PCEs plus NCEs at the 24 hour time point indicating that this dose produced a reduction in red blood cell proliferation. Females in the same group, however, did not experience a reduction in red blood cell proliferation and combined data for both sexes in this group did not demonstrate significant differences relative to the negative control group. All other R089674 treatment groups did not demonstrate reductions in red blood cell proliferation at either the 24 or 48 hour sampling times. In contrast, 40 mg/kg cyclophosphamide-treated animals at the only sampling time (48 hours) for this group demonstrated significantly reduced red blood cell proliferation.

Incidence of micronucleated PCEs: None of the R089674-dose groups demonstrated increased micronucleated PCEs relative to the vehicle control group at either the 24 or 48 hour sampling time. In contrast, the 40 mg/kg cyclophosphamide-dose group demonstrated significantly increased micronucleated PCEs. Results for males and females were similar for all the control and treatment groups and values for the negative control group fell within the historical range.

Toxicokinetic study: R089674 was quickly metabolized to R090692 which was quantified in each of the R089674 dose groups confirming exposure to the test article.

7.4 Other Genetic Toxicity Studies

Study title: R089674: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre® Fluctuation Technique

Study no.:	N125233/1
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 19, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, batch # ZR089674PFA011, Purity of 99.4%.

Key Study Findings

In the absence of S9 activation, none of the R089674 concentrations produced significant increases in mutant frequencies at any concentration including cytotoxic doses. In the presence of S9 activation, a small but significant increase in mutant frequency was observed at the highest dose (500 μg/ml) tested in the first experiment. However, in a follow-up experiment, no significant increases in mutant frequency were observed even at a higher concentration of R089674 (550 μg/ml).

Methods

Strains:	L5178Y TK+/- mouse lymphoma cells
Concentrations in definitive study:	Definitive Experiment 1: a. Without activation: 0, 31.25, 62.5, 125, 250, and 375 μg/ml. b. With S9 activation: 0, 62.5, 125, 250, and 500 μg/ml. Definitive Experiment 2: a. Without activation: 0, 50, 100, 200, 300, and 350 μg/ml. b. With S9 activation: 0, 200, 300, 400, 500, and 550 μg/ml.
Basis of concentration selection:	The concentrations selected for the definitive study were based on the results of a range-finding assay. Two independent studies were conducted each with a different concentration range. In the toxicity range-finding experiment, six doses of R089674 separated by two-fold intervals and ranging from 68.75 to 2200 μg/ml were tested. The top two concentrations (1100 and 2200 μg/ml) produced 100% cytotoxicity and the top surviving dose was 550 μg/ml which yielded 0.56% and 31.98% relative survival in the absence and presence of S9 activation respectively.
Negative control:	Purified water diluted 10 fold in treatment media (RPMI A, RPMI 10 or RPMI 20)
Positive control:	Without S9 activation; 4-nitroquinoline-t-oxide (NQO) and With S9 activation: benzo(a)pyrene (BP)
Formulation/Vehicle:	Purified water diluted 10 fold in media.
Incubation & sampling time:	Cells were incubated for three hours with solvent, different concentrations of R089674, or positive control solutions in the presence or absence of S9 activation. Cells were then washed followed by plating for survival measurements or further incubation in flasks for growth through the TK+ mutation expression period. The survival plates were incubated for 8-9 days until they were scored for survival. Expression cell cultures were maintained in flasks for 2 days, then subcultured and plated for

viability and 5-trifluorothymidine (TFT) resistance.

Study Validity

The study was considered valid because the following criteria were met:

1. The mutation frequencies in the negative control cultures fell within the normal range (above 60 mutants per 10^6 viable cells, but not more than three times the historical mean value).
2. At least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value).
3. The plating efficiencies of the negative control cultures were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

Results

In the absence of S9 activation, the highest concentration in Experiment 1, 375 μg/ml, produced excessive cytotoxicity, (< 10% survival) and thus a second experiment (Experiment 2) was conducted with a modified dose-range in order to test a top concentration which did not produce undue cytotoxicity. For both experiments, in the absence of S9 activation, no statistically significant increases in mutant frequencies were observed following treatment with R089674 at any dose level tested in Experiment 1 or 2.

In the presence of S9 activation in Experiment 1, a small but significant increase in mutant frequency was observed at the highest tested dose (500 μg/ml). However, in Experiment 2, no significant increases in mutant frequency were observed even at a higher concentration of R089674 (550 μg/ml). Based on the lack of reproducibility, the small increase in mutant frequency in Experiment 1 was considered a chance event without biological significance.

8 Carcinogenicity

No carcinogenicity studies were conducted. A waiver for carcinogenicity evaluation was submitted for IND 66884 on July 11, 2006 and granted on September 13, 2006.

9 Reproductive and Developmental Toxicology

Segment I (male and female rats), Segment II (rats and rabbits), and Segment III (rats) reproductive and developmental toxicity studies were conducted with R89674.

Toxicokinetic evaluations were performed in conjunction with the Segment II rat and rabbit studies.

9.1 Fertility and Early Embryonic Development

Study title: R089674: Experiment No. 3971; male and female fertility study in Wistar rats (Segment I).

Study no.:	N122467/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	September 30, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # ZR089674PFA011, the purity was not reported.

Key Study Findings

R089674 at doses of ≤ 60 mg/kg/day in male rats and ≤ 20 mg/kg/day in female rats did not lead to mortality, adverse clinical signs, or adverse fertility effects. R089674-related clinical signs including poor condition and rough coats were noted in female rats in the high-dose group receiving 60 mg/kg/day R089674, and the dose was lowered to 40 mg/kg/day before mating. Ultimately 20/24 females died or were sacrificed before mating in the high-dose group. None of the maternal or litter parameters were adversely affected at doses ≤ 20 mg/kg/day in female rats. The maternal and litter parameters were difficult to interpret in the 60(40) mg/kg/day group since only 4/24 females survived in this group until mating.

Methods

Doses:	0 (control), 5 mg/kg/day (low), 20 mg/kg/day (medium), 60(40) mg/kg/day (high)
Frequency of dosing:	Once per day; 28 days prior to mating and during mating for males; 14 days prior to mating, during mating and up to the 8th day of the pregnancy for females.
Dose volume:	0.8 ml/100 g body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	R089674 was dissolved in demineralized water and HCl. The vehicle was demineralized water and HCl.
Species/Strain:	SPF Wistar rats
Number/Sex/Group:	Four groups of 24 males and 24 females
Satellite groups:	None
Study design:	See table below. Males in the high-dose group received 60 mg/kg/day throughout the dosing period. Females in the high-dose group initially received 60 mg/kg/day; however, after a few days of dosing, some females died and the high dose for females was reduced to 40 mg/kg/day before mating. During the following days dosing was stopped for some females in this dose group, but only 4/24 females survived until the end of the study. After a two week pre-mating period, females were mated on a one-to-one basis with males from the same treatment group. Each morning following mating, a vaginal smear was prepared from each female and examined for the presence of spermatozoa. Females demonstrating a positive vaginal smear were separated from the males and remained isolated until sacrifice on Day 15 of the pregnancy. The day on which evidence of copulation was found was designated as Day 1

	of pregnancy. The mating period lasted for a maximum of 21 days.
Deviation from study protocol:	A formal summary of protocol deviations was not provided in this report. A deviation that was noted was that due to female mortality before mating in the high-dose group, the dose in this group for females was reduced from 60 mg/kg/day to 40 mg/kg/day and for some females in this group dosing was stopped in the following days.

Table 42: Study Design for Study No. N122467/1

Colour code	Treatment (mg/kg/day)	Concentration formulation (mg/ml)	Number per group	Identity number of females	Identity number of males
White	Control	0	24	1 - 24	201 - 224
Red	5	0.63	24	31 - 54	231 - 254
Yellow	20	2.5	24	61 - 84	261 - 284
Green	40/60	5/7.5	24	91 - 114	291 - 314

Observations and Results

Mortality

No mortality was observed in male rats dosed up to 60 mg/kg/day or female rats dosed up to 20 mg/kg/day. In females dosed at 60(40) mg/kg/day, 20 out of 24 died or were sacrificed during the dosing period before mating.

Clinical Signs

All animals were observed at least once per day for signs of waning health, abnormal behavior, unusual appearance, occurrence of untoward clinical effects, manifestation of toxic or pharmacological responses, morbidity, and mortality.

No clinical signs were observed in any of the male rats. During the premating period, poor appearance was observed in 21/24 female rats in the 60(40) mg/kg/day group and rough coats were observed in 22/24 female rats in the same group. Some females in this group were also thin and demonstrated red stained external nares or wet urogenital regions.

Body Weight

Individual body weights were determined weekly for the males and females during the premating period and on Days 1, 9, and 15 of pregnancy for females.

Male rats receiving 20 mg/kg/day R089674 demonstrated significantly increased body weights and body weight gain relative to control males, but the effect was not dose-dependent and not considered relevant by the Sponsor. Male rats in the 5 and 60 mg/kg/day groups did not demonstrate reduced body weight or reduced body weight gain compared to the control group. Similarly, body weight, body weight gain, and corrected maternal weight gain were comparable between all the R089674-treatment groups and the control group for the surviving female rats.

Food Consumption

Food consumption was measured weekly for individual male and female rats during the premating period and for females during their pregnancies (Days 1-8 and Days 9-14).

Food consumption was significantly increased in males in all of the R089674 dosing groups; however, as there was no dose-response relationship, and a similar effect was not noted in female rats, the Sponsor did not consider these effects to be relevant. There were no R089674-related adverse effects on food consumption for the surviving female rats.

Toxicokinetics

Toxicokinetics were not performed for this study.

Stability and Homogeneity

The concentration and stability of R089674 was evaluated up to one month after its preparation and the results are summarized in Table 43 below.

Table 43: Concentration and Stability of R089674 Dosing Solutions

		Nominal concentration of R089674 (mg/ml)			
Date of		0.63	2.5	5	7.5
preparation	analysis	Concentration found in % of nominal concentration			
Sep 24, 1996	Sep 26, 1996	99.7	98.4	-	98.7
Sep 24, 1996	Oct 24, 1996	99.8	98.8	-	99.2
Oct 17, 1996	Oct 21, 1996	-	-	99.0	-

Necropsy

Female rats were euthanized before examination of their uterine contents on Day 15 of their pregnancy. Male rats were euthanized after successful mating. Each animal was dissected and examined for evidence of disease or adverse reactions to treatment. Any macroscopic pathological changes were noted. The following parameters were noted for the female genital tract: weight of the gravid uterus, number of corpora lutea, number of implantation sites, number of live and dead fetuses, and number of resorptions.

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

Copulation and fertility rates: Copulation rates (the number of animals with successful copulation/ the number of mated animals) and fertility rates (the number of pregnant females/ the number of females with successful copulation) were comparable between groups.

Precoital interval: The time elapsing between the initial pairing and the record of mating was recorded. The precoital interval was lower in the control group (1.0 days) compared to the 5 mg/kg/day (4.0 days), 20 mg/kg/day (4.0 days) and 60(40) mg/kg/day (3.5 days) R089674 groups, but the difference was only significant for the 20 mg/kg/day group.

Weight of the gravid uterus: The weight of the gravid uterus was not adversely affected in rats dosed up to 60(40) mg/kg/day.

The number of implantations and number of copora lutea: The number of corpora lutea was not adversely affected by R089674 treatment.

Pre- and post-implantation loss: There were no relevant adverse effects in pre- and post-implantation losses.

Litter Data: The number of live and dead fetuses and the number of resorptions were individually recorded at sacrifice on the morning of the 15th day of pregnancy. There were no relevant adverse effects on the number of live and dead fetuses, the mean litter size or the number of resorptions in any of the R089674-treatment groups. No teratogenic effects were observed.

Reviewer Comments: *It should be noted that 20/24 females in the high-dose 60(40) mg/kg/day group died before mating. Therefore the measured fertility and offspring parameters for this group are based on four females. This report was also deficient in that the purity of the test substance (R089674) was not reported and a summary of the protocol deviations and deviations from GLP compliance was not provided.*

9.2 Embryonic Fetal Development

Study title: R089674: Experiment No. 3969; Study for effects on embryo-fetal development in SPF Wistar Rats (Segment II).

Study no:	N122419/1
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium.
Date of study initiation:	August 26, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # ZR089674PFA011, purity was not identified.

Key Study Findings

Maternal toxicity in the form of slightly reduced body weight, clinical symptoms (poor condition, rough haircoat, red vaginal discharge) and mortality were observed in 15/24 pregnant females in the high-dose group receiving oral 80 mg/kg/day R089674 for a few days followed by 40 mg/kg/day for the remainder of the dosing period. However, all other maternal measurements including caesarian section data were normal, and no adverse effects on embryo-fetal development or teratogenic effects were produced by any of the R089674 doses.

Methods

Doses:	0 (control), 5 (low), 20 (medium), and 40* (high) mg/kg/day (*See study deviations).
Frequency of dosing:	Daily from Day 6 to Day 16 of pregnancy.
Dose volume:	0.8 ml/100 g body weight
Route of administration:	Oral gavage
Formulation/Vehicle:	Demineralized water and HCl
Species/Strain:	SPF Wistar rats
Number/Sex/Group:	Four groups of 24 female rats
Satellite groups:	8 female SPF Wistar rats were assigned to a concurrently performed toxicokinetic study
Study design:	See below. Females were mated with stock males. Females demonstrating a sperm-positive vaginal smear after mating were assigned to treatment groups following a randomization procedure, and this day was assigned as Day 0 of the pregnancy. Animals were sacrificed on Day 21 of pregnancy.
Deviation from study protocol:	A formal summary of protocol deviations or deviations from GLP compliance was not included in this report. Due to early mortality and adverse clinical signs, the high dose of 80 mg/kg/day R089674 was reset to 40 mg/kg/day for the rest of the dosing period. Consequently, in the high-dose group, four, two, twelve, and six female rats were dosed with 80 mg/kg/day for four, three, two and one day respectively.

Table 44: Study Design for Study No. N122419/1

Dosage groups	mg/kg body weight/day	Identity number of female rats
C: Control (white)	0	1 - 24
L: Low (red)	5	31 - 54
M: Medium (yellow)	20	61 - 84
H: High (green)	40	91 - 114

Observations and Results

Mortality

No mortality was observed in rats receiving ≤ 20 mg/kg/day R089674. In the high-dose group receiving 80(40) mg/kg/day, 15/24 of the rats experienced R089674-related mortality. Most (14/15) of these rats demonstrated pale livers, and some demonstrated dilated urinary bladders.

Clinical Signs

All animals were individually observed at least once per day for signs of waning health, abnormal behavior, unusual appearance, occurrence of untoward clinical effects, manifestations of toxic or pharmacological response, morbidity, and mortality.

Rats receiving ≤ 20 mg/kg/day R089674 did not exhibit any clinical signs. Poor condition and rough coats were observed in approximately half of the rats in the high-dose group (80(40) mg/kg/day). All affected animals died within one or two days after the first symptoms. In 10/24 rats in the high-dose group, red vaginal discharge was noted.

Body Weight

Body weights were obtained on Days 0, 6, 9, 13, 17 and 21 of pregnancy.

None of the R089674 doses adversely affected body weight. The corrected mean maternal weight gain was slightly decreased at 80(40) mg/kg/day, and this change was entirely attributed to a slightly decreased maternal body-weight gain.

Food Consumption

Individual records were maintained for food consumption during the following periods of pregnancy: Days 0-5, Days 6-8, Days 9-12, Days 13-16, and Days 17-20.

There were no adverse R089674-related effects on food consumption.

Toxicokinetics

A satellite group consisting of 8 female SPF Wistar rats was assigned to a concurrently performed toxicokinetic study (Study No. N122427/2). The rats were randomly assigned to each of the four treatment groups on day 16 of the pregnancy. Blood samples (processed to plasma) were obtained at 1 (all animals), 2 (20 mg/kg/day dose animals only), 4 (all animals), and 8 (20 mg/kg/day dose animals only) hours after dosing. Plasma concentrations of R089674 and its metabolite R090692 were analyzed individually using an HPLC method.

The plasma concentrations of R089674 were mostly below the quantification limit of 0.025 µg/ml. The mean plasma concentrations of R090692 at the 1 hour sampling timepoint were 0.230, 1.33, and 1.78 µg/ml for the 5, 20, and 80(40) mg/kg/dose levels and the $AUC_{1\text{-}8\ hrs}$ was 2.44 µg x h/ml for the 20 mg/kg dose group. The individual and mean concentrations are summarized in Table 45 below.

Table 45: Individual and Mean Concentrations (µg/ml) of R090692 in Pregnant Female Rats After Dosing on Day 16.

Time post -dose	*5 mg/kg/day*			*20 mg/kg/day*			*40 mg/kg/day*[a]	
(h)	**55**	**56**	**Mean**	**86/87**	**86/88**	**Mean**	**115**	**116**
1	(b) (4)		0.230	(b) (4)		1.33	(b) (4)	NS[b]
2	-	-	-			*0.600*		-
4	(b) (4)		0.052			0.250		NS
8	-	-	-			*0.063*	-	-
$AUC_{1\text{-}8\ h}$ µg.h/ml			-			2.44		

a the high dose was reduced on the 5th day from 80 to 40 mg/kg/day.

b NS: no sample. Rat #116 died.

Stability and Homogeneity

The concentration and stability of R089674 was evaluated up to one month after its preparation and the results are summarized in Table 46 below.

Table 46: Concentration and Stability of R089674 in Each Dose Group

		Nominal concentration of R089674 (mg/ml)			
Date of		0.63	2.5	5	10
preparation	analysis	Concentration found in % of nominal concentration			
Aug 27, 1996	Aug 29, 1996	99	99.2	-	99.9
Aug 27, 1996	Sept 27, 1996	98.4	98.8	-	99.8
Sep 4, 1996	Sept 10, 1996	-	-	96.4	-

Necropsy

On the morning of the 21st day of pregnancy, surviving females were euthanized and autopsied for evidence of disease or adverse reaction to treatment, and all macroscopic pathological changes were noted. The ovaries from all sacrificed animals were removed and weighed. In addition, a number of parameters associated with female fertility and fetal development were assessed as noted below.

The weight of the ovaries was comparable between groups.

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

The weight of the gravid uterus was recorded, and the dams were examined for the number of corpus lutea, live and dead fetuses, early and late resorptions, and pre- and post-implantation loss. Any abnormal condition in the uterus that could have contributed to embryonic death was also noted.

The weight of the gravid uterus was comparable between groups. None of the R089674 doses produced adverse effects on the number of live and dead fetuses, the mean litter size, and the number of early and late resorptions. Also the number of implantations and the number of corpora lutea and pre- and post-implantation loss were comparable between groups.

Offspring (Malformations, Variations, etc.)

All live fetuses were individually weighed, evaluated for gender, and carefully examined for external abnormalities. Half of the fetuses in each litter were processed for skeletal examination and half were processed for visceral examination.

No R089674-related fetal malformations were observed. The number of fetal malformations was comparable between groups, and no major abnormalities were found in the 20 and 80(40) mg/kg dosage groups.

Study title: R089674: Experiment No. 3970; Study for effects on embryo-foetal development in albino rabbits (Segment II).

Study no:	N122418/2
Study report location:	Electronic transmission
Conducting laboratory and location:	Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium
Date of study initiation:	October 8, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R089674, Batch # ZR089674PFA011, purity of 99.4%

Key Study Findings

R089674 administered by oral gavage at doses of 10, 40, and 80 mg/kg/day to female Albino rabbits once daily from Day 6 to Day 18 of pregnancy was well tolerated, did not alter any litter parameters in the mother rabbits, and did not produce any teratogenic effects in the offspring. A slight maternal toxicity was noted for the 40 and/or 80 mg/kg/day doses in the form of reduced maternal weight gain and food consumption and decreased serum chloride and increased serum blood urea nitrogen, creatinine, and potassium.

Methods

Doses:	0 (control), 10 mg/kg/day (low), 40 mg/kg/day (medium), 80 mg/kg/day (high)
Frequency of dosing:	Once per day on Days 6 through 18 of pregnancy
Dose volume:	The dose volume was not reported.
Route of administration:	Oral gavage
Formulation/Vehicle:	R089674 dissolved in demineralized water and HCl/ vehicle of demineralized water and HCl.
Species/Strain:	72 Albino rabbits
Number/Sex/Group:	18 females/group
Satellite groups:	Eight animals (2/group) were assigned to a concurrently performed toxicokinetic study; however, the results of this study were not reported.
Study design:	See Table 47 below
Deviation from study protocol:	A formal summary of protocol deviations was not provided with this report.

Table 47: Study Design for Study No. N122418/2

Dosage group	mg/kg bwt/day	Identity number rabbits females
C: Control	0	1 - 18
L: Low	10	19 - 36
M: Medium	40	37 - 54
H: High	80	55 - 72

Observations and Results

Mortality

All animals that died or were sacrificed due to reasons of animal welfare during the course of the experiment were noted.

No R089674-related mortality was observed.

Clinical Signs

All animals were observed at least once a day for signs of waning health, abnormal behavior, unusual appearance, untoward clinical effects, manifestations of toxic and pharmacological response, morbidity, and mortality.

No relevant R089674-related adverse effects were noted.

Body Weight

Individual body weights were recorded on Days 0, 6, 9, 12, 15, 19, 22, 25, and 28.

Body weight patterns were comparable for all the groups. A slight non-significant decrease in the corrected mean maternal weight gain was seen in the groups dosed at 40 and 80 mg/kg body weight.

Food Consumption

Food consumption was calculated for individual animals during the following periods of pregnancy: Days 0-5, Days 6-11, Days 12-18, and Days 19-27.

A slight non-significant decrease in food consumption was noted in the groups dosed at 40 and 80 mg/kg body weight during the dosing period.

Toxicokinetics

Eight animals (2/group) were assigned to a concurrently performed toxicokinetic study, and the results were reported in the study report for Study No N122426/2. On Day 18, serial blood samples were obtained and processed to plasma from all rabbits at 1, 2, 4, 8, and 24 hours after the start of dosing. Plasma concentrations of R089674 and R090692 were analyzed with an HPLC-method.

Plasma concentrations of R089674 were ≤ the quantification limit at the 10 and 40 mg/kg dose levels at all sample times. For the 80 mg/kg dose level, R089674 dose levels were 4-5 times the quantification limit (0.010 μg/ml) at 1 and 2 hours after dosing. The T_{max} was one hour in 5/6 animals receiving R089674. The R090692 mean C_{max} values were 5.06, 12.3, and 17.3 μg/ml, and the mean AUC_{0-24h} values were 10.2, 36.2, and 93.6 μg x hr/ml for the 10, 20, and 80 mg/kg/day dose levels respectively. R090692 C_{max} values increased in a less than dose- proportional manner, and AUC_{0-24h} values increased in an approximately dose-proportional manner. The individual and mean values are summarized below in Table 48.

Table 48: Individual and Mean Plasma Concentrations (µg/ml) of R090692 in Pregnant Rabbits Following Dosing on Day 18 of Pregnancy.

		10 mg/kg/day			*40 mg/kg/day*			*80 mg/kg/day*		
Time post -dose (h)		*31*	*32*	*Mean*	*49*	*50*	*Mean*	*67*	*68*	*Mean*
1		(b) (4)		5.06	(b) (4)		12.3	(b) (4)		17.0
2				1.93			6.22			12.9
4				0.422			3.04			8.69
8				0.082			0.452			2.92
24				0.020			0.113			0.239
C_{max}	µg/ml	5.34	4.78	5.06	11.7	12.8	12.3	18.9	15.7	17.3
T_{max}	h	1	1	1	1	1	1	1	2	1.5
$t_{1/2}$	h	10.1	5.6	7.8	6.3	9.0	7.7	4.2	4.6	4.4
$AUC_{0\text{-}24\ h}$	µg.h/ml	11.1	9.3	10.2	31.6	40.8	36.2	78.7	108	93.6

Stability and Homogeneity

The stability of test solutions was assessed approximately 1.5 months after solution preparation. The results indicating little or no compound degradation are summarized in the Sponsor table, Table 49, below.

Table 49: Concentration and Stability of R089674 Formulations

		Nominal concentration of R089674 (mg/ml)		
Date of		2	8	16
preparation	analysis	Concentration found in % of nominal concentration		
Oct 7, 1996	Oct 9, 1996	98.8	99.5	99.8
Oct 7, 1996	Nov 20, 1996	97.4	97.8	98.1

Serum Analysis

Serum analysis was performed on all surviving animals on Day 15 of pregnancy. A standard panel of serum parameters was examined.

R089674 dosed at up to 10 mg/kg/day had no adverse effects on any of the examined serum parameters. At 40 mg/kg/day, decreased serum chloride and increased blood urea nitrogen was observed. At 80 mg/kg/day, these changes were more pronounced, and decreased serum sodium, and increased serum creatinine and potassium was also

observed. The serum sodium value in the 80 mg/kg/day group was within the historical range and not considered toxicologically relevant.

Necropsy

On the morning of the 28th day of pregnancy, all surviving females were sacrificed, an autopsy was performed and all macroscopic pathological changes were noted. The spleen was removed and weighed, and a number of maternal fertility and fetal development parameters were assessed as noted below.

The absolute and relative weight of the spleen was comparable between groups.

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

The following maternal parameters were assessed: the weight of the gravid uterus, number of corpora lutea, live and dead fetuses, early and late resorptions, and number of implantations. Any abnormal condition in the uterus was also noted.

The weight of the gravid uterus was comparable between groups. The mean litter size, the number of live and dead fetuses per dam, and the number of resorptions per dam were comparable between all groups. Also, the number of implantations and the number of corpora lutea were comparable between all groups as was the pre- and post-implantation loss.

Offspring (Malformations, Variations, etc.)

Following maternal sacrifice, multiple fetal development parameters were assessed. All live fetuses were individually weighed and carefully examined for external anomalies. The neck, thoracic cavity, and abdominal cavity of all fetuses from each litter were dissected and examined for visceral anomalies using a modified Staples technique. The fetal sex was recorded. Half of the fetuses from each litter were decapitated and their heads were examined using a modified Wilson technique. The torsos and intact fetuses were subsequently examined for skeletal malformation.

There were no R089674-related adverse effects on the weight or sex ratio of fetuses in any of the groups. Also, there was not an increase in the number of malformations, minor abnormalities, or variations in the R089674-treated groups compared with the control group.

9.3 Prenatal and Postnatal Development

Study title: Oral (gavage) developmental and perinatal/postnatal reproduction toxicity study of R89674 in rats including a postnatal behavioral/functional evaluation.

Study no:	AAL00006
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R89674, Lot # 05P0189, Purity of 99.5%

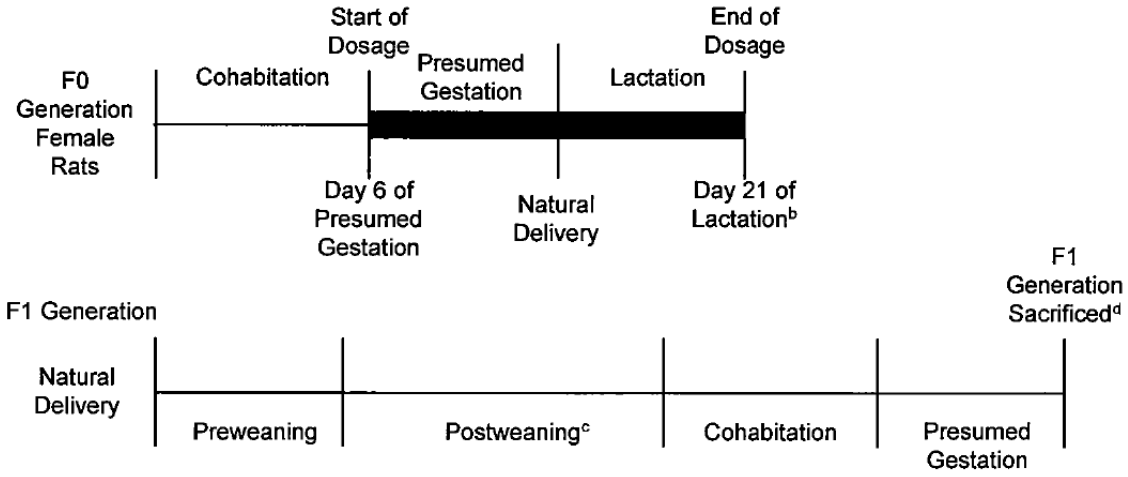
Key Study Findings

The maternal NOAEL for R89674 was considered to be 20 mg/kg/day as the 30 mg/kg/day dose produced reductions in body weight gain during the gestation period. The reproductive NOEL in dams was 30 mg/kg/day and there were no adverse effects on reproduction in the F_0 generation. The NOAEL for offspring viability and growth was 5 mg/kg/day because pup body weights were reduced at 20 and 30 mg/kg/day.

Methods

Doses:	0, 5, 20, and 30 mg/kg/day
Frequency of dosing:	daily
Dose volume:	8 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	R89674 was dissolved in deionized water/ the vehicle was deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	25 female per group for the F_0 generation and 25 males and females per group for the F_1 generation.
Satellite groups:	none
Study design:	F_0 generation rats: See Figure 3 below. Rats were administered R089674 or the vehicle once daily from Gestation Day (DG) 6 through Lactation Day (DL) 20 (or DG 24 for rats that did not deliver a litter). Rats were dosed once per day at approximately the same time; however, dams in the process of delivering pups were not dosed until completion of parturition. Consequently some dams missed one daily dose, but no dam missed more than one daily dose.
Deviation from study protocol:	The report contains a “Statement of the Study Director” which stipulates that no deviations occurred that affected the quality or integrity of the study.

Figure 3: Schematic Representation of the Study Design for Study No. AAL00006.



b. F_0 generation rats sacrificed on Day 21 of lactation.
c. Behavioral and functional assessments.
d. Fetal evaluations (all fetuses – external examinations).

Table 50: Study Design for F_0 Generation Rats.

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rats	Assigned F0 Generation Rat Numbers
I	0 (Vehicle)	0	8	25	401 - 425
II	5	0.63	8	25	426 - 450
III	20	2.5	8	25	451 - 475
IV	30	3.75	8	25	476 - 485, 10160[b], 487 - 500

a. The test article was considered 100% active for the purpose of dosage calculations.
b. Rat 486 was removed from study before dosage administration on DG 6 due to body weight loss and replaced with rat 10160.

Table 51: Study Design for F_1 Generation Rats.

Maternal Dosage Group	Maternal Dosage (mg/kg/day)	Number of Rats Per Sex	Assigned F1 Generation Rat Numbers	
			Male Rats	Female Rats
I	0 (Vehicle)	25	1101 - 1125	1201 - 1225
II	5	25	1126 - 1150	1226 - 1250
III	20	25	1151 - 1175	1251 - 1275
IV	30	25	1176 - 1200	1276, 994[a], 1278 - 1300

a. F1 generation female rat 1277 was found dead on postweaning day 2 and was replaced with F1 generation female rat 994.

Observations and Results

F_0 Dams

Survival

Rats were observed for viability at least twice each day of the study.

All rats survived until scheduled sacrifice.

Clinical Signs

Rats were observed for clinical signs and general appearance weekly during acclimation and on DG 0. The rats were also examined for clinical signs, abortions, premature deliveries, and deaths before and approximately 60 minutes after each dose administration and on the day of sacrifice.

No R89674-related clinical signs were observed during the gestation or lactation periods at R89674 doses ≤ 30 mg/kg/day.

Body Weight

Body weights were recorded weekly during the acclimation period, on DG 0, daily during the dosage period, and at sacrifice.

Compared to the vehicle control group, body weight gains were significantly reduced on DGs 6-9 and for the entire gestation dosage period (DGs 6-20) in the 30 mg/kg/day dosage group. Body weight gains for the entire gestation dosage period decreased in a R89674 dose-dependent manner with the 5, 20, and 30 mg/kg/day dose groups demonstrating 99.2, 90.7, and 88.0% weight gain respectively compared to the vehicle control group. In contrast, lactation body weights and body weight gains were unaffected by R89674 doses of ≤ 30 mg/kg/day.

Feed Consumption

Feed consumption was recorded on DGs 0, 6, 9, 12, 15, 18, 20, and 25, and Lactation Days (DLs) 1, 4, 7, 10, and 14.

Feed consumption was not affected by R89674 doses as high as 30 mg/kg/day during the gestation and lactation periods.

Uterine Content

Following necropsy, the number and distribution of implantation sites was recorded. Rats were also evaluated for maternal behavior on DLs 1, 4, 7, 14, and 21; adverse

clinical signs during parturition; duration of gestation; litter sizes, and pup viability at birth.

Pregnancy occurred in 23/25 mated female rats in all of the groups. All of the pregnant dams delivered litters. There was no significant differences among the four groups for duration of gestation, implantation sites per litter, dams with stillborn pups, gestation index, average live litter sizes, stillbirths, surviving pups per litter, the lactation index, percent of male pups, or live litter size at weighing. The viability index was significantly reduced in the 30 mg/kg/day group with 13 pups dying in this group on DL 1-4 compared to 5 deaths in the vehicle control group for this period. However, 5 of the 13 deaths in the 30 mg/kg/day group occurred in the same litter suggesting maternal care may have affected this measurement.

Necropsy Observation

After completion of the 21-day postpartum period, female rats were sacrificed and examined for gross pathology of the thoracic, abdominal, and pelvic viscera. Rats that did not deliver a litter were sacrificed on DG 25 and examined for gross pathology.

No gross lesions were revealed by necropsy.

Toxicokinetics

Toxicokinetics were not performed for this study

Stability and Homogeneity

The seven-day stability for several test samples were measured and determined to be > 93% of target for all samples. The concentration and stability of R89674 test samples are summarized in Table 52 below.

Table 52: Concentration and Stability of R89674 Test Samples

Sample Batch #	Target Concentration	Sample Description	Assay Results (mg/mL)	% of Target (b) (4)
A	0 mg/mL	Prestudy / Initial		
A	0 mg/mL	7 day stability		
A	0 mg/mL	Start of dosage		
A	0 mg/mL	End of dosage		
B	0.63 mg/mL	Prestudy / Initial		
B	0.63 mg/mL	7 day stability		
B	0.63 mg/mL	Start of dosage		
B	0.63 mg/mL	End of dosage		
C	2.50 mg/mL	Start of dosage		
C	2.50 mg/mL	End of dosage		
D	3.75 mg/mL	Prestudy / Initial		
D	3.75 mg/mL	7 day stability		
D	3.75 mg/mL	Start of dosage		
D	3.75 mg/mL	End of dosage		

Other

No other F_0 evaluations were performed.

F_1 Generation

Survival

Beginning on the day of birth, each litter was evaluated for viability at least twice daily during the preweaning and postweaning periods.

All F_1 generation rats aside from a few dying in the first four days after birth survived to the scheduled sacrifice. As noted above, the viability index was reduced in the 30 mg/kg/day R89674 group, but ≈ 40% of the offspring deaths occurred in a single litter, suggesting maternal care may have played a role.

Clinical Signs

Clinical observations were recorded once daily during the preweaning period, and once weekly during the postweaning period.

No clinical signs considered to be related to R89674 administration were reported.

Body Weight

Pup body weights were recorded on DLs 1, 4, 7, 14, and 21. During the postweaning period, body weights for the male rats were recorded weekly and on the day of sacrifice. Body weights for female rats were recorded weekly during the post weaning period and on DGs 0, 6, 9, 12, 15, 18, and 21.

The average pup weight was significantly reduced on DLs 1 and 4 in the 20 (7.9% and 9.9% respectively) and 30 mg/kg/day (7.9% and 8.8% respectively) groups. During the postweaning period body weights and body weight gains of male and female F_1 rats were unaffected by maternal doses of R89674. Statistically significant changes did occur in some of the R89674-dose groups relative to the vehicle control group, but the changes were not considered related to maternal administration of R89674 because the differences were not dose-dependent.

Feed Consumption

Feed consumption for male and female rats was recorded weekly during the postweaning period except during cohabitation. Feed consumption for female rats was also measured on DGs 0, 6, 9, 12, 15, 18, and 21.

Absolute and relative feed consumption during the postweaning, precohabitation, and gestation periods were unaffected by maternal doses of R89674 ≤ 30 mg/kg/day. The statistically significant changes that did occur for some of the R89674 dose groups relative to the vehicle control group, were not considered related to maternal administration of R89674 because the differences were not dose-dependent.

Physical Development

Female pups were evaluated for the age of vaginal patency beginning on Day 28 postpartum. Male rats were evaluated for the age of preputial separation beginning on Day 39 postpartum.

The average day of preputial separation or vaginal patency was comparable among the four dose groups.

Neurological Assessment

Beginning at Day 24 ± 1 postpartum, one male and one female rat from each litter was evaluated in a passive avoidance test for learning, and short- and long-term retention. Beginning at Day 70 ± 2 postpartum, one male and one female rat from each litter was evaluated in a water-filled M-maze for overt coordination, swimming ability, learning and memory.

There were not significant differences in the measurements of learning, short-term retention, long-term retention, or response inhibition in the F_1 generation male and female rats evaluated in the passive avoidance test. Similarly, no significant differences

occurred in watermaze performance of the F_1 generation male and female rats regarding learning, short-term retention, long-term retention, or response inhibition.

Reproduction

At approximately 90 days of age, the F_1 generation rats within each dosage group were randomly assigned (excluding sibling rats) to cohabitation, one male per female rat for a maximum of 17 days. Female rats with positive vaginal smears and/or copulatory plug were considered to be at DG 0 and assigned to individual housing. Female rats not mated within the first 14 days of cohabitation were assigned alternate male rats from the same dosage group that had mated.

There were no significant effects on the mating and fertility parameters evaluated in the F_1 generation male and female rats. Measured parameters including: the number of cohabitation days; the number of rats that mated; the fertility index (number of pregnancies/number of female mating rats); the number of rats with confirmed mating during the first, second, and/or third weeks of cohabitation; and the number of pregnancies per number of rats in cohabitation did not significantly differ among the four groups.

Necropsy Observation

After completion of the cohabitation period, all male rats were sacrificed and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. Testes and epididymides were excised and paired organs were weighed, fixed, and retained.

All female F_1 rats were sacrificed on DG 21, and a Caesarian section and gross examination of the thoracic, abdominal, and pelvic viscera was performed. No necropsy observations in male or female rats related to maternal administration of R89674 occurred.

Uterine Content

The number and distribution of corpora lutea were recorded. The uterus of each rat was excised and examined for pregnancy, number, and distribution of implantation sites, live and dead fetuses, and early and late resorptions. Each fetus was weighed and examined for sex and gross external alterations.

No Caesarean-sectioning or litter parameters were affected by maternal dosages of R89674 ≤ 30 mg/kg/day. The litter averages for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were comparable among the four dosage groups. No F1 dam had a litter consisting of only resorbed conceptuses, and there were no dead fetuses. All placentae appeared normal. The only fetal gross external alterations were cleft palates occurring in one offspring in the 5 and 20 mg/kg/day

maternal dose groups, but due to its low incidence, this alteration was considered unrelated to maternal administration of R89674.

10 Special Toxicology Studies

No other toxicity studies examining antigenicity, immunotoxicity, mechanistic studies, dependence, or metabolic studies were conducted.

11 Integrated Summary and Safety Evaluation

R89674 (alcaftadine, R089674) is a histamine H_1, H_2, and H_4 receptor antagonist intended for use as a topical ophthalmic treatment for allergic conjunctivitis. In pharmacodynamic studies, R89674 was shown to bind histamine H_1 and H_2 receptors with nM affinity, maintain conjunctival epithelial integrity, prevent vascular leak, and inhibit early- and late- phase allergic inflammation. In addition, the safety pharmacology, pharmacokinetics, ocular, and systemic toxicity, genetic toxicity, and reproductive toxicity of R89674 were assessed in multiple studies.

Safety pharmacology studies examined central nervous system activity in rats and cardiovascular and behavioral activity in dogs. In rats, altered EEG activity suggestive of a sedative effect occurred following the oral dose of 2.5 mg/kg R89674, but not after lower but pharmacologically efficacious doses of 0.63 and 0.16 mg/kg. In an initial study in dogs, R89674 did not produce behavioral effects or consistent changes in most monitored cardiovascular parameters (heart rate, diastolic and systolic blood pressure, pressure rate product, cardiac relaxation, cardiac output, stroke volume, total systemic resistance, coronary artery blood flow, and most ECG parameters). However, a significant decrease in LV dp/dt max/p was observed at 15 minutes and half an hour after drug administration but not at the one hour T_{max} timepoint for conscious dogs receiving a single oral dose of 0.63 mg/kg R89674. This change was not noted in other follow-up studies in dogs. In a second study examining the effects of R89674 on behavioral and cardiovascular parameters in dogs, limited behavioral effects including urination and/or agitation were noted in 3/7 study dogs receiving oral 2.5 mg/kg R89674. In this study, R89674 tended to increase cardiac output with significant differences from vehicle-treated animals at 15 and 45 minutes after dosing, but did not alter other cardiac parameters including ECG-interval durations. ECG measurements in dogs receiving repeated-oral doses of R89674 in one-month and six-month studies demonstrated significant shortening of the PQ interval at the highest study doses of 20 mg/kg (one-month study) and 40 mg/kg (six-month study) which was considered R89674-related. As the exposure values for the high doses were much higher than those expected following clinical topical ocular administration of daily 0.25% R89674, PQ interval shortening is not an expected consequence of clinical ocular administration and was not reported in any of the clinical safety studies.

The mass balance distribution of R89674 following ocular administration was measured in a single-dose pharmacokinetic study in rabbits. Less than 10% of [^{14}C]R089674

administered topically to both eyes of male New Zealand White rabbits distributed to the examined ocular tissues (aqueous humor, eyelids, conjunctiva, cornea, iris-ciliary body, lens, optic nerve, retina/choroid, sclera, vitreous humor) suggesting much of the administered dose quickly drained to nasopharangeal passages or was diverted to fur. Among the ocular tissues, the highest mean C_{max} concentrations of radioactivity within the first eight hours of administration were observed in the aqueous humor, iris-ciliary body, cornea, and eyelids. Plasma radioactivity levels 30 minutes after dosing were more than 3% of the administered dose, but declined to levels below the limit of quantification within four hours. Over 60% of the radioactivity was recovered in the urine.

The plasma toxicokinetics of R89674 and its metabolite R90692 following ocular administration were also examined as part of the 6-month repeated-ocular dose toxicology study in rabbits. In this study, plasma levels of R89674 were only detectable 5 minutes after the first administration then fell below the lower limit of detection. However, R90692, the R89674 metabolite, was detectable at the five minute sampling timepoint and subsequent timepoints for all doses. On Days 1, 28, 91, and 182, R90692 C_{max} values following the first daily dose for each dose group increased in a roughly dose-dependent manner, and were relatively constant throughout the dosing period, suggesting little or no accumulation of R90692 occurred with any of the dose regimens. After the first ocular dose, plasma exposure for the high-dose regimen (0.5% TID) produced C_{max} values of 115 and 110 ng/ml and $AUC_{(0\text{-}t)}$ values of 150 and 110 ng x hr/ml in male and female rabbits respectively. Also the plasma $t_{1/2}$ values for R90692 were short, ranging from approximately 1 to 3 hours.

R89674 was not absorbed well following oral administration with an estimated oral bioavailability of < 7% in rats and approximately 15% in dogs. In both rats and dogs, R89674 plasma levels rapidly declined following intravenous or oral administration as R89674 was metabolized to its carboxylic acid metabolite, R90692. In conjunction with a single intravenous administration of R89674, the plasma $t_{1/2}$ values for R89674 were approximately 0.1 and 0.9 hours, and $t_{1/2}$ values for R90692 were approximately 2 and 3 hours for rats and dogs respectively. Following 6 months of oral daily dosing, R90692 appeared to accumulate in rats, but R89674 and R90692 did not accumulate in dogs. In both rats and dogs, the apparent volumes of distribution of R89674 were much greater than blood volumes for the respective species indicating extravascular distribution.

In rats, ^{14}C-R89674 tissue distribution proceeded rapidly following oral administration with maximum concentrations in most tissues occurring within one hour of dosing. Tissue concentrations of radioactivity were as high as 25 fold greater than in plasma, and the highest concentrations were found in the small and large intestines, and in the urinary bladder followed by stomach, liver, esophagus, trachea, prostate, kidney, and seminal vesicles. The lowest concentrations were found in subcutaneous fat, testicles, and brain. Radioactivity declined rapidly, and no irregular tissue retention or accumulation was observed. Following 6 months of daily oral R89674 dosing in dogs, tissue concentrations of R90692 were higher in tissue than in plasma and several fold higher than those of R89674, but R90692 tissue to plasma concentration ratios

decreased with increasing dose. The highest tissue concentrations of R89674 occurred in the adrenals followed by kidney and lung. The highest tissue concentrations of R90692 were found in kidney and liver followed by lung, fat, and heart. The lowest concentrations were found in muscle, adrenal glands, and brain.

R89674 and R90692 demonstrated low to moderate plasma protein binding in all tested species including humans suggesting a low potential of drug-drug interactions based on protein binding. Dog, mouse, and human plasma proteins bound 41%, 52%, and 40% of R89674 *in vitro* in plasma samples. The plasma protein binding of R90692 was more variable with 12-14% binding in rats and mice, 20% in dogs, 28% in rabbits and 63% in humans. In purified plasma protein binding experiments, approximately 27% and 10% of R89674 bound human serum albumin (HSA) and α1-acid glycoprotein (α1-AGP) respectively. In comparison, a greater percentage of R90692 bound HSA (72%) but only negligible amounts of R90692 bound α1-AGP. Blood distribution of R89674 was similar for human and dog blood, with blood to plasma concentration ratios of approximately 1.4. In dog and human blood, the largest fraction of R89674 distributed to blood cells (62-66%) followed by 15% bound to plasma proteins, and 20-23% remaining unbound in water. Less R90692 remained in blood with blood to plasma concentration ratios of 0.63 in human blood and 0.75-0.89 in the blood of dogs, rats, mice, and rabbits. In human blood, the largest fraction of R90692 bound to plasma proteins (≈ 52%), and approximately 16% bound to red blood cells with 32% remaining unbound in plasma water. In the blood of dogs, rats, mice, and rabbits, the largest fraction of R90692 remained unbound in plasma water (49-62%) with 7-23% bound to plasma proteins, and 20-39% bound to blood cells.

Following a single oral administration of 5 mg ^{14}C-R89674 in rats, the predominant metabolite was R90692 accounting for 45% (female) and 49% (male) of the dose. Eight other metabolites accounted for 1-6% of the dose, with 5/8 metabolites identified as to structure. No qualitative or quantitative gender differences in the metabolism of R89674 were noted. Human metabolism of R89674 was examined in human hepatocytes and liver microsomal fractions, and the only significant metabolite observed was R90692. In human hepatocytes more so than in any other tested species, much of R89674 was not metabolized with 38% and 76% remaining unchanged in suspension and plated hepatocyte cultures respectively. Much less R89674 metabolism was measured in human liver microsomes compared to that observed in 12000 x g supernatant fractions of human hepatocytes indicating the R89674 is primarily metabolized by cytosolic enzymes.

Following ocular administration in rabbits, more than 60% of R89674 was excreted in urine. Consistent with a low oral bioavailability, following oral administration to rats, the primary route of excretion was through feces (87-92%) followed by urine (11-15%).

Neither R89674 nor R90692 greatly inhibited the activity of the major CYP-450 isozymes (CYPA1, CYPA2, CYP2A6, CYP2C19, CYP2C8, CYP2C9, CYP2C10, CYP2D6, CYP3A4, and CYP4A). These results are in agreement with the minor degree of R89674 metabolism occurring via CYP-450 activity. Some degree of selective CYP-

450 isozyme inhibition was observed *in vitro* in human liver microsomes and *ex vivo* in liver microsomes obtained from rats orally dosed for 6-months with R89674. However, because only about 4% of the overall metabolism of R89674 is mediated by CYP-450 enzymes, these effects are not expected to greatly impact the metabolism of other drugs. The drug-drug interaction potential of R89674 was directly evaluated by incubating R89674 with human liver microsomes or human liver cytosolic fractions in the absence and presence of drugs known to be metabolized by different CYP-450 isozymes. Three drugs, ketotifen, loratidine, and erythromycin substantially inhibited the minor degree of R89674 metabolism occurring in human liver microsomes, but only slightly affected the predominant metabolic pathways in liver cytosolic fractions.

In single-dose studies in mice and rats, high doses of oral or intravenous R89674 caused clinical signs including tremors, convulsions, sedation, paralysis, and mortality. The approximate lethal dose following oral administrations was 60-80 mg/kg in mice and 320-640 mg/kg in rats. The approximate lethal doses following single-dose intravenous administration was 30 mg/kg in mice and 40 mg/kg in rats. A single-topical ocular dose administration of 0.0625% R89674 did not cause eye irritation or any signs of systemic toxicity in rabbits.

In a 14-day repeated-dose ocular toxicology study in rabbits, R89674, administered by the topical ocular route at doses as high as 0.5% TID did not cause significant ocular or systemic toxicity. Similarly, in a pivotal 6-month repeated-dose study in rabbits, no overt clinical signs of R89674-related ocular or systemic toxicity occurred at topical ocular R89674 doses of ≤ 0.5% administered TID. Also no changes in ophthalmology, clinical chemistry, hematology, or coagulation parameters were associated with R89674 administration. The NOEL was considered to be 0.5% (TID), and this dose produced R90692 plasma C_{Max} values of 53.9 (male) and 67.9 (female) ng/ml and $AUC_{(0\text{-}t)}$ values of 49.3 (male) and 72.1 (female) ng x hr/ml after six months of dosing.

One-month and six-month repeated oral-dose toxicology studies were conducted in rats and dogs. In both rats and dogs, toxicity appeared to be progressive with more pronounced toxicity in the six-month studies compared to the one-month studies. Toxicity in rats was more pronounced than in dogs, and the apparent target organ for toxicity in rats was the liver. Female rats in both the one-month and six-month studies demonstrated higher R90692 exposure levels and more pronounced toxicity than males, but a similar pattern did not occur in dogs.

In a 6-month oral repeated-dose study, slight to moderate toxicity occurred in rats at R89674 doses of ≥ 20 mg/kg/day. Toxicity was most pronounced in females in the high-dose group receiving 80/40/60 mg/kg/day, and this correlated with substantially higher C_{max} and $AUC_{0\text{-}t}$ values for females versus males in this group. Among the toxic effects were alteration of several hematological parameters (slight decreases in hematocrit, hemoglobin, and red blood cells, slight increases in white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and thrombocytes), and slight to moderate alterations in several serum values at 3 and/or 6 months including decreased total protein, albumin, and creatinine and increased serum potassium, aspartate

aminotransferase, cholesterol, inorganic phosphate, phospholipids, total bilirubin, alkaline phosphatase, and alanine aminotransferase. The serum chemistry alterations suggest the liver was a target organ for toxicity. Liver weights were increased in male and female rats, and in females liver histopathology included hepatic atrophy and diffuse hyperplasia of oval cells and bile ducts. The NOAEL dose for this study was considered to be 5 mg/kg/day and R90692 AUC_{0-t} values associated with this dose were 0.627 (male) and 0.493 (female) μg x h/ml.

In a 6-month oral repeated-dose study in dogs, a few, relatively minor, toxicological effects occurred in the high-dose group (40 mg/kg/day) including transient salivation, rough haircoat, increased incidences of focal alopecia, shortened PQ interval, and a slight increase in systolic blood pressure. Body weight and weight gain were also decreased in the high-dose group. In conjunction with this change, a number of organs were decreased in weight; however, the organ weight changes were generally not accompanied by histopathological changes. Some laboratory values were affected in the high-dose group including decreased red blood cell volume, decreased serum calcium, cholesterol, and phospholipids, and increased serum blood urea nitrogen. The NOAEL was considered to be 10 mg/kg/day and the R89674 and R90692 $AUC_{0-\infty}$ values for this dose were 1.12 and 29.6 μg x h/ml respectively.

Based on the results of the repeated-dose toxicology studies in animals, daily clinical ocular administration of 0.25% R89674 does not appear to pose a major concern for ocular or systemic toxicity. In both the 14-day and 6-month repeated-dose ocular studies in rabbits, no ocular or systemic toxicity occurred and the NOEL values were the highest administered doses of 0.5% R89674/eye TID. Also R89674 and R90692 did not accumulate after 6 months of dosing in the second study. As shown in Table 53, these values support the ocular safety of the proposed clinical daily dose. When the 0.5% TID ocular NOEL doses in rabbits are normalized to human vitreal volume or conjunctival surface area, they provide 28- and 7- fold safety margins compared to the proposed human clinical dose.

Table 53: Safety Evaluation for the Ocular Toxicity of R90692 (the Active Metabolite of R89674) Based on Eye Volume and Eye Surface Area.

Route and Study	Species	NOEL (mg/kg)	HED (based on vitreal volume) [b]	Safety Margin (based on vitreal volume)[c]	HED (based on conjunctival surface area)[d]	Safety Margin (based on conjunctival surface area)[c]
14-Day ocular study	rabbit	0.5% TID 200 µg/dose[a] 600 µg/eye/day	2.4 mg/ eye/day	28	600 µg/eye/day	7
6-month ocular study	rabbit	0.5% TID 200 µg/dose[a] 600 µg/eye/day	2.4mg/ eye/day	28	600 µg/eye/day	7

[a] Dose volume of 40 µl/rabbit eye

[b] The human and rabbit vitreal volumes are approximately 4 ml and 1 ml respectively.

[c] Clinical human daily dose = 34 µl of 0.25% R89674/eye/day = 85 µg/eye/day.

[d] Humans and rabbits have approximately the same conjunctival surface area.

Topical ocular administration of R89674 is not expected to cause serious systemic toxicity. No systemic toxicity occurred in the 14-day or 6-month topical ocular studies in rabbits. In the 6-month rat and dog repeated-dose oral studies where some R89674-related changes in hematology and clinical chemistry did occur as well as histopathology of the liver, the exposure levels associated with the NOAEL doses were much higher than those measured clinically. As shown in Table 54, the R90692 AUC values corresponding to the NOAEL doses in the 6-month rat and dog studies provide safety margins of 40 and 22 for male and female rats respectively and 2789 for dogs relative to the clinical R90692 $AUC_{0\text{-last}}$ of 10.613 ng x h/ml measured after ocular dosing in the clinical pharmacokinetic protocol # 05-003-09. Similarly, as summarized in Table 55, the human equivalent dose (HED) values based on surface area normalization of the rat and dog NOAEL values provide safety margins of 329 and 2222 for the rat and dog NOAELs respectively. These results suggest the proposed clinical dose and regimen present minimal concerns for systemic toxicity.

Table 54: Safety Evaluation for the Systemic Toxicity of R90692 (the Active Metabolite of R89674) Based on Plasma Exposure (AUC).

Route and Study	Species	NOAEL (mg/kg)	AUC (μg x h/ml)	Safety Margin based on AUC*
6-month oral study	rat	5 mg/kg/day	0.421 (male) 0.236 (female)	40 (male) 22 (female)
6-month oral study	dog	10 mg/kg/day	29.6	2789

*AUC in human for R90692: $AUC_{0\text{-last}}$ = 10.613 ng x hr/ml for 0.25% R89674/eye/day administered to both eyes.

Table 55: Safety Evaluation for the Systemic Toxicity of R90692 (the Active Metabolite of R89674) Based on Surface Area Conversion.

Route and Study	Species	NOAEL (mg/kg)	HED (μg/kg/day) [a]	Safety Margin (based on human clinical dose) [b]
6-month oral study	rat	5 mg/kg/day	800 μg/kg/day	329
6-month oral study	dog	10 mg/kg/day	5400 μg/kg/day	2222

[a] Normalization based on body surface area. Conversion factors of 0.16 for rats and 0.54 for dogs.

[b] Clinical human daily dose = 0.25% R89674/eye/day administered to both eyes. Assuming bilateral doses of 34 μl of 0.25% R89674 per day = 170 μg/day or for a 70 kg human, 2.43 μg/kg/day

The genetic toxicity of R89674 was examined in a full panel of *in vitro* and *in vivo* genetic toxicology assays. R089674 at concentrations as high as 5000 μg/plate was negative for mutagenicity in *Salmonella typhimurium* strains TA1538, TA98, TA1537, TA1535, and TA100 in an Ames tests. Similarly, the major metabolite of R89674, R90692, was negative for mutagenicity in the TA98 and TA100 strains at concentrations up to 5000 μg/plate in an Ames test. *In vitro*, R89674 as well as (b) (4) at concentrations of 10, 100, and 1000 mg/ml did not increase chromosome aberrations in a chromosome aberration assay in mammalian cells. In a thymidine kinase mutation assay, R89674 did not produce significant increases in mutant frequencies even at cytotoxic doses in lymphoma L51178Y cells. *In vivo*, in two micronucleus tests in mice, R89674 did not cause a significant increase in the number of micronucleated polychromatic erythrocytes. These studies satisfy what is recommended by the genetic toxicology guidances (ICH-S2A and ICH-S2B) and indicate minimal genotoxicity potential for R89674.

The Sponsor requested a waiver for carcinogenicity studies in an amendment to IND 66884 (document submission number 31). The request was granted based on: the

negative mutagenicity results in the extensive panel of genetic toxicology studies noted above; an expectation of intermittent clinical dosing; low carcinogenicity potential for the antihistamine class of drugs as a whole; a lack of carcinogenicity concerns for R89674 or its major metabolite, R90692, based on structure-activity analysis; an absence of pre-neoplastic lesions in the oral repeated-dose toxicology studies; no indication of long-term retention in tissues; and low systemic exposure following topical ocular administration of R89674.

In order to determine its potential for reproductive and developmental toxicity, R89674 was examined in Segment I, II, and III studies. In a Segment I fertility study in Wistar rats, the majority of females (20/24) but none of the males in the 60 mg/kg/day dose group experienced mortality again demonstrating a pattern of increased R89674-related toxicity in females compared to males. The four remaining females in the 60 mg/kg/day group survived until the end of the study after the dose was lowered to 40 mg/kg/day, and did not demonstrated reduced fertility. R89674 at doses of ≤ 60 mg/kg/day in male rats and ≤ 20 mg/kg/day in female rats did not lead to mortality, adverse clinical signs, or adverse fertility effects. Also in the 6-month oral repeated-dose study in rats, organ weights for testes and ovaries were not changed even in the high-dose groups. In dogs in the 6-month oral repeated-dose study, testes weights were not changed, but ovary weights were decreased in the high-dose (40 mg/kg/day) group in conjunction with lower body weights.

In a Segment II embryo-fetal toxicity study in Wistar rats, slightly reduced body weight, clinical symptoms (poor condition, rough haircoat, red vaginal discharge) and mortality were observed in more than half of pregnant females orally administered 80 mg/kg/day R89674 for a few days followed by dosing with 40 mg/kg/day. The surviving animals and all animals in the lower dosage groups demonstrated normal litter parameters including caesarian section data. Also, no adverse embryo-fetal development or teratogenic effects occurred in any of the R89674-dose groups. Similarly, R89674 administered by oral gavage at doses of 10, 40, and 80 mg/kg/day to female Albino rabbits once daily from Day 6 to Day 18 of pregnancy did not alter any litter parameters in the mother rabbits, and did not produce any teratogenic effects in the offspring. In this study, no mortality, but a slight maternal toxicity was noted for the 40 and/or 80 mg/kg/day doses in the form of reduced maternal weight gain and food consumption and decreased serum chloride and increased serum blood urea nitrogen, creatinine, and potassium.

In the Segment II study in rats, none of the pregnant rats died in the 20 mg/kg/day dose group, but 15/24 pregnant female rats experienced R89674-related mortality when dosed with 80 mg/kg/day R89674 for a few days followed by dosing with 40 mg/kg/day through Pregnancy Day 16. By comparison, non-pregnant female rats experienced some mortality when dosed for nine days with 80 mg/kg/day R89674, but ceased dying after the dosing was stopped for four days, then resumed at 40 followed by 60 mg/kg/day in the 6-month oral repeated-dose toxicology study. In the Segment I rat study, some female rats died when initially dosed with 60 mg/kg/day R89674, and ultimately 20/24 of the female rats died in this group before mating even after the dose

was lowered to 40 mg/kg/day, and dosing was stopped for some animals. These results suggest that pregnant rats are not substantially more sensitive than non-pregnant female rats to the toxic effects of orally administered R89674. In the Segment II study in rabbits, none of the pregnant rabbits receiving up to 80 mg/kg/day R89674 experienced mortality suggesting a greater sensitivity in rats.

In a developmental and perinatal/postnatal reproduction study in Crl:CD(SD) rats, the maternal NOAEL for R89674 was considered to be 20 mg/kg/day as the 30 mg/kg/day dose produced reductions in body weight gain during the gestation period. The reproductive NOEL in dams was 30 mg/kg/day and there were no adverse effects on reproduction in the F_0 generation. The NOAEL for offspring viability and growth was 5 mg/kg/day because pup body weights were reduced at 20 and 30 mg/kg/day.
These Segment I, II, and III studies provide satisfactory information regarding the reproductive and developmental toxicity of R89674, and indicate that daily topical ocular administration of 0.25% R89674 has a low potential to limit male or female fertility, cause fetal toxicity, or produce teratogenicity.

While the batch specification limits for solvent impurities contained in the R89674 drug substance all fell within the limits established in ICH guidance “Guidance for Industry Q3C Impurities: Residual Solvents”, several organic impurites were tested in impurity qualification studies according to the ICH guidance: “Guidance for Industry ICH 3A: Impurities in New Drug Substances.” The tested impurities included (b) (4)
These isolated impurities or crude R89674 containing higher levels of impurities were combined with neat R89674 drug substance in order to test the neat drug substance with a higher level of impurities. Test substances composed of R89674 spiked with 0.25-0.5% concentrations of (b) (4), or crude R89674 or the combination of 0.26% (b) (4) and 0.52% (b) (4) were evaluated for genetic toxicity. Test substances were examined in valid Ames’ tests and *in vitro* chromosomal aberration tests with uniformly negative results for mutagenicity or chromosomal damage. In addition, 0.5% crude R89674 enriched with 0.5% of (b) (4) degradation product was tested in a 14-day repeated-dose ocular toxicity study in rabbits and 0.003% (b) (4) and (b) (4) were evaluated in a similar study. In both studies, the impurities did not produce significant ocular or systemic toxicity.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22134	ORIG-1	VISTAKON PHARMACEUTICALS LLC	(b) (4) OPHTHALMIC SOLUTION

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES S WILD
06/02/2010

WENDELYN J SCHMIDT
06/02/2010
I concur with the findings of the reviewer that the pharmacology and toxicology data is complete, and that the NDA can be approved from the pharmacology/toxicology perspective.

Comments on N22134

From: Abigail Jacobs, AD

Date: May 6, 2010

NDA 22134 (b) (4) ophthalmic solution alcaftadine

1. There are no pharm/tox issues with this NDA.

2. I have made some editorial suggestions for the review. I have discussed these with the reviewer and he will address them as appropriate.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22134	ORIG-1	VISTAKON PHARMACEUTICALS LLC	ALCAFTADINE OPHTHALMIC SOLUTION O.25%

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/s/

ABIGAIL ABBY C C JACOBS
07/16/2010