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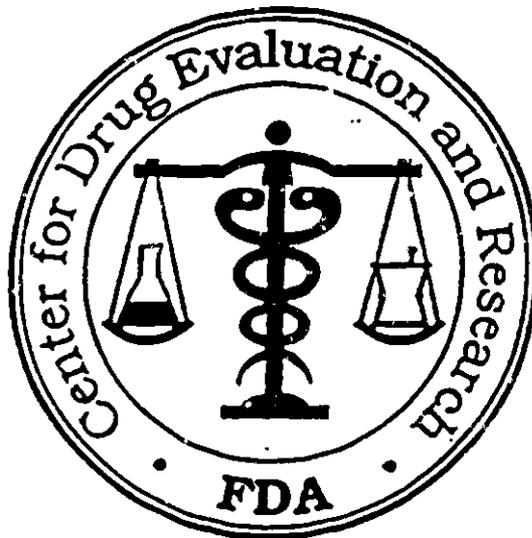
1 OF 3

20629

NDA 20-629

**DENAVIR™
(penciclovir cream), 1%**

Treatment of herpes labialis



DIVISION OF ANTIVIRAL DRUG PRODUCTS

CSO: JOHN MAHONEY

301-827-2335

NDA 20-629

SEP 24 1996

SmithKline Beecham
Attention: Edward M. Yuhas, Ph.D.
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101

Dear Dr. Yuhas:

Please refer to your October 16, 1995 new drug application submitted under section 505 (b) of the Federal Food, Drug, and Cosmetic Act for Denavir™ (penciclovir cream), 1%.

We acknowledge receipt of your amendments dated:

January 16, 1996	June 12, 1996 (2)	July 2, 1996
April 19, 1996	June 13, 1996	July 23, 1996
April 29, 1996	June 14, 1996 (2)	August 1, 1996
May 6, 1996	June 26, 1996	August 8, 1996
May 17, 1996	June 27, 1996	September 9, 1996
May 28, 1996	July 1, 1996	September 13, 1996

This new drug application provides for the treatment of recurrent herpes labialis in adults.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the September 9, 1996 draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the September 9, 1996 draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product miss branded and an unapproved new drug.

Please submit fifteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-629. Approval of this FPL is not required before it is used.

We also acknowledge your phase 4 commitment included in your September 13, 1996 letter.

Should additional information relating to the safety or effectiveness of the drug becomes available, revision of the FPL may be required.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

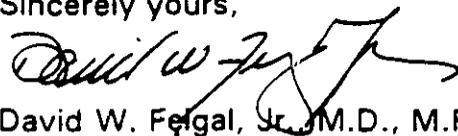
Please submit one market package of the drug when available.

Under section 736 (a)(1)(B)(ii) of the Prescription Drug User Fee Act of 1992, this letter triggers the remaining 50% of the fee assessed for this application. You will receive an invoice for the amount due within the next month. Payment will be due within 30 days of the date of the invoice.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

Should you have any questions, please contact Mr. John Mahoney, Consumer Safety Officer at (301) 827-2335.

Sincerely yours,

 9-25-96

David W. Feigal, Jr., M.D., M.P.H.
Acting Director
Office of Drug Evaluation IV
Center for Drug Evaluation and Research

— FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE
ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE
PUBLIC.

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-629 Trade (generic) names Denavir (penciclovir cream), 1%

Check any of the following that apply and explain as necessary, on the next page:

- 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.
- 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(C) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.
 - a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.
 - b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate).
- 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).
 - a. The applicant has committed to doing such studies as will be required.
 - (1) Studies are ongoing.
 - (2) Protocols have been submitted and approved.
 - (3) Protocols have been submitted and are under review.
 - (4) If no protocol has been submitted, on the next page explain the status of discussions.
 - b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.
- 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.
- 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: _____



Signature of Preparer

9/11/96

Date

cc: Orig NDA
HFD-530/Div File
NDA Action Package

DEBARMENT CERTIFICATION

Pursuant to section 306(K)(1) of the Federal Food, Drug and Cosmetic Act, the applicant certifies that, to the best of its knowledge and belief, the applicant did not and will not use in any capacity, in connection with this application, the services of any person listed pursuant to section 306(e) as debarred under subsections 306(a) or (b) of the Act.

000008

Division of Antiviral Drug Products
Food and Drug Administration
Rockville MD 20857

Date: September 18, 1996

To: David W. Feigal, M.D., M.P.H.
Acting Director, Office of Drug Evaluation IV

From: Donna J. Freeman, M.D. *DSF 9/18/96*
Acting Director, Division of Antiviral Drug Products

Subject: NDA 20-269
1% Topical Penciclovir Cream (*Denavir*)
SmithKline Beecham Pharmaceuticals

This memorandum accompanies the reviews and related materials for NDA 20-269 for 1% topical penciclovir cream, Denavir, for the treatment of herpes labialis in immunocompetent adults. I concur with the consensus of the reviewers that this NDA should be approved. This approval will represent the first product approved for the treatment of herpes labialis in the United States.

The IND for topical penciclovir was filed in 1993, and the major clinical trials were undertaken shortly thereafter. The NDA application was submitted on October 16, 1995, and was considered acceptable for filing following a review team meeting. The applicant and the FDA had worked together prior to this application to agree on the nature and methods for analysis that would be most appropriate.

Herpes labialis in the immunocompetent adult is a self-limited disease that for most patients is inconvenient but not disabling. Some patients have very frequent lesions and severe recurrences, and the availability of therapy for them may provide clear clinical benefit. As the clinical and statistical reviewers have noted, the demonstration of a treatment effect in these fairly large placebo-controlled studies has been achieved with statistical significance, but the effect is modest in the overall population. There is a suggestion that the benefit may be more noticeable for those patients with lesions of longer duration, as has been discussed in the statistical and medical reviews.

Please see the Team Leader's memorandum for comments on the analyses and the way the treatment effect has been described in the labeling. It should also be noted that since that memorandum was written there has been further discussion of the section of the label with regard to use in pregnancy and in nursing mothers. While the wording used may not be particularly clinically relevant in the topical treatment of herpes labialis, the standard cautions have been retained. The information is relevant to the data derived from systemic exposure to penciclovir and the related prodrug, famciclovir, and the inclusion of this wording is consistent with the labeling for other topical drug formulations, even in instances where topical dosing does not lead to any systemic drug exposure.

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research

DATE: August 27, 1996

FROM: Rachel E. Behrman, M.D. M.P.H. *RD 8/27/96*
Team Leader, Division of Antiviral Drug Products

SUBJECT: NDA 20-629

TO: Donna J. Freeman, M.D.
Acting Director, Division of Antiviral Drug Products

The review team for NDA 20-629 is pleased to recommend that penciclovir cream be approved for the treatment of herpes labialis in adults. This recommendation is based on the results from two adequate and well controlled studies that demonstrated a modest, but robust, clinical benefit associated with penciclovir therapy. Patients randomized to active drug healed approximately one half day more quickly than patients randomized to placebo. Applications site reactions were infrequent.

There are no outstanding issues, either preclinical or clinical, that impact on the proposed approval action. However, there are several points that warrant comment.

Primary efficacy analysis

The primary efficacy analysis was based on duration of critical lesions stage. The discussions that preceded this decision are detailed in the "Regulatory History" section of the medical officer review. It is important to note that an equally valid analysis plan would have included some measure of aborted lesions (that is lesions that never progress to the papule stage).

Labeling comments

In the clinical trials section, mean duration of time to healing is presented. This is not intended to be seen as a precedent; it simply appeared to be the most accurate representation of the data.

In the pregnancy comments, the common statement about risk benefit assessment has been omitted. This is because when considering a topical agent, with little or no systemic absorption intended to treat a self limited disease of little consequence, the typical statement was neither appropriate nor informative. Similarly, there is no suggestion that a nursing mother consider discontinuing nursing.

Acknowledgment

The review team (Dr. G. Chikami, Dr. P. Flyer, Dr. B. Davit, Dr. G. Sherman, Dr. M. Seggal, Dr. D. Morse and Mr. J. Mahoney) are to be congratulated on completing this review in a timely manner and on a smooth and productive NDA review process.

MEDICAL REVIEW OF NDA 20-629

Date Submitted: October 16, 1995
Date Received: October 16, 1995
Date Assigned: October 23, 1995
Draft MOR Completed: August 26, 1996
MOR Completed: September 3, 1996

Applicant: SmithKline Beecham Pharmaceuticals
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101-7929

Drug: Chemical: 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine
Generic: penciclovir
Trade: Denavir™

Formulation: 1% penciclovir in Cetomacrogol cream base

Indication: Treatment of recurrent herpes labialis in adults

Related NDA/INDs: NDA 20-363;

Abstract

SmithKline Beecham has submitted a New Drug Application for Denavir™ (penciclovir cream, 1%) for the treatment of recurrent herpes labialis in adults. The core of the clinical data to support this NDA consists of two phase III, randomized, placebo-controlled, double-blind clinical studies of 1% penciclovir cream for the treatment of recurrent herpes labialis in immunocompetent adults. In addition two dermal irritancy studies that were conducted with 5% penciclovir cream, using the formulation proposed for marketing, were included to provide additional data to support the safety of the drug for the proposed use. The two phase III clinical trials demonstrated that 1% penciclovir cream was associated with a statistically significant but modest decrease in the duration of herpes labialis lesions (approximately one half day) and a similar decrease in the duration of lesion pain. Results from the phase III studies and the dermal irritancy studies demonstrated that topical application of 1% penciclovir is safe for the proposed use. Based on these results, the reviewer has concluded that the application is approvable.

Background

Recurrent herpes simplex labialis is a common disease that is characterized by repeated attacks of vesicular eruptions most commonly on the lips and perioral skin. It is primarily due to HSV type 1 (HSV-1). HSV type 2 (HSV-2) may occasionally cause primary infection, but recurrent orolabial disease due to HSV-2 is rare. Transmission of HSV-1 is by direct mucocutaneous contact

with infected secretions and the usual route of entry is via penetration of the oral mucosa. The primary infection of the oral cavity may be either asymptomatic or manifested by gingivostomatitis. Following primary infection, HSV-1 is thought to ascend through sensory nerve axons and establish chronic, latent infection in sensory ganglia, including those of the trigeminal, cervical and vagus nerves.

Recurrent herpes labialis most likely represents the consequence of reactivation of latent virus in the sensory ganglia, followed by migration of the virions via the nerve fibers to the skin. Reinfection of the epidermis occurs rapidly, most likely by a process of multifocal inoculation from a single infected neuron, with maturation to fully developed vesicular herpes labialis lesions occurring within 8 hours of onset of the papular stage. It is hypothesized that only 2-3 cycles of virus replication are required at each microfocus before coalescence of the foci leads to a clinically apparent lesion. As the infection develops and spreads to adjacent cells, immune factors such as the neutralizing activity of antibody and complement, lysis of infected keratinocytes and suppression of viral replication by lymphocyte-derived interferon, come into play. The variable outcomes of the disease may be influenced by the quantity of virus delivered to the skin, the opportunity for viral replication and the intensity of the immune response.

The frequency of recurrent outbreak is extremely variable, ranging from rare episodes every five to 10 years, to monthly or more frequent outbreaks among a small proportion of individuals. The severity of recurrences is also extremely variable, ranging from prodromal symptoms on the lips without lesion formation to 'classical' herpetic lesions with vesicle, ulcer and crust formation requiring five to 15 days for resolution. In approximately 25 percent of recurrences "classical" lesions do not develop (aborted episodes), with about half of these being limited to prodromal symptoms with or without erythema ("false prodromes"), and the other half progressing to the papule stage before resolving. In clinical studies, prodromal symptoms of pain, burning, or itching at the site of the subsequent eruption preceded herpes labialis in 46% to 60% of lesions. Patients with prodromes have been reported to have more severe lesions than patients who do not experience prodromes.

Clinical trials for the treatment of recurrent herpes labialis have been undertaken with a variety of compounds. In two small trials of 49 and 30 patients respectively, some benefits were reported for a 5% acyclovir modified aqueous cream formulation. In these trials, analyses of per protocol populations showed that acyclovir treatment was associated with reductions in time to complete healing of one to two days when compared to placebo. However, in two further placebo-controlled trials, no statistically significant benefit attributable to acyclovir cream could be detected. Oral acyclovir has also demonstrated some activity in the treatment of herpes labialis. Other topical agents have not been so extensively evaluated. Idoxuridine (IDU) in a topical formulation with dimethyl sulfoxide (DMSO) was shown in one trial to reduce the time to healing and the mean duration of pain, with the best results being obtained in patients who began treatment in the prodromal or erythema lesion stages. Similar results have been reported for topical foscarnet sodium.

In the United States and Canada there are no approved treatments available for herpes labialis in immunocompetent patients. Topical 5% acyclovir ointment is approved for the treatment of limited, non-life threatening mucocutaneous herpes simplex infection in immunocompromised patients. In Europe, 5% acyclovir cream is approved for the treatment of recurrent herpes labialis in immunocompetent patients.

Regulatory History

The clinical development of the topical formulation of penciclovir was initiated in February 1993 with the submission of IND. The clinical program has consisted of the following studies: 1) a study to assess the percutaneous absorption of topical penciclovir through abraded skin in healthy volunteers; 2, five studies to assess the irritancy and sensitization potential of topical penciclovir in healthy volunteers; and 3) two phase III double-blind, placebo controlled trials to determine the safety and efficacy of 1% penciclovir cream for the treatment of recurrent herpes labialis in immunocompetent patients.

On January 17, 1995 a pre-NDA meeting was held to review SmithKline Beecham's proposed package for an NDA for topical penciclovir. Several issues were raised regarding the definition of primary efficacy endpoint in the phase 3 clinical trials and the proposed plan for analysis. The proposed primary endpoint and the proposed analysis plan were not acceptable. The primary endpoint of subjects who had lost crust by day eight would have excluded subjects whose disease had resolved prior to crust formation. In addition, the proposed definition of the intent-to-treat population was not acceptable because it would excluded a large number of subjects from the analysis. It was stated that the agency prefers all subjects randomized included in the intent-to-treat analysis. In a telephone conference on February 21, 1995, this position was clarified. It was stated that for the intent-to-treat analysis the agency expects that all subjects with the condition of interest would be included in the analysis. This would include subjects who had recurrences but did not initiate therapy. These subjects would not have follow-up information on lesion duration. To include these subjects in the analyses, it was agreed that the following procedures would be used:

- a) assign all a duration of zero;
- b) assign all the median value of the primary efficacy parameter;
- c) assign all the maximum value of the primary efficacy parameter plus one day.

The primary endpoint was further clarified in a teleconference on April 27, 1995. During that teleconference representatives of the applicant and the division agreed that the primary endpoint would be a duration analysis and that the time to event analysis will be considered secondary.

Related Regulatory Actions

Topical penciclovir 1% cream, under the Trademark Vectavir™, received approved for marketing for the treatment of herpes labialis in the United Kingdom on February 28, 1996 and in Denmark on March 28, 1996.

Famciclovir, the oral prodrug of penciclovir, was approved for marketing in the United States for the treatment of herpes zoster at a dose of 500 mg TID for 7 days on June 29, 1994. It was also approved for the treatment of recurrent genital herpes at a dose of 125 mg BID for 5 days on September 29, 1995.

CLINICAL STUDIES

The applicant conducted two phase III studies to demonstrate the safety and effectiveness of 1% topical penciclovir for the treatment of herpes labialis infection in immunocompetent patients. These studies are summarized in Table 1. Both of the trials were multicenter, vehicle-controlled, double-blind studies in immunocompetent adults with recurrent herpes labialis. Eligible subjects were randomized at the time of enrollment to either 1% penciclovir cream or vehicle control. Subjects were instructed to initiate therapy with study medication within one hour of noticing the first sign or symptom of a recurrence and to continue treatment every 2 hours while awake. Study medication was to be applied for 4 days. The subjects were to report to the clinic for assessments within 24 hours of initiating study medication. Assessments of the primary endpoint (duration of critical lesion stages) were to be made by the investigators for at least 4 days or until the loss of crust. The patients also recorded self-assessments of the primary and secondary endpoints 4 times daily until lesions had completely healed. A more detailed description of the study protocols is provided in Appendix I.

Table 1 - Phase III Clinical Trials				
	Study 024		Study 025	
Dates	4/21/93-7/8/94		3/18/93-3/14/94	
Location	United States - 31 centers		Europe - 34 centers Canada - 8 centers Singapore - 1 center	
Design	randomized, double-blind, patient-initiated comparison of penciclovir to placebo for treatment of recurrent herpes labialis			
Formulation	1% penciclovir Cetomacrogol cream placebo - Cetomacrogol cream vehicle			
Regimen	Study drug patient-initiated within one hour of noticing the first sign or symptom of a herpes labialis recurrence and continue every 2 hours (while awake) for 4 days			
Follow-up assessments	Clinic visit for assessment of lesions and symptoms within 24 hours of initiating treatment; daily clinic visits for at least 4 days or until loss of crust; patient self-assessments 4 times daily until lesions had completely healed, following loss of crust, QOD visits until skin was assessed as normal; daily viral cultures obtained during vesicle/pustule and ulcer stages			
	Penciclovir	Placebo	Penciclovir	Placebo
# Subjects randomized	1103	1106	1177	1187

Analysis Plan

After discussions between the applicant and the division (see Regulatory History) it was

agreed that the primary endpoint would be the duration of critical stage lesions based on the investigator assessments. The duration was calculated by subtracting the date at which the condition was first reported (at or after the start of study medication) from the date at which the condition was last reported, then adding one day. Subjects who never reported the condition (had not reported a papule, vesicle, ulcer/soft crust or hard crust) and had seen the investigator on at least four occasions were assigned a duration of zero.

For the intent-to-treat analysis it was agreed that all subjects with the condition of interest would be included in the analysis. This would include subjects who had recurrences but did not initiate therapy, did not return to clinic or were lost to follow-up, or no efficacy assessments were performed. These subjects would not have follow-up information on lesion duration. To include subjects without follow-up data in the analyses, the following procedures were used:

- a) Q1 - assign all a duration equal to the first quartile duration derived from all subjects with a known duration;
- b) Median - assign all a duration equal to the median duration derived from all subjects with a known duration;
- c) Q3 - assign all a duration equal to the third quartile duration derived from all subjects with a known duration.

Subjects with incomplete data (start date, but no end date for lesions) were assigned the maximum observed duration plus one day.

The duration of the critical was analyzed by the permutation test, stratifying by investigational center. The analyses were carried out using the statistical package StatXact Turbo V2.11. Due to large number of subjects included in the analysis, a monte-carlo sample was used to derive an estimate of the exact p-value.

RESULTS - EFFICACY

Subject Disposition

The disposition of the subjects who were enrolled in the clinical studies is shown in Table 2. 1573 (71%) of the subjects enrolled in study 024 and 1484 (63%) enrolled in study 025 had a recurrence and were known to have initiated treatment with study medication. In study 024, there were 76 subjects in the penciclovir group and 71 subjects in the placebo group who had known recurrences of herpes labialis but either did not initiate treatment with study medication or did not return for clinic visits. The numbers of subjects who had a recurrence in study 025 but did not initiate study medication was higher, 117 in the penciclovir group and 108 in the placebo group.

	Table 2 - Subject Disposition			
	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Randomized	1103	1106	1177	1187
Initiated treatment	782	791	734	750
Completed treatment	732	734	684	695
Withdrew	50	57	50	55
Did not initiate treatment	321	315	443	437
No recurrence	125	126	283	266
Lost to follow-up	84	84	38	44
Recurrence, but did not initiate	71	66	117	108
Other	36	34	5	19
Recurrence, but did not return	5	5		

Study populations analyzed

The intent-to-treat population was defined as all patients randomized except those who did not apply study medication were known not to have had a recurrence. The second population was the known treated population which included all patients who were known to have applied study medication. The table below shows the categories of subjects included in these populations. In both studies, subjects who were known to have initiated study medication comprised approximately 80% of the intent-to-treat population. This was similar in each of the treatment arms. The largest two categories of subjects who did not initiate therapy in study 024 were subjects lost to follow-up and subjects who had a recurrence but did not initiate therapy. In study 025, the largest category was subjects who had a recurrence but did not initiate therapy.

	Table 3 - Populations Analyzed			
	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Intent-to-treat population	978	980	894	921
Known treated population (Initiated treatment)	782	791	734	750
Did not initiate treatment (included in intent-to-treat population)	196	189	160	171
Recurrence - did not initiate	71	66	117	108
Recurrence - did not return	5	5	5	19
Lost to follow-up	84	84	38	44
Other	36	34		

Completeness of Follow-up

Follow-up data on lesion duration were obtained only on those subjects who initiated study medication and who returned for follow-up clinic visits. This group comprised 76% to 78% of the subjects in the studies. Subjects who had a recurrence but did not initiate treatment with study medication, had a recurrence but did not return for follow-up clinic visits, were lost to follow-up or

did not initiate study medication for other reasons did not have any follow-up data collected. This group comprised 22% to 24% of the intent-to-treat population in both studies (Table 4). All of these categories were similar in both of the treatment arms in each study.

	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Subjects with complete data	744	743	701	710
Lesion start date but no end date Duration assigned max+1=19days	13	25	14	14
Had <4 visits, did not have critical lesion stages; incomplete data Assumptions applied*	25	23	19	26
# (%) with incomplete data	38 (5%)	48 (6%)	33 (4%)	40 (5%)
Applicant's known treated population	782	791	734	750
No data - Assumptions applied* (Subjects who did not initiate therapy, excluding those that did not have a recurrence - see Table 1)	196	189	160	171
# (%) with incomplete or missing data	234 (24%)	237 (24%)	193 (22%)	211 (23%)
FDA defined intent-to-treat population	978	980	894	921

Comparability of the Treatment Groups at Baseline

For both studies, the subjects enrolled population consisted of primarily white females with a mean age of approximately 40 years. The studies and the treatment groups in each study were similar in regard to these characteristics (Table 5).

	Study 024				Study 025			
	Intent-to-treat		Known treated		Intent-to-treat		Known treated	
	Pcv n=978	Placebo n=980	Pcv n=782	Placebo n=791	Pcv n=894	Placebo n=921	Pcv n=734	Placebo n=750
Gender								
Male	27%	27%	25%	30%	26%	22%	27%	22%
Female	73%	71%	75%	70%	74%	78%	73%	78%
Age (yrs)								
Mean	39	40	40	40	38	37	39	37
Range	18-78	18-82	18-78	18-82	18-84	18-74	18-84	18-71
Race								
White	95.7%	95.6%	96.0%	96.0%	96.9%	97.0%	97.0%	96.9%
Black	0.7%	0.9%	0.5%	0.8%	0.2%	0.2%	0	0.3%
Other	3.6%	3.5%	3.5%	3.3%	2.9%	2.8%	3.0%	2.8%

Table 6 shows the baseline disease characteristics of population for both studies. The populations across studies and the treatment groups in each study were similar in regard to disease history.

Table 6 - Baseline Disease Characteristics (Intent-to-treat population)				
	Study 024		Study 025	
	Penciclovir n=978	Placebo n=980	Penciclovir n=894	Placebo n=521
Duration of herpes labialis (yrs)				
Mean	23.1	22.9	19.2	19.6
Range	1-70	1-72	0-60	1-60
# episodes during previous 12 months				
Mean	5.6	5.6	5.7	5.6
Range	1-36	0-36	1-50	0-50
# episodes per year				
Mean	6.2	6.1	6.2	6.1
Range	2-36	2-36	1-99	2-50
# subjects who had prodromes				
Always	641 (65.5%)	661 (67.5%)	594 (66.4%)	637 (69.2%)
Most of the time	298 (30.5%)	294 (30.0%)	273 (30.5%)	259 (28.1%)
Half of the time	39 (4.0%)	24 (2.5%)	26 (2.9%)	24 (2.6%)
Occasionally			0	1 (0.1%)
Never			1 (0.1%)	0
# subjects who had classical lesions				
Always	593 (60.6%)	632 (64.6%)	593 (66.3%)	624 (67.8%)
Most of the time	352 (36.0%)	314 (32.1%)	275 (30.8%)	274 (29.8%)
Half of the time	33 (3.4%)	33 (3.4%)	25 (2.8%)	23 (2.5%)
Occasionally			1 (0.1%)	0
# subjects who had false prodromes				
Never	631 (64.5%)	617 (63.0%)	569 (63.3%)	587 (63.7%)
Occasionally	354 (35.3%)	359 (36.7%)	321 (35.9%)	324 (35.2%)
Always	2 (0.2%)	1 (0.1%)	2 (0.2%)	1 (0.1%)
Half of the time	0	2 (0.2%)	1 (0.1%)	6 (0.7%)
Most of the time			1 (0.1%)	3 (0.3%)

Table 7 shows the patient-assessed lesion stage at the time of initiation of study medication. In both studies approximately 50% to 55% of the subjects initiated study medication at the erythema stage or earlier. An additional 26% to 34% of the subjects initiated study medication at the papule stage. The disease stage at the time of initiation of study medication was similar across the treatment groups in both studies.

	Study 024		Study 025	
	Penciclovir n=782	Placebo n=791	Penciclovir n=734	Placebo n=750
Normal skin	6 (0.8%)	3 (0.4%)	4 (0.5%)	4 (0.5%)
Prodrome	274 (35.0%)	281 (35.5%)	251 (34.2%)	257 (34.3%)
Erythema	137 (17.5%)	114 (14.4%)	161 (21.9%)	170 (22.7%)
Papule	253 (32.4%)	272 (34.4%)	197 (26.8%)	195 (26.0%)
Vesicle/pustule	91 (11.6%)	95 (12.0%)	104 (14.2%)	105 (14.0%)
Ulcer/soft crust	6 (0.8%)	8 (1.0%)	1 (0.1%)	5 (0.7%)
Hard crust	0	1 (0.1%)	2 (0.3%)	0
Unknown	15 (1.9%)	17 (2.1%)	14 (1.9%)	14 (1.9%)

A comparison of the baseline characteristics of the all subjects who had a recurrence (intent-to-treat) and those subjects who were known to have initiated study medication (known treated) shows that these groups were similar in regard to these characteristics. This suggests that the subjects who had a recurrence and did not initiate treatment with study medication were not different from the subjects who had a recurrence and did initiate treatment. This is supported by information on the reasons that subjects did not initiate study medication, which was collected in study 024 and recorded in monthly telephone logs (Table 8). The reasons for not initiating study medication are shown in the table below. The reasons were similar for both treatment groups and did not appear to be related to the disease process.

	Penciclovir	Placebo	Missing
Unable to go to clinic	81	81	2
Too late in the day	42	23	
Past prodrome	14	25	
Inappropriate location	9	19	
Medication not available	6	4	
Other	2	2	

Treatment Compliance

Dosing information was collected in the patient diaries. The mean number of applications were similar across the treatment groups in both studies (Table 9).

Table 9 - Number of Applications per Day (Mean)				
	Study 024		Study 025	
	Penciclovir n=756	Placebo n=763	Penciclovir n=734	Placebo n=750
Day 1	6.3	6.3	5.8	5.8
Day 2	9.5	9.6	8.8	8.6
Day 3	8.9	8.9	8.2	8.2
Day 4	8.7	8.7	8.0	8.1
Day 5	4.0	4.1	4.6	4.8
Overall (Days 1 to 4)	8.1	8.4	7.7	7.7

Duration of Critical Stage Lesions

The results of the applicant's analyses for the intent-to-treat population in studies 024 and 025 are shown in Table 10. The results from the analyses using each of the different assumptions for the inclusion of subjects with incomplete or missing data are shown.

Table 10 - Duration of Critical Lesion Stages (Intent-to-treat)					
		Study 024		Study 025	
		Penciclovir n=978	Placebo n=980	Penciclovir n=894	Placebo n=921
Assumption: Q1	Mean (days)	4.2	4.7	4.2	4.5
	Median	3	4	3	3
	p value*	<0.0001		0.041	
Assumption: Median	Mean (days)	4.4	4.9	4.4	4.8
	Median	4	4	4	4
	p value*	<0.0001		0.0325	
Assumption Q3	Mean (days)	4.9	5.3	4.8	5.2
	Median	5	6	5	6
	p value*	<0.0001		0.0220	

*p value from the permutation test.

For both studies, there was a statistically significant difference between the two treatment groups in each of the analyses that the applicant performed. The difference in the mean durations of the critical stage lesions ranged from 0.3 to 0.5 days. The difference in the median durations ranged from 0 to 1 day.

Comment: The imbalance in the number of subjects for whom there was incomplete data and were assigned a duration of 19 days in study 024 would bias the analysis in favor of penciclovir. In this case, the applicant's analyses are likely to overestimate the treatment effect. The other categories of subjects for whom lesion durations were assumed were balanced across the treatment arms for both studies. This issue is addressed in the FDA

analyses (see below).

The analyses performed for the know treated population is shown in Table 11. In the first row, only those subjects for whom complete data were available and no assumptions were made in regard to lesion duration were included. For subjects with partial follow-up information, assumptions were applied as for the intent-to-treat population.

		Table 11 - Duration of Critical Lesion Stages (Known treated)			
		Study 024		Study 025	
		Penciclovir n=757	Placebo n=768	Penciclovir n=715	Placebo n=724
Assumption: None	Mean (days)	4.5	5.2	4.5	5.0
	Median	4	4	4	4
	p value*	<0.0001		0.0295	
		n=782	n=791	n=734	n=750
Assumption: Q1	Mean (days)	4.5	5.1	4.5	4.9
	Median	4	4	4	4
	p value*	<0.0001		0.0300	
Assumption: Median	Mean (days)	4.5	5.1	4.5	4.9
	Median	4	4	4	4
	p value*	0.0005		0.0335	
Assumption: Q3	Mean (days)	4.6	5.2	4.6	5.0
	Median	4	5	4	4
	p value*	<0.0001		0.0305	

*p value from permutation test

Similar to the analyses performed for the intent-to-treat population, there was a statistically significant difference in the duration of lesions between the penciclovir and the placebo groups in both of the studies. The observed difference in the mean duration ranged from 0.4 to 0.7 days. The difference in the median duration ranged from 0 to 1 day.

FDA Analysis of the Duration of Critical Lesion Stages

The distribution of lesion duration for studies 024 and 025 are shown in the tables on page 16 and page 17 of the FDA Statistical Review and Evaluation (Dr. Paul Flyer). As shown in the graphs, the distribution of the lesion durations for the subjects randomized to the penciclovir groups in both studies is shifted to the left, indicating that subjects randomized to treatment with topical penciclovir had shorter lesion durations. The differences in the distributions are more apparent at the longer times. The differences are less apparent around days 3 to 5. This distribution suggests that subjects who had longer healing times were more likely to have benefitted from treatment with 1% penciclovir cream.

The FDA's approach to the estimation of the treatment effect in the presence of missing data was to set the difference between the treatment arms for subjects with missing data to zero and then to combine the difference with the estimated difference for subjects with complete data using weights proportional to the number of subjects in each subgroup (see Statistical Review and Evaluation for this NDA). The results from this approach are likely to represent a lower bound on the estimated treatment effect. The results are shown in the Table 12. Both the analysis of the intent-to-treat population and the know treated population are presented in the table.

	Table 12 - FDA Analyses of Lesion Duration			
	Study 024		Study 025	
	Difference (placebo-penciclovir)	95% CI	Difference (placebo-penciclovir)	95% CI
Know treated population*	0.39	0.17, 0.61	0.36	0.08, 0.64
Intent-to-treat population	0.42	0.18, 0.66	0.32	0.08, 0.56

*Includes subjects lost to follow-up.

For both studies, the results of these analyses showed a statistically significant difference in the mean duration of critical stage lesions for the analysis of the intent-to-treat population and the know treated population. For study 024 this difference was 0.42 days with a 95% CI of 0.18 to 0.66 and for study 025 the difference was 0.32 with a 95% CI of 0.08 to 0.56. These differences are similar to the results reported by the applicant in their analyses where the difference in the mean durations for the intent-to-treat population ranged from 0.3 to 0.5 days.

Lesion Pain

Assessment of lesion pain was a secondary endpoint in the protocol. The subjects recorded their assessment of pain on a diary card. Subjects were to record their evaluation of lesion and lesion pain 4 times a day at approximately six hour intervals. The actual time of the assessment was to be recorded with the scores. The following rating scale was used:

- 0 No pain
- 1 The patient is vaguely aware of the cold sore. The affected area is tender when touched, but does not interfere with daily activities.
- 2 The subject is aware of the cold sore at all times.
- 3 The discomfort interferes with daily activities (i.e., eating, drinking, talking). There is enough discomfort to cause sleep disturbance. The discomfort may seem to be an unbearable irritation.

The duration of pain was measured from the first report of lesion pain by the subject at or after initiation of therapy to the last report of pain by the subject. Not all subjects were included in the analyses of the duration of lesion pain. Only subjects who initiated therapy and who recorded at least one pain assessment were included. Subjects who recorded "no pain" throughout the study were assigned a duration of zero. Table 13 shows the number of subjects in each of the studies who were included in the analysis of the duration of pain, broken down by treatment group. The number

subjects who recorded no pain was similar across the treatment groups.

	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Subjects who initiated therapy	782	791	734	750
Subjects included in analysis of duration of lesion pain	766	774	720	736
Subjects who recorded no pain	39	38	52	54

At the time of the initiation of study medication the subjects in the treatment groups in both studies were comparable in regard to the severity of lesion pain. These results are shown in Table 14. Most subjects had mild lesion pain at the time they initiated study medication.

		0	1	2	3	Missing
Study 024	Penciclovir	167	454	130	14	338
	Placebo	160	472	129	13	332
Study 025	Penciclovir	180	407	126	6	458
	Placebo	176	429	120	11	451

In both studies subjects were instructed to initiate therapy within one hour of noticing signs or symptoms of a recurrence and to record the assessment of lesion pain at the time of initiating therapy. Subjects were not specifically instructed to record signs or symptoms prior to initiating study medication, therefore there is little information on the time between when the subject first noticed signs or symptoms of a recurrence and when they actually started study medication. Some subjects did record assessment of lesion pain prior to initiating study medication. These data are shown in the following Table 15. While it does not appear to be a difference in the time between when the subjects first noticed symptoms and when study medication was initiated, there were too few subjects who recorded this information to draw any conclusions.

	Study 024		Study 025	
	Penciclovir n=82	Placebo n=96	Penciclovir n=149	Placebo n=172
Mean (days)	0.08	0.09	0.07	0.07
Median (days)	0.03	0.03	0.02	0.02
Range	0.0-0.6	0.0-0.6	0.0-0.7	0.0-0.8

The duration of lesion pain is shown in Table 16. In both studies there was approximately a one half day difference in the duration of lesion pain.

	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Mean duration of lesion pain	4.2 days	4.7 days	3.6 days	4.2 days
Difference	0.5 days		0.6 days	
Median duration of lesion pain	3.0 days	3.6 days	2.3 days	2.9 days
Difference	0.6 days		0.6 days	

Comment: The one half day difference in lesion pain between the treatment groups in both studies is consistent with the one half day difference seen in the duration of the critical stage lesions. The duration of pain is shorter than the lesion duration. This would be expected since pain would be primarily associated with the prodrome, papule, vesicle/pustule, and ulcer/soft crust stages of the lesions and not the hard crust stage, which is included in the duration of the critical stage lesions.

Time to Cessation of Viral Shedding

In both studies, daily samples for viral cultures were to be obtained during the vesicle/pustule and ulcer stages (stages 4 and 5). Viral cultures were performed at either a central laboratory or at a local virology laboratory. The time to cessation of viral shedding was defined as the first assessment at which a negative culture was reported (and a positive culture was not reported at any subsequent assessment) after the initiation of study medication. Only those patients with at least one positive culture at or after study medication were included in the analysis. The results are shown in Table 17.

	Study 024		Study 025	
	Penciclovir n=515	Placebo n=532	Penciclovir n=418	Placebo n=460
Time to cessation (days)	3	3	3	3
p value (log rank)	0.003		0.0002	

In study 024, only 515 of the penciclovir group and 532 of the placebo group had a positive culture. 60% of subjects had a positive culture at their final virology and were censored in the analysis. Similarly in study 025, 418 in the penciclovir group and 460 in the placebo group had a positive culture. 50% of the subjects had a positive culture at their final virology assessment and were censored in the analysis. To address the issue of the large amount of censoring due to the high proportion of subjects who had only a single positive culture, the applicant analyzed the subsets of subjects with at least two cultures taken and subjects with at least 3 cultures taken. In study 024,

47% and 26% respective had a positive culture at the last viral culture taken. In study 025, 39% and 24% of the subjects had a positive culture at their last assessment. The results of these analyses were similar to those conducted on all subjects with a positive culture.

Comment: Because HSV cultures were positive on only a subset of the subjects in both studies and because of the high degree of censoring that resulted from the subjects who had a positive culture at their last evaluation, the estimates of the time of viral shedding in both studies may not provide an accurate estimate of the duration of viral shedding. In the analyses of the subgroups of subjects who had at least two or at least three cultures taken, there was still a substantial percentage of subjects who were censored because of a positive culture at the last assessment. In addition, these groups had fewer subjects. Therefore, these analyses may also not provide a accurate estimate of the duration of viral shedding.

Analyses of Early Versus Late Treatment Initiation

Early treatment was defined as initiation of study medication at the prodrome or erythema stages and late was defined as the papule stage or later. This classification was based on the patient-assessed lesion stage at the time closest to the initiation of study medication. The analyses of lesion duration were based on the investigator-assessed data. The applicant performed analyses applying the same set of assumptions used in the primary analyses for the duration of critical stage lesion: assumption - none; assumption - Q1; assumption - median; assumption Q3. The results from the three analyses were similar, so only the analysis using assumption - none will be presented. These results are shown in Table 18.

Table 18 - Duration of Critical Lesion Stages: Early versus Late								
	Study 024				Study 025			
	Early		Late		Early		Late	
	Pcv n=01	Placebo n=390	Pcv n=347	Placebo n=170	Pcv n=406	Placebo n=420	Pcv n=364	Placebo n=300
Mean (days)	4.4	4.9	4.6	5.3	4.4	4.5	4.6	5.6
Median (days)	4	4	4	5	4	4	4	5
p-value*	0.0205		0.0030		0.8970		0.0020	

*p-values from permutation test stratified by investigator

Compliers Versus Non-Compliers

Compliance with study medication was defined as having at least six doses each day each of the first four days. The known treated subject population was used for these analyses. For the analyses of duration of critical lesion stages, the applicant applied the same assumptions as in the primary analyses of this endpoint for the inclusion of those subjects with incomplete data. Only the analyses in which no assumptions were made will be presented below (Table 19).

Table 19 - Duration of Critical Lesion Stages: Compliant versus Noncompliant								
	Study 024				Study 025			
	Compliant		Non-compliant		Compliant		Non-compliant	
	Pcv n=618	Placebo n=626	Pcv n=139	Placebo n=142	Pcv n=484	Placebo n=464	Pcv n=223	Placebo n=260
Mean (days)	4.4	5.0	4.9	5.6	4.5	5.2	4.6	4.5
Median (days)	4	5	4	4	4	5	3	4
p-value*	<0.0001		0.0430		0.0015		0.930	

*p-values from permutation test stratified by investigator

Comment: The baseline characteristics of the subgroups analyzed in the comparison of early versus late initiators of study medication and subjects who were compliant with application of study medication versus those who were not compliant is show in the tables in Appendix II. The subgroups were similar in regard to demographic characteristics and disease history. The analyses of early versus late initiation of study medication and subjects who applied study medication six or more times a day are based on subgroups that were not prespecified and therefore not accounted for as characteristics in the randomization. The differences observed between these subgroups may have been the result of a factor other than the randomization and therefore, conclusions on the effectiveness of the study medication based on the observed differences may not be valid.

Summary of Efficacy

The two phase III studies submitted by the applicant in support of the NDA are adequate and well controlled. The randomization appears to have resulted in treatment groups that were comparable at baseline. The self-initiation aspect of the design did not appear to introduce bias into the study design as judged by the similarity in baseline characteristic of those subjects who had a recurrence and initiated study medication and those subjects who had a recurrence and did not initiate study medication, and the apparent unrelatedness of reasons given by the subjects for not initiating treatment with study medication and the disease process. Both applicant's and FDA's analysis of the primary endpoint demonstrated a shorter duration of lesions in penciclovir group when compared to the control group. The estimates of the treatment effect ranged from 0.3 to 0.7 days, depending upon the assumptions used and the populations analysed. However, regardless of the assumptions applied or the population analyzed, in both studies the differences were statistically significant. The analysis of duration of pain produced an estimate of difference in duration of one half day which was consistent with the effect on lesion duration.

The applicant also presented analyses of the suppression of viral shedding, the lesion duration in subjects who initiated treatment with study medication early versus those who initiated treatment late, and the lesion duration in subjects who complied with instructions for the application of study medication versus those who did not. As discussed in the review, no clear conclusions may be drawn from these analyses

RESULTS - SAFETY

Background

In clinical trials conducted with famciclovir at doses of 250 mg TID to 750 mg TID for the treatment of herpes zoster and 125 mg BID to 500 mg TID for the treatment of herpes genitalis, the most frequently reported adverse events were headache and nausea. The incidence of headache was approximately 23% in these studies and the incidence of nausea ranged from 10% to 12%.

Drug Exposure

The expected systemic exposure to penciclovir with topical application of the 1% cream is small. When penciclovir 1% cream was applied repeatedly for 4 days to normal skin with occlusion in 12 healthy male volunteers (Study 39123/108), no penciclovir was detected in plasma or urine. Using the lower limit of quantitation of the assay for penciclovir in urine ($10\mu\text{g/mL}$), an estimate of the maximum systemic exposure to penciclovir from this treatment regimen would be approximately 0.5 mg/day.

All subjects who applied at least one dose of study medication were included in the safety analyses performed for both studies 024 and 025. Table 20 shows the number of subjects included in the safety data base and the duration of exposure. The mean and median duration of exposure to penciclovir in both studies was approximately 4 days. The intensity of drug exposure is shown in Table 8 (Treatment Compliance). The mean number of applications per day was approximately 8 in both studies.

	Study 024	Study 025
	Penciclovir	Penciclovir
Included in safety analyses	782	734
Mean duration of exposure	3.8 days	3.7 days
Median duration of exposure	4 days	4 days
Range	1 to 7 days	1 to 9 days

Discontinuations Due to Adverse Events

Of subjects who initiated treatment with study medication, 96% in study 024 and 97% in study 025 completed 4 days of therapy. The primary reason of discontinuation of study medication were protocol violations. The data for both studies is shown in Table 21.

	Table 21 - Discontinuations			
	Study 024		Study 025	
	Penciclovir	Placebo	Penciclovir	Placebo
Included in safety analyses	782	791	734	750
Completed 4 days of therapy	751 (96%)	756 (96%)	713 (97%)	724 (97%)
Did not complete therapy	31	35	21	26
Protocol violation	22	23	18	23
Other	4	4	3	1
Lost to follow up	3	3	0	2
Adverse event	1	3		
Withdrawn by SB	1	2		

Few subjects discontinued study medication because of an adverse event. In study 024, three subjects in the penciclovir group and three in the placebo group were withdrawn from the study because of adverse experiences. In study 025 only one subject was withdrawn from the study because of an adverse experience. These cases are summarized in the Table 22.

Study	Subject	Onset of AE	Reason for Withdrawal
024	0527 - penciclovir	after 3 doses	Severe pain from lesion; resolved 2 days after onset; herpes labialis
	1194 - penciclovir	4 days after initiation of medication	Dissociative disorder; admitted to psychiatric hospital
	0450 - penciclovir	2 days after last dose	Severe abdominal pain; appendicitis diagnosed
	0024 - placebo	day 3 of treatment	Rash, swelling of eye lids; pruritis
	0566 - placebo	day 2 of treatment	Worsening of herpes labialis; subject used one day of study medication then switched to an OTC product
	0791 - placebo	day 3 of treatment	Pneumonia; resolved after course of erythromycin
025	1300 - placebo	day 4 of treatment	Mild malaise

Deaths

Only three deaths were reported in subjects who were enrolled in either study and none were attributed to study medication. In study 024 no deaths were reported on study medication or within 30 days following the last dose of study medication. One death was reported for a subject who never started study medication. The subject committed suicide two months after being randomized into the study. In study 025, two deaths were reported for subjects randomized in the study. One subject committed suicide 16 days after the last dose of study medication (penciclovir). The subject had applied penciclovir for 5 days and had completed the study as planned. No other adverse events were reported for this subject. A death was reported in another subject who had been randomized but never initiated study medication. The subject committed suicide three months after enrollment in the study.

Adverse Events

The Table 23 lists adverse events reported on therapy or within 30 days of the last dose of study medication. Adverse events are included regardless of treatment attribution.

	Table 23 - Adverse Events			
	Study 024		Study 025	
	Penciclovir n=782	Placebo n=791	Penciclovir n=734	Placebo n=750
Total with an AE	118 (15%)	146 (19%)	184 (25%)	205 (27%)
Headache	28 (3.6%)	22 (2.8%)	76 (10.4%)	89 (11.9%)
Application site reaction	-	-	18 (2.5%)	23 (3.1%)
Lymphadenopathy	18 (2.3%)	17 (2.1%)	-	-
Pharyngitis	7 (0.9%)	9 (1.1%)	15 (2.0%)	7 (0.9%)
URTI	10 (1.3%)	9 (1.1%)	12 (1.6%)	7 (0.9%)
Hypesthesia	-	-	11 (1.5%)	18 (2.4%)
Herpes simplex	-	-	10 (1.4%)	9 (1.2%)
Nausea	9 (1.1%)	10 (1.3%)	10 (1.4%)	6 (0.8%)
Dysmenorrhea	-	-	9 (1.2%)	12 (1.6%)
Abdominal pain	-	-	7 (1.0%)	4 (0.5%)
Pain	-	-	7 (1.0%)	10 (1.3%)
Rhinitis	-	-	6 (0.8%)	9 (1.2%)
Sinusitis	7 (0.9%)	11 (1.4%)	-	-

In study 024, two serious adverse events were reported in subjects within 30 days of the last dose of study medication, both in the penciclovir group. One subject developed abdominal pain diagnosed as appendicitis two days after the last dose of penciclovir and another subject experienced an episode of a personality disorder requiring hospitalization 4 days after the initiation of penciclovir. Both of these adverse events were considered to be unrelated to application of study medication. In study 025, two serious adverse events were reported, one in each treatment group. One subject (penciclovir) was hospitalized for a repeat stapedectomy for otosclerosis. The event was considered to be unrelated to study medication. The second subject (placebo) experienced a severe allergic reaction four days after the start of study medication. The subject was hospitalized overnight and treated with epinephrine and diphenhydramine. The reaction was considered to be related to study medication.

Comments:

1. Adverse events were more commonly reported in study 025 than in study 024. In study 025 the overall rate was highest for Canada. However, the profile of adverse events was similar to the overall profile for study 025. Headache was the most frequently reported adverse event

(17.7% in the [redacted] group and 18.7% in the placebo group), followed by application site reactions, which were reported in 4.4% of the penciclovir group and 5.5% of the placebo group.

2. More adverse events were reported for women than for men. The overall rate was 21.8% for women in the penciclovir group versus 14.6% in men. Headache was the most commonly reported adverse event for both men and women, occurring in 8.0% of women and 3.8% of men.

Local Adverse Events

Adverse events that occurred at the site of study medication application were coded under a number of different terms. They included application site reaction, pain, pruritis, hypesthesia, paresthesia, taste perversion, herpes simplex, rash and skin exfoliation. These events are shown in Table 24.

	Table 24 - Local Adverse Events			
	Study 024		Study 025	
	Penciclovir n=782	Placebo n=791	Penciclovir n=734	Placebo n=750
All local adverse events	9 (1.2%)	26 (3.3%)	56 (7.6%)	72 (9.6%)
Application site reaction	2	4	18	23
Hypesthesia/Local anesthesia	1	3	12	18
Herpes simplex	1	5	10	9
Pain	1	1	7	10
Paresthesia	2	4	1	1
Taste perversion	0	5	3	0
Rash (erythematous)	0	1	2	2
Pruritis	1	1	0	2
Skin discoloration	-	-	0	1
Skin exfoliation	-	-	1	1
Allergic reaction	-	-	0	3
Allergy	-	-	2	2
Dry skin	1	0	-	-
Contact dermatitis	0	1	-	-
Tongue paralysis	0	1	-	-

Comment: Overall, reported local adverse events occurred at 1.2% to 3.3% in study 024 and 7.6% to 9.6% in study 025. The rates were similar between the treatment groups in both studies.

Drug Interactions

The applicant compared the incidence of adverse events reported in subjects who were taking

the ten most frequent concomitant medications while the subjects were applying study medication (1% penciclovir cream or vehicle control). The ten drugs included in the analyses were paracetamol, ethinylestradiol, acetylsalicylic acid, ibuprofen, norethisterone, estrogen (conjugated), vitamins, levonorgestrel, levothyroxine sodium, and pseudoephedrine hydrochloride. There were no apparent differences in the incidence of adverse events reported between the treatment groups in subjects who were taking any of these concomitant medications. The applicant concluded that the data support the absence of any apparent drug-drug interactions when topical penciclovir is co-administered with medications commonly used by subjects with herpes labialis.

Comment: No formal drug interactions studies have been performed, therefore, there are not data on whether there may be a drug interaction between topical penciclovir and any concomitantly administered drugs. The number of subjects included in the comparison of adverse events for each of the concomitant medications varied and ranged from approximately 220 per treatment group for paracetamol, the most commonly used concomitant medication, to approximately 40 per treatment group for pseudoephedrine hydrochloride. For most comparisons, the numbers were too small to allow the conclusion that no significant drug-drug interaction could be drawn.

Special Toxicity Studies

Of the five special toxicity studies that were conducted to determine the dermal irritancy and potential for sensitization, only two, 042 and 044, used the carrier in the proposed formulation for marketing, cetomacrogol 1000, cetostearyl alcohol, liquid paraffin, propylene glycol, purified water, white soft paraffin. The other three studies were done with formulations that were considered for development. With these formulations, both the drug product (containing penciclovir) and the vehicle caused significant irritation. As a result these formulations were dropped from clinical development. Only the results from studies 042 and 044 will be discussed in this review. The designs of these two studies are shown in the Table 25.

Table 25 - Special Toxicity Studies Using Formulation Proposed for Marketing		
	39123/042	39123/044
	Double-blind, placebo-controlled	Double-blind, placebo-controlled
Dates	4/6/92-4/30/92	6/15/92-8/10/92
Objectives	1. Dermal irritancy 2. Sensitization potential	Preliminary comparative dermal irritancy
Regimen	Repeat application with occlusion	Repeat application with occlusion
Drug	5% penciclovir, Cetomacrogol formulation	5% penciclovir, Cetomacrogol formulation 5% acyclovir cream
N	90	19
Population	Healthy volunteers	Healthy volunteers
Age - Mean Range	36 yrs 18-58	35 yrs 21-48
Gender - Female Male	24 66	11 8

Results

A total of 108 subjects were enrolled in the two studies. The proportion of subjects who showed an irritancy response (visual irritancy score ≥ 1.0 for at least one of the patches for that treatment) are shown in Table 26.

	Pcv only	Placebo only	Pcv & placebo	Acv only	Acv & placebo	Pcv & acv	Pcv, acv & placebo	Total
N	108	108	108	19	19	19	19	108
# with irritancy response	19 (18%)	14 (13%)	21 (19%)	6 (32%)	1 (5%)	4 (21%)	7 (37%)	72 (67%)

The applicant stated in their report that 51 of 108 (47%) subjects exposed to penciclovir had a visual irritancy score of 1.0 or greater as compared to 43 of 108 (40%) of subjects exposed to placebo and 18 of 19 (95%) of the subjects exposed to acyclovir. Although the number of subjects exposed to acyclovir was much smaller than to penciclovir and placebo, acyclovir 5% cream resulted in a greater severity of irritancy and a larger proportion of subjects with an irritancy response.

	Penciclovir	Placebo	Acyclovir
N	108	108	19
Mean (SD)	0.2 (0.2)	0.1 (0.2)	1.0 (0.3)
Median (range)	0.1 (0.0 to 0.9)	0.0 (0.0 to 0.9)	1.1 (0.2 to 1.5)

The mean visual irritancy scores for all subjects in studies 042 and 044 are shown in Table 27. The mean scores were similar for penciclovir and vehicle. No subject in study 042 had a maximal score greater than 1.0 for either penciclovir or vehicle. In study 044, one subject had a maximal irritancy score of 2.0 for penciclovir. For acyclovir, eight of 19 subjects had a maximal irritancy score of 2.0.

Since the mechanism for sensitization to an agent involves a systemic, immunological component, sensitization data on both current and previous formulations were analyzed. In terms of its sensitization potential, 2 of 260 subjects challenged showed equivocally positive reactions to penciclovir. These responses were felt to be due to mild irritancy and the applicant concluded that penciclovir is unlikely to induce sensitization.

Phototoxicity

Phototoxic and photoallergic reactions are most commonly due to light in the UVA range, however, UVB may occasionally be involved. The UV absorption spectrum of penciclovir has a maximum at 252 nm and shows no absorption in the UVA band and some absorption in the UVB band. It would be expected therefore, that penciclovir would have little potential for phototoxicity

or photoallergenicity. The applicant has conducted a phototoxicity and photoallergenicity study in guinea pigs with famciclovir which showed no evidence of phototoxicity or photoallergenicity. The applicant also submitted the initial report of a clinical study in which phototoxicity was assessed in 12 healthy subjects in Japan (six randomized to topical 1% penciclovir cream and six to placebo). No evidence of phototoxicity was seen.

Summary of Safety

The overall safety profile for 1% topical penciclovir was similar to that of the vehicle control. There were few adverse events reported and none were considered to be related to study medication. The most frequently reported adverse event in both phase 3 studies was headache. The incidence was for the penciclovir and placebo groups in both study 024 and study 025. The incidence of local adverse events was 1% to 3% in study 024 and 8% to 10% in study 025. The incidences was similar across the treatment groups. Overall in the two phase 3 clinical studies, 1% topical penciclovir was well tolerated. The results of the two dermal irritancy studies that were conducted with 5% penciclovir in the Cetomacrogol formulation showed that the proposed marketing formulation of penciclovir cream does not cause significant dermal irritation and is not likely to cause dermal sensitization. The information submitted also supports the conclusion that topical penciclovir is not likely to cause phototoxicity.

CONCLUSIONS

To support the efficacy of Denavir for the treatment of recurrent herpes labialis in immunocompetent adults, the applicant has submitted the results from two phase III, randomized, placebo-controlled, double-blind studies. After review of the study reports and of the analyses of the data conducted by the FDA Biostatistical Reviewer, this medical reviewer has concluded that the studies are adequate and well-controlled and that the studies demonstrate that Denavir is associated with a statistically significant, but modest, decrease in the duration of herpes labialis lesions. This decrease is approximately one half day in both of the studies. The studies also demonstrated an approximately one half day decrease in lesion associated pain in the subjects who were treated with Denavir, consistent with the effect seen on lesion duration. In summary, the data presented support the conclusion that Denavir been show to have a statistically significant, but modest effect in the treatment of recurrent herpes labialis in immunocompetent adults.

To support the clinical safety of Denavir for the treatment of recurrent herpes labialis in immunocompetent adults, the applicant has submitted the results from the two phase III trials, two irritancy studies with the formulation proposed for marketing and data from an ongoing study of the phototoxicity of 1% topical penciclovir in healthy volunteers in Japan. The safety data from the phase III clinical trials demonstrated that, except for headache, adverse events were reported in 3% or less of the subjects and that in regard to the frequency of reported adverse events, the treatment groups were comparable. Local adverse events were comparable between the treatment groups in both of the phase III clinical trials. The irritancy studies conducted with the formulation proposed

for marketing demonstrated that the 1% penciclovir Cetomacrogol cream formulation did not have significant irritancy potential and did not induce sensitization. The information on the UV absorption spectrum of penciclovir, the results of the phototoxicity study conducted in guinea pigs, and the available data from the clinical study conducted in Japan support the conclusion that the 1% penciclovir cream is unlikely to be associated with phototoxicity. In summary, the data presented support the conclusion that Denavir is safe for the intended use.

The clinical trials contained in the NDA support the conclusion that 1% topical penciclovir is safe and has a statistically significant, but modest effect on the duration of herpes labialis lesions. Because of the self-limited nature of herpes labialis in the normal host and the relatively short duration of the disease process, demonstration of larger decreases in the duration of the disease process may be difficult. Therefore, though modest, the demonstrated average decrease in the duration of the disease process represents a reasonable clinical benefit and given there are no approved antiviral products for the treatment of herpes labialis in the normal host, it is the conclusion of this medical reviewer that this NDA is approvable.

LABEL REVIEW

The proposed package insert included in NDA was reviewed. Comments were conveyed to the applicant and an agreement was reached on the format and content of the package insert. (Please refer to the final draft label included in the NDA package.)

RECOMMENDATIONS

It is the recommendation of the medical reviewer that this New Drug Application for Denavir™ for the indication of the treatment of recurrent herpes labialis in adults be approved.

Gary K. Chikami
 Gary K. Chikami, M.D.
 Medical Officer, HFD-530

Concurrence:

HFD-530/MTL/RBehrman/MB 9/5/96
 HFD-530/ADivDir/DFreeman 9/18/96

cc:

NDA 20-629

HFD-340

HFD-530/DivFile

HFD-530/Feigal

HFD-530/Freeman

HFD-530/Behrman

HFD-530/Mahoney

HFD-530/Chikami

Appendix I - Phase III Clinical Trial Design

The primary objective of the studies was to compare the safety and efficacy of 1% penciclovir cream with placebo for patient-initiated treatment of an acute, recurrent episode of herpes simplex labialis. The efficacy assessment as stated in the protocol was the proportion of subjects who had lost crust by day 8. The loss of crust was used to represent the end of clinically significant lesion stages (vesicle/pustule, ulcer/soft crust, and/or hard crust) for classical lesions. Assessments were to be made by the both subjects and investigators.

The secondary efficacy assessments included: the duration of pain for subjects with classical lesions with pain; time to loss of crust, measured as duration of the clinically significant lesion stages for classical lesions; the maximum lesion area of classical lesions; the proportion of subjects who develop classical herpes labialis lesions and proportion of subjects who develop aborted herpes labialis lesions; the proportion of subjects experiencing lesion pain; the duration of viral shedding among subjects with positive cultures; the proportions of subjects who have positive viral cultures for HSV; the duration of individual lesion stages (vesicle/pustule, ulcer/soft crust, and/or hard crust).

The entry criteria included: males or females aged 18 years or older in good general health; subjects with a clinically diagnosed history of recurrent herpes simplex labialis infection; recurrences at a rate of three or more per year with greater than 50% of the episodes preceded by prodromal symptoms and resulting in classical lesions; an accepted method of birth control for female subjects of child bearing potential.

Subjects who met the entry criteria were randomized to either penciclovir 1% w/w in cetomacrogol cream base or placebo (cetomacrogol cream base). Study medication was to be initiated therapy within 1 hour of recognizing the initial symptoms and/or signs of a recurrence of their herpes labialis. Subjects were to apply study medication every 2 hours while awake for 4 days. A minimum of 6 applications were to be applied on the first day. Subjects were required to report to the clinic within 24 hours of initiation study medication. Daily clinic visits were to continue until loss of crust. Thereafter, the subjects were to be seen in clinic every other day until the skin was assessed as normal. Subjects who initiated therapy based on prodromal symptoms were to followed for only four visits if no lesion developed. Subjects whose lesions progressed to the papule stage after four consecutive visits continued every other day visits until the skin was assessed as normal. At each visit, symptom and lesion assessments were performed. Daily viral cultures were performed during the vesicle/pustule and ulcer stages.

Assessments of symptoms and lesion characteristics were to be recorded by the subjects in a diary every six hours. The information in the diary was transcribed into the case report form by the investigator. Physician assessments were performed at each clinic visit. The anatomical location was documented on a orofacial diagram contained in the case report form. The duration of the lesions stages (prodrome, erythema, papule, vesicle/pustule, ulcer/soft crust, hard crust, residual swelling/dry flaking and normal skin) was assessed by clinical findings and patient history. Lesions that developed within the first 24 hours were considered part of the primary lesion complex

regardless of distance from the primary lesion. After 24 hours, lesions appearing within 1 centimeter of the primary lesions were considered part of the primary lesion complex. Lesions appearing greater than one centimeter from the primary lesion were considered new lesions. The maximum lesion area for the primary lesion complex was determined by measuring the greatest length, then measuring the greatest perpendicular width. Only the raised area was included in the measurement. If there were lesions or parts of a lesion at different stages, the stage covering the largest surface area was followed for assessment. Lesion stages were assigned the following codes:

- 0 No lesion/symptoms
- 1 Prodrome
- 2 Erythema
- 3 Papule
- 4 Vesicle/pustule
- 5 Ulcer/soft crust
- 6 Hard crust
- 7 Residual swelling/dry flaking
- 8 Normal skin (including erythema)

The following rating scale was used by the subjects to assess lesion pain:

- None No pain
- Mild The patient is vaguely aware of the cold sore. The affected area is tender when touched, but does not interfere with daily activities.
- Moderate The subject is aware of the cold sore at all times.
- Severe The discomfort interferes with daily activities (i.e., eating, drinking, talking). There is enough discomfort to cause sleep disturbance. The discomfort may seem to be an unbearable irritation.

Daily viral cultures were obtained during the vesicle/pustule and ulcer stages (stages 4 and 5). Viral cultures were performed at either a central laboratory or at a local virology laboratory. The protocol for performing viral cultures were reviewed by the virology laboratories of the applicant prior to initiation of the study. All herpes simplex isolates were stored at -70°C and sent to a central laboratory.

The primary efficacy variable was defined as the proportion of subjects losing hard crust by day 8 and the primary comparison was to be between the penciclovir-treated group and the placebo-treated group. The proportion of subjects with healing by day 8 was to be analyzed using the Cochran-Mantel-Haenszel test, stratified by center. The null hypothesis of no difference between the treatment groups was to be tested against the alternative hypothesis with a nominal type 1 error of 5% (two tailed).

For the proposed analyses the protocol defined two study populations. The "intent-to-treat" population was defined as all subjects who used coded study medication. The protocol-evaluable

population was defined as subjects who: fulfilled all protocol requirements; applied at least six doses of study medication each separated by at least two hours for four successive days; developed classical lesions; initiated treatment in the prodrome/erythema stage.

Secondary efficacy variables included: the duration of pain experienced by subjects with classical lesions who had pain; time to loss of crust, expressed as the duration of clinically significant lesion stages for classical lesions; maximum area of the classical lesions; proportion of subjects who developed classical lesions and the proportion of subjects who develop aborted lesions; proportion of subjects experiencing lesion pain; duration of viral shedding among subjects with positive cultures; proportion of subjects with positive viral cultures for HSV; duration of lesion stages for classical lesions.

Evaluation of safety data was to include all subjects who received a least one dose of study medication. The evaluation included time to onset, severity, study medication and investigator reported relationship of the clinical event to study medication. Subjects were evaluated at each clinic visit for the occurrence of any adverse event. Relation to study medication will be assessed as unrelated, probably unrelated, possibly related and related. The severity of adverse events were graded according to the following scale:

Mild	An adverse event easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
Moderate	An adverse event which is sufficiently discomforting to interfere with normal everyday activities.
Severe	An adverse event which prevents normal everyday activities.

Differences in protocols for Study 024 and Study 025

The protocols for studies 024 and 025 were similar except for minor differences noted below.

1. Exclusion Criteria -

Study 024 included the exclusion criteria that subjects who had participated in a previous famciclovir or penciclovir study would be excluded from the study. This was not specified in the protocol for study 025.

2. Monthly Phone Calls - Both protocols specified that subjects enrolled in the studies would be contacted on a monthly basis to remind them that they were participating in a clinical trial. However, the following instructions were included in the protocol for study 024, but not in study 025:

a) If recurrence occurs in the evening or during the night or if it is first noticed in the papule or later stage, it was recommended to the subject that medication be started at the next recurrence.

b) Subjects were asked if they have had a recurrence, and if so, was study medication initiated. If the subject initiated study medication and did not call for an appointment, he/she

was to be queried as to why not.

c) Specific instructions were given in regard to storage of study medication and inspection of study medication for changes due to improper storage.

3. Clinical Observations and Procedures -

a) The protocol for study 024 specified that subjects were to be reminded not to apply study medication immediately before the clinic visit. This was not stated in the protocol for study 025.

b) Subjects whose lesions had progressed to the papule stage were to continue alternate day visits until the skin was assessed as normal. This was not specified in the study 025 protocol. Appendix A of study 025 specifies that for patients whose lesions did not progress further than the papule stage no further visits beyond visit 4 were required.

4. Assessments of Lesions -

In study 024 if a lesion two distinct stages or there were two lesions in different stages, the lesion or part of the lesion that covers the largest surface area was followed for assessment purposes. This was not specified in the study 025 protocol.

5. Laboratory Procedures -

In study 024 the method of birth control used by female patients of child bearing potential was to be recorded in the case report form. This was not specified in the study 025 protocol.

6. Storage -

More detailed information on storage of study medication was provided in the protocol for study 024 and instructions were to be provided to the subjects if the study drug became inhomogeneous.

7. Statistical Methods, Comparison of Interest -

In study 024, the demographic characteristics history of herpes simplex infections of patients who initiate treatment was to be compared with those who had not initiated therapy. This comparison was not specified in the protocol for study 025.

APPENDIX II - BASELINE CHARACTERISTICS OF SUBGROUPS ANALYZED

Early Versus Late Initiators

	Study 024				Study 025			
	Early		Late		Early		Late	
	Pcv n=411	Placebo n=395	Pcv n=350	Placebo n=376	Pcv n=412	Placebo n=427	Pcv n=304	Placebo n=300
Mean age (yrs)	39.3	38.6	39.4	40.2	38.3	37.4	39.2	37.3
Gender								
Female	304	284	264	252	293	323	231	249
Male	107	111	86	124	119	104	73	56
Race								
Black	1	2	2	3	0	1	0	1
White	399	382	334	362	404	413	290	297
Other	11	11	14	11	8	13	14	7

	Study 024				Study 025			
	Early		Late		Early		Late	
	Pcv n=411	Placebo n=395	Pcv n=350	Placebo n=376	Pcv n=412	Placebo n=427	Pcv n=304	Placebo n=300
Duration of herpes labialis	23.3	21.8	23.5	24.1	19.0	19.8	19.4	19.0
# episodes during last year	5.6	6.0	6.0	5.4	5.8	5.7	5.8	5.5
# episodes per year	6.3	6.5	6.5	5.9	6.3	6.1	6.1	6.0
# subjects who had prodromes								
Always	275	266	210	255	286	295	203	206
Most	120	120	123	110	118	126	91	91
Half of time	16	9	17	11	8	6	10	8
# subjects with classical lesions								
Always	247	250	210	245	278	287	200	205
Most	147	136	130	114	124	133	94	91
Half of time	17	9	10	17	10	7	9	9
Occasionally							1	
# subjects who had false prodromes								
Always							1	1
Most of time			1				1	3
Half of time				1		2	1	3
Occasionally	144	147	133	140	144	143	118	112
Never	267	248	216	235	268	282	183	186

Compliant Versus Noncompliant

	Study 024				Study 025			
	Compliant		Noncompliant		Compliant		Noncompliant	
	Pcv n=620	Placebo n=629	Pcv n=162	Placebo n=162	Pcv n=489	Placebo n=466	Pcv n=245	Placebo n=284
Mean age (yrs)	39.0	39.2	41.4	40.6	39.2	37.7	37.6	36.5
Gender								
Female	466	438	117	114	372	370	163	213
Male	154	191	45	48	117	96	82	71
Race								
Black	2	1	2	5	0	1	0	1
White	597	607	154	152	474	452	238	275
Other	21	21	6	5	15	13	7	8

	Study 024				Study 025			
	Compliant		Noncompliant		Compliant		Noncompliant	
	Pcv n=620	Placebo n=629	Pcv n=162	Placebo n=162	Pcv n=489	Placebo n=466	Pcv n=245	Placebo n=284
Duration of herpes labialis	23.1	22.7	25.0	24.3	19.6	19.7	18.4	18.9
# episodes during last year	5.7	5.7	5.9	5.5	5.8	5.6	5.7	5.6
# episodes per year	6.4	6.2	6.5	6.1	6.2	5.9	6.1	6.2
# subjects who had prodromes								
Always	393	439	108	100	322	318	166	193
Most	202	174	46	58	142	137	76	85
Half of time	25	16	8	4	15	11	3	6
# subjects with classical lesions								
Always	371	405	101	107	330	316	155	161
Most	226	205	56	47	147	138	78	89
Half of time	23	19	5	8	11	12	8	4
Occasionally					1			
# subjects who had false prodromes								
Always	1				1			1
Most of time						1	1	2
Half of time		1			1	2		4
Occasionally	223	235	57	57	176	165	93	97
Never	396	393	105	105	311	298	151	180

APPENDIX III - Design of Studies 042 and 044

Study 042

Study was a double-blind, placebo-controlled (within subject comparison), repeat insult patch test. Subjects received nine repeat topical patch applications over a period of 22 days. Dosing was on days 1, 3, 5, 8, 10, 12, 15, 17, and 19. On each dosing day, subjects received two topical applications, one loaded with penciclovir 5% cream and the other loaded with placebo. Patches were left in place for 48 to 72 hours. A randomized code allocated a treatment to either the left or right side of the subject's upper back for the duration of the study. Once the initial patch was applied, the corners of each patch were marked with a waterproof marker to facilitate re-application to the same site. Patches were occluded with waterproof tape. To assess sensitization potential, applications of penciclovir 5% cream and placebo were applied at new sites under occlusive patches on day 36 (after a 14 day washout period). Patches were left in place for 24 hours before assessment.

The patch sites were assessed for irritancy and sensitization by visual inspection using a rating scale for erythema and edema. The assessment was made 15 minutes after the removal of the patch from the prior application and was made by the same blinded evaluator throughout the study. The assessor was not involved in the application of the study medication.

The following scale was used to rate the skin reaction:

- 0 No visible reaction. (Included superficial responses such as glazing, peeling, cracking)
- 1 Mild erythematous reaction. Faint pink to pink over 25% or more of the patch site
- 1E Mild erythematous reaction with papules and/or edema
- 2 Moderate erythematous reaction (definite pink to red erythema similar to sunburn)
- 2E Moderate erythematous reaction with papules and/or edema
- 3 Strong erythematous reaction (beet red)
- 3E Strong erythematous reaction with marked edema, papules and/or few vesicles
- 4 Severe reaction with erythema, edema, papules and vesicles (may be weeping)
- 5 Bullous reaction

The visual assessment scores for dermal irritancy and sensitization responses from any subject receiving at least one application of each treatment were considered valid for the analyses of clinical safety and tolerability. The irritancy and sensitization phases were analyzed separately. A subject was considered a responder if they had a score of greater than or equal to 1.0 at any time.

Study 044

The study was a double-blind, placebo-controlled, randomized, within-subject comparison of penciclovir 5% cream (Cetomacrogol cream base), placebo (Cetomacrogol cream base) and acyclovir 5% cream. Subjects received 9 repeat topical applications of each study medication with occlusion on days 1, 2, 3, 4, 5, 6, 8, 9, and 10. Each patch remained in place for 24 to 48 hours. A randomized code was used to allocate the treatments to the left, middle or right upper back. Visual assessments of the application sites were made at least 15 minutes after removal of each patch. The assessments were made by two independent blinded assessors. The assessors remained the same throughout the study and were not involved in the application of the study medications. The following scale was used to grade the reactions observed at the application sites:

- 0 No visible reaction (including superficial skin responses e.g., glazing, peeling, cracking)
- 0.5 Mild erythematous reaction over less than half of the patch site
- 1 Mild erythematous reaction. Faint pink
- 1.5 Moderate erythematous reaction. Definite pink
- 2 Moderate erythematous reaction. Definite red (similar to sunburn)
- 3 Strong erythematous reaction. Beet red.
- 4 Severe reaction with erythema, edema, papules and vesicles (may be weeping)
- 5 Bullous reaction

After each visual assessment, skin blood flow was measured using an Oxford laser doppler flowmeter. Blood flow was recorded over 30 seconds and an average of the output signal was recorded. Control measurements were obtained at an adjacent site on the subject's upper back.

The average of the two scores for each assessor was calculated for each assessment. A responder was defined as a subject with at least one score greater than or equal to 1.0 over the nine patches for that treatment. The number of responders to each treatment alone and to all three treatments was tabulated. The maximum score over all nine patches for each subject and each treatment was calculated and a frequency table was constructed for the maximum score by treatment.

To determine whether the proportion of responders was influenced by treatment, Cochran's Q test was performed using the average scores. The average score over all nine patches for a particular treatment for each subject and treatment was calculated. These average patch values were analyzed using the Wilcoxon matched pairs method of each of the pair wise comparisons with penciclovir.

Statistical Review and Evaluation

SEP 17 1996

NDA#: 20-629

APPLICANT: SmithKline Beecham Pharmaceuticals

NAME OF DRUG: Denavir™ (Penciclovir Cream) 1%

INDICATION: Treatment of Recurrent Herpes Simplex Labialis Infection

DOCUMENTS REVIEWED: V. 1.110, 1.122, 1.136, 1.147
Responses to Request for Information: 5/13/96, 6/12/96

MEDICAL INPUT: HFD-530: G. Chikami

A. Introduction

Two studies have been considered for the proposed indication for the treatment of recurrent herpes simplex labialis infection: 024 and 025. These studies followed patients over the course of lesions produced by herpes simplex labialis infections. Assessments were made both by the medical investigators and by patients using a diary. Lesions were scored over time using the following classification system.

<u>Stage</u>	<u>Score</u>
no lesion or symptoms	0
prodrome	1
erythema	2
papule	3
vesicle/pustule	4
ulcer/soft crust	5
hard crust	6
residual swelling/dry flaking	7
normal skin (including erythema)	8

The duration of critical lesion stages (papules, vesicles/pustules, ulcers/soft crusts and hard crusts), as assessed by the investigator, was identified during discussions between the medical division and the applicant as being of primary interest. It was agreed that duration would be defined as the number of days of any stage of a critical lesion. The use of critical lesions as the primary endpoint differs from the protocol specified primary endpoint where classical lesions were defined as being one of the following: vesicles/pustules, ulcers/soft crusts and hard crusts. The patient's assessment of critical lesion stages was treated as supportive.

During preNDA discussions with the applicant, two analysis populations were considered. The first population is made up of all subjects known to have started study therapy and the second excludes only subjects known not to have had a recurrence regardless of therapy status. The first analysis

population (known treated) was defined in the protocol while the second was requested by the Division of Antiviral Drug Products (DAVDP) as an intent-to-treat (ITT) population.

B. Summary of the Designs

Study 024

Protocol Title: "A Prospective, Randomized, Double-Blind, Multicenter, Patient-Initiated Study to Compare the Efficacy of Topical 1% Penciclovir Cream with Placebo in Patients with Recurrent Herpes Simplex Labialis Infection" (11/25/92)

This is a double-blind study of patient-initiated therapy for the treatment recurrent herpes labialis infections. To be eligible, patients were to historically have at least three recurrences per year. Approximately 2,000 subjects were to be randomized to one of the following treatment arms:

Penciclovir	1% penciclovir cream; or
Placebo	placebo cream.

Randomization was to take place within each of 35 centers located in the United States.

After randomization, patients returned home with their assigned medication and were to begin therapy within 1 hour of the first sign or symptom of herpes labialis. Treatment was to be applied topically every 2 hours for four days to the affected or perioral areas. It was expected that 232 subjects per arm would initiate therapy.

Patients were to have had their first clinic visit within 24 hours of initiating therapy. Daily visits were to be made until loss of crusts. Thereafter, alternate day visits were to be made until complete healing. Patients initiating therapy on the basis of prodromal/erythema were to be followed for only four consecutive visits if no lesions occurred.

Each patient was to be contacted at least once a month by a study nurse to determine if a recurrence had taken place. If therapy was not initiated, the reason for this was recorded by the study nurse.

The primary efficacy analysis was to be based upon the proportion of patients who have lost crusts (vesicle, pustule, ulcer, soft crust, or hard crust) by day 8. This assessment was to be made both by the patient and the investigator.

The protocol specifies that an intent-to-treat analysis would be conducted. The Cochran-Mantel-Haenszel test was to be used to compare the treatments for the primary endpoint with stratification by investigator.

Study 025

Protocol Title: "A Prospective, Randomised, Double-Blind, Multicentre, Patient-Initiated Study to Compare the Efficacy of Topical 1% *Penciclovir* Cream with Placebo in Patients with Recurrent Herpes Simplex Labialis Infection" (8/12/92)

This is a double-blind study of patient-initiated therapy for the treatment recurrent herpes labialis infections. To be eligible, patients were to have had 3 or more recurrences per year. Approximately 2,000 subjects were to be randomized to one of the following treatment arms:

Penciclovir	1% penciclovir cream; or
Placebo	placebo cream.

Randomization was to take place within each of approximately 40 centers located worldwide.

Patients returned home after randomization with their assigned medication and were to begin therapy within 1 hour of the first sign or symptom of herpes labialis. The assigned treatment was to be applied topically every 2 hours for four days to the affected or perioral areas. It was expected that 232 subjects per arm would initiate therapy and be evaluable (50% develop herpes labialis, 75% comply with protocol, and 75% develop classical lesions and 75% initiate early treatment).

The schedule of follow-up and definition of primary endpoints were consistent with study 024. The protocol for study 025 has a more extensive description of secondary endpoints than the protocol for study 024. Pain was to be assessed by the patient using diary cards to be completed every six hours with the following rating scale: none, mild, moderate and severe. Viral cultures were to be obtained during the blister (vesicle/pustule) and ulcer stages.

The protocol specifies that an intent-to-treat analysis would be conducted. The Cochran-Mantel-Haenszel test was to be used to compare binary outcome variables with stratification by investigator. Outcome measures based upon duration were to be analyzed using the Wilcoxon rank sum test.

B. Applicant's Results

Study 024

The study report is based upon subjects enrolled between 4/21/93 and 7/8/94 in 31 centers. Two-thousand two-hundred nine subjects were randomized with 1573 known to have initiated therapy. The remaining 636 are known to have not initiated therapy or are of unknown treatment status. The following table provides the distribution of subjects randomized by treatment (using permuted blocks of size 8) and follow-up status. There is no apparent difference between the treatment groups in the distribution of subjects with respect to follow-up.

Subject Disposition (n)

	Penciclovir	Placebo
Total Randomized	1103	1106
Initiated Therapy	782	791
1) Complete Follow-up	744	743
2) Incomplete Follow-up	38	48
No Therapy Recorded	321	315
3) No Recurrence	125	126
4) Lost to Follow-up	84	84
5) Recurrence	76	71
6) Other	36	34

Source: Tables 3, 4, 14 V. 1.110

Of the subjects that initiated study therapy, approximately 50% initiated therapy prior to developing a papule (based upon patient assessment) while the remaining 50% initiated therapy at or after the papule phase. The treatment arms showed little difference in the time of initiation (Table 9, V. 1.110).

As discussed earlier, both the known treated and ITT analyses exclude subjects known to have not had a lesion. The known treated and ITT analyses exclude 29% and 11% of the subjects randomized, respectively. Of those subjects included in the ITT population, 20% were excluded to create the known treated population.

The application presents a comparison at baseline between the known treated population and the ITT population (Table 8, V. 1.110). Subjects not initiating therapy (i.e., subset of the ITT population) tended to have fewer lesions during the previous year as well as fewer episodes per year. The average was about 1/2 episode fewer per year for both treatment arms. Other clinical conditions were comparable at baseline between the known treated population and the remaining subjects in the ITT population (Table 11, V. 1.110).

As no endpoint data were collected for subjects not initiating therapy, the study report contains a number of analyses based upon excluding subjects with missing data or assigning one of the following values to subjects with no duration data: zero, lower quartile, median, upper quartile and maximum+1. Values were assigned without regard to treatment status. Three additional strategies were also employed which treated the study arms differently (other approaches were included in the NDA, but the results are consistent with the approaches described and will not be discussed further). The first assigned a value of the maximum+1 to the subjects receiving penciclovir and a value of 0 to subjects assigned to placebo. This is a "worst case" analysis. The second assigns the median to the penciclovir subjects and 0 to the placebo subjects. The third assigns the third quartile to penciclovir and the first quartile to placebo. These strategies were developed with the cooperation of the DAVDP prior to an examination of the study results.

Duration of Critical Lesions

As shown previously, a number of subjects who initiated therapy had incomplete follow-up information. The NDA breaks these subjects into two groups (Table 14, V. 1.110): 35 subjects (13 and 22 for penciclovir and placebo, respectively) who had a critical lesion start date with unknown

end date and 48 subjects (25 and 23 for penciclovir and placebo, respectively) for whom treatment was initiated without a critical lesion. The imputation strategy previously described was used for the 48 subjects in the second group. For the 35 subjects who initiated therapy but had critical lesions at the time follow-up was discontinued, the maximum over subjects + 1 with complete follow-up was imputed for all analyses.

The following table summarizes the results of the comparisons between the treatment groups for the various ways of imputing missing data described previously. The first row of this table contains the results for subjects known to have received therapy with either complete follow-up or the maximum+1 imputed (these subjects are labeled the "exclude" approach because subjects with no lesion data are excluded from the analysis). The remaining rows of this table contain the results for the intent-to-treat population. Note, that all but three comparisons favor penciclovir when the mean is used to compare the treatments.

Duration of Critical Lesions

Imputation Approach	p-value	Mean		Median	
		Penciclovir	Placebo	Penciclovir	Placebo
Exclude	<.001	4.5	5.2	4	4
1st Quartile	<.001	4.2	4.7	3	4
Median	<.001	4.4	4.9	4	4
3rd Quartile	<.001	4.9	5.3	5	6
Max+1/0*	<.001	7.1	4.0	5	4
Median/0**	.014	4.4	4.0	4	4
Q3/Q1***	.234	4.9	4.7	5	4

Source: V. 1.110, Table 15, V. 1.135, Table 5

*maximum+1 assigned to penciclovir and 0 assigned to placebo

**median assigned to penciclovir and 0 assigned to placebo

***third quartile assigned to penciclovir and first quartile assigned to placebo

The p-values in the above table were calculated using StatXact (Version 2.11). A Monte Carlo approach was used to calculate the permutation p-value (i.e. no scoring was used as would be done to calculate the log-rank or Wilcoxon test). This procedure is based upon comparing the total over subjects for one treatment arm to that which would have been expected if there was no treatment difference. Stratification based upon investigator was used.

Duration of Pain

The duration of pain analyses were limited to subjects initiating therapy and recording data in their diaries at some point during follow-up. For each subject, the number of days with mild to severe pain was determined by counting the days where any pain was recorded in the patient's diary. Only values recorded after the initiation of therapy were included in the analysis. If a patient was experiencing pain as of the last visit, the number of days was set to the maximum+1 found using subjects with complete data. A test of significance was conducted based upon the number of days with pain using the Mann-Whitney rank test stratified by investigator. There was approximately .5 days less pain for the penciclovir arm with a p-value <.001. The following table summarizes the results of these analyses.

Duration of Pain - Patient Assessment (Diary)

	Penciclovir	Placebo
Number Starting Therapy	782	791
Diary Not Used*	16	17
Included in Analysis	766	774
mean	4.2	4.7
median	3.0	3.6
interquartile range	1.5, 5.3	1.9, 6.1

Source: V. 1.110, Table 19

NDA indicates 32 such subjects in total, table values found by subtraction

The applicant also presented analyses based upon the time until loss of pain using a Cox model and log-rank test. This analysis differs from that conducted for the duration of pain in that subjects recording no pain were excluded from the analysis (38 placebo and 39 penciclovir). Additionally, the time until loss of pain corresponds to the diary entry where no pain was first recorded rather than the day of last recorded pain. The results of the analysis based upon time to loss of pain is consistent with the duration of pain analysis. A significant reduction in the time to the end of pain was reported for penciclovir ($p=.001$) with a hazard ratio of 1.22 (95% C.I.: 1.09-1.36).

Time to Cessation of Viral Shedding

A time to event analysis (Cox regression analysis stratified by center with treatment as the covariate) was conducted for the time until the last positive culture. An "event" was determined to have occurred as of the last positive culture when the positive culture was followed by a negative culture. Only subjects starting therapy and having at least one positive culture were included in this analysis. Any subject with a positive culture at the last study visit (i.e., no negative culture) was treated as censored. The following table contains the disposition of subjects for this analysis.

Viral Assessment

	Penciclovir	Placebo
Number Starting Therapy	782	791
No Positive Culture on Study	267	259
Positive Culture on Study	515	532

Approximately 60% of subjects with a positive culture on study had a positive culture as of their last visit. A number of these subjects had only a single culture available (i.e. the culture result used for inclusion in the viral shedding analysis). As such, these subjects could never become negative for this analysis. Because of this, the application contains additional analyses including only subjects with at least two and also with at least three culture results. The main study report describes the results for all subjects with at least one culture and at least three cultures. The analysis based upon subjects with at least 2 cultures is reported as consistent with the other analyses and will not be discussed further.

The following table summarizes the results of the Cox regression analysis conducted for the time until the cessation of viral shedding. Based upon this analysis the applicant has concluded that penciclovir is associated with a shortening of the time until the cessation of shedding.

Cessation of Shedding

	Ever Positive		Ever Positive & ≥ 3 cultures	
	Penciclovir	Placebo	Penciclovir	Placebo
n	515	532	223	232
median	3	3	3	3
interquartile range	2, 3	2, 4	2, 3	2, 4
Cox hazard ratio	1.35		1.26	
95% C.I.	1.10, 1.64		1.26, 1.59	
p-value	.003		.048	

Source: Table 21, V. 1.110

Study 025

The study report for study 025 is based upon subjects enrolled between 3/18/93 and 8/14/94 in 43 centers from 10 countries (Canada, United Kingdom, Poland, Belgium, Netherlands, France, Switzerland, Sweden, Denmark and Singapore). Two-thousand three-hundred sixty-four subjects were randomized with 1484 known to have initiated therapy and 880 not known to have initiated therapy. The following table provides the distribution of subjects randomized by treatment (using permuted blocks of size 4) and follow-up status. There is no apparent difference between the treatment groups in the distribution of subjects with respect to follow-up.

Subject Disposition (n)

	Penciclovir	Placebo
Total Randomized	1177	1187
Initiated Therapy	734	750
1) Complete Follow-up	701	710
2) Incomplete Follow-up	33	40
No Therapy Recorded	443	437
3) No Recurrence	283	266
4) Lost to Follow-up	38	44
5) Recurrence	122	127

Source: Tables 4, 5, 15 V. 1.136

Of the subjects that initiated study therapy, approximately 57% initiated therapy prior to developing a papule (based upon patient assessment), 41% initiated therapy at or after the papule phase and 2% were of unknown initial stage. The treatment arms showed little difference in the time of initiation (Table 10, V. 1.136).

Duration of Critical Lesions

As was discussed for study 024, two analysis populations were considered. The first population is made up of all known to have begun study therapy and the second excludes only subjects known not to have had a recurrence regardless of therapy status. These two approaches exclude 37% and 23%

of the subjects randomized, respectively. Of those subjects included in the ITT population, 18% were excluded to create the known treated population.

The application presents a comparison between subjects at baseline between the known treated population and the ITT population (Table 9, V. 1.136). Subjects not initiating therapy tended to have fewer episodes during the previous year as well as fewer episodes per year. The average was about 1/3 episode fewer per year for both treatment arms. Other presenting conditions (table 12, V. 1.136) were lower for the known treated population (41% had some presenting condition) than subjects not initiating therapy (32%).

As with study 024, no endpoint data were collected for subjects not initiating study therapy. The same strategies were used in study 025 as were previously described for study 024. Similarly, StatXact (Version 2.11) was used to carry out the statistical analyses using a Monte Carlo approach to calculating the permutation p-value for duration

The following table summarizes the results of the comparisons between the treatment groups as was previously presented for study 024. Note, all but the last three comparisons favor penciclovir.

Duration of Critical Lesions

Imputation Approach	p-value	Mean		Median	
		Penciclovir	Placebo	Penciclovir	Placebo
Exclude	.030	4.5	5.0	4	4
1st Quartile	.041	4.2	4.5	3	3
Median	.033	4.4	4.8	4	4
3rd Quartile	.022	4.8	5.2	5	6
Max+1/0*	<.001	7.4	3.9	5	3
Median/0**	.012	4.4	3.9	4	3
Q3/Q1***	.045	4.8	4.5	5	3

Source: Table 16, V.1.136 and Table 5, V. 1.158

*maximum+1 assigned to penciclovir and 0 assigned to placebo

**median assigned to penciclovir and 0 assigned to placebo

***third quartile assigned to penciclovir and first quartile assigned to placebo

Duration of Pain

The duration of pain analyses were limited to subjects initiating therapy and recording data in their diaries at some point during follow-up. For each subject, the number of days with mild to severe pain was determined by counting the days where any pain was recorded in the patient's diary. As with study 024, only those values recorded after the initiation of treatment were included in the analysis. If a patient was experiencing pain as of the last visit, the number of days was set to the maximum+1 found using subjects with complete data (i.e., followed to the cessation of pain). A test of significance was conducted based upon the number of days with pain using the Mann-Whitney rank test stratified by investigator. There was an approximately .5 day reduction in the duration of pain associated with the use of penciclovir with a p-value <.001. The following table summarizes the results of these analyses.

Duration of Pain - Patient Assessment (Diary)

	Penciclovir	Placebo
Number Starting Therapy	734	750
Diary Not Used	14	14
Included in Analysis	720	736
mean	3.6	4.2
median	2.3	2.9
interquartile range	1.0, 4.2	1.2, 5.2

Source: Table 20, V. 1.136

As for study 024, the applicant also presented analyses based upon the time until loss of pain using a Cox model and log-rank test. The analysis based upon time to loss of pain is consistent with the duration of pain analysis. A significant reduction in the time to the end of pain was reported for penciclovir ($p < .001$) with hazard ratio of 1.26 (95% C.I.: 1.12-1.41).

Time to Cessation of Viral Shedding

As for study 024, a time to event analysis was conducted using the data from study 025 for the time until the last positive culture. Only subjects starting therapy and having at least one positive culture were included in this analysis. Any subjects with a positive culture at the last study visit were treated as censored. The following table contains the disposition of subjects for this analysis.

Viral Assessment

	Penciclovir	Placebo
Number Starting Therapy	734	750
No Positive Culture on Study	316	290
Positive Culture on Study	418	460

Approximately 50% of subjects with a positive culture on study had only a single culture available (i.e. the culture result used for inclusion in the viral shedding analysis). As such, these subjects could never become negative for this analysis. Because of this, the application contains additional analyses including only subjects with at least two and also with at least three culture results. The main study report describes the results for all subjects with at least one culture and at least three cultures. The analysis based upon subjects with at least 2 cultures is reported as consistent with the other analyses and will not be discussed further.

The following table summarizes the results of the Cox regression analysis conducted for the time until the cessation of viral shedding. Based upon this analysis the applicant has concluded that penciclovir is associated with a shortening of the time until the cessation of shedding.

Cessation of Shedding

	Ever Positive		Ever Positive & ≥3 cultures	
	Penciclovir	Placebo	Penciclovir	Placebo
n	418	460	208	243
Median	3	3	3	3
Interquartile Range	2,3	2,4	2, 3	2, 4
Cox Hazard Ratio	1.47		1.46	
95% C.I.	1.20, 1.80		1.16, 1.85	
p-value	<.001		.002	

Source: V. 1.136, Table 22

Summary of the Applicant's Analyses

The applicant has presented a number of statistical analyses of studies 024 and 025 to demonstrate that topical penciclovir is associated with a reduction in the time until the healing of lesions brought about by herpes labialis. These analyses compensated for incomplete follow-up using a variety of different approaches. These approaches generally showed topical penciclovir to be associated with a reduction in the time until healing of approximately .5 day. Secondary analyses were presented for the duration of pain as well as the time of viral shedding. These analyses also showed approximately a .5 day reduction in the number of days with pain and viral shedding.

D. Reviewer's Comments

1) Assessment of the Applicant's Analyses

Duration of Critical Lesions

Comparison of the Imputation Strategies

The applicant's analyses were based upon a stratified permutation test with stratification by investigator. Though not stated explicitly, the applicant has compared the two treatment arms on the sum of the durations over subjects conditioning upon the number of subjects assigned to each treatment by investigator. This procedure is algebraically equivalent to comparing the treatment arms using mean duration.

The applicant presented analyses for the duration of critical lesions for both the known treated and the FDA requested intent to treat population. For both populations, a number of imputation strategies for missing data were used.

The majority of the imputation strategies assigned the same value for missing data regardless of treatment assignment. These analyses demonstrated that if the same values are imputed for both penciclovir and placebo, penciclovir has a shorter mean duration of critical lesions. This is true for both studies 024 and 025.

The remaining imputation strategies imputed longer durations for the penciclovir arm. These analyses favored placebo. This change in the direction of the treatment effect reflects the number of subjects missing data both due to loss to follow-up and subjects not starting therapy.

Based upon the pattern of missing data, it appears that the imputation strategies which differentially penalize penciclovir relative to placebo may be excessively biasing the treatment comparison. As this was a double-blind study, there is no evidence to suggest that subjects failing to start medication differ between the two arms. This implies that for subjects never starting medication, there was likely to have been no treatment effect rather than an effect in favor of placebo.

If the conclusion that the analyses treating penciclovir and placebo the same for imputation are the preferred approaches, it is not clear which of these imputation strategies leads to the best estimate of the treatment effect. It should be noted that though the imputation strategies lead to different estimates of the mean duration for each treatment arm, the mean differences between the treatment arms are comparable for each imputation strategy treating penciclovir and placebo the same. Slight differences are present due to the small differences in follow-up between the two treatment arms. The calculation of a single treatment effect for the ITT population will be discussed later in this review.

Choice of a Population: Known Treated versus ITT

The choice of which population, known treated versus ITT, best represents the treatment effect is unclear. There has been a long standing tradition in clinical trials that the intent to treat population should always be analyzed and that the ITT population should include all subjects randomized to therapy. This issue is complicated for the studies contained in this application by the fact that treatment was only to be initiated by the subject if a lesion developed.

The principal of including all subjects randomized in the ITT population has been modified in anti-infective trials. In these trials, confirmation of the disease of interest can not be made at the time of randomization but it is necessary to begin therapy as soon as a clinical diagnosis is made (i.e., presumptive disease). Subjects for which laboratory confirmation is not established are later excluded from the ITT analysis. This approach is consistent with the principles outlined by Tsiatis (1990, Intent-to-Treat Analysis, JAIDS) in which conditions existing prior to randomization can be used to validly exclude subjects from an ITT analysis.

The analogous population for the present trials are those subjects never experiencing a lesion. These subjects have been excluded from the ITT analyses conducted for these studies. The rationale for this exclusion is that development of a lesion will not be affected by the act of randomization and is only a function of each patient's condition at the time of randomization. The ITT population is therefore made up of those subjects with a herpetic lesion for which treatment should have been started.

The known treated population described in the application excludes subjects from the ITT population who were not known to have started therapy. The exclusion of these subjects is more problematic than subjects never developing a lesion. It was specified in each protocol that each of these subjects should have initiated therapy. It is conceivable that the types of lesions experienced by these subjects may have differed from lesions for which patients initiated therapy. The difference between the response to penciclovir and placebo may differ for the types of lesions and it may not be reasonable to assume that the patient experience for one population can be extrapolated to the other.

The population covered by the "known treated" subgroup can therefore only be described as being made up of subjects with recurrent herpes who initiated therapy under the conditions imposed by the clinical trials. This population is somewhat poorly specified since no information is available as to why some subjects initiated therapy while other failed to initiate therapy. Still, the double-blind nature of these studies and the apparent similarity between the treatment groups suggest that the applicant's analyses may have produced valid treatment comparisons for this subpopulation.

Assignment of the Maximum+1

The application contains a number of imputation strategies for dealing with subjects with no follow-up data. A different approach was used for the relatively small number of subjects with partial follow-up data. These subjects were known to have had a lesion and started therapy, but were lost to follow-up prior to resolution of the lesion. In every analysis presented in the application, subjects with partial follow-up were assigned a value of the maximum+1. For study 024, this appears to have exaggerated the treatment effect since 13 subjects for the penciclovir arm were treated this way while 22 were treated this way for the placebo arm (the original application has 25, but this was later corrected to 22). The treatment effect for subjects with complete follow-up is .52 days (4.31 vs. 4.83) while the treatment effect when the value of 16 is imputed is .64 (4.51 vs. 5.15). This shows that imputation for these subjects has increased the treatment effect by approximately 20%. Given this pattern, it would have been preferable to have used an imputation strategy which did not implicitly increase the treatment advantage for penciclovir due to the imbalance in study 024 for this group of subjects. No such problem was evident for study 025 in which both arms have the same number of subjects with known critical lesions. Analyses will be provided later in this review to eliminate this potential bias in favor of penciclovir introduced for subjects with partial follow-up data.

Duration of Pain

The analyses based upon the patient assessed duration of pain are subject to the same discussion as for the duration of healing. Additionally, as a secondary endpoint, the interpretation of tests of significance is difficult.

Analyses were conducted both in terms of the duration of pain and the time until the cessation of pain. These analyses both suggest that penciclovir is associated with a reduction in the time until pain is resolved. This correspondence is not surprising given the self limiting nature of the condition and the definition of the endpoints. The two endpoints differ only by the time between the visit with the last recorded pain and the following visit. The Mann-Whitney test evaluates whether the average rank of duration differs while the log-rank test compares average log-rank scores (based upon a transformation of the ranks) between the treatments. Since the tests are in basic agreement for the present data, the main issue to consider is the choice of the most appropriate summary measure to use for the difference between treatments: difference in mean durations, difference in median durations, difference in mean time to no pain, difference in median time to no pain or hazard ratio in time to no pain.

The hazard ratio does not appear to be a desirable summary statistic. The hazard ratio is generally used as a relative measure for the risk having a negative outcome at any point in time. For the present situation, this would be the risk for healing. This necessitated excluding subjects without pain. An additional issue is that the hazard ratio is typically used in situations where complete follow-up is impossible due to administrative censoring and where an underlying model is assumed

to aid in summarizing the treatment effect. For the present data, complete follow-up is generally available and the assumption of an underlying model may not be necessary since measures of central tendency (mean or median) are available. These measures have the advantages of allowing all subjects with data to be included in the analysis rather than excluding subjects with no pain.

The difference in the mean durations for study 024 for subjects with complete data (excludes 93 subjects set to maximum+1 in the applicant's analysis) is .58 (3.26 vs. 3.84) while the difference in medians is .66 (2.78 vs. 3.44). The analogous differences for the time until no pain (excludes "censored" subjects) are .60 (3.86 vs. 4.46) and .61 (3.39 vs. 4.00). This shows that the difference between treatments for both duration and time are approximately .6 regardless of the measure of central tendency used to summarize the data. Since the duration of pain analysis incorporates subjects with no pain, the analysis of the mean (or median) for duration may be preferred over the Cox model to avoid excluding subjects.

The difference in the mean durations for study 025 for subjects with complete data (excludes 77 subjects assigned maximum+1 in the applicant's analysis) is .48 (2.80 vs. 3.28) while the difference in medians is .44 (2.21 vs. 2.65). The analogous differences for the time until no pain are .51 (3.55 vs. 4.06) and .40 (2.90 vs. 3.30) for the mean and median, respectively. As for study 024 since the duration of pain incorporates subjects with no pain, the analysis of mean (or median) duration) may be preferred to the Cox model analysis.

The application has only presented analyses for subjects with at least partial data and imputation has not been performed (other than assigning the maximum+1 to subjects with partial data) for either the known treated or ITT populations as was done for the duration of healing. Estimates incorporating missing data, as was done for the duration of healing, will be presented later in this review.

It was noted previously (summary of the study results) that the applicant only included data for pain after treatment was initiated. At the request of DAVDP, the applicant submitted additional analyses that included all diary entries regardless of the timing of therapy. These analyses are essentially identical to the analyses originally submitted. Therefore, these additional analyses will not be discussed further.

Viral Shedding

The protocols specified that subjects were to have culture results for blister (vesicle/pustule) and ulcer stages (stages 4-5).

Study 024

The data set provided by the applicant shows that 3,512 visits had lesions at stages 4 or 5. Of these, 2,788 or 79% had a culture result (either positive or negative). Stage 4 lesions were the most consistently cultured (97%; 1385/1433) with 77% of those cultured found to be positive. Stage 5 lesions were less frequently cultured (67%; 1403/2079) with 40% of those cultured found to be positive. A number of lesions were cultured for other than stage 4 and 5 lesions. Stage 6 lesions were infrequently cultured (6%; 140/2447) with 21% of those cultured found to be positive. Two-hundred twenty-four culture results are available for stages 1, 2 and 3. Of these, 46% were positive.

The applicant's 5/13/96 response to an FDA request for information describes the results of a review for 79 patients who had 25% or fewer of their stage 4 or 5 lesions cultured. It was found that these

subjects were cultured only at their first or second clinic visit and were not subsequently cultured. No reason was given for this deviation from the protocol in the CRFs reviewed by the applicant.

Study 025

Similarly for study 025, the data set provided by the applicant contains 2,983 visits with lesions at stages 4 or 5. Of these, 2,441 or 82% had a culture result. Lesions at stage 4 were consistently cultured (94%; 1199/1277) with 69% found to be positive. As in study 024, stage 5 lesions were less consistently cultured (73%; 1242/1706) with 62% found to be positive. A small percentage of stage 6 lesions were cultured (13%; 320/2497) with 18% found to be positive. There were a limited number of culture results available for stage 1, 2 and 3 lesions (277) and of these 47% were positive.

Studies 24 and 25 have extensive culture data for subjects only at the time of stage 4 and 5 lesions. There were consistently fewer culture results available for stage 5 lesions. There was also clear evidence that the risk of a positive culture depends upon the stage of lesion. The applicant has attempted to use this data to characterize the time to the cessation of shedding and demonstrate a reduction in the time to the cessation of shedding by the use of topical penciclovir. Cox models were the primary analysis technique used to assess the time to cessation of shedding. Observations were "censored" when measurements were no longer available. Since the availability of culture results is dependent on the stage of the lesion and the risk of shedding is associated with the lesion stage, this censoring is informative. This informative censoring violates one of the key assumptions of the survival analysis, and implies that the applicant's analyses may not be valid.

Since the survival analysis used by the applicant is seriously flawed due to the availability of culture data, it is of interest to consider the possibility of using an alternative technique to assess the impact of the treatment upon shedding. The possibility of conducting such analyses is extremely limited because complete data is only available for stage 4 lesions. Analyses limited to stage 4 lesions may be misleading. This is the case because the limited data for the other stages of lesions demonstrate that shedding consistently occurs for stages other than stage 4. This implies that the present data and analyses are unlikely to adequately represent the actual distribution of shedding. In the absence of data representing the actual distribution of the time until the cessation of shedding, the claims based upon viral shedding are not supported by clear statistical evidence.

2) Statistical Reviewer's Analyses

Introduction

As discussed above, the applicant's analysis raised a number of statistical issues. One such issue is the estimation of the treatment effect in the presence of missing data. The application contains a number of analyses which suggest that if it is assumed that subjects missing data are treated the same for both treatment arms, there is still a statistically significant reduction in the duration of healing. In the following, estimates of the treatment effect are presented which estimate the overall treatment effect by explicitly setting the treatment effect to zero for subjects missing data (rather than implicitly as was done in the NDA by assigning the same value to individual subjects for both treatment arms). As was mentioned previously, the applicant's use of the maximum+1 for imputation had a disproportionate impact upon the estimated treatment effect. The estimates presented below reduces the impact of this group of subjects.

The application presents analyses for the duration of pain which are based only upon subjects with at least partial data. The imputation strategies, with the exception of the assignment of the maximum+1, were not carried out for subjects with missing data. In the following, subjects with missing data for the duration of pain are incorporated into the analysis using the same approach as will be introduced for the duration of healing analyses.

Duration of Critical Lesions

The following table contains an accounting of subjects with respect to the duration of healing. This table shows subjects with incomplete follow-up as well as the distribution for duration for subjects with complete data. The distribution of subjects does not exactly match that presented earlier in the description of the study results. A number of subjects have been reclassified based upon the data set provided by the applicant. Ten subjects previously listed as having a recurrence with no therapy (out of the 147 such subjects) have been reclassified as lost to follow-up because they were known to have had a recurrence, but the data file indicates that they never returned for a clinic visit rather than never started therapy. Therefore, the treatment status has been treated as unknown for these subjects and they have been classified as lost to follow-up in the analysis. Additionally, 3 subjects were mistakenly listed in the NDA as having incomplete follow-up when they actually had complete follow-up.

Study 024: Distribution of the Duration of Lesion by Treatment for the ITT Population

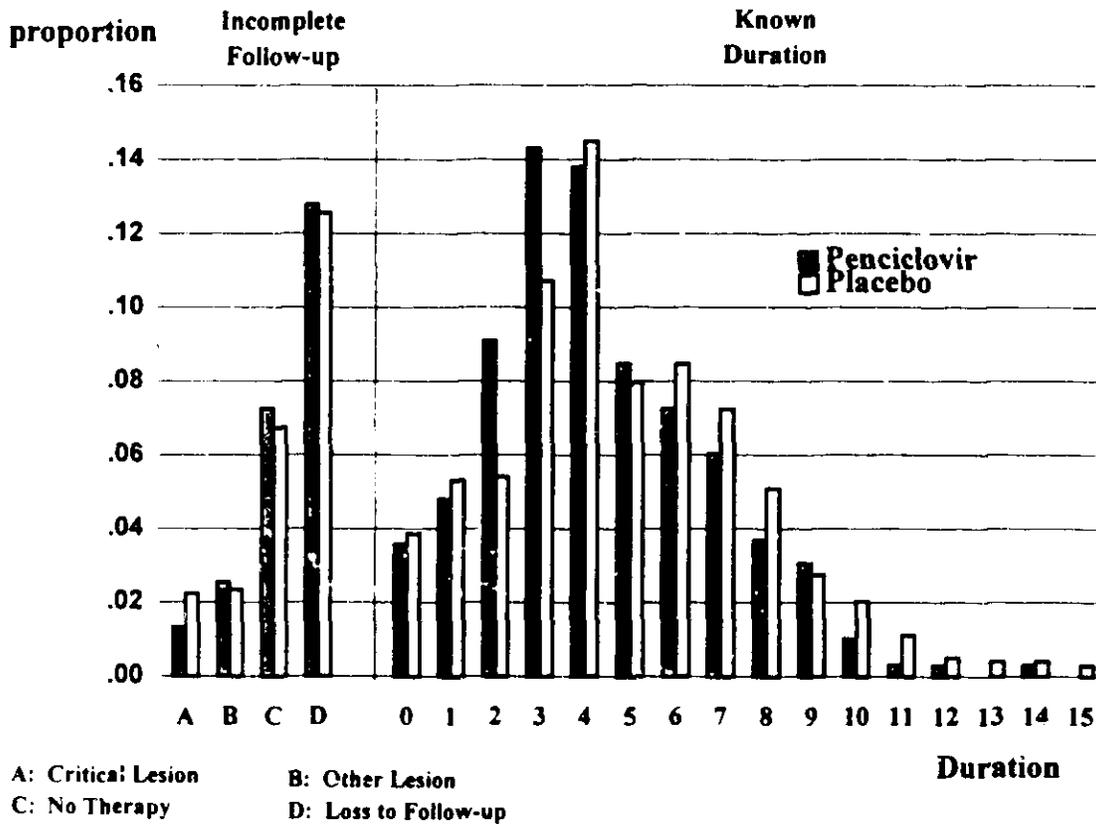
	Penciclovir	Placebo
Total Subjects	978	980
No therapy	71	66
Incomplete Follow-up	163	168
Lost to Follow-up*, Other	125	123
Critical Lesion**	13	22
Other Lesion	25	23
Known Duration (days)	744	746
0	35	38
1	47	52
2	89	53
3	140	105
4	135	142
5	83	78
6	71	83
7	59	71
8	36	50
9	30	27
10	10	20
11	3	11
12	3	5
13	0	4
14	3	4
15	0	3

*includes subjects with recurrence, but no other follow-up

**assigned value of 16 in the applicant's analysis

The information contained in this table is graphed below. This graph contains the proportion of subjects with each outcome displayed in the table. It can be seen that over 20% of subjects have incomplete follow-up. Of subjects with complete data, the distribution for duration can be seen to be concentrated at around 3 or 4 days for both treatments. There is a shift toward 0 for penciclovir relative to placebo with noticeably more subjects at 2 and 3 days for penciclovir than for placebo. As discussed previously, the comparison of mean (or median) duration is complicated by the amount of missing data. Based upon the distribution of duration, it can be seen that the amount of missing data is relatively large in comparison to the shift seen for subjects with data.

Study 024



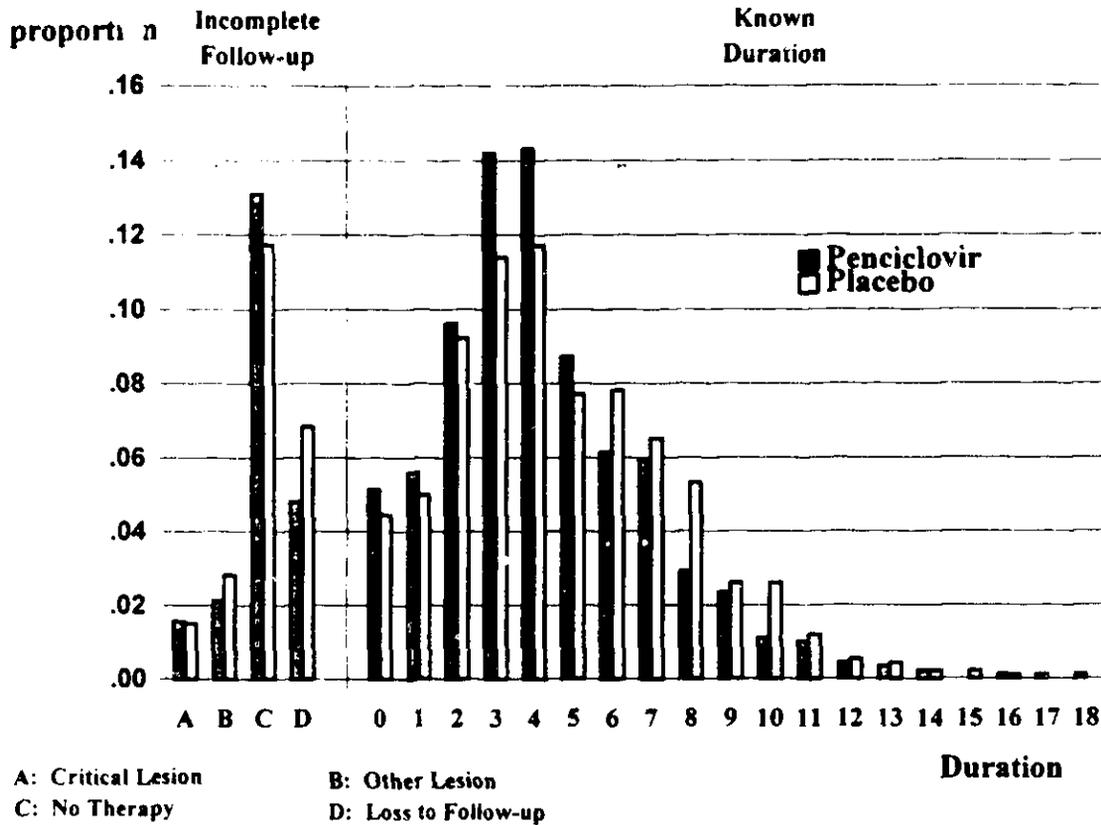
Similar results are presented below for study 025.

Study 025: Distribution of the Duration of Lesion
by Treatment for the ITT Population

	Penciclovir	Placebo
Total	894	921
No therapy	117	108
Incomplete Follow-up	76	103
Lost to Follow-up*	43	63
Critical Lesion	14	14
Other Lesion	19	26
Known Duration (days)	701	710
0	46	41
1	50	46
2	86	85
3	127	105
4	128	108
5	78	71
6	55	72
7	53	60
8	26	49
9	21	24
10	10	24
11	9	11
12	4	5
13	3	4
14	2	2
15		2
16	1	1
17	1	
18	1	

*includes subjects with recurrence, but no other follow-up

Study 025



The applicant presented a number of analyses attempting to compensate for the missing data. For each of these analyses, subjects with unknown duration had a value of the maximum+1 imputed. For the other types of missing data, sensitivity analyses were conducted based upon imputing a number of different values for the missing data. These sensitivity analyses were conducted both for the ITT as well as the known treated populations. The following table lists the important subgroups formed on the basis of degree of follow-up and the imputation strategies employed by the applicant.

Subgroup	Strategy	
	Known Treated	ITT
Started Therapy:		
Incomplete Follow-up Critical Lesion	Impute Max+1	Impute Max+1
Incomplete Follow-up No Critical Lesion	Sensitivity*	Sensitivity*
No Known Therapy		
Loss to Follow-up, Other	Exclude	Sensitivity*
No Therapy	Exclude	Sensitivity*

*sensitivity=various imputation strategies

In this manner, the applicant provided a number of estimates of the treatment effect for both the known treated and the ITT populations. The applicant does not identify a single estimate as being the "optimal" one. In the following, a method of producing a single treatment effect estimate is introduced. As mentioned previously, it appears that for the present application the assumption of no treatment effect for subjects not initiating therapy is most appropriate. Rather than following the applicant's approach of implicitly introducing a treatment effect of 0 by imputing the same value for each subject missing data, the proposed approach will algebraically introduce zero treatment effects for each subgroup as a whole. In this manner, a single overall treatment effect in the presence of missing data is produced.

For the moment, ignore subjects with incomplete follow-up and those lost to-follow-up. The remaining subjects either initiated therapy or were known to have had a lesion but chose not to initiate therapy. Algebraically, the overall estimate of the treatment effect can be shown to be a weighted combination of the treatment effects in these two groups. The following table shows the means for each of the subgroups formed on the basis of treatment group and treatment status (i.e. follow-up status). In this table, the means and sample sizes are taken over strata (center).

Subgroup	Penciclovir		Placebo		Total	
	n	mean	n	mean	n	mean
Treated	N_{11}	\bar{x}_{11}	N_{12}	\bar{x}_{12}	$N_{1.}$	$\bar{x}_{1.}$
Not Treated	N_{21}	\bar{x}_{21}	N_{22}	\bar{x}_{22}	$N_{2.}$	$\bar{x}_{2.}$
Total	$N_{.1}$	$\bar{x}_{.1}$	$N_{.2}$	$\bar{x}_{.2}$	$N_{..}$	$\bar{x}_{..}$

The means for the ITT population for penciclovir and placebo are represented by $\bar{x}_{.1}$ and $\bar{x}_{.2}$, respectively. It can be seen that these means are made up of the weighted combination of the subgroup means. For example, the ITT mean for penciclovir can be written as

$$\bar{x}_{.1} = [N_{11}\bar{x}_{11} + N_{21}\bar{x}_{21}] / N_{.1}$$

This can be seen to be a combination of the known mean for the treated subgroup and the unknown mean (i.e. clinical data not collected) for the untreated subgroup.

Even though the overall means for the ITT population can't be estimated from the present data, it may be possible to estimate the treatment effects (i.e. difference between the treatment means) by making a number of modest assumptions. Given the double blind nature of the study, it may be safely assumed that if the untreated subjects had been followed with respect to critical lesions, the penciclovir and placebo groups in this subgroup would have had comparable outcomes since neither treatment was taken. This implies that the treatment effect for the untreated subgroup is 0 (i.e., means for the untreated groups are equal). To use this assumption, the overall treatment effect can be approximated by the average of the subgroup treatment effects:

$$\bar{x}_{.1} - \bar{x}_{.2} \approx [N_{1.}/N_{..}][\bar{x}_{11} - \bar{x}_{12}] + [N_{2.}/N_{..}][\bar{x}_{21} - \bar{x}_{22}]$$

This quantity is an approximation because the sample sizes assigned to each treatment are slightly unequal. These differences are so small that the above approximation will be quite good. Therefore,

since it is assumed that the second difference is equal to 0, the estimated ITT treatment effect reduces to the following which is a function of the known treatment means

$$\bar{x}_{.1} - \bar{x}_{.2} \approx d = [N_1 / N_{..}] [\bar{x}_{11} - \bar{x}_{12}] .$$

This is a proportionate reduction in the treatment effect for the treated subjects based upon the proportion of subjects initiating therapy.

In calculating a test of significance, the applicant adopted a randomization test in which permutations were made within center conditioning on the number of subjects observed for each treatment arm. An approximation (i.e., not using an exact test) to this test can be found by the use of the stratified, permutation variance. To adapt this approximate test to the above estimator of the treatment effect, the following estimator of variance can be used:

$$V[d] = [N_1 / N_{..}]^2 V[\bar{x}_{11} - \bar{x}_{12}] ,$$

where the stratified, permutation variance is used for the variance of the difference. Note that this variance contains no contribution for subjects for which the treatment effect has been set to zero.

The StatXact software used by the applicant is written in terms of totals rather than means. To maintain consistency with the computer software used by the applicant, the variance of the difference has been written in terms of the variance of the simple total over subjects for the penciclovir group. The permutation variance for the difference can be written as a function of the variance of the total found over strata:

$$V[\bar{x}_{11} - \bar{x}_{12}] = \left(\frac{N_{1.}}{N_{11}N_{12}} \right)^2 V[T] ,$$

where T is the total used to conduct the test of significance (i.e., the sum of the durations for one of the treatment arms). The variance of T following this approach is

$$V[T] = \sum_{i=1}^m \frac{N_{11i}N_{12i}}{N_{1.}} S_i^2 ,$$

where i indicates the stratum (i.e., center), m is the number of strata and S_i^2 is the variance for the i-th stratum found by pooling the two treatment groups.

As was mentioned previously, the above formula have been developed for subjects classified as either having complete data or never started therapy. The above approach can be modified to take into account subjects with incomplete follow-up and those lost to follow-up. In these analyses, the treatment effect is explicitly set to 0 for all subjects lacking complete follow-up regardless of treatment status and the stage of the lesion. This approach is consistent with the applicant's imputation strategies in which the treatment effect is implicitly set to 0 by using the same value for penciclovir and placebo. The main cause of the numerical difference between the results from this approach and the applicant's approach is that the applicant assigned a value of the maximum+1 to more penciclovir than placebo subjects. The analyses presented in the following remove the bias in favor of penciclovir introduced by imputing the maximum+1.

A distinction is made between subjects lacking complete follow-up because medication was never started versus other subjects who started therapy and are lacking follow-up. For subjects never starting therapy it is being assumed that the true treatment effect is 0. Because of this there is no

variance associated with the estimated treatment effect. For the remaining subjects, it can not be assumed that the underlying treatment effect is truly 0. Instead, a treatment effect can not be estimated and for the purpose of estimating the overall mean the treatment effect has been treated as 0. Since variability would have been present if data were available, this treatment effect of 0 will be treated as an estimate and variability will be introduced into the overall estimate of the treatment effect based upon the variance exhibited by subjects with data.

The definition of the treated population as used by the applicant raises the issue of how to treat subjects for which the treatment status is unknown. The exclusion of these subjects by the applicant makes the "known treated" analyses open to possible bias. For this reason, subjects with unknown treatment status will be included in the "treated" subgroup with an estimate of 0 treatment effect. The following table summarizes the two populations for which estimates will be produced.

Subgroup	Treatment Effect
ITT	
Complete Follow-up	Average Diff.
Incomplete Follow-up Critical Lesion	0
Incomplete Follow-up No Critical Lesion	0
Loss to Follow-up, Other	0
No Therapy	0
"Treated"	
Known No Therapy	Exclude

To estimate the average treatment effect, the following table is created over strata with the first subscript referring to the treatment and the second referring to the subgroup. In keeping with the permutation framework adopted by the applicant, the overall numbers are treated as constants and the placebo quantities can be found by subtraction.

Subgroup	Penciclovir			Overall	
	Sample	Total	V(Total)	Sample	Total
Full Follow-up	N_{11}	T_{11}	V_{11}	$N_{.1}$	$T_{.1}$
Incomplete Follow-up	N_{12}	unknown	unknown	$N_{.2}$	unknown
Known No Treatment	N_{13}	unknown	unknown	$N_{.3}$	unknown
Total	$N_{1.}$	unknown	unknown	$N_{..}$	unknown

Based upon this table, the overall estimate of the treatment effect for the ITT population is estimated as:

$$d_{ITT} = w_1 \left(\frac{T_{11}}{N_{11}} - \frac{T_{\cdot 1} - T_{11}}{N_{\cdot 1} - N_{11}} \right) + w_2(0) + w_3(0),$$

where $w_1 = N_{\cdot 1} / N_{\dots}$, $w_2 = N_{\cdot 2} / N_{\dots}$, and $w_3 = N_{\cdot 3} / N_{\dots}$.

If d_1 and d_2 represent the treatment differences for the first and second subgroups, the variance for the overall treatment effect is

$$V[d_{ITT}] = w_1^2 V(d_1) + w_2^2 V(d_2).$$

The variance for the first difference is the one described earlier, but the variance for the second can not be directly estimated since the required data has not been collected. Assuming that the unit variance is the same in groups 1 and 2, the unit variance from group 1 can be substituted into the formula for the variance using the following:

$$V(d_2) = \left(\frac{N_{\cdot 2}}{N_{12}(N_{\cdot 1} - N_{12})} \right)^2 \sum_{i=1}^m \frac{N_{12i}(N_{\cdot 2i} - N_{12i})}{N_{\cdot 2i}} S_i^2,$$

where the i -th subscript refers to the i -th stratum and the variance comes from the i -th stratum for first subgroup. As this approach is based upon a conditional permutation test, all centers for which both treatments are not represented within a subgroup are deleted. The number of subjects affected by this rule will be discussed as the formula are applied.

Study 024

The following table contains the duration data for all subjects included in the ITT population.

Subgroup	Penciclovir			Overall	
	Sample	Total	V(Total)	Sample	Total
Full Follow-up	744	3208	2531.64	1490	6811
Incomplete Follow-up*	157	-	-	320	-
Known No Treatment**	69	-	-	128	-
Total	970	-	-	1938	-

*Excludes 11 subjects in strata without both treatments

**Excludes 9 subjects in strata without both treatments

Based upon the numbers contained in the above table the estimated treatment effect is

$$\begin{aligned} d_{ITT} &= .77(.52) + .17(0) + .07(0) \\ &= .39. \end{aligned}$$

The corresponding variance for this difference is

$$V[d_{ITT}] = .77^2(.0182) + .17^2(.0783)$$

$$= .0129$$

which corresponds to a standard error of .1135. The mean treatment effect in the applicant's analysis for the ITT population range from .50 to .52 (based upon the data set provided by the applicant using the median, first quartile and third quartile). The difference between the estimates provided by the applicant and the present analysis arise primarily from not assigning the maximum +1 in the present analysis.

Excluding subjects known to have never begun treatment, the estimated treatment effect is

$$\begin{aligned} d_T &= .82(.52) + .18(0) \\ &= .42. \end{aligned}$$

The corresponding variance for this difference is

$$\begin{aligned} V[d_T] &= .82^2(.0182) + .18^2(.0768) \\ &= .0148 \end{aligned}$$

which corresponds to a standard error of .1215. The mean treatment effect provided by the applicant for the known treated population varies from .61 to .64. The difference between the estimates provided by the applicant and the present analysis come from both not assigning the maximum+1 and the inclusion of subjects of unknown treatment status.

Study 025

The following table contains the duration data for all subjects included in the ITT population

Subgroup	Penciclovir			Overall	
	Sample	Total	V(Total)	Sample	Total
Full Follow-up*	701	2968	2625.84	1409	6289
Incomplete Follow-up**	70			167	
Known No Treatment***	110			214	
Total	881			1790	

- *Excludes 2 subjects in strata without both treatments
- **Excludes 12 subjects in strata without both treatments
- ***Excludes 11 subjects in strata without both treatments

Based upon the numbers contained in the above table the estimated treatment effect is

$$d_{ITT} = .32.$$

The corresponding variance for this difference is

$$V[d_{ITT}] = .0149$$

which corresponds to a standard error of .1220.

Excluding subjects known to have never begun treatment, the estimated treatment effect is

$$d_T = .36.$$

The corresponding variance for this difference is

$$V\{d_T\} = .0192$$

which corresponds to a standard error of .1386.

2) Analysis of Pain

Study 024

The following table contains the distribution of subjects, excluding those known not have had a lesion, with respect to the ascertainment of pain for study 024. In the applicant's analysis, subjects with any diary data were used in the analysis of duration. Those with partial data were scored as having the maximum+1 and those with no pain were set to 0. There are 4 more such subjects for penciclovir than placebo which will tend to decrease the treatment effect. Subjects with no diary data were excluded from the analysis.

Patient Disposition with Respect to Pain Measurements

	Penciclovir	Placebo
Total	978	980
Subgroup		
Used Diary		
Complete Data	679	692
Partial Data	48	44
No Pain	39	39
No Diary Data		
No Diary Entry	16	16
Lost to Follow-up	84	84
No Treatment	76	71
Other	36	34

Estimates of the overall treatment effect for the duration of pain can be constructed by treating the treatment effect as 0 as was done for the duration of healing. For the intent to treat population, all subjects with partial data or no data will be treated as having a treatment effect of 0. This leads to an estimated treatment effect for the duration of pain of

$$.43 = .58 \frac{679 + 39 + 692 + 39}{978 + 980}$$

Excluding subjects known to have not started treatment the mean duration becomes

$$.46 = .58 \frac{679 + 39 + 692 + 39}{902 + 909}$$

These estimates are quite close to the estimate contained in the NDA of approximately .46 (Table 19, V. 1.110) found using only subjects with at least partial data. The closeness of the ITT and

applicant's analysis using only a subset of the subjects reflects the competing operation of two factors. The first is the assignment of the maximum+1 in the applicant's analysis which works against penciclovir since there are four more subjects assigned the maximum+1 for penciclovir. The second factor is the inclusion of subjects with completely missing data as having a treatment effect of 0. When these two factors are simultaneously taken into account to form the ITT estimates above, the net effect is to produce an estimate similar to that contained in the original submission.

Study 025

The following table contains the same type of data as was just presented for study 024. It can be seen that there is an imbalance in the number of subjects with partial data. This suggests that the applicant's analysis of the treatment effect may be biased since it is based upon assigning these subjects the maximum+1.

Patient Disposition with Respect to Pain Measurements

	Penciclovir	Placebo
Total	894	921
Subgroup		
Used Diary		
Complete Data	582	585
Partial Data	34	43
No Pain	52	54
No Diary Data		
No Diary Entry	14	14
Lost to Follow-up	38	44
No Treatment	122	127

Estimates of the overall treatment effect for the duration of pain can be constructed by treating the treatment effect as 0 as was done for the duration of healing. For the intent to treat population, all subjects with partial data or no data will be treated as having a treatment effect of 0. This leads to an estimated treatment effect for the duration of pain of

$$.34 = .48 \frac{582 + 52 + 585 + 54}{894 + 921}$$

Excluding subjects known to have not started treatment the mean duration becomes

$$.39 = .48 \frac{582 + 52 + 585 + 54}{780 + 782}$$

These estimates are in contrast to the estimates of approximately .6 (Table 20, V. 1.136) found using only subjects with at least partial data. The difference between the applicant's estimate of the treatment effect (.6) and the estimate based upon subjects with complete data (.48) reflects the impact of the assignment of the maximum+1 for more subjects on the placebo arm.

D. Statistical Reviewer's Overall Assessment

The applicant is seeking an indication for topical penciclovir based upon a statistically significant improvement in healing, pain and viral shedding. Based upon preNDA discussions between the DAVDP and the applicant, it was agreed that healing would be considered the primary outcome and pain and viral shedding would be viewed as secondary. This implies any claims based upon pain and viral shedding would simultaneously require that topical penciclovir be associated with an improvement in healing. In this manner, the need for adjustments for multiple endpoints was eliminated for the duration of healing. It was further agreed at these meetings that healing would be summarized primarily in terms of the duration of healing which was defined as the number of days an investigator recorded any of the following lesion stages: papules, vesicles/pustules, ulcers/soft crusts and hard crusts.

The following table summarizes the applicant's analyses for the duration of healing as well as the additional analyses conducted for this review. For ease of presentation, only the applicant's imputation based upon the median are shown. The differences between the treatments based upon the imputation of the median are consistent with the other imputation techniques. It can be seen that penciclovir is associated with a reduction in the mean duration of healing for both the applicant's and additional analyses regardless of the population considered. The major difference between the applicant's and additional analyses is in terms of the magnitude of the estimated treatment difference between penciclovir and placebo. The applicant's estimate of the treatment effect for study 024 in particular is an overestimate due to the applicant's use of the maximum+1 for a number of subjects. Additionally, the additional analysis treated subjects missing data as if the treatment effect was 0 leading to a likely underestimate of the treatment effect. This suggests that the additional analysis is likely to have produced a lower bound on the estimate of the result which would have been obtained if all subjects had been followed with respect to duration of healing.

Mean Duration of Healing

	Study 024		Study 025	
	Difference Placebo - Penciclovir	95% C.I.**	Difference Placebo - Penciclovir	95% C.I.**
Applicant				
Treated	.7	***	.5	***
ITT	.5	***	.4	***
FDA				
Treated*	.39	.17-.61	.36	.08, .64
ITT	.42	.18-.66	.32	.08, .56

*includes subjects lost to follow-up

**based upon test inversion using permutation variance

***not available

The results for the duration of pain are consistent with the results for the duration of healing. There is approximately an average 1/2 day decrease in the amount of time with recorded pain. These results are summarized below. The applicant did not conduct imputation analyses for this variable. As such, the applicant's results contained in the table include only subjects with complete or partial data. Subjects with partial data were set to a value of the maximum+1 in the applicant's analyses.

Treatment Effect (Mean/days) for Patient Assessed Pain

Study 024	Study 025
Placebo - Penciclovir	Placebo - Penciclovir
.5	.6

The applicant's analyses for the cessation of shedding are based upon only a small subset of days with a lesion. Only subjects with stage 4 lesions were consistently cultured though it is apparent that shedding occurs consistently for other lesion stages. As such, the applicant's results are inadequate to sufficiently characterize viral shedding to allow for meaningful statistical comparisons between the treatment groups.



Paul Flyer, Ph.D.
Mathematical Statistician

Concur: Dr. Kammerman *JK 9/17/96*

cc:

Archival NDA # 20-629

HFD-530

HFD-104/Ms. Sage (via team links)

HFD-530/Dr. Freeman (via team links)

HFD-530/Dr. Chikami

HFD-530/Dr. Behrman

HFD-530/Mr. Mahoney

HFD-725/Dr. Kammerman

HFD-725/Dr. Flyer

HFD-725/Dr. Harkins

HFD-725/Ms. Shores

HFD-344/Dr. Lisook

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This review contains 27 pages.

CLINICAL PHARMACOLOGY BIOPHARMACEUTICS REVIEW

SEP 13 1996

NDA: 20-629

REVIEWER: Barbara M. Davitt, Ph.D.

DRUG: DENAVIR™ (penciclovir cream 1%)

SUBMISSION DATE: 10/16/95, 8/2/96

APPLICANT: SmithKline Beecham

REVIEW DRAFT: 6/24/96, 7/1/96, 8/9/96

TYPE: NME

REVIEW FINAL: 9/13/96

SUMMARY: The applicant provided adequate information to enable the evaluation of pharmacokinetics of penciclovir administered topically in a 1% cream (DENAVIR™) to healthy subjects. Findings from one *in vivo* and one *in vitro* study, both of which studied the to-be-marketed cream formulation, suggest that penciclovir is not systemically absorbed when administered topically as the 1% cream. Penciclovir could not be detected in plasma and urine in a study in which the 1% cream formulation was applied to tape-stripped skin of healthy male volunteers (N = 12) at a daily dose 67 times greater than the recommended daily dose. In the *in vitro* study, in which the 1% cream containing [¹⁴C]penciclovir was applied to human intact and tape-stripped skin samples, distribution of penciclovir into the stratum corneum, epidermis and dermis was low (< 1%), with almost all the recovered radioactivity (> 99%) found on the skin surface. Drug-drug interactions of topical penciclovir due to metabolism or to displacement from plasma proteins are considered unlikely (see Background section below). The clinical pharmacology of topical penciclovir has not been studied in pediatric patients, pregnant women, or nursing mothers, and it is recommended that this be stated in the product label. Results of studies characterizing the pharmacokinetics of IV-administered penciclovir were submitted to NDA 20-363 and are summarized in the Clinical Pharmacology/Biopharmaceutics (CPB) review dated 5/31/94.

BACKGROUND: The following information is summarized from the CPB review of NDA 20-363 and from Studies 39123/005 and 007 which were submitted to NDA 20-629. When administered by the IV route, penciclovir pharmacokinetics were linear over the range 0.5 to 20 mg/kg. The volume of distribution was approximately 1 L/kg, suggesting distribution into a volume exceeding total body water (0.6 L/kg). In the rat, the highest tissue concentrations of [¹⁴C]penciclovir were found in kidney and urinary bladder. *In vitro* protein binding of penciclovir was low in human plasma; protein binding averaged about 10% at penciclovir concentrations ranging from 2 to 20 µg/mL. Findings from *in vivo* and *in vitro* studies suggest that penciclovir undergoes very little metabolism and is rapidly eliminated, primarily by the renal route. Penciclovir had no effect on hepatic drug metabolizing activity in the rat, and was not a substrate or inhibitor of human cytochrome p450 3A (CYP3A) *in vitro*. In a mass balance study, in which a single 5 mg/kg dose of [¹⁴C]penciclovir was given IV to healthy male subjects (N = 3), no major metabolites of penciclovir were detected in plasma or urine. Mean total recovery of radioactivity in urine and feces was 97% over 192 hr. The urine was the major route of elimination, representing a mean of 94% of the radiolabeled dose, and 87-100% of the urinary radioactivity was due to parent penciclovir. Penciclovir total clearance was 0.5 L/hr/kg, with renal clearance accounting for 87% of total clearance. The elimination half-life was about 2 hr.

Studies 39123/005 and 007 have not previously been reviewed but will not be reviewed at this time for the following reasons: (1) Penciclovir pharmacokinetics have previously been reviewed in the CPB review of NDA 20-363; and (2) it appears that penciclovir is not topically absorbed when administered as the 1% cream.

RECOMMENDATION: The Human Pharmacokinetics and Bioavailability Section of NDA 20-629 has met the requirements of the Code of Federal Regulations 320.

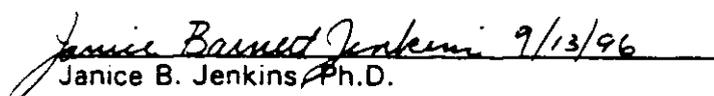
Biopharm Day: July 8, 1996

Participants: Drs. Nick Fleischer, Mei-Ling Chen, Vinod Shah, John Lazor, Mehul Mehta, Janice Jenkins, and Mark Seggel

 9/13/96

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Concurrence:

 9/13/96

Janice B. Jenkins, Ph.D.
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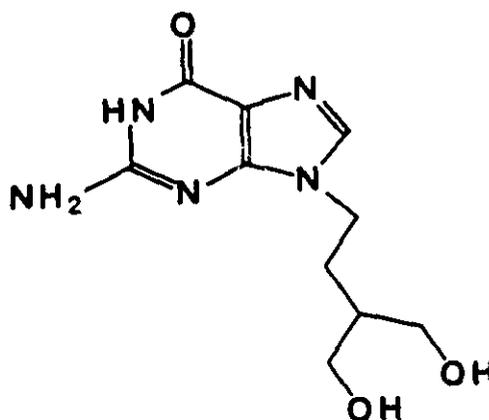
cc:	HFD-530	NDA 20-629 /MO/GChikami /CSO/JMahoney
	HFD-340	/Vishwanathan
	HFD-205	/FOI
	✓HFD-880	/DPEIII/DivFile /DPEIII/JJenkins /DPEIII/BDavit
	✓HFD870	/Drug Files/CBott (Parklawn 13B-31)

REVIEW

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I. CHEMISTRY

Chemical Name (CAS):	6H-purin-6-one, 2-amino-1,9-dihydro-9-[4-hydroxy-methyl]butyl]
IUPAC Name:	9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine
Molecular Formula:	C ₁₀ H ₁₃ N ₅ O ₃
Molecular Weight:	253.26
Structure:	



pH and pK_a Values:	3.2 and 9.8 (by spectrophotometric titration at 25°C) The pH of a saturated aqueous solution of penciclovir (< 2 mg/mL) was 5.6.
Solubility:	At 20°C: Methanol: 0.2 mg/mL 1,2-propanediol: 1.3 mg/mL pH 2.1 buffered solution: 10.0 mg/mL pH 6.9 buffered solution: 0.9 mg/mL pH 9.6 buffered solution: 1.7 mg/mL At 37°C: pH 7.0 buffered solution: 3 mg/mL
Partition Coefficient:	n-octanol water (phosphate buffer) partition coefficient at pH 7.5 was 0.024 (logP = -1.62). Determined by shake-flask method at room temperature.
Melting Point:	Penciclovir melts at 278°C with decomposition.

Polymorphism: Penciclovir has been recrystallized from a number of solvents: dimethylacetamide, dimethylformamide, acetic acid, water, and an aqueous solution of the sodium salt precipitated with CO₂. Only one crystalline anhydrous form of penciclovir has been detected. When precipitated from a slightly alkaline aqueous solution, a monohydrate with a moisture content of 6.6% water was produced. Drying the monohydrate at temperatures > 60°C produced a mixed amorphous/anhydrate phase which slowly converted to pure anhydride on storage (by 15 days). Penciclovir prepared by the proposed process is always in the anhydrate form (routinely monitored by IR spectroscopy and water content).

Hygroscopicity: Penciclovir is not hygroscopic. No increase in moisture content was observed after 6 months storage at 75% relative humidity, 30°C.

Potential Isomerism: Structural isomerism is a possibility. Penciclovir has the side chain attached at the 9-position of the purine ring. The 7-substituted version can also exist. However, this impurity has not been shown to be present in any batches to date.

II. FORMULATION:

The formulation is a cream containing 1% penciclovir in a white cream base (10 mg penciclovir per gram cream). A full batch size will be approximately sufficient for

The composition of a penciclovir cream (based on a fill weight as appropriate) is:

Name of Ingredient	% w/w	2 g Tube Unit Composition (mg)	2 g Tube Working Ranges	5 g Tube Unit Composition (mg)	5 g Tube Working Ranges	Function	Specification
Active constituent							

Penciclovir is manufactured, tested for release and stability at the following SmithKline Beecham Pharmaceuticals facility: SmithKline Beecham Pharmaceuticals, Clarendon Road Worthing, West Sussex BN14 8QH, United Kingdom

III. INDICATIONS AND USAGE:

The product is indicated for the treatment of cold sores (herpes labialis).

IV. DOSAGE AND ADMINISTRATION:

The product is supplied in 2 and 5 gram tubes containing 10 mg penciclovir per gram. Apply once every 2 hours during waking hours for a period of 4 days.

V. PHARMACOKINETICS:1. *IN VIVO*

Study 39123/108: AN OPEN STUDY TO DETERMINE THE PERCUTANEOUS ABSORPTION OF PENCICLOVIR THROUGH ABRADED SKIN FOLLOWING APPLICATION OF REPEAT DOSES OF PENCICLOVIR CREAM (1% W/W) TO HEALTHY VOLUNTEERS (Volume 1.31, pp 1-464)

Objective: The objective of this open, uncontrolled study was to determine the percutaneous absorption of penciclovir through abraded skin following application of repeat doses of penciclovir cream (1% w/w).

Subjects: Healthy male volunteers (N = 12). See Appendix I for individual demographics.

Drug Formulation: Batch No. W93017, Formula J, Lot No. GBD 40 (fa) batch size, manufactured 3/93. See Appendix I for composition. The composition of Batch No. W93017/W031 is identical to that of the to-be-marketed formulation, with the exception that penciclovir used in the clinical program and will not be used for production of a full batch. A full batch size is The formulation was:

Formula Code J

Active Constituent: (% w/w)

Study Design: The study involved 4 successive dosing days. On each dosing day the subjects received 3 topical applications of 6.0 mL penciclovir cream, each 8 hours apart. The cream was applied via one non-absorbant patch (24 cm²) to abraded skin on the upper back. The total daily dose was 180 mg of penciclovir. The upper dorsal skin was abraded prior to the first application on Days 1 and 3 by tape-stripping until the skin showed a moist glare; skin was wiped clean with a moist tissue prior to each application.

Sampling: Blood and urine samples for pharmacokinetic analysis were taken following the first topical application on Days 1 and 4. Blood sampling times were predose and at 1, 2, 4, 8, 16 and 24 hr post-dosing. Urine samples were taken from 0-4, 4-8, 8-16, and 16-24 hr post-dosing.

Assay:

On Days 1 and 4, concentrations of penciclovir in all plasma and urine samples from all 12 subjects were below the See Appendix I for penciclovir concentrations in individual samples. It was assumed that since systemic penciclovir is predominantly renally excreted (91% of the IV-administered penciclovir dose was excreted in urine as parent drug), the amount of drug determined in urine reflects absorption of penciclovir. If urinary concentrations of penciclovir had reached the of the analytical assay, systemic absorption of penciclovir in individual subjects would have ranged from of the administered dose. See attached individual urine volume data.

At the reviewing Medical Officer's request, this reviewer estimated the absolute bioavailability of 180 mg penciclovir applied topically as three divided daily doses. If plasma concentrations of penciclovir had reached the of the analytical assay at all sampling times, the absolute bioavailability of topically applied penciclovir would be This value was calculated in the following manner. First, data from Study 30123/007 (Entitled "Study to investigate the pharmacokinetics and tolerance to the antiviral compound BRL 39123 following its administration, by IV infusion, to human volunteers", Vol 1.32 of NDA 20-629) were used to estimate a cumulative AUC_{0-24h} of about 9 µg*hr/mL for 60 mg penciclovir given q8h. Next, assuming that the plasma concentration of penciclovir was at all sampling times, an AUC_{0-24h} of 2.45 µg*hr/mL was calculated for the topically-administered penciclovir in Study 30213/108. The bioavailability of the topical dose was taken as the AUC_{0-24h} ratio of topical:IV. There were a number of assumptions underlying these calculations: (1) the relationship between AUC and dose is linear over IV penciclovir doses ranging from (2) penciclovir concentrations at all time periods following topical administration were µg/mL; and (3) penciclovir pharmacokinetic data are consistent across studies.

The topical penciclovir dose recommended in the package insert is From the urinary excretion data, it can be estimated that this regimen would result in a daily exposure of of penciclovir. From the AUC data, it is estimated that the daily exposure to penciclovir would be The two estimates agree well, although the estimates are based on a number of assumptions. Note that these estimated exposures are

many-fold lower than the daily penciclovir exposure estimated in response to the recommended oral famciclovir (penciclovir prodrug) regimen for herpes zoster

Conclusion: Penciclovir could not be quantified in plasma and urine following application as a topical cream (1%, w/w) to abraded and occluded skin of 12 healthy volunteers at a daily dose 67 times greater than the recommended daily dose.

Table 11.1

DEMOGRAPHY DETAILS OF STUDY BRL39123/108

Sub No	Sex	Age (Yrs)	Height (cm)	Weight (kg)	Race
001					
002					
003					
004					
005					
006					
007					
008					
009					
010					
011					
012					
	Mean	32	180	76.0	
	SDev	7	4	7.2	
		12	12	12	
		24	175	66.0	
	Max	53	189	93.0	

. = No data available

[DEMOG_STD_P:V2.3]

[02FEB95 06:55]

Table 11.15

Table 11.15: Concentrations of penciclovir (ug/mL) in plasma samples collected on days 1 and 4 following repeated topical administration of penciclovir cream (1% w/w) for 4 days

Collection period (h)	Subject number											
	1	2	3	4	5	6	7	8	9	10	11	12
Day 1 Predose												
Day 1 1h												
Day 1 2h												
Day 1 4h												
Day 1 8h												
Day 1 16h												
Day 1 24h												
Day 4 Predose												
Day 4 1h												
Day 4 2h												
Day 4 4h												
Day 4 8h												
Day 4 16h												
Day 4 24h												
Day 4 48h												

NC: Not Quantifiable, concentrations below the lower limit of quantification (0.10 ug/mL)

Table 11.16

Table 11.16: Concentrations of penciclovir (ug/mL) in urine samples collected on days 1 and 4 following repeated topical administration of penciclovir cream (1% w/w) for 4 days

		Subject number											
Day	Collection period (h)	1	2	3	4	5	6	7	8	9	10	11	12
1	0-4												
1	4-8												
1	8-16												
1	16-24												
4	0-4												
4	4-8												
4	8-16												
4	16-24												

NQ: Not Quantifiable, concentrations below the lower limit of quantification (10 ug/mL)

Table C1 : Concentrations of penciclovir (ng/mL) determined in plasma QC samples

Batch	Nominal 0.25ng/ml.	Nominal 2.5ng/mL	Nominal 8.0ng/ml.
PB-01	0.22	2.47	8.04
	0.25	2.46	7.67
PB3	0.27	2.59	8.97
	0.26	2.57	8.07
PB-04	0.27	2.53	7.60
	0.26	2.46	7.98

Table C2 : Concentrations of peniclovir (ug/mL) determined in urine QC samples

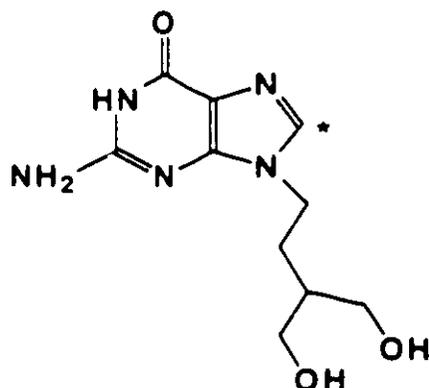
Batch	Nominal 10ug/mL	Nominal 400ug/mL	Nominal 1000ug/mL
U01	8.16	401.44	1052.13
	10.04	361.96	990.63
U02	10.12	350.05	1000.24
	10.98	363.69	970.94

2. IN VITRO

Study BF-1019/B.L.-039123/1: *IN VITRO* PERCUTANEOUS ABSORPTION AND CUTANEOUS DISTRIBUTION OF [¹⁴C]BRL 39123 ACROSS INTACT AND TAPE-STRIPPED HUMAN SKIN (Vol. 1.38, pp 120-148)

Objectives: Evaluation of the percutaneous absorption and distribution of [¹⁴C]BRL 39123 (in a 1% w/w cream formulation) across intact and tape-stripped skin *in vitro*. This study was performed for the applicant by a

Formulation: The penciclovir cream (1% w/w) was identical to that of the to-be-marketed formulation. added to the formulation was from Lot No. 50457-66. Specific activity was Radiochemical and chemical purities were respectively. The location of the [¹⁴C] label with respect to the penciclovir chemical structure is shown below by the asterisk:



Study Design: Cryopreserved human skin was obtained from the organ procurement center. Twelve skin samples were tested. All samples were dermatomed to a thickness of approximately 250 μ m, then half of the dermatomed skin samples (N = 6) were tape-stripped to partially remove the stratum corneum layer. Several 11 mm diameter disks were made from each skin sample with a cork boring tool. Skin disks were mounted into Bronaugh flow-through diffusion cells with the epidermal surface facing the upper chamber (Reference 1). The space below the skin contained receptor media set to a flow rate of approximately 1.5 mL/h. Samples were topically dosed with 0.1 g of cream to provide 1 mg of [¹⁴C]penciclovir per disk, and incubated for up to 24 hr.

Assay: For all samples, residual applied cream, skin layers, and receptor fluid collections were assayed for total radioactivity by

Sampling: Receptor fluid was collected at 0.5, 1, 2, 3, 4, 6, 8, and 24 hr intervals during the 24-hr incubation period in order to determine the absorption of drug-related material. The distribution of drug-related material through the skin layers was determined after 0.5, 3, 6, and 24 hr of incubation.

Data Analysis: Percutaneous absorption data, flux rates across skin, and amounts of drug-related material in the skin layers were calculated. For calculations, the following assumptions were made: (1) epidermal thickness of 0.0045 cm; (2) dermal thickness of 0.0135 cm; (3) each layer had a density of $1\text{g}/\text{cm}^3$. Percutaneous absorption data were expressed as percentages of recovered dose. Flux rates across the skin were expressed as μg -equivalents of penciclovir/ cm^2/hr . Amounts of drug-related material present in the skin were calculated both in terms of percentages of recovered dose and concentrations in μg -equivalents of penciclovir/g.

Results: Tables of mean \pm S.D. values for flux rates, elimination into receptor fluid, distribution into dermis and epidermis, and percentage of dose in the various skin layers are presented in Appendix I. The percutaneous absorption flux rate (Table 1, attached) was greater in tape-stripped skin than in intact skin. The total permeation of radioactivity through both intact and tape-stripped skin was low; of the recovered radioactivity, respectively, were found in the receptor fluid over the 24 hr period (Table 2, attached). Concentrations of drug-related material remained relatively constant during the first 6 hr after penciclovir cream application, but had increased by 24 hr (Table 3, attached). At all times investigated, the concentrations were higher in the epidermal and dermal layers of tape-stripped skin than in those of intact skin. However, the total amounts of drug-related material relative to dose were low in all of the skin layers and almost all of the recovered radioactivity (>99%) was found on the skin surface in the recovered cream (Table 4, attached).

Conclusion: Distribution of penciclovir into the epidermis and dermis was low *in vitro* following topical application of [^{14}C]penciclovir (1% w/w) cetomacrogol base cream to intact and tape-stripped human skin. Almost all the recovered radioactivity (>99%) was found on the skin surface. Penciclovir concentrations in the various skin layers and penetration of penciclovir through skin were higher in tape-stripped skin than in intact skin.

Table 1: Mean flux rates of drug-related material at various times after application of [¹⁴C]BRL 39123 cream to intact and tape-stripped human skin *in vitro*

Time period (Hours)	Flux rate (ug/cm ² /h)	
	Intact	Tape-Stripped ^d
0-0.5	< 0.001	0.067 ± 0.059
0.5-1	0.002 ± 0.003	0.097 ± 0.114
1-2	0.003 ± 0.004	0.099 ± 0.099
2-3	0.004 ± 0.003	0.092 ± 0.091
3-4	0.006 ± 0.006	0.085 ± 0.065
4-6	0.013 ± 0.017	0.118 ± 0.078
6-8	0.018 ± 0.029	0.175 ± 0.117
8-24	0.018 ± 0.031	0.244 ± 0.248

Values are means ± standard deviation (N = 6).

Table 2: Mean elimination of radioactive material into receptor fluid at various times after application of [¹⁴C]BRL 39123 cream to intact and tape-stripped human skin *in vitro*

Timepoint (Hours)	Percentage of recovered radioactivity in receptor fluid	
	Intact Skin	Tape-Stripped Skin
0.5	< 0.01	0.02 ± 0.04
1	0.01 ± 0.01	< 0.01
3	0.02 ± 0.02	0.01 ± 0.01
6	0.01 ± 0.02	0.03 ± 0.03
24	0.02 ± 0.04	0.31 ± 0.24

Values are means ± standard deviation (N = 6).

Table 3: Mean distribution of radioactive material in epidermal and dermal layers of intact and tape stripped human skin samples *in vitro* at various times following application of [¹⁴C]BRL 39123 cream

Timepoint (Hours)	Concentration (ug equivalents of [¹⁴ C]BRL 39123/g of skin layer)			
	Epidermis		Dermis	
Intact Human Skin				
0.5	8.58 ±	5.95	8.07 ±	4.17
1	7.87 ±	5.05	2.27 ±	1.42
3	22.25 ±	20.84	7.40 ±	8.78
6	11.47 ±	8.46	5.99 ±	4.07
24	67.32 ±	36.65	22.31 ±	14.86
Tape-Stripped Human Skin				
0.5	37.13 ±	49.03	33.03 ±	66.22
1	8.76 ±	3.00	3.11 ±	1.87
3	31.69 ±	14.43	11.06 ±	11.90
6	30.01 ±	16.16	10.02 ±	6.68
24	390.90 ±	348.81	217.43 ±	333.45

Values are means ± standard deviation (N = 6).

Micrograms of [¹⁴C]BRL 39123 per gram of skin were determined by calculating the micrograms of [¹⁴C]BRL 39123 per cubic centimeter of each skin layer and assuming the density of each layer was 1 g per cm³.

Table 4: Amounts of radioactive material on the surface and in layers of intact and tape-stripped human skin *in vitro* at various times following application of [¹⁴C]BRL 39123 cream

Timepoint (Hours)	Percentage of recovered radioactivity			
	Surface	Stratum Corneum ^a	Epidermis	
			Dermis	
	Intact Human Skin			
0.5	99.957 ± 0.021	0.033 ± 0.018	0.002 ± 0.001	0.006 ± 0.003
1	99.959 ± 0.027	0.031 ± 0.027	0.002 ± 0.001	0.002 ± 0.001
3	99.892 ± 0.090	0.075 ± 0.073	0.008 ± 0.008	0.008 ± 0.010
6	99.875 ± 0.058	0.101 ± 0.058	0.003 ± 0.002	0.006 ± 0.004
24	99.872 ± 0.056	0.066 ± 0.023	0.019 ± 0.012	0.020 ± 0.014
	Tape-Stripped Human Skin			
0.5	99.923 ± 0.093	0.027 ± 0.024	0.009 ± 0.011	0.024 ± 0.056
1	99.975 ± 0.007	0.019 ± 0.006	0.002 ± 0.001	0.002 ± 0.002
3	99.883 ± 0.044	0.080 ± 0.033	0.010 ± 0.004	0.011 ± 0.010
6	99.883 ± 0.062	0.068 ± 0.048	0.011 ± 0.005	0.011 ± 0.008
24	99.293 ± 0.419	0.097 ± 0.047	0.117 ± 0.105	0.180 ± 0.254

Values are means ± standard deviation (N = 6).

^a Values obtained by tape-stripping of intact skin and further tape-stripping of tape-stripped skin (where the stratum corneum was partially removed)

VI. IN VITRO RELEASE:

In-vitro release studies were conducted during product development using a Franz cell system with artificial membranes as described by Shah, V.P. et al (Reference No. 2).

Introduction: This method was developed to determine the *in vitro* release rate (steady-state flux) of penciclovir from the 1% (w/w) topical cream formulation, formula code J.

Method: The *in vitro* diffusion system consisted of 12 Franz cells with magnetic stirrers, maintained at 32°C using a temperature controlled circulating bath. The receptor phase was distilled water. A Celgard 3500 membrane was cut into 3x3 cm squares. From 150-200 mg of cream was applied to a 1.7 cm² area on each membrane. The cell was assembled and the release test initiated. Receptor phase was sampled at 10 minute intervals. A 0.5 mL sample was taken at each interval, and replaced with distilled water. Penciclovir concentrations in the receptor were determined by

Sampling intervals: 10, 20, 30, 40, 50, 60 minutes.

Calculations: The release rate of penciclovir from the formulation was defined as the slope of penciclovir released per cm² of membrane (Q) plotted against time^(x):

$$\text{Penciclovir } (\mu\text{g}) \text{ in receptor cell} = \text{Penciclovir (Precp}(n)\text{)content } (\mu\text{g/mL}) * \text{Volume of the receptor cell (mL)}$$

Penciclovir released per cm² of membrane taking into account the dilution of the receptor phase during the sampling procedure:

$$\text{Penciclovir (in } \mu\text{g/mL)} = [(Precp(n)) + (Precp(n-1) * 0.5/Vol) + (Precp(n-2)*0.5/Vol)]$$

Where: Precp(n) = Penciclovir (μg) in the receptor cell at time n
Vol = Volume of the receptor cell

Alternatively, the following calculation is used to account for dilution of the receptor phase as part of the sampling procedure:

$$C_{recep@n} = C_{recep@n-1} + [C_{sam(n)} - C_{sam(n-1)} * (Vol-0.5/Vol)]$$

$$\text{Penciclovir released per cm}^2 \text{ of membrane} = (C_{recep@n} * Vol) / 1.77$$

Where:

C_{recep@n} = Concentration of penciclovir in receptor phase ($\mu\text{g/mL}$) at time n
C_{sam(n)} = Concentration of penciclovir ($\mu\text{g/mL}$) at time n (Assay value)
Vol = Volume of the receptor cell

Results:**Penciclovir 1% w/w Cream: *In-Vitro* Release**

<u>Cream Batch</u>	<u>Penciclovir Flux</u> ($\mu\text{g}/\text{cm}^2/\text{min}^{1/2}$)
Lab Scale	
Batch Ref 35727-136	18.3
Pilot Scale (clinical supply)	
after 36 months at 20-25°C:	
Batch W92007	18.1
Batch W92054	17.7
Batch W92056	21.6
Production scale (process qualification)	
after 6 months at 20-25 °C:	
Batch 275010	17.9
Batch 275040	17.4
Batch 275060	21.4
after repeated freeze/thaw cycling:	
Batch 275010	21.7
Batch 275060	23.0

The 1%w/w penciclovir cream is formulated such that the aqueous phase is saturated with drug. Penciclovir solution concentration in the aqueous phase is ca. 0.6% w/w, equivalent to ca. 0.4% w/w in the cream as a whole. At a strength of 1%w/w, there is sufficient undissolved penciclovir present to ensure the penciclovir concentration remains at saturation, thus optimizing the thermodynamic activity of penciclovir which drives the percutaneous penetration process.

The applicant stated that scale up of the manufacturing process was accompanied by small increases in the particle size of the undissolved penciclovir. The above data show similar release rates for batches at all scales of manufacture and for production scale batches after freeze/thaw cycling (largest particle size), suggesting that the release rate is not affected by the differences in the particle size of undissolved penciclovir due to scale-up or by Ostwald ripening. The applicant proposed that a specification for penciclovir particle size in the cream is unnecessary based on the above data and in view of the consistent particle size of undissolved penciclovir produced by the cream manufacturing process.

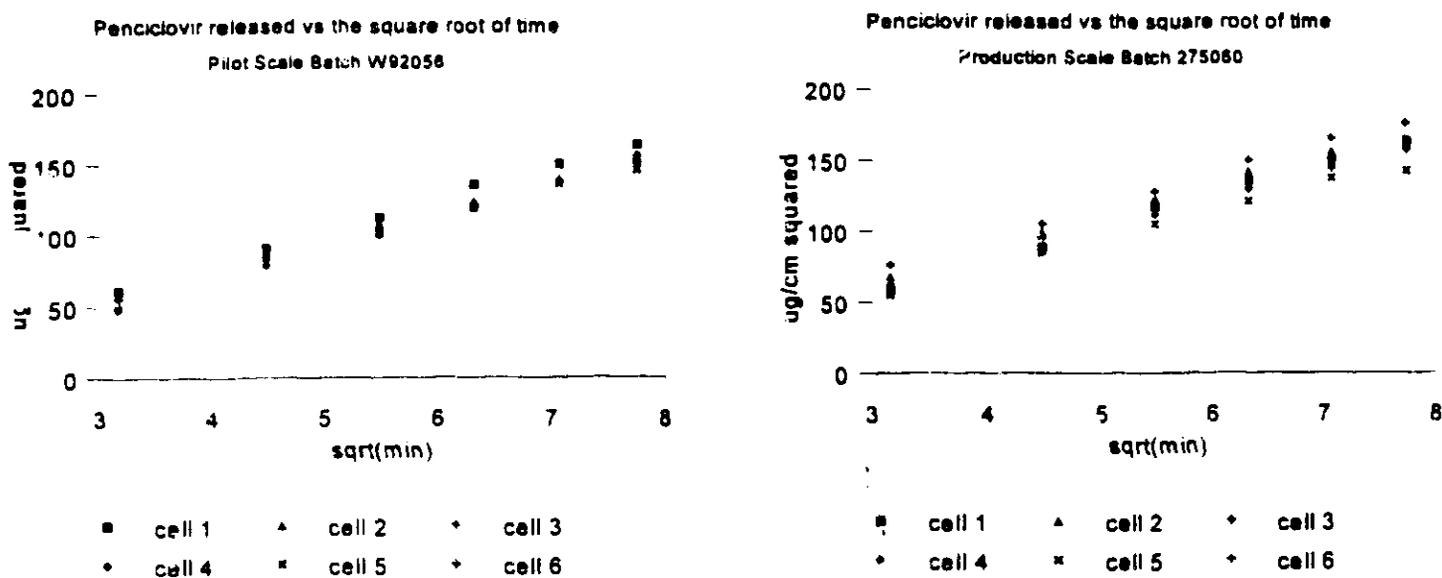
Pivotal clinical efficacy studies 39123/024 and 39123/025 used product manufactured via micronized penciclovir, added to the oil phase. For large scale production, this requirement is avoided by complete dissolution of penciclovir during compounding. The use of micronized or unmicronized penciclovir during manufacturing did not affect the *in vitro* penciclovir release rate or the particle size (assessed by microscopy) of the undissolved penciclovir in the final product.

Therefore, the use of micronized or unmicronized penciclovir did not affect the drug availability from a 1% w/w cream. The Reviewing Chemist, Dr. Mark Seggel, concurred that drug availability from the cream will not be affected by micronization.

The applicant concluded from the particle size and *in-vitro* release data that there is no significant difference in drug availability between the clinical and the proposed commercial product. This reviewer concurs with the applicant's conclusion.

Individual release data and plots of Q vs time and Q vs the square root of time (attached) were requested from the applicant in writing on 6/21/96 and received by the agency on 6/28/96. These data are located in Appendix I.

Following are two representative plots of Q vs time^{1/2}, one from a pilot batch and one from a production scale batch:



VII. ANALYTICAL METHODOLOGY:

The Study Reports BF1013/BRL-039123 /1 (for the assay of penciclovir in plasma) and BF1016/BRL-039123/1 (for the assay of penciclovir in urine) have previously been reviewed and summarized in the CPB review of NDA dated 9/25/95. Assay sensitivity, linearity, precision, and accuracy were acceptable to this reviewer.

VIII. REFERENCES:

1. Bronaugh RL, Stewart RF, Simon M. Methods for *in vitro* percutaneous absorption studies VII: use of excised human skin. *J. Pharm. Sci.*, 1986, 75,

1094-1097.

2. Shah VP, Elkins JS, Williams RL. In vitro drug release measurement for topical glucocorticoid creams. *Pharmacoepial Forum*, 1993, 19, 551-563.

IX. LIST OF STUDIES NOT REVIEWED:

The following studies were submitted to NDA 20-629 but not reviewed:

Study No.: 39123A/009 (HP/87/21)

Study to investigate the tolerance to three intravenous doses of BRL 39123A when administered at 8 hourly intervals over a period of 24 h to human volunteers. Volume 1.36, page 000246

Study No.: 39123A/007 (HP/86/69)

Study to investigate the pharmacokinetics of and tolerance to the antiviral compound BRL 39123A following its administration by intravenous infusion to human volunteers. Volume 1.32, page 000002

Study No.: 39123A/011 (HP/87/42)

Study to investigate the pharmacokinetics of and tolerance to the antiviral compound BRL 39123 following its administration by intravenous infusion to human volunteers. Volume 1.32, page 000161

Study No.: 39123A/017/BP/001/

A study to compare the pharmacokinetics of BRL 39123 in healthy elderly male subjects with the pharmacokinetics in healthy young male subjects following administration of a single intravenous dose of BRL 39123A at 5 mg pfa/kg. Volume 1.33, page 000002

Study No.: 39123A/053

A randomised, double blind, placebo controlled crossover study to assess the dose proportionality of pharmacokinetic parameters of penciclovir following a single intravenous infusion of 2.5, 5.0 and 7.5 mg/kg and to assess the cardiovascular effects of penciclovir and a single intravenous dose of acyclovir 10 mg/kg in healthy male and female volunteers. Volume 1.34, page 000002

Study No.: 39123A/008 (HP/86/146R)

Study to investigate the pharmacokinetics of and tolerance to the antiviral compound BRL 39123A following its repeat administration by intravenous administration to human volunteers. Volume 1.36, page 000002

Study No.: 39123A/010 (HP/87/22)

Study to investigate the pharmacokinetics of and tolerance to the antiviral compound BRL 39123A following its repeat administration by intravenous infusion to human volunteers. Volume 1.36, page 000333

X. LABELING COMMENTS:

The following comments refer to the draft annotated labeling of Oct. 10, 1995. Comments were conveyed to the applicant in writing on 6/24/96. The draft label of 9/6/96 is attached. In all cases, the applicant corrected the text to be consistent with the following recommendations.

p 6, Clinical pharmacokinetics

CHANGE section to read: "Measurable penciclovir concentrations were not detected in plasma or urine of healthy male volunteers (N = 12) following single or repeat application of the 1% cream at a dose of 180 mg penciclovir daily (approximately 67 times the estimated usual clinical dose)."

DELETE THE REMAINDER OF THIS SECTION

p 6, Pediatric patients

CHANGE section to read: "The systemic absorption of penciclovir following topical administration of *NAME* has not been evaluated in patients < 18 years of age."

p 9, Carcinogenesis, mutagenesis, impairment of fertility

CHANGE first sentence to read: "Measurable penciclovir concentrations were not detected in plasma or urine of healthy male volunteers following topical administration of the 1% cream (see Clinical Pharmacokinetics)."

p 9, Pregnancy

DELETE THE FIRST SENTENCE

p 10, Nursing mothers

DELETE THE FIRST SENTENCE

p 11, OVERDOSAGE

CHANGE first sentence to read: "Since penciclovir is poorly absorbed following oral administration, adverse reactions related to penciclovir ingestion are unlikely."

XI. PROPOSED LABELING:

See the attached following pages. As of this writing, the most current proposed package insert is the draft of 9/6/96.

- FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE PUBLIC.

Pharmacology Review of NDA 20-629

DENAVIR

(Penciclovir 1% Cream for the treatment of Herpes Labialis)

April 1996

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20629

2 OF 3

PHARMACOLOGIST'S REVIEW

NDA: 20-629

Date Submitted: 16 Oct. 1995
Date Review Assigned: 16 Oct. 1995
Date Review Completed: 18 April 1996
Reviewer: David E. Morse, Ph.D.
HFD-530

SPONSOR: SmithKline Beecham Pharmaceuticals
1 Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101 (215) 751-3868

DRUG: BRL 39123 (Penciclovir)¹
6H-Purin-6-one, 2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]

STRUCTURAL FORMULA:
CAS #: 3 09-25-1
EMPIRICAL FORMULA: C₁₀H₁₄N₅O₃
MOLECULAR WEIGHT: 253.26
SOLUBILITY: ~3% w/w in H₂O (20°C at pH 7)



BRL39123

FORMULATION: 1% cream for topical use
INDICATION: Treatment of Herpes Labialis

RELATED INDs/NDAs:
IND
IND
NDA 20-363 - Famciclovir (Oral)

DEFINITIONS:²

INTRODUCTION

The sponsor is requesting approval to market Penciclovir 1% cream (DENA VIR®) for the topical treatment (every 2 hours for 4 days) of Herpes Labialis infection in non-immunocompromised patients. Both pre-clinical and clinical safety data were included in the submission of Oct. 1995. The subsequent sections of this document include a review of the submitted toxicology data. The proposed product label for topical penciclovir (DENA VIR®) is presented in a separate memorandum.

BACKGROUND

Penciclovir is a guanine nucleoside analogue, and is the major 'activated' form of drug found in systemic circulation following the oral administration of famciclovir (FAMVIR®; see NDA 20-363, approved 1994). BRL 39123, following entry into infected cells, is phosphorylated by viral and cellular encoded thymidine kinases into the corresponding mono-, di-, and tri-phosphate nucleotides. It exerts its antiviral activity through interference with viral DNA synthesis. The initial phosphorylation reaction is purported to be accomplished only by the viral induced thymidine kinase of herpes infected cells.

¹ CAS Registry Number: CAS-39809-25-1

² BRL 39123 - Penciclovir, BRL 42810 - Famciclovir

NON-CLINICAL TOXICOLOGY SUMMARY

The toxicology and oncogenicity of penciclovir and famciclovir were evaluated in a series of acute through chronic administration studies in mice, rats, dogs and/or monkeys. In addition, studies of potential adverse genetic, reproductive, immunologic and dermal irritancy were performed. A brief summary of each of the major study areas follows, while detailed reviews of each topic area are included in the Appendices (A-H) of this document.

Summary of the General Toxicology of Penciclovir

Penciclovir (BRL 39123) was evaluated in several toxicity studies (1-90 days) in the mouse, rat, dog and monkey. Study results indicate that when administered intravenously in the rat, dog and monkey, the primary toxicities of BRL 39123 appear related to local vascular/perivascular irritation, the kidneys, testes, thymus/spleen, and the heart and lungs. Toxic effects were generally dose related, although there was some indication of idiosyncratic responses and increased sensitivity to damage among animals treated at low doses. Mild to moderate inflammatory responses, occasionally including signs of hemorrhage, oedema, and myositis, were evident at the site of injection in multiple species. Histologic changes in the kidney appeared to be associated with changes in clinical chemistries and urinalytic parameters, and thus appear to be monitorable. In the heart and lungs, the intravenous administration of penciclovir appeared to be associated with coronary artery pathology, acute changes in ECG waveforms (reductions in P-R and Q-T intervals), tachycardia, and periplueral effusions. In the testes, histopathologic changes generally included degeneration of the seminiferous tubules and hypospermatogenesis. Involution of the thymus and splenomegaly were noted but were generally unrelated to changes in circulating lymphoid cell characteristics. Lastly, minimal to moderate decreases in several hematologic parameters including hematocrit, hemoglobin and red blood cell counts were sometimes associated with the administration of penciclovir. Most of the responses appeared to regress following cessation of drug administration.

See Appendix A for a detailed review of the submitted toxicology studies for penciclovir.

Summary of the Dermal Irritancy Potential of Penciclovir

Multiple toxicity studies were conducted to evaluate the dermal, ocular and intravaginal irritancy potential, and contact sensitization effects of penciclovir cream. In summary, rats, rabbits and/or guinea pigs were exposed to one or more applications of penciclovir cream (of various formulations) to shaved regions (abraded and unabraded) of the dorsum, into the vagina or, to the eye/conjunctiva. In general, the application of any of the penciclovir containing creams caused minimal to mild dermal irritation responses with occasional edema at the site of application. However, only slightly less severe and/or similar reactions were evident in most test systems following the application of any of the placebo/vehicle "drug" formulations. Intravaginal application of penciclovir was found to be non-irritating when tested in the rabbit. In contrast, application of the test compound to the conjunctivae of the rabbit eye, produced mild-moderate redness and chemosis (inflammation) in all animals at 1 and 24 hours post-exposure with resolution of these effects by 48-72 hours.

See Appendix B for a detailed review of the submitted dermal irritancy studies for penciclovir.

Summary of the Immunotoxic Potential of Penciclovir

Penciclovir was tested in multiple in vivo and in vitro assays for immunotoxic and/or immunostimulant/hypersensitization effects. In the rat, despite a slight degree of splenomegaly and thymic atrophy evident after 28 days of IV dosing, the study results did not indicate that BRL 39123A caused any immune system suppression. In the guinea pig, the repeated administration of penciclovir cream (proposed market formulation and multiple investigatory pre-market formulations) failed to elicit a dermal hyper-sensitization response. Also in the guinea pig and rat, 'sensitized' animals showed no evidence of anaphylaxis when challenged with either penciclovir or famciclovir. In vitro studies of penciclovir and/or famciclovir showed only minimal interaction/covalent binding between either compound and human serum proteins or albumin.

See Appendix C for a detailed review of the submitted immunotoxicology studies for penciclovir.

Summary of the Reproductive Effects of Penciclovir

The sponsor has conducted standard Segment I-III reproductive toxicology studies in the rat and rabbit. Results of the studies revealed minimal or no effects of intravenous penciclovir on fetal abnormalities and viability, multigenerational development and reproduction, reproductive performance of treated animals and, fertility of treated females. In contrast, chronic dosing studies conducted with famciclovir (see NDA 20-363, review date June 1994) showed that long-term exposure resulted in significant adverse effects on the male reproductive organs (including testicular atrophy and seminiferous tubule degeneration), and induced profound adverse effects on sperm production and morphology. Furthermore, with long-term famciclovir exposure fertility among treated animals was significantly decreased and pre-implantation losses were increased. The recovery of sperm production appeared related to the time off-dose, with approximately equal numbers of animals showing full, partial or no recovery of sperm production after multiple (approx. 13 cycles) spermatogenic cycles. Similar testicular and hypospermatogenic effects have been noted in animals with other members of this chemical class, however, these effects have not been demonstrated in human patients.

See Appendix D for a detailed review of the reproductive toxicology studies conducted with penciclovir.

Summary of the Oncogenic Potential of Penciclovir

Penciclovir, either as the topical or intravenous product has not been evaluated for carcinogenic potential. However, famciclovir (the oral pro-drug form of penciclovir) has been tested in rodents and found to increase the incidence of mammary adenocarcinomas in female rats (a common tumor in aging female rats), without significantly altering other tumor frequencies. Drug exposure in the female rat was approximately 1.5x human exposure for the oral product when used in accordance with the product label (maximum recommended human dose of 500 mg t.i.d.; inter-species dose comparisons based on 24 hr AUC). Given the short duration and low level of exposure to penciclovir after topical application of the cream product (4 days dosing; estimated applied dose of 0.05 mg/kg/day), it appears unlikely that the use of penciclovir topical cream (DENA VIR®) poses a significant risk of increased tumors in man. In accordance with 21 CFR 201.56 (d)(3) and 21 CFR 201.57 (f)(6), it is recommended that the 'systemic carcinogenicity' data for famciclovir not be included in the product label for topical penciclovir (DENA VIR®), since no absorption of penciclovir following topical dosing has been demonstrated (see the Biopharmacology Review for NDA 20-629).

See Appendix E for a discussion of the oncogenic potential of penciclovir (and famciclovir).

Summary of the Genetic Toxicology of Penciclovir

Penciclovir was evaluated for potential genotoxic effects in a standard battery of assays, which included: a) assays for unscheduled DNA synthesis in HeLa cells in vitro, b) bacterial and mammalian cell gene mutation assays in vitro and, c) in vitro and in vivo assays for chromosomal aberrations. The results of several assays suggest that penciclovir has some clastogenic activity, although these effects were generally coincident with signs of cellular toxicity and thus suggest that the responses might be due to non-specific cellular effects and not direct DNA-drug interaction. Similarly, the increased mutation rate noted in the mouse lymphoma assay occurred at relatively high drug concentrations which inhibited cell replication and growth.

See Appendix F for a detailed review of the genetic toxicology studies conducted with penciclovir.

Summary of the General Pharmacology of Famciclovir

Penciclovir was tested in a battery of standard in vivo and in vitro bioassays to determine the general pharmacologic effects of the compound. Study data indicate that penciclovir was relatively inactive in the production of central or peripheral nervous system effects, hematologic or metabolic effects except at high dose levels. Primary responses noted following the bolus intravenous administration of penciclovir were generally confined to transient cardiovascular effects (prolongation of the P-R and Q-T intervals) and, renal tubular injuries caused by insolubility.

See Appendix G for a detailed review of the general pharmacology studies conducted with penciclovir.

PHARMACOKINETICS AND MOLECULAR PHARMACOLOGY

Summary of the Pharmacokinetics and ADME Profile of Penciclovir

The sponsor has conducted multiple pharmacokinetic and ADME (absorption, distribution, metabolism and excretion) studies with penciclovir in support of the NDA. Similar studies were previously reported for famciclovir (BRL 42810: the oral pro-drug form of penciclovir) and have been reviewed in NDA 20-363, June 1994. Following from the chemical similarity of the compounds and the extensive co-development/testing of these drugs, the following PK/ADME summary includes information for both compounds. A brief summary of the findings from these studies is included in the following paragraphs.

General Background: BRL 39123 (Penciclovir) is a substituted nucleoside analogue of guanine. It is suggested that the drug is selectively phosphorylated intracellularly to the mono-, di- and triphosphate, by virally and cellularly encoded thymidine kinases. The initial phosphorylation reaction is purportedly accomplished only by the viral induced thymidine kinase of herpes infected cells. BRL 39123 tri-phosphate, it is suggested, exerts its antiviral activity through incorporation in the herpes virus genome and/or through interference with viral DNA synthesis. Penciclovir is inactive prior to conversion to BRL 39123 tri-phosphate.

Percutaneous Absorption: Multiple studies of the percutaneous absorption of penciclovir (some using the market formulation for the topical cream) were

conducted in rats, rabbits and in vitro. These studies included both intact and abraded skin systems. In rodents, when [¹⁴C] penciclovir cream was applied to intact skin, the majority of the administered radioactivity (approx. 88% (+1.6)) was retained at the application site at the end of the 24 hour exposure period. Urine, faecal and carcass levels of radioactivity were all below the limit of reliable detection (< 0.01% of the administered dose). Total recovery of radioactivity was approximately 92%. In contrast, when applied (6 hours) to the abraded skin of rabbits there was an average 3-5% percutaneous absorption. However, as with intact skin, the majority of the drug product was retained on/in the skin of the application site and on the dressing materials. Small, but measurable, amounts of radio-activity (and by inference - BRL 39123) were retained in the skin of the application site and were still being excreted in the urine and faeces of each rabbit at the end of the 96 hour follow-up period.

In an in vitro test system using 'intact' or tape-stripped human skin, the study results suggested that transport of BRL 39123 was increased approx. 3-fold by the removal of the stratum-corneum layer of the skin. Further, the transport and incorporation of radioactivity across the tissue specimens increased in extent during the 24 hour assessment period, for both intact and tape-stripped tissues. Similar to the results obtained in whole animals, the transport of BRL 39123 across the component layers of the skin was low (3-5%), although not as that noted in vitro (0.1% vs. 1-5%). Overall, regardless of the test system used, the percutaneous absorption of penciclovir appears to be relatively low (5% or less of the administered dose).

Oral Administration: Absorption of [¹⁴C]famciclovir following oral dosing in the mouse, rat, dog and rabbit was generally between 50-100% (doses up to 4000, 250 and 1000 mg/kg in the rat, dog and rabbit), with peak plasma levels (C_{max}) occurring approximately 1 hour after dosing in all species. By comparison, the oral availability of penciclovir was somewhat lower at approximately 20-50% of the administered dose. BRL 39123 (penciclovir) was the primary metabolite found in the circulation of all species following the oral administration of famciclovir. The data clearly indicate that famciclovir undergoes substantial first pass metabolism in the liver, and is almost undetectable in the systemic circulation. In the monkey, there was some evidence of the metabolism of penciclovir to the 8-hydroxy derivative (BRL 44072). Overall, the pharmacokinetic profile for famciclovir is actually that for penciclovir, which will be discussed almost exclusively throughout the subsequent paragraphs.

Intravenous Administration: In both the rat and dog, the intravenous administration of 10 mg/kg of BRL 39123 resulted in peak drug levels immediately post-infusion with blood concentrations in the range of 10.9-16.1 ug/ml (mean of 13.8 ug/ml). In the rat, blood drug levels declined in a monophasic manner with a half-life of approximately 20-40 minutes, while in the dog drug levels declined in a biphasic manner with an initial half-life of 25 minutes and terminal half-life of 2.9 hrs. In the dog, $AUC_{(0-t_{inf})}$ values ranged from 29-40.4 ug.hr/ml following a 10 mg/kg IV dose of BRL 39123, with a clearance rate of 3.6-4.2 L/hr., and volume of distribution of 11-15 liters (approximately equal to body weight). The concentration of drug in whole blood was approx. 50% of that in plasma (due to volume dilution), and suggested only limited association between radioactivity and red blood cells (generally 10% or less). No evidence of accumulation of drug related material was observed during repeat dose studies. Peak plasma (C_{max}) levels and systemic exposure (AUC) to penciclovir and its precursors could not be reliably measured during dietary intake of the test compound by rats.

Tissue Distribution: Following the oral administration of [¹⁴C]famciclovir (40 mg/kg) to rats, the tissues with relatively high concentrations of drug

related product included the GI tract, liver, kidneys, thyroid/parathyroid, seminal vesicles, aorta and skin. Relatively low concentrations were observed in neural tissue. Concentrations of radioactivity declined rapidly following the acute or daily administration of [¹⁴C]famciclovir, with little evidence of drug accumulation. A somewhat slower decline in radioactivity was noted in the testes of the rat.

Tissue Distribution-Fetal Drug Exposure: Administration of [¹⁴C]famciclovir to pregnant rats and rabbits demonstrated that placental passage and fetal exposure to drug related material occurred at levels nearly comparable to maternal exposure. In the rat fetus, penciclovir and BRL 42359 (the 6 deoxy precursor of penciclovir) accounted for the majority of the [¹⁴C] radiolabeled material, whereas in the rabbit, BRL 42359 and BRL 48959 were identified as the major metabolites in addition to penciclovir. Penciclovir was rapidly secreted in the milk of lactating rats after an oral dose of [¹⁴C]famciclovir (40 mg/kg). Milk concentrations were considerably higher than those observed in plasma.

Excretion: Following the oral administration of [¹⁴C]BRL 42810 or the intravenous administration of [¹⁴C]BRL 39123A to rats, mice, and dogs, nearly all of the administered radioactivity was recovered in urine and faeces (65-85% and 10-20%, respectively), during the subsequent 72-96 hours. Drug elimination was quite rapid with over 90% of the excreted radioactivity being eliminated within the first 6 or 24 hours. The terminal plasma half-life of penciclovir was estimated at less than 1 hour in the rat and at about 2 hours in the dog. In all species studied, TLC and HPLC analyses performed on urine specimens indicated that >95% of the urinary radioactivity co-eluted with a BRL 39123 control sample. All other radioactive peaks accounted for a small fraction of the urinary radioactivity.

Miscellaneous: Ex vivo studies revealed only low level binding of penciclovir (and its precursors) to plasma proteins from either the rat (11-24%) or dog (12-22%) when tested at concentrations of 2-20 mg/ml. The distribution of drug related material in rat blood was approximately even between the plasma and cells, whereas in the dog there was evidence of nearly complete exclusion of the drug from blood cells (i.e., the drug product was found almost exclusively in the plasma of the dog). In both species, the volume of distribution for drug related materials was estimated at approximately 1 L/kg.

Comparison of Pharmacokinetic and ADME Data in Animals and Man:

In man, as in all species tested, the bioavailability of famciclovir was high (approx. 75%) with oral administration at doses up to 750 mg (approx. 15 mg/kg). Rapid conversion of famciclovir to penciclovir (the predominant drug form detected in the systemic circulation) and BRL 42359 was evident in man, as it had been in the mouse, rat, dog and monkey. This metabolic conversion appears to be mediated by a cytosolic aldehyde oxidase, and is independent of the P450 system. As seen in preclinical evaluations, the drug kinetics in human subjects were dose independent. Renal excretion of penciclovir was the primary route of drug elimination in man, as had been demonstrated in the preclinical animal studies. Plasma clearance of penciclovir was estimated at 0.37 L/h/kg following a 5 mg/kg IV dose, which was similar to that observed in the dog (0.33 L/h/kg) after a 25 mg/kg IV dose and was 1/3-1/7 the rate noted in the rat (0.9-1.7 L/h/kg) following an IV dose of 40 mg/kg. Active tubular secretion of drug related material is suggested in all species evaluated. In man, as in the dog, the estimated plasma elimination half-life of penciclovir was approximately 2 hours. The volume of drug distribution in man was estimated at 1 L/kg (similar to the rat and dog), with plasma protein binding of approximately 6-16%.

In conclusion, when evaluated in multiple test systems, the percutaneous absorption of penciclovir appears to be relatively low (5% or less of the administered dose). Further, the pharmacokinetic and metabolic profiles for penciclovir (and for famciclovir) as determined in multiple animal species suggests strong similarities to the data obtained in man. Together, these findings support the use of the animal toxicology studies conducted with penciclovir and famciclovir for estimating the safety profile of penciclovir when administered to humans.

See Appendix H for a detailed review of the pharmacokinetics and ADME studies conducted with penciclovir.

SUMMARY

The sponsor has requested approval to market penciclovir (DENA VIR®) for the topical treatment (4 days dosing; estimated total applied dose of 0.05 mg/kg/day) of Herpes Labialis sores in non-immunocompromised adults. Both pre-clinical safety and clinical efficacy/safety data were included in the submission. The preceding sections of this document and the attachments include a review of the submitted toxicology data and the proposed product label for (DENA VIR®).

Briefly, pre-clinical safety studies indicate that the primary toxicities associated with intravenous penciclovir are related to local vascular irritation, renal inflammation, testicular atrophy, thymus/spleen involution, and cardiac/pulmonary effects. Intravenous penciclovir appeared associated with coronary artery pathology, acute changes in ECG waveforms (reductions in P-R and Q-T intervals), tachycardia, and periplueral effusions. In general, the toxic responses associated with IV penciclovir appeared to regress following cessation of drug administration. Standard Segment I-III reproductive toxicology studies revealed minimal or no effects of intravenous penciclovir on fetal abnormalities and viability, multigenerational development and reproduction, reproductive performance of treated animals and, fertility of treated females. Study data indicate that penciclovir was relatively inactive in the production of central or peripheral nervous system effects, and hematologic or metabolic effects except at high dose levels.

Results of genotoxicity assays suggest that penciclovir has some clastogenic activity (generally at high doses coincident with signs of cellular toxicity), and increased the rate of mutation in the mouse lymphoma assay. No genotoxic effects were evident in the remaining tests. Penciclovir has not been tested for carcinogenic potential. However, famciclovir has been tested in rodents and found to increase the incidence of mammary adenocarcinomas in female rats. However, based on the short duration of treatment (4 days), the low level of exposure to penciclovir with topical application of the cream product (applied dose of 0.05 mg/kg/day), and the lack of any demonstrated systemic absorption of the drug product (Biopharmacology Review for NDA 20-629), it seems unlikely that penciclovir cream (DENA VIR®) poses a significant risk of increased tumors in man. Therefore, in accordance with 21 CFR 201.56 (d)(3) and 21 CFR 201.57 (f)(6), it is recommended that the 'systemic carcinogenicity' data for famciclovir not be included in the product label for topical penciclovir (DENA VIR®), since no absorption of penciclovir following topical dosing has been demonstrated.

In general, the application of any of the penciclovir containing creams caused minimal to mild dermal irritation responses with occasional edema at the site of application. No evidence of the development of hypersensitivity or contact sensitization responses were noted with the topical drug product.

The proposed product label for topical penciclovir (DENAVID[®]) is presented in a separate memorandum.

CONCLUSIONS

The preclinical toxicity data suggest that it is reasonably safe to approve the marketing of topical penciclovir (BRL 39123; DENAVID[®] 1% cream) for the short-term treatment of Herpes Labialis infection in immunocompetent adults.

RECOMMENDATIONS FOR PHASE IV COMMITMENTS



David E. Morse, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/Dir (Act.) /DFreeman *DF 1/21/6*
HFD-530/SPharm/JGFarrelly *JDF 4/23/96*
HFD-530/Pharm/DMorse

Disk: HFD-530

cc:

HFD-530/NDA 20-363
HFD-530/Division File
HFD-340/
HFD-502/
HFD-530/CSO/JMahoney
HFD-530/Pharm/DMorse
HFD-530/MO/
HFD-530/Chem/MSeggel
HFD-530/Micro/
HFD-345/

SUMMARY OF THE GENERAL TOXICOLOGY STUDIES:

Penciclovir (BRL 39123) was evaluated in several toxicity studies (1-90 days) in the mouse, rat, dog and monkey. Study results indicate that when administered intravenously in the rat, dog and monkey, the primary toxicities of BRL 39123 appear related to local vascular/perivascular irritation, the kidneys, testes, thymus/spleen, and the heart and lungs. Toxic effects were generally dose related, although there was some indication of idiosyncratic responses and increased sensitivity to damage among animals treated at low doses. Mild to moderate inflammatory responses, occasionally including signs of hemorrhage, oedema, and myositis, were evident at the site of injection in multiple species. Histologic changes in the kidney appeared to be associated with changes in clinical chemistries and urinalytic parameters, and thus appear to be monitorable. In the heart and lungs, the intravenous administration of penciclovir appeared to be associated with coronary artery pathology, acute changes in ECG waveforms (reductions in P-R and Q-T intervals), tachycardia, and periplueral effusions. In the testes, histopathologic changes generally included degeneration of the seminiferous tubules and hypospermatogenesis. Involution of the thymus and splenomegaly were noted but were generally unrelated to changes in circulating lymphoid cell characteristics. Lastly, minimal to moderate decreases in several hematologic parameters including, hematocrit, hemoglobin and red blood cell counts were sometimes associated with the administration of penciclovir. Most of the responses appeared to regress following cessation of drug administration.

COMPILATION OF EFFECTS AND DISCUSSION:

Penciclovir (BRL 39123: the di-deacetylated analogue of famciclovir) was tested in several acute through 90 day repeat dose toxicity studies in the mouse, rat, dog and monkey. A summary of the studies submitted to this NDA, along with a general discussion of penciclovir pharmacology and related studies of penciclovir as submitted to the IND is presented below.

BRL 39123A (penciclovir) is a substituted nucleoside analog of guanine and is the di-deacetylated analogue of famciclovir (see NDA 20-363). BRL 39123 represents the predominant drug moiety found in systemic circulation following oral administration of famciclovir.

When administered as a bolus intravenous infusion, the median lethal dose of BRL 39123 in the mouse and rat was 1200 and 700-1,000 mg/kg, respectively. Deaths appeared to be related to an extensive CNS activation, as indicated by seizures, tremor and convulsions. The minimum lethal dose following oral administration was greater than 5,000 mg/kg in both rats and mice. Also noted in these studies was a frequent association of mottled appearing heart and/or lung tissues and peripleural effusions following bolus drug infusions.

Histologic indications of moderate inflammatory responses with mixed cell infiltrates were evident in several monkeys, dogs and rats at the vascular injection site of BRL 39123. Reactions occasionally included signs of hemorrhage, oedema, and myositis. These adverse effects of IV penciclovir administration are likely associated with the high pH (approx. 10.5) of the dosing solutions used in the treatment of the 'high-dose' groups from several of the studies. Similar vein irritation effects have been noted in a clinical trial referenced in this report.

In mature animals of multiple species (mouse, rat, dog and monkey), the kidney appeared as a primary site of toxicity following the repeated IV administration of penciclovir. Histologic evidence of damage included hyperplasia and/or necrosis of the epithelial tissues of the renal tubules and collecting ducts. Occasional dilatation of the renal tubules and renal pelvis

were also evident. Clinical and urine chemistries suggest that significant toxic effects are evident within 1-4 weeks of dose initiation. Further, the toxic effects on the kidney were not clearly dose related, but were in part idiosyncratic (evidenced by the distribution and severity of histologic and urine chemistry changes). Recovery following drug cessation was evident although not always complete.

In several studies there was evidence of extensive crystal (birefringent) formation coincident with tissue damage/necrosis in the renal tubules and collecting ducts. This effect appeared to be related to the dose and possibly also the rate of drug administration. Because of the high rate of renal excretion and limited solubility of BRL 39123 it is possible that the drug concentration in renal glomerular filtrate may exceed the limit of solubility thereby resulting in crystal formation. Furthermore, the formation of drug crystals in the renal tubules is likely to cause physical trauma to the surrounding tissues and promote the histopathologic changes described above.

Testicular atrophy was noted in rats and dogs treated with BRL 39123 for 28 days or longer. These effects had not been seen in the rat when drug was administered for 10 days. Histopathological changes in the testes included degenerative changes in the seminiferous tubules. Partial recovery was evident in some rats following an off-dose recovery period. Testicular and seminiferous tubule atrophy with hypospermatogenesis has been evident with several other nucleoside analogues, and has been associated with significant reductions in fertility (in animals) and occasional sterility (in animals and man).

Atrophy of the thymic gland was noted in rats treated with 40-160 mg BRL 39123/kg/day for 28 days. Similarly, in some studies the intravenous administration of penciclovir induced significant atrophy of the thymus and spleen among male and female dogs treated at 100 mg (pfa)/kg/day and, in thymus weight of female animals treated at 50 mg/kg/day. Reductions in total white cell counts or in several subtypes of white cells were sometimes evident. These data suggest that penciclovir may have specific toxic effects on the immune system; although this effect was not demonstrated in a special immunotoxicity study conducted in rats administered famciclovir (the oral precursor of penciclovir) for 28 days (see Appendix B).

In addition, the administration (IV) of penciclovir appears to cause a significant degree of tachycardia (15-40% increase), and reductions in the P-R (15-23%) and Q-T (10-15%) intervals in multiple species (dog and monkey). In both species, the heart rate and conduction changes were closely associated with drug infusion, generally being evident within 5 minutes and lasting about 30-60 minutes (although decreases in the P-R interval were detected at up to 6 hours following drug infusion [the final assessment interval] in the dog). These effects were evident in multiple dose groups in each study conducted with a sensitive species.

While not specifically evaluated, the rate of drug infusion may effect the heart rate changes observed, as evidenced by the somewhat smaller changes seen in one primate study in which the infusion rate was significantly slowed versus a previous study (i.e., infusion of 2 ml/kg over 7 min. versus an infusion of 2 ml/kg over 6 seconds). The mechanism of the rate enhancing effect of penciclovir, whether direct (receptor interaction) or indirect (sympathetic agonist release or changes in ion levels), is not defined at this time.

Coronary artery pathology (intimal hyperplasia and/or necrosis with inflammatory cell infiltration) was noted in multiple high and intermediate dose treated animals from the 90 day study conducted in dogs. While the sponsor contends that the coronary artery lesions were not drug related, given the

distribution of effected animals in the high and intermediate dose groups and the significant chronotropic effect of IV penciclovir, it is not reasonable to conclude that the cardiac artery lesions were unrelated to the test compound. This potential drug effect warrants further evaluation.

— Lastly, minimal to moderate decreases in several hematologic parameters were evident in multiple species following the repeat administration of intravenous penciclovir, and generally included changes in hematocrit, hemoglobin and red blood cell counts. The lack of any histologic evidence of a change in bone marrow cellularity suggests that hematologic effects of penciclovir may be mediated through a mechanism other than myelosuppression. Most of the responses appeared to regress following cessation of drug administration.

Individual reviews are contained in the following pages.

Toxicity Studies Summary:**Acute Toxicity: 1-7 Days****A) Mouse:**

1) **BRL 39123A: An Acute Study In Mice By The Intravenous Route**, Study ID T85019/39123A/M/IV/A, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 11 July 1985, Compound: BRL 39123A, Batches: GBD1 (86.2% pure).

B) Rat:

2) **BRL 39123A: An Acute Study In Rats By The Intravenous Route**, Study ID T85018/39123A/R/IV/A, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 11 July 1985, Compound: BRL 39123A, Batches: GBD1 (86.2% pure).

3) **BRL 39123A: An Investigative Single-Dose Study In Male Rats By The Intravenous Route**, Study ID T89022/39123A/R/IV /SDS, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 8 June 1989, Compound: BRL 39123A, Batch: GBD18 (86% pfa).

Subchronic Toxicity Studies: Oral**A) Mouse:**

4 and 5) **BRL 39123: A 4 Day Oral Range Finding Repeat Dose Study in Mice**, Study ID T86600/39123/M/PO/RFS/4D, and **BRL 39123: A 7 Day Oral Repeat Dose Study in Mice**, Study ID T86303/39123/M/PO/RDS/7D, GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 17 Jan. and 11 Feb., '86, BRL 39123, batch GBD 16.

B) Rat:

6) **BRL 39123: A 4 Day Oral Range Finding Repeat Dose Study in Rats**, Study ID T85616/39123/R/PO/RFS/4D, GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 29 Jul., '85, BRL 39123, batch GBD 7.

C) Dog:

7 and 8) **BRL 39123: A 4 Day Range Finding Repeat Dose Study in Dogs by the Oral Route Followed by a 7 Day Off Dose Period**, Study ID T85621/39123/D/PO/RFS/4D, and **BRL 39123: A 7 Day Oral Repeat Dose Study in Dogs**, Study ID T86302/39123 /D/PO/RDS/7D, GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 12 Aug., '85 and 30 Jan., '86, BRL 39123, batches GBD 8 and GBD 16.

Subchronic Toxicity: 1-13 Weeks**A) Rat:**

9) **BRL 39123A: A 10 Day Intravenous Range Finding Repeat Dose Study in Rats**, Study ID T85617/39123A/R/IV/RFS/10D, GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 30 Jul., '85, BRL 39123A, batch GBD 1.

10) **BRL 39123A/Acyclovir: A 10 Day Investigative Repeat Dose Study in Male Rats by the Intravenous Route**, Study ID T86304 /39123A/Acyclovir/R/IV/RDS/10D, GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 14 Jan., '86, BRL 39123A, batch GBD5, and Acyclovir.

Lot FS9753.

11) BRL 39123A: A 28 Day Intravenous Repeat Dose Study in Rats Followed By a 14 Day Off Dose Period, Study ID T85327/39123A/R/IV/RDS/28D. GLP, Beecham Pharm., Stock, Essex, U.K., Study Initiation: 14 Oct., '85, BRL 39123A, batch GBD 5.

12) BRL 39123A: A 13 Week Intravenous Repeat Dose Study in Rats Followed By a 4 Week Off-Dose Period, Study ID T93575/BRL-039123A/R/IV/RDS/13W. GLP, Beecham Pharm., The Frythe, Welwyn, Herts, U.K., Study Initiation: 21 May '93, BRL 39123A, batch W93045.

B) Dog:

13) BRL 39123A: A 14 Day Intravenous Range Finding Repeat Dose Study in Dog Followed By a 14 Day Off Dose Period, Study ID 033/850627TG/39123A/D/IV/RF-RDS/14D. GLP, no audit report, Study Initiation: 8 Jul., '85, BRL

39123A, batch GBD 1.

14) BRL 39123A: A 28 Day Intravenous Repeat Dose Study in Beagle Dogs With a 14 Day Off Dose Period, Study IDs 034 /850911TG/39123A/D/IV/RDS and 034/850911TG/39123A/D/IV /RDS/Supplement. GLP, Study Initiation: 13 Sept., '85, BRL 39123A,

batches GBD 1-6.

15) BRL 39123A: A 13 Week Intravenous Repeat Dose Study in Dogs, Study ID T93647/39123A/D/IV/RDS/13W. GLP, Beecham Pharm., The Frythe, Welwyn, Herts, U.K., Study Initiation: 16 Aug. '93, BRL 39123A, batch W93120.

C) Monkey:

16) BRL 39123A: A 14 Day Intravenous Range Finding Study in Cynomolgus Monkeys, Study ID BRL 1162/87268. non-GLP,

Study Initiation: 4 Nov., '86, BRL 39123A, batch GBD 9.

17) BRL 39123A: Toxicity in Cynomolgus Monkeys by Repeated Intravenous Administration for 4 Weeks (28 Days), Study ID BRL 1181/88674. GLP,

10 Nov., '87, BRL 39123A, batch GBD 16.

18 and 19) BRL 42810: A 14 Day Intravenous Range Finding Repeat Dose Study in Cynomolgus Monkeys (Incorporating BRL 39123A and BRL 29906), Study ID BRL 1179/88479, and BRL 42810: A 14 Day Intravenous Range Finding Repeat Dose Study in Cynomolgus Monkeys (Incorporating BRL 39123A and BRL 29906). Histopathology of Injection Sites, Study ID BRL 1179/88479/Addendum. non-GLP,

Study Initiation: 3 Nov., '87, BRL 39123A, batch GBD 15, BRL 42810, batch CT14245, BRL 29906, batch CT13962.

Toxicity Studies Reviews:

Acute Toxicity: 1-7 Days

A) Mouse:

1) BRL 39123A: An Acute Study In Mice By The Intravenous Route, Study ID T85019/39123A/M/IV/A, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 11 July 1985, Compound: BRL 39123A, Batch: GBD1 (86.2% pure).

Doses Tested: 980, 1400, 2000 mg/kg

Dose Volume and Route: 10 ml/kg, IV, 0.05 ml/sec.

Vehicle or Control: sterile H₂O

Species, Strain, Sex, Age, WT: Male and Female CD-1 mice, weight range 21-29 grams, 3 animals/sex/dose.

Test Conditions: Single IV administration with 14 day follow-up. Body weight

on days 1, 2, 8 and 15. Gross pathological evaluation at death or study termination.

Mortality: Male - 0, 3, 3; Female - 0, 2, 3 by dose groups. All deaths occurred within 1 minute of dosing.

Signs: Tremors/twitching, stretching and relaxation of limbs, rigidity, loss of righting reflex, limb weakness and gasping noted in animals treated at the intermediate and high doses. Stretching of the hindlimbs, supination and ataxia was noted among surviving animals. Injection site irritation was noted in surviving animals. Weight gain was transiently reduced among surviving animals on day 2.

Macroscopic Pathology: A dose related reddening of the lungs was noted in animals dying on day 1.

Comments: 1) Acute LD₅₀ (IV) for BRL 39123A is estimated at 1200 (1100-1400) mg/kg for male and female CD-1 mice.

2) Deaths following IV dosing were accompanied by multiple CNS activation effects (i.e., altered extensor muscle activity and spinal reflexes [righting]). Similar effects have been noted following the IV administration of BRL 42810, which undergoes rapid conversion to BRL 39123.

3) No NOEL dose for CNS activation following IV BRL 39123A was noted in the male or female CD-1 mouse.

4) Target organs for toxicity following IV administration of BRL 39123A, appear to include the lungs and CNS. These effects are closely related to the effects noted following the IV administration of BRL 42810, which is rapidly metabolized to BRL 39123.

B) Rat:

2) **BRL 39123A: An Acute Study In Rats By The Intravenous Route**, Study ID T85018/39123A/R/IV/A, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 11 July 1985, Compound: BRL 39123A, Batch: GBD1 (86.2% pure).

Doses Tested: 490, 700, 1000 mg/kg

Dose Volume and Route: 10 ml/kg, IV, 0.05 ml/sec.

Vehicle or Control: sterile H₂O

Species, Strain, Sex, Age, WT: Male and Female SD rats, weight range 120-149 grams, 3 animals/sex/dose.

Test Conditions: Single IV administration with 14 day follow-up. Body weight on days 1, 2, 8 and 15. Gross pathological evaluation at death or study termination.

Mortality: Male - 0, 0, 2 + 1; Female - 0, 0, 2 by dose groups. All deaths occurred within 1 minute of dosing, except for one male animal treated at 1000 mg/kg which was found dead on day 3.

Signs: Tremors, supination, ataxia, hindlimb weakness and gasping noted in animals treated at the dose of 1000 mg/kg. Stretching, hindlimb weakness, ataxia and prostration was noted among surviving animals at doses ≥ 700 mg/kg. Reduced activity was noted among some animals at all treatment doses. Injection site irritation was noted in animals treated at doses ≥ 700 mg/kg. Weight gain was transiently reduced among surviving animals on day 2 (males at doses ≥ 700 mg/kg; females at all doses).

Macroscopic Pathology: Reddening of the lungs was noted in animals dying on day 1. Red speckling of the thymus was noted among females treated at ≥ 1000 mg/kg. In the male animal found dead on day 3 post-dose, the right kidney was enlarged and hard and the lower intestine filled with green/black fluid.

Comments: 1) Acute LD₅₀ (IV) for BRL 39123A is estimated between 700-1000 mg/kg for male and female SD rats.

2) Deaths following IV dosing were accompanied by multiple CNS activation effects (i.e., altered extensor muscle activity and spinal reflexes [ataxia]). Similar effects have been noted following the IV administration of BRL 42810, which undergoes rapid conversion to BRL 39123.

3) Target organs for toxicity following IV administration of BRL 39123A, appear to include the lungs, kidneys, thymus and CNS. These effects are closely related to the effects noted following the IV administration of BRL 42810, which is rapidly metabolized to BRL 39123.

3) **BRL 39123A: An Investigative Single-Dose Study In Male Rats By The Intravenous Route**, Study ID T89022/39123A/R/IV /SDS, non-GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 8 June 1989, Compound: BRL 39123A, Batch: GBD18 (80% pfa).

Doses Tested: 160 mg/kg

Dose Volume and Route: 10 ml/kg, IV, 3 ml/min.

Vehicle or Control: sterile 0.9% saline

Species, Strain, Sex, Age, WT: Male Crl: COBS, CD(SD)BR rats, weight range 218-310, 5 animals.

Test Conditions: Single IV administration to male SD rats. Six hour post-dose collection of urine. Urine chemistry assessment and histopathologic examination of kidney tissue taken at end of collection period.

Mortality and Clinical Signs: There were no premature deaths or clinical signs noted.

Microscopic Pathology: Significant numbers of rectangular and birefringent crystalline deposits were noted in the medulla, papilla and cortex of 4/5 treated animals. Crystalline bodies were also seen in the urine. Chemical analysis of the crystalline material suggests that it was composed of BRL 39123A.

Comments: 1) As noted previously, the kidneys appear to be a site of toxicity following IV BRL 39123A administration. This effect may, in part, be due to the deposition of crystalline material within the kidney tubules with resultant irritation of renal tissues.

2) The deposition of crystalline bodies and resultant renal irritation has been noted previously with several other of the nucleoside analogues. This effect appears to be related to the limited solubility of the nucleosides in aqueous solutions. Enhancement of renal filtration rate through pre-dose fluid loading has previously been reported to reduce the incidence and/or progression of renal irritation/toxicity associated with the nucleosides.

Subchronic Toxicity Studies: Oral

A) Mouse:

4 and 5) **BRL 39123: A 4 Day Oral Range Finding Repeat Dose Study in Mice**, Study ID T86600/39123/M/PO/RFS/4D, and **BRL 39123: A 7 Day Oral Repeat Dose Study in Mice**, Study ID T86303/39123/M /PO/RDS/7D, (Submission 000, Vol. 13, Page 36).

Status: GLP, no audit report for 4 day study

Study Initiation: 17 Jan. and 11 Feb., '86

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batch GBD 16, 99.2% pure

Doses Tested: 0 and 1000 mg/kg for the 4 Day study; 20, 100, and 1000 mg/kg for the 7 Day study

Dose Volume and Route: 0.1 ml/10 g, PO
Solvent: 1% aqueous methyl cellulose
Species, Strain, Sex: male and female Crl:CD-1 mice
4 Day study; 18-22 grams, age 4 weeks, 5 animals/sex/dose
7 Day study; 22-27 grams, age 5 weeks, 20 animals/sex/dose
(animals were housed 5 per cage)

Test conditions: BRL 39123 was administered once each day by intragastric lavage as a freshly prepared suspension. Mortality, physical signs, body weight, food and water consumption, were monitored daily for 7 days prior to dosing and throughout the dosing interval. Urinalysis, clinical chemistry and hematologic assessments were made near the end of the dosing interval in the 7 Day study. Gross and microscopic pathology (kidney and spleen [7 Day study], only) was assessed at the termination of dosing.

There were no premature deaths in either study. There were no abnormal physical signs observed at any dose of BRL 39123 tested, except for an increased incidence (11/20) of bites, scratches and scabs in the peri-anal region of the high dose male animals. Body weight, food and water consumption were not consistently effected by the administration of BRL 39123 at any dose tested (larger effects were observed following initiation of the daily gavage).

Near the end of the 7 Day dosing interval, platelet counts were slightly decreased (10%), while total white cell and lymphocyte counts were increased (10-50%) in the intermediate dose males and females, respectively. Serum potassium levels were slightly increased (0.5 mM/l) in males and decreased (0.6 mM/l) in females from the low and intermediate dose groups. Bilirubin levels were increased (50-100%) in low and intermediate dose females and high dose males.

Spleen (7 Day study only) and kidney weights were increased among the BRL 39123 treated animals. Histology revealed a significant increase in extramedullary hemopoiesis in the spleen of drug treated animals, and evidence of renal tubule dilation with acidophilic casts. In addition, 3/40 animals from the high dose group showed signs of focal hemorrhages at the corneal/scleral junction. There were no other gross or microscopic abnormalities observed at necropsy.

- Comment:
- 1) BRL 39123 appears to adversely impact the kidneys, even when administered for only a short interval of 4-7 days.
 - 2) Evidence of an increased incidence of focal hemorrhage (3/40 animals from the high dose group) at the corneal /scleral junction is particularly worrisome, as these animals were not the same as those showing the greatest decrease in platelet number. Close monitoring of all follow-up studies showed be performed to look for this effect.
 - 3) The increased incidence of peri-anal scabs, bites and sores seen among the high dose males may be due to fights among the group housed animals, which in turn may have been caused by drug induced irritability. Alternatively, the sores may have been due to a local drug-induced irritation of the skin and resultant increases in grooming/scratching.

B) Rat:

6) BRL 39123: A 4 Day Oral Range Finding Repeat Dose Study in Rats, Study ID T85616/39123/R/PO/RFS/4D, (Submission 000, Vol. 13, Page 171).

Status: GLP

Study Initiation: 29 Jul., '85

Study Site: Beecham Phar., Stock, Essex, U.K.
Compound Tested: BRL 39123, batch GBD 7 (99% pure)
Doses Tested: 0, 40, and 1,000 mg/kg
Dose Volume and Route: 1.0 ml/100 g, PO
Solvent: 1% aqueous methyl cellulose
Species, Strain, Sex: male and female COBS CD (SD) Br rats, age approximately 4-5 weeks, weight range 76-100 grams, 5 animals/sex /dose condition

Test conditions: BRL 39123 was administered by intragastric lavage once per day for 4 days. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Blood chemistries, hematologic assays, and gross and microscopic pathology (kidney only) were assessed at the termination of dosing.

There were no premature deaths or adverse physical signs observed during the study. Body weight gain was reduced in low (20%) and high (10%) dose males, and high (60%) dose females, whereas low dose females displayed a large increase (approx. 80%) in weight gain. There was no obvious reason for the unusual weight gain among low dose females, as the food and water intake of males and females at both dose levels were within 10% of control values.

Platelet counts were slightly increased among the high dose females (976 vs. 777 [control] $\times 10^9$ cells/l), while blood-urea-nitrogen levels were reduced in females from both treatment groups (5.2-5.6 vs. 7.0 [control] mM/l). Absolute and relative kidney weights were increased slightly (5-10% vs. control) in the BRL 39123 treated females at the end of dosing. There were no additional signs of gross or microscopic pathology assessed at the termination of dosing.

Comment: 1,000 mg/kg was selected as an appropriate high dose for additional longer-term toxicity studies in the rat.

C) Dog:

7 and 8) BRL 39123: A 4 Day Range Finding Repeat Dose Study in Dogs by the Oral Route Followed by a 7 Day Off Dose Period, Study ID T85621/39123/D/PO/RFS/4D, and BRL 39123: A 7 Day Oral Repeat Dose Study in Dogs, Study ID T86302/39123/D/PO/RDS/7D, (Submission 000, Vol. 13, page 235).

Status: GLP

Study Initiation: 12 Aug., '85 and 30 Jan., '86
Study Site: Beecham Phar., Stock, Essex, U.K.
Compound Tested: BRL 39123, batches GBD 8 and GBD 16, 99.3% and 99.2% pure, respectively
Doses Tested: 0, 20, 60 and 200 mg/kg, (200 mg/kg 4 day study)
Dose Volume and Route: gelatin capsule, PO
Solvent: none
Species, Strain, Sex: male and female beagle dogs, 7-8 months old, and weighing 7-12 kg, 2 animals/sex/group (1 male and 1 female in the 4 day study).

Test conditions: Test animals were randomly assigned to the 4 test groups. Drug was administered orally (capsule) once each day for 4 or 7 days. In the 4 Day study, dosing was followed by a 7 day 'off-dose' recovery period. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at baseline and during the dosing interval (7 day study only). Gross and microscopic pathology was assessed following termination of the studies.

There were no premature deaths in either study. There were no adverse physical signs, changes in body weight or changes in food and water consumption in either study. Ophthalmoscopy revealed no drug related changes in the retina, lens or corneas of treated animals. One male animal from the high dose group

(200 mg/kg) showed an increase in heart rate (approximately 40 beats /minute) 2 hours after drug administration on day 7. No other changes in ECG (all animals and doses tested) were noted in association with the oral administration of BRL 39123.

Hematologic parameters were not consistently effected following oral administration of BRL 39123. However, an individual male animal (200 mg/kg) displayed a minimal decrease in total white cell and lymphocyte counts, while one female from the low dose group (20 mg/kg) showed an increase in eosinophil count (all changes were at or near the boundary of the historical control range).

Albumin was decreased somewhat (3-6 g/l) in all dosed females, although the effect was not dose related. Plasma globulins were increased slightly (2-6 g/l) in the 2 low dose males, 2/4 intermediate dose animals and 3/4 high dose animals. An increase in urine volume and decrease in urine osmolality was evident in the high dose males and one intermediate dose male. These effects were intermittently evident in all dosed females.

At necropsy, serous filled and/or atrophic acini of the salivary glands were evident in 3/4 intermediate and high dose males. A single intermediate dose female showed areas of congestion and reddening of the lamina propria of the colon. Changes in organ weights were sporadically distributed among the test and control animals and did not appear related to experimental treatment. There were no histological differences evident in the kidneys of treated and control animals.

Comment: Oral administration (4-7 days) of BRL 39123 at doses up to 200 mg/kg appears to have minimal toxic effects in the beagle dog. Decreases in albumin, and increases in serum globulins are evident following BRL 39123 treatment but do not appear to be dose related.

Subchronic Toxicity: 1-13 Weeks

A) Rat:

9) BRL 39123A: A 10 Day Intravenous Range Finding Repeat Dose Study in Rats, Study ID T85617/39123A/R/IV/RFS/10D, GLP, no audit record, Study Initiation: 30 Jul., '85, Study Site: Beecham Pharm., Stock, Essex, UK, Compound Tested: BRL 39123A, batch GBD 1 (86.2% pure free acid [pfa]).

Doses Tested: 0, 10 and 80 mg/kg/day (pfa)

Dose Volume and Route: 1.0 ml/100 g bodyweight, IV injection

Solvent and Control: sterile saline

Species, Strain, Sex: Rat, Crl: COBS^(R) CD^(R) SD Br, 15 male and 15 female, age 4-5 weeks, initial weight range 76-100 grams, 5 animals/sex/dose condition.

Test conditions: Test animals were randomly assigned to give approximately equal initial group mean body weights. BRL 39123A was administered intravenously (1.0 ml/100 grams of body weight; rate 0.05 ml/sec) as a freshly prepared solution in sterile saline. Drug administration was continued once per day for 10 days. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Hematology and clinical chemistries, gross and microscopic pathology (kidney only) were assessed at study termination.

There was a 15% decrease in mean body weight gain among males in the 80 mg/kg treatment group as compared with controls. However, food consumption was not significantly altered by BRL 39123 at either dose tested. Water consumption was increased slightly among males and females in the high dose group (13 and 11%, respectively), and in males administered 10 mg/kg/day (8% increase).

Hematologic parameters measured at study termination showed a non-significant decrease in prothrombin time and APTT (1.1 and 2.3 sec, respectively) in males from the high dose group. These same animals also displayed an increase in neutrophil count ($1312 \times 10^6/l$) versus the control (756×10^6), although total white cell counts for all groups were comparable. BUN was slightly increased (non-significant) in 4 males and 2 females from the 80 mg/kg/day treatment group. There was a dose related increase in LDH among BRL 39123 treated females (statistically significant at the 80 mg/kg/day treatment level; 123 versus 89.7 [control] g/l). All other clinical chemistry and hematologic parameters were similar between treatment groups.

Hyperplasia of the epithelial layer of the collecting ducts was seen in 3 male and female animals from the high dose group. The hyperplasia was associated with basophilia, increased mitotic figures, and occasional acidophilic intracytoplasmic droplets. Necrosis of the proximal tubules was also noted in 1 or 2 animals of each sex.

Comment: In the rat, as in the dog, the primary toxicity associated with the intravenous administration of penciclovir is hyperplasia and/or necrosis of the epithelial tissues in the renal tubules and collecting ducts. It is likely that the increase in water intake seen among the high dose treated rats was associated with collecting tubule damage and an osmotic diuresis. While there was no histologic evidence of renal tubule damage among animals treated with penciclovir at 10 mg/kg/day, the increased water consumption by treated males might be indicative of early renal tubule damage.

10) BRL 39123A/Acyclovir: A 10 Day Investigative Repeat Dose Study in Male Rats by the Intravenous Route, Study ID T86304/39123A /Acyclovir/R/IV/RDS/10D, GLP, incomplete audit report, Study Initiation: 14 Jan., '86, Study Site: Beecham Pharm., Stock, Essex, UK, Compound Tested: BRL 39123A, batch GBD5-86.6% (pure free acid [pfa]), and Acyclovir, Lot FS9753.

Doses Tested: BRL 39123 - 0, 80, and 160 mg/kg (pfa),

Acyclovir - 80 mg/kg (pfa)

Dose Volume and Route: 1.0 ml/100 g, IV, 3.0 ml/min.

Solvent and Control: sterile saline

Species, Strain, Sex: Rat, Crl: COBS^(R) CD^(R) SD Br, 40 males, age 4-5 weeks, initial body weight 98-122 grams, 10 male animals/dose condition.

Test conditions: Animals were divided into 4 test groups. BRL 39123 solution, Acyclovir, or saline was infused into the tail vein once per day for 10 consecutive days. Physical signs, body weight, food and water consumption, mortality, blood chemistry, hematology, and urinalyses were performed at intervals throughout the study. Gross and microscopic (kidney only) pathology was assessed at termination.

Bruising and irritation was occasionally noted in animals from all test groups at the site of injection. There were no other physical signs observed in the treated or control animals. Mean body weight gain was decreased by 19% and 32% among animals in the acyclovir and 160 mg/kg BRL 39123 groups, respectively. Weight gain among animals in the low dose BRL 39123 group did not differ from the control. Food consumption was reduced (15%) and water intake increased (75%) by treatment with acyclovir and high dose BRL 39123. Low dose BRL 39123 treatment increased water consumption somewhat (20%) but did not effect food consumption. The ratios of food intake/weight gain, and water intake/food consumption were increased by acyclovir and high dose BRL 39123 treatment.

A statistically significant reduction in prothrombin time (11.7 versus 12.4 sec) was evident in males from the high dose BRL 39123 group versus control. Acyclovir treated animals displayed an approximately 10% decrease in hemoglobin and RBC counts versus the control. BUN and creatinine were

increased (100 and 25% over control, respectively) in animals treated with acyclovir and high dose BRL 39123. Chloride ions were increased by 2-3 mM in BRL 39123 treated animals, while inorganic phosphate was increased by .6 mM in acyclovir treated animals. All other clinical chemistry and hematologic parameters were similar between treatment groups.

Urine volume was increased (25-100%) and osmolality decreased (15-50%) by treatment with BRL 39123 at either dose. During the initial 6 hours after dosing, BRL 39123 (160 mg/kg) reduced the urinary excretion of potassium and creatinine, while increasing the urinary excretion of alkaline phosphatase, and sodium and chloride ions. Acyclovir treatment initially reduced urine osmolality, creatinine elimination, and potassium excretion. The drug induced effects on urinary parameters were smaller after 10 days of dosing than on day 1.

Hyperplasia of the epithelial layer of the collecting ducts and distal tubules was seen in animals from the high dose BRL 39123 group. The hyperplasia was associated with basophilia, increased mitotic figures, and occasional casts and dilated tubules. Similar effects were noted in acyclovir, but not low dose BRL 39123 treated animals. Birefringent, crystalline material was noted in the cortex and papilla of 50% of acyclovir treated, and 100% of high dose BRL 39123 treated animals. Hyperplasia and basophilia of the collecting ducts was noted in only 3 animals from the low dose BRL 39123 group. No crystalline material was observed in frozen sections from these animals.

Comments: 1) It appears that BRL 39123A may be somewhat less likely to form crystalline deposits in distal collecting tubules than is acyclovir; a similar incidence and distribution of crystalline material having been observed following administration of 80 and 160 mg/kg (pfa) of acyclovir and BRL 39123A, respectively.

2) It is likely that the extent of crystal formation in the kidney tubules following administration of BRL 39123 is dependent on urine pH, and the degree of hydration and urine flow of the animal. The sponsor should consider these factors in further testing of the drug.

11) BRL 39123A: A 28 Day Intravenous Repeat Dose Study in Rats Followed By a 14 Day Off Dose Period, Study ID T85327/39123A/R/IV /RDS/28D, GLP, Study Initiation: 14 Oct., '85, Study Site: Beecham Pharm., Stock, Essex, UK, Compound Tested: BRL 39123A, batch GBD 5 (86.6% pure free acid [pfa]).

Doses Tested: 0, 10, 20, 40 and 160 mg/kg/day (pfa)

Dose Volume and Route: 1.0 ml/100 g bodyweight, IV injection

Solvent and Control: sterile saline

Species, Strain, Sex: Rat, Crl: COBS^(R) CD^(R) SD Br, 50 male and 50 female, age 4-5 weeks, initial weight range 76-111 grams, 10 animals/sex/dose condition. An additional 10 male and 10 female animals were assigned to a 14 day post-drug recovery period. All animals were housed in groups of 5.

Test conditions: Test animals were randomly assigned to 1 of 5 treatment groups. BRL 39123A was administered intravenously via the dorsal tail vein. Drug administration was continued once per day for 28 days. An additional five male and five female animals from the control and high dose treatment conditions were assigned to a fourteen day recovery period. Physical signs, body weight, food and water consumption, and mortality were monitored. Hematology and clinical chemistries, gross and microscopic pathology (selected tissues only from the low-intermediate drug doses), and ophthalmologic status were assessed at the end of dosing or recovery.

One male animal from the 10 mg/kg/day treatment group was sacrificed in extremis on day 25 of the study. There were no other premature deaths. There was a dose-related increase in the incidence and severity of bruising,

swelling and scab formation at the tail vein injection site. In addition, there was evidence of poor grooming with staining of the fur, ocular and nasal discharge, and reddening of the extremities (high dose only) in a small number of animals from each of the treatment groups. All signs regressed during the off-dose period. There were no ophthalmologic effects observed with the administration of IV penciclovir.

Body weight gain was reduced among male (35%) and female (14%) animals from the high dose group. Body weight gain was reduced in males at the low and intermediate doses (approximately 10%), but was not consistently effected in females. During recovery (off-dose phase) body weight gains were increased among males (21%) and females (49%) previously treated with 160 mg/kg/day versus the controls. Food consumption was slightly reduced (4 grams/day) in males from the high dose group, and was not consistently altered in males at other doses or in female animals. Water intake was markedly increased (15-30 ml/animal/day) in males and females in the high dose group. The increase in water consumption was maintained throughout the period of drug administration and at reduced levels (approx. 10 ml/animal/day) during recovery.

As in the 10 day repeat dose study, there were significant decreases in prothrombin time and APTT (approximately 1 and 2.5 sec, respectively) in males from the high dose group. Similar (although not significant) reductions were seen in female animals. In both males and females from the high dose group there were statistically significant reductions in hemoglobin, hematocrit, and red blood cell counts, with females also showing an increase (increase of 200×10^9 cells/l) in platelet count. The percentage of reticulocytes was increased (control 1.3%, versus high dose 3.2%) in a dose related manner among treated males. BUN, creatinine and phosphorus were significantly increased by high dose penciclovir treatment (male and female). Plasma calcium levels were raised somewhat by penciclovir administration at 40 and 160 mg/kg/day. Hematocrit and RBC counts remained decreased in males and females, and BUN levels remained elevated in males at the end of the recovery phase of the study. All other hemato-logic and clinical chemistry values showed recovery after drug discontinuation.

Urine volume was increased (approx. 2x the control), and osmolality decreased among males and females in the high dose group during dosing. These parameters generally showed recovery following drug discontinuation.

At the termination of drug administration, marked increases were evident in the absolute weight of the kidneys and spleen of high dose treated animals (male and female). In addition, slight increases in heart, liver and lung weights were noted in high dose females, and adrenal weight in males. Thymus weights were reduced in males and females from the 40 and 160 mg/kg/day groups, with testicular weights also reduced in males from the 160 mg/kg/day group. An increase (not significant) in uterine weight was noted in females from all dose groups, but did not appear to be dose related. At the end of the drug recovery period, testicular weights remained depressed, while kidney, adrenal and spleen weights remained elevated in high dose animals. An increase in thymic weight was noted in males and females.

Microscopic examination of kidney tissue revealed a pattern of renal tubule distention, with hyperplasia of the epithelium and development of casts. Fibrosis and inflammatory cell infiltrates were also evident in animals from the high dose group. Epithelial hyperplasia and inflammatory cell infiltrates were also noted in the urinary bladder of 3 high dose females and 1 male animal. These effects showed some degree of regression following drug cessation, although residual damage was still evident at 2 weeks post-dosing. At the end of dosing, males from the high dose group showed occasional depletion of spermatogonia and spermatocytes with seminiferous tubule degeneration, and reduced testicular weight. These degenerative effects were

more prominent at the end of the off-dose period with only sertoli cells remaining in some tubules. Signs of regeneration (proliferation of spermatogonia and spermatocytes) was also evident.

As noted above, thymic atrophy was noted in animals from several of the dosed groups. Microscopic indications of thymic involution were seen in all treatment groups as noted in the following incidence table. Evidence of thymic regression was noted in 2/5 females and 0/5 males from the former high dose group at the end of the drug recovery period.

Incidence of Thymic Atrophy		
Dose Group	Male (N=10/group)	Female
0 mg/kg/day	0	0
10 mg/kg/day	3	5
20 mg/kg/day	3	4
40 mg/kg/day	6	6
160 mg/kg/day	6	10

The remaining histologic abnormalities seemed evenly distributed among the study groups and, therefore were probably unrelated to the test compound. However, an unusually high incidence of granulomas, thrombi, purulent emboli, and perivascular and peri-bronchiolar hyperplasia was evident in the lungs of animals from all groups (including the controls). Further, inflammatory cell infiltrates were also seen in the liver parenchyma of several animals. These signs may be indicative of infections due to the introduction of pathogens during IV infusions or, an increased incidence of opportunistic infection following immune suppression or dysfunction. (The low dose male which was sacrificed during treatment showed several of these signs, along with hemorrhages in the lungs.)

Comments: 1) The primary toxicity associated with the intravenous administration of penciclovir is hyperplasia and/or necrosis of the epithelial tissues in the renal tubules and collecting ducts. It is likely that the increase in water intake seen among treated rats was associated with collecting tubule damage and an osmotic diuresis. Microscopic evidence of renal tubule damage which was seen at the end of the recovery phase of the study suggests that drug-induced renal damage may be slow to regress (or may be irreversible) following drug cessation. Changes in the plasma levels of creatinine, BUN and phosphorous may have been due to the kidney damage.

2) Testicular and thymic atrophy was noted in animals from the 160 and 40 mg/kg/day groups. These effects had not been seen in the rat when drug was administered for 10 days. Testicular and seminiferous tubule atrophy with hypo-spermatogenesis has been evident with several other nucleoside analogues, and has been associated with significant reductions in fertility and occasional sterility. Indications of partial recovery were evident following 14 days off-drug. Whether complete recovery of testicular function will occur is unknown and should be evaluated during reproductive toxicity testing. Thymic atrophy may be an indication of immunologic toxicity, therefore, as the drug continues to be developed particular attention should be maintained on this topic.

12) BRL 39123A: A 13 Week Intravenous Repeat Dose Study in Rats Followed By A 4 Week Off-Dose Period, Study ID T93575/BRL-039123A/R/IV/RDS/13W.

Status: GLP

Study Initiation: 21 May '93

Study Site:

Compound Tested: BRL 39123A, batch W93045

Doses Tested: 0, 10, 30 and 80 mg/kg/day (as pure free acid)

Drug was administered once each day as an intravenous infusion, all dosing solutions were determined to be within 10% of the nominal dose

Dose Volume and Route: 10 ml/kg, IV (caudal vein), 3 ml/min.

Solvent and Control: sterile saline

Species, Strain, Sex: 16 male and 16 female Sprague-Dawley rats (CrI: CD(SD)BR), age approximately 7 weeks (at dose initiation), weight range: male, 168-234 g; female, 146-199 g.

Test Conditions: Test animals were randomly assigned to the 4 test groups (16 animal/sex/group). Drug was administered (IV) once a day for 13 weeks, followed by a 4 week off-dose recovery period. Physical signs, body weight, food and water consumption, and mortality were monitored daily during drug administration. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at intervals throughout the study. Gross and microscopic pathology was assessed at the termination of the dosing or 'off-dose' interval.

Mortality: Three high dose animals (2♂ and 1♀) died or were sacrificed in extremis during the dosing period. Both male animals appeared to die of causes unrelated to the test compound (1 accidental injury and 1 multifocal lymphoma), while the female animal succumb during a convulsion immediately following dosing (week 3). There were no other premature deaths among the treatment or control animals.

Clinical Signs, Body Weight and Food/Water Consumption: Irritation at the site of injection (bruising, inflammation and/or necrosis) was noted in all study groups, although the incidence and severity of the effects increased with the administered dose (likely related to the high pH of the dosing solution). Except for the one case of post-drug administration convulsion as noted above, there were no other clinical signs which appeared related to the administration of BRL 39123A. All signs regressed during the off-dose period. There were no ophthalmologic effects observed with the administration of IV penciclovir.

Body weight gain was increased slightly during dosing among high-dose treated female animals (13%), resulting in an approx. 5% difference in absolute body weight at the conclusion of dosing (these effects were not statistically significant). A concurrent 5-10% increase in food consumption was noted during weeks 5-13 among the same animals. Body weight, weight gain and food consumption was similar among all other study groups, whether during the treatment or off-dose recovery periods. Water intake was markedly increased (up to 15-30 ml/animal/day) in males (51%) and females (17%) in the high dose group. The increase in water consumption was maintained throughout the period of drug administration and at reduced levels (approx. 10 ml/animal/day) during recovery. There were no other consistent effects on water consumption among the remaining treatment groups.

Hematology, Clinical Chemistry and Urinalysis: In both males and females from the high dose group there were progressive and statistically significant reductions (5-15%) in hemoglobin, hematocrit, and red blood cell counts which were evident during the period of drug treatment (weeks 5-11). Somewhat smaller reductions in these same red cell parameters were noted among the intermediate and low dose (statistically significant) treated animals. Slight increases in platelet and reticulocyte counts were evident among intermediate and high dose treated animals. BUN (50%), creatinine (6-22%; progressive with the duration of dosing), beta and gamma globulin were significantly increased by high dose penciclovir treatment (male and female). Serum AST and/or ALT levels were sporadically elevated in animals from the high dose group. Serum

sodium and chloride levels were increased slightly during the period of drug treatment among high dose male and female animals, with potassium levels also increased among high-dose males. In contrast, serum calcium and phosphorus levels were slightly increased among high and intermediate dose treated males animals but decreased among female animals from the same dose groups. Generally, all of the hematologic and clinical chemistry values showed recovery after drug discontinuation.

Urine volume was increased (approx. 50% over the control), and osmolality decreased among males and females in the high dose group during dosing. Urinary excretion of sodium, potassium and chloride ions were also sporadically increased among intermediate and high dose treated animals (males and females) throughout treatment. Atypical birefringent crystals and red and white blood cells were noted in the urine of most high dose treated male animals and sporadically noted among animals (male and female) from other dose groups. These parameters generally showed recovery following drug discontinuation.

Gross and Microscopic Pathology: At the termination of drug administration, marked increases were evident in the relative weight of the kidneys of high dose treated animals (41% in males and 22% in females). In addition, slight increases in liver weights (10% without evidence of significant histopathology) were noted in high dose females. At the end of the drug recovery period, kidney weights remained elevated in high dose animals (38% in males and 13% in females), along with increased heart weight among high dose treated males, and increased adrenal weights among high dose treated animals and low dose treated males.

Microscopic examination of kidney tissue revealed a pattern of renal tubule distention, with hyperplasia of the epithelium and development of casts among high dose treated animals and male animals from the intermediate dose group. Fibrosis, inflammatory cell infiltrates and epithelial hyperplasia were noted in the urinary bladder many affected animals. These effects showed some degree of regression following drug cessation, although residual damage was still evident at 4 weeks post-dosing. No histologic evidence of renal damage was noted at the conclusion of the off dose recovery period among the low dose treated animals or female animals from the intermediate dose group.

A slight increase in the frequency and/or severity of injection site inflammatory/necrotic reactions was noted among drug treated males animals and high-dose treated female animals at the conclusion of the dosing period. These local reactions were generally not evident at the conclusion of the off-dose period. All remaining histologic abnormalities seemed evenly distributed among the study groups and, therefore were probably unrelated to the test compound.

Comments. 1) Renal tubular degeneration with necrosis and/or regeneration was the primary toxicity associated with the intravenous administration of penciclovir. These effects were evident among all high dose treated animals and among the intermediate dose male animals. The renal injury appears consistent with physical trauma resulting from the formation of drug crystals in the renal tubules following glomerular filtration. Microscopic examination of collected urine from intermediate and high dose treated animals revealed frequent instances of atypical birefringent crystals and renal tubule casts. Similar "insolubility" related renal toxicities have been evident with several other nucleoside analogues, and appear associated with elevated C_{max} values and glomerular filtrate drug concentrations achieved following bolus drug infusions.

2) It appears likely that the increase in water intake, and changes in serum/urine creatinine and electrolytes, as seen among

the drug treated rats was associated with the collecting tubule damage and an osmotic diuresis.

3) Microscopic evidence of renal tubule damage which was seen at the end of the recovery phase of the study suggests that drug-induced renal damage may be slow to regress or may be irreversible following drug cessation.

4) As noted previously, sporadic increases in serum AST and/or ALT levels were seen among the high dose treated animals. Furthermore, at the conclusion of the drug dosing interval, a slight (10%) but statistically significant increase in mean liver weight was seen among the high dose treated female animals. Since there was no corresponding morphologic hepatopathy, the source and cause of these serum chemistry changes remains undetermined.

5) It should be noted that complete histologic evaluations were performed only on tissues from the control and high dose treatment groups, while selected tissues were examined from animals in other treatment groups.

B) Dog:

13) BRL 39123A: A 14 Day Intravenous Range Finding Repeat Dose Study in Dog Followed By a 14 Day Off Dose Period, Study ID 033 /850627TG/39123A/D/IV/RF-RNS/14D GMP no audit report, Study Initiation: 8 Jul., '85, Study Site: Compound Tested: BRL 39123A, batch GBD

1 (86.2% pure free acid [pfa]).

Doses Tested: 50 mg/kg (pfa), single infusion or split dose (25 mg/kg B.I.D.)

Dose Volume and Route: 2.0 ml/kg, IV, 10 ml/min

Solvent and Control: sterile saline

Species, Strain, Sex: 2 male (9-11 kg) and 2 female (8-10 kg) beagle dogs.

Test conditions: Test animals were randomly assigned to test groups. BRL 39123A was administered intravenously once (dose = 50 mg/kg) or twice/day (two equal doses of 25 mg/kg, 6 hours apart), as a freshly prepared solution in sterile saline. Administration was continued for 14 days and was followed by a 14 day 'off-dose' period prior to necropsy. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Blood chemistries, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at approximately weekly or biweekly intervals throughout the study. Gross pathology was assessed at study termination.

There were no premature deaths during the study. Physical signs included lip-licking during or immediately after drug administration, slight tremors (15 min. - 3 hours after dosing) in the 50 mg/kg single dose animals, and occasional soft feces with traces of mucus and/or blood in animals on either dose regimen. Tachycardia (30-75% increase over pre-drug measures) was evident from 5-30 minutes following dosing for both animals in the 50 mg/kg group, and the female animal in the 25 mg/kg (2x) group. Transient shortening of the P-R interval and elevation of the T wave was sporadically evident in both female animals immediately following dosing (within 5 minutes).

There were no observable effects of drug administration on body weight, food and water consumption, or ophthalmologic findings. There were no apparent treatment related changes in urinalysis, blood chemistries or hematologic parameters during the 14 days of drug administration. However, a slight increase in hematocrit and mean corpuscular volume was evident in the male from the 25 mg/kg (B.I.D.) group at the termination of the 'off-dose' period.

Several gray-white nodules were observed in the lungs of both male animals at

the time of necropsy. Examination of the nodules suggested their presence was associated with a parasitic infection. Reddening of the mucosa of the distal jejunum, and the presence of a small clot attached to the mucosa, was noted in the male animal from the 50 mg/kg single dose group. No other gross pathologies or organ weight changes were evident at the time of necropsy.

Comments: 1) The intravenous administration of penciclovir appears to be associated with a significant tachycardia (up to 75% over pre-administration rate). Changes in the P-R and Q-T intervals were also evident in the ECGs from both female animals. The heart rate and conduction changes were closely associated with the administration of penciclovir and generally dissipated within 30 minutes following the drug infusion. The occurrence of ECG changes only in the female animals (regardless of the dosing schedule) suggests that they may be more sensitive to this effect of penciclovir than are males.

2) Penciclovir, when administered intravenously at 25 (B.I.D.) or 50 mg/kg for 14 days, appears to be relatively devoid of toxic effects in the dog (other than ECG changes). However, the lack of a 'no-treatment' control group in this study makes definitive determination of drug effects impossible.

14) BRL 39123A: A 28 Day Intravenous Repeat Dose Study in Beagle Dogs With a 14 Day Off Dose Period, Study IDs 034/850911TG/39123A /D/IV/RDS and 034/850911TG/39123A/D/IV/RDS/Supplement. GLP. Study Initiation: 13 Sept., '85, Study Site: Compound Tested: BRL 39123A,

batches GBD 1-6 (85.1-86.6% pure free acid [pfa]).

Doses Tested: 0, 10, 20, 50, and 100 mg/kg/day (pfa), divided into equal split doses of 0, 5, 10, 25 and 50 mg/kg (B.I.D.)

Dose Volume and Route: 2.0 ml/kg, IV, 10 ml/min

Solvent and Control: sterile saline

Species, Strain, Sex: 19 male and 19 female beagle dogs, 7-8 months old, and weighing 9-13 kg.

Test conditions: Test animals were randomly assigned to 5 test groups (3 animals/sex/group, with 2 additional animals of each sex in the control and high dose groups). Drug was administered intravenously twice/day with 6 hours between doses. Drug dosing was continued for 28 days. Two male and 2 female animals each from the control and high dose groups were placed on a 14 day 'off-dose' period prior to necropsy. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at weekly or biweekly intervals throughout the study. Gross and microscopic pathology was assessed at the termination of the dosing or 'off-dose' interval.

There were no deaths in the study. As in the previous study, emesis and soft feces containing blood and/or mucus were the most frequently observed signs associated with drug administration, although the effects did not appear dose related. Lip licking was observed immediately following dosing in animals from all treated and control groups. Conjunctivitis was noted in 4 animals; 2 high dose, 1 high intermediate dose, and 1 control group animal. There were no treatment related changes in ophthalmic status.

Body weight gain was reduced somewhat among males and females from the 3 highest dose groups. While drug effects on weight gain were not statistically significant at any treatment levels, body weight gain differences were most evident when comparing the high dose females with controls. Food and water intake were not consistently effected by treatment with penciclovir at any dose. Blood chemistry, urinalysis and hematologic parameters were not effected by drug treatment, except for a reduction in total white cell counts among

males and females (25-30%) at the high dose and males (20%) in the high intermediate dose group.

As noted in the dose range-finding study, intravenous administration of penciclovir was associated with statistically significant increases in heart rate (30-40% above pre-infusion rates in males and females from the high dose group), and reductions in the P-R (15-23%) and Q-T (10-15%) intervals. Heart rate and conduction changes were closely associated with drug infusion, beginning within 5 minutes and lasting approximately 30 minutes. These effects were evident in both male and female animals in the high and high intermediate dose groups, with female animals demonstrating somewhat larger responses.

At necropsy there was evidence of increased kidney weights among male animals at all treatment doses (15% increase high dose versus control). Thymus and spleen weights were reduced (nearly 60 and 30% versus controls, respectively) among males and females in the high dose groups, with thymus weights also being reduced in females in the high intermediate dose condition. All additional organ weight effects appeared randomly distributed among the test and control animals and therefore unrelated to drug administration.

Decreased testicular weights (30% decrease versus control) were noted in males from the high and high intermediate dose groups. Testicular atrophy was associated with a slight increase in polynucleated cells in high dose animals versus control and all other drug doses, with effects being more prominent at the end of the off-dose period. All remaining histopathologies appeared randomly distributed among the test animals.

Comments: 1) Administration (IV) of penciclovir appears to cause a significant degree of tachycardia (30-40% above pre-infusion rates in males and females from the high dose group), and reductions in the P-R (15-23%) and Q-T (10-15%) intervals. The heart rate and conduction changes were closely associated with drug infusion, beginning within 5 minutes and lasting approximately 30 minutes. These effects were evident in all high and high-intermediate dose animals, with female animals demonstrating somewhat larger responses (suggesting that female animals may be more sensitive to this effect than are males).

2) In the present study, significant kidney damage (increased weight [15% increase in the high dose group] and abnormal histology) was evident among male animals at all treatment doses. Decreased testicular weights (30% decrease versus control) were also noted in males from the high and high intermediate dose groups. It is possible that the kidney and testicular damage seen in the present study may be gradual or delayed effects of penciclovir administration, as these effects were not evident in the dose range-finding study. However, because the preceding study failed to include a no treatment control group, a definitive conclusion regarding delayed onset and/or progression of toxicity is not possible at this time.

3) Intravenous penciclovir induced significant atrophy of the thymus and spleen among males and females in the high dose groups (approximately 60 and 30% reductions in organ weights versus controls, respectively), with thymus weights also being reduced in females in the high intermediate dose condition. Reductions in total white cell counts were also evident among males and females at the high dose and males in the high intermediate dose group. These data suggest that penciclovir may have specific toxic effects on the immune system. The sponsor should be advised to consider this point in the further development of the drug.

15 3A: A 13 Week Intravenous Repeat Dose Study in Dogs, Study ID
T9 3A/D/IV/RDS/13W.

Study GLP

Study Initiation: 16 Aug. '93

Study Site: Beecham Pharm., The Frythe, Welwyn, Herts, U.K.

Compound Tested: BRL 39123A, batch W93120 (99.7% pfa)

Doses Tested: 0, 10, 30 and 100 mg/kg/day (pfa), divided into equal split doses of 0, 5, 15 and 50 mg/kg (BID), all dosing solutions were determined to be within 10% of the nominal dose

Dose Volume and Route: 2.0 ml/kg, IV, 10 ml/min

Solvent and Control: sterile saline

Species, Strain, Sex: 12 male and 12 female beagle dogs, 10.5-11.5 months old, weight range: male, 11-15.1 kg; female, 10.2-13.4 kg.

Test conditions: Test animals were randomly assigned to one of 4 test groups (3 animals/sex/group). Drug was administered intravenously twice/day with 6 hours between doses. Drug dosing was continued for a minimum of 90 days. Physical signs, body weight, food and water consumption, and mortality were monitored daily. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed. Blood samples were taken on the first and last day of dosing for drug level measurements. Morphologic pathology (gross and microscopic) was assessed at the termination of the dosing interval.

Mortality: There were no deaths in the study.

Clinical Signs, Body Weight and Food Consumption: Emesis following drug administration was noted on multiple occasions in 2/3 males and 2/3 females from the high dose group, and in individual animals from the intermediate and low dose groups. In addition, bruising and/or swelling at the site of drug administration was noted in one female animal from each drug treatment group and in one low dose male animal. There were no other clinical signs which appeared related to the IV administration of BRL 39123A.

A slight reduction (approx. 0.5 kg) in body weight and/or weight gain was evident among all of the high dose female animals and 2/3 of the intermediate dose treated female animals during the early and latter weeks of dosing, respectively. Corresponding with the reductions in body weight, food consumption was slightly reduced (<15%) among the high and intermediate dose treated female animals. There were no apparent drug related effects on body weight, weight gain or food consumption among male animals or the low dose treated female animals, showing reduced weight during the last few weeks of dosing.

Ophthalmoscopy: There were no apparent drug related effects.

Hematology, Clinical Chemistry and Urinalysis: Five of the 6 high dose treated animals (2♂ and 3♀) showed slight (5%) increases in both mean corpuscular volume and hemoglobin concentration, with the males also showing a slight increase (approx. 10%) in reticulocyte count, during week 12 of treatment. Also evident during week 12 of drug administration was an approximately 50% reduction in the number of circulating basophils among the high dose treated male and female animals. There were no other apparent drug related effects on any hematologic parameter.

Changes in blood chemistry were confined to single male and female animals from the high dose treatment group, which demonstrated a modest increase in serum urea (50%) and decrease in serum cholesterol (30%), respectively. Mild bilirubinuria was noted occasionally among all study groups (including the controls), and was unlikely to have been related to drug treatment. There were no other apparent drug related changes in serum or urine chemistry.

Electrocardiography: As noted in multiple previous studies in the dog, the

intravenous administration of penciclovir was associated with significant increases in heart rate (15-30% above pre-infusion rates), and reductions in the P-R (15-27%) among males and females from the high dose group. Smaller reductions in the P-R interval were occasionally evident among female animals from the intermediate dose group. Heart rate and conduction changes were closely associated with drug infusion, beginning within 5 minutes (the first post-infusion assessment time-point), and frequently lasting up to one hour post-infusion. Among the high dose treated males, there were occasional instances of residual changes in the P-R interval at 6 hours post-infusion (i.e., immediately prior to the second of the twice-daily drug infusions).

Comments: 1) As stated above, similar drug related effects on heart rate and cardiac conduction parameters have been evident in earlier studies in which penciclovir was administered by bolus IV infusion. Unfortunately, in the present study report the sponsor has failed to provide information regarding possible changes in the Q-T interval (a cardiac parameter which was altered in the previous studies) and/or 'strip-charts' of the cardiac waveforms. This information should be requested from the sponsor.

2) In the present study, measurements of the ECG were performed ONLY prior to and during the first of the twice daily drug infusions. No apparent attempt was made to evaluate residual drug effects on the ECG more than 6 hours following drug administration or, to examine possible interactions of the altered ECG with the second of the daily drug infusions (i.e., increased or decreased responsiveness and, duration of the responses beyond 6 hours post-dose). Failure on the part of the sponsor to include these longer-duration measures in the present study suggests either, an inattentiveness to the ongoing study results/implications, or an unwillingness to modify an ongoing protocol. The lack of information related to the duration of the cardiac effects and/or any potential 'second-dose' effect is a potentially important issue related to the intravenous use of penciclovir and warrants further investigation.

Gross and Microscopic Pathology: Decreased testicular weights (approx. 30-50% decrease versus control) were noted in males from the high dose group at the time of necropsy. Testicular degeneration in the affected males was associated with marked atrophy of the seminiferous tubules, degeneration and/or absence of the germinal epithelium, and absence of sperm in the epididymides. The atrophy was occasionally associated with a slight increase in polynucleated cells in the testes of high dose animals. Individual male animals from the intermediate and low dose treatment groups also showed reduced testicular weights, however since the values were within the historical control range for the test site these effects can not be clearly related to the administration of penciclovir.

An arteriopathy of the coronary arteries was noted in 2/3 and 1/3 female animals dosed at 100 or 30 mg/kg/day, respectively, and in 1/3 male animals dosed at 100 mg/kg/day. The coronary artery lesion were characterized by hyperplasia and/or necrosis of the intimal cell layer and inflammatory cell (lymphocytic and/or mononuclear cells) infiltrates. Occasional reddening and/or thickening of the A-V valve was noted in several of the animals.

Comment: The sponsor contents that the coronary artery lesions were of an idiopathic "type", spontaneous in origin and were not related to drug treatment. However, it is the opinion of this reviewer that, given the distribution of effected animals only in the high and intermediate dose groups and in consideration of the significant chronotropic effect of IV penciclovir, it is not reasonable to

conclude that the cardiac artery lesions were unrelated to the test compound.

Macro- and microscopic evidence of injection site irritation was evident in the majority of the test animals, including the controls. All remaining gross and/or microscopic pathologies appeared randomly distributed among the test animals.

Toxicokinetics: Systemic (AUC) and peak plasma (C_{max}) drug levels were determined on days 1 and 91 of dosing. A summary of the study findings are presented in the following table.

AUC and C_{max} by Drug Dose (AUC _{0-inf} : $\mu\text{g}\cdot\text{hr}/\text{ml}$; C_{max} : $\mu\text{g}/\text{ml}$)						
Treatment	Day	Male AUC	C_{max}	Female AUC	C_{max}	
10 mg/kg	1	16.1	15.1	14.1	10.6	
	91	16.6	13.5	17.4	16.9	
30 mg/kg	1	44.2	40.5	47.8	39.7	
	91	57.5	71.2	57.8	67.0	
100 mg/kg	1	223.4	166.5	161.7	212.0	
	91	217.1	223.6	202.6	257.9	

Maximum plasma drug levels were generally detected during the first post-infusion measure (5 minutes), and declined rapidly thereafter with an approximate half-time of 1.6-2.0 hours. For males and females from all dose groups, the plasma drug levels typically remained above the threshold for quantification at the 6 hour post-dose sample. While mean plasma C_{max} and AUC values for all animals increased from day 1 to 91, these increases were generally less than 2 fold and do not appear to reflect significant changes in drug disposition and elimination.

Comments: 1) Administration (IV) of penciclovir appears to cause a significant degree of tachycardia (15-30% increase over pre-infusion rates in males and females from the high dose group), and reductions in the P-R (15-23%) interval. Furthermore, in a previous 1 month study in beagle dogs, it was determined that IV penciclovir decreased the duration of the Q-T (10-15%) intervals. In both studies, the heart rate and conduction changes were closely associated with drug infusion, being evident within 5 minutes (the first post-infusion assessment point) and lasting generally 30-60 minutes (although decreases in the P-R interval were detected at up to 6 hours following drug infusion [the final assessment interval] in the current study). These effects were evident in the high and intermediate dose animals, with male animals showing somewhat larger responses (suggesting that male animals may be more sensitive to this effect than are females).

2) In the present study, measurements of the ECG were performed ONLY prior to and during the first of the twice daily drug infusions. No apparent attempt was made to evaluate residual drug effects on the ECG more than 6 hours following drug administration or, to examine possible interactions of the altered ECG with the second of the daily drug infusions (i.e., increased or decreased responsiveness and, duration of the responses beyond 6 hours post-dose). In addition, the sponsor has failed to provide information

regarding possible changes in the Q-T interval (a cardiac parameter which was altered in the previous studies) and/or 'strip-charts' of the cardiac waveforms. This information should be requested from the sponsor.

Failure on the part of the sponsor to include longer-duration measures in the present study (either by initial study design or by modification of the study protocol), results in a potentially serious toxicologic finding being uninterpretable and/or undefined. In particular, the lack of information related to the duration of the cardiac effects and/or any potential 'second-dose' effect is a potentially important issue related to the intravenous use of penciclovir. This effect warrants further investigation.

3) Coronary artery pathology (intimal hyperplasia and/or necrosis with inflammatory cell infiltration) was noted in 2/3 and 1/3 female animals dosed at 100 or 30 mg/kg/day, respectively, and in 1/3 males dosed at 100 mg/kg/day. While the sponsor contends that the coronary artery lesions were not drug related, given the distribution of effected animals in the high and intermediate dose groups and the significant chronotropic effect of IV penciclovir, it is not reasonable to conclude that the cardiac artery lesions were unrelated to the test compound. This potential drug effect warrants further evaluation.

C) Monkey:

16) BRL 39123A: A 14 Day Intravenous Range Finding Study in Cynomolgus Monkeys, Study ID BRL 1162/87268, non-GLP, Study Initiation: 4 Nov., '86, Study Site: Compound

Tested: BRL 39123A, batch GBD 9 (86.0% pure free acid [pfa]).

Doses Tested: 50, 100 (2x 50), 100, and 200 mg/kg/day (pfa), all doses were administered as a single daily intravenous infusion except as follows: one group receiving 100 mg/kg/day received the dose divided into two equal doses of 50 mg/kg approximately 6 hours apart, and the remaining 100 and 200 mg/kg/day dose groups which received the daily dose divided into two equal doses (6 hours apart) on the first day of dosing

Dose Volume and Route: 2.0 ml/kg, IV, 1 ml/6 sec

Solvent and Control: sterile saline

Species, Strain, Sex: 4 male and 4 female cynomolgus monkeys (Macaca fascicularis), estimated age 2-4 years, and weighing 1.8-3.5 kg.

Test conditions: Test animals were randomly assigned with 1 animal/sex/group. Drug was administered intravenously once or twice/day, with 6 hours between doses when applicable. Drug dosing was continued for 14 days. Animals from the 100 and 200 mg/kg (single daily infusion) groups were placed on a 14 day 'off-dose' period prior to necropsy. Physical signs, body weight, food and water consumption, and mortality were monitored twice weekly during baseline and recovery phases of the study, and daily during drug administration. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at intervals throughout the study. Gross and microscopic pathology was assessed at the termination of the dosing or 'off-dose' interval.

There were no deaths in the study. Hypoactivity and huddled posture were the most frequently observed clinical signs occurring in 5 of 8 animals. Loose/liquid feces were evident in 2 female animals from the 50 and 2x 50 mg/kg groups during dosing. A 5-20 beat/minute increase in heart rate immediately following drug administration at all doses was the only change evident in the ECG. There were no treatment related changes in ophthalmic status.

Body weight was reduced somewhat (6-12%) among males and females from all dose groups during the 2 weeks of drug administration. Following drug cessation in the 2 recovery groups (i.e., the 1x 100 and 200 mg/kg/day treatment groups) body weight gradually returned to or near baseline levels. Food intake was decreased in 7/8 animals during drug administration, and returned to normal during the off-dose period. Water consumption was increased slightly during treatment (as compared with the pretreatment baseline) in animals from the 50 and 100 mg/kg/day (single dose) groups, but was unaffected in the remaining animals. Urine volume was increased while specific gravity and osmolality were reduced in 7/8 animals after 14 days of dosing. Renal tubule inflammation with epithelial hyperplasia and basophilia was evident at necropsy in the majority of animals.

Hemoglobin, hematocrit and RBC counts were generally reduced in 6/8 animals at the end of drug dosing. Progression and partial recovery from the hematologic effects of penciclovir were seen in the 200 mg/kg/day group following drug cessation. There was no evidence of a change in bone marrow cellularity at the time of necropsy. Changes in blood chemistry evident at the end of the 14 day dosing interval included increases in creatinine and urea in most animals, and decreases in plasma inorganic phosphorus in females from the 100 and 200 mg/kg/day groups. Additional blood chemistry and hematologic differences appeared randomly distributed among the treatment groups and unrelated to the onset and cessation of drug dosing.

- Comments:
- 1) Histologic, clinical chemistry and urinalysis data collected during the study suggest that the kidney is a primary site of toxicity with penciclovir. Significant increases in urine volume, decreases in osmolality and specific gravity, and increases in plasma urea and creatinine were evident in all dose groups. These changes were generally apparent at 10-14 days of dosing, with gradual recovery following drug cessation. Epithelial hyperplasia and renal tubule inflammation with basophilia was evident at necropsy in the majority of animals.
 - 2) Decreases in several hematologic parameters including, hematocrit, hemoglobin and red blood cell counts, were associated with the administration of penciclovir. The lack of any histologic evidence of a change in bone marrow cellularity suggests that hematologic effects of penciclovir may be mediated through a mechanism other than myelosuppression.
 - 3) Intravenous administration of penciclovir induced a 5-20 beat/minute increase in heart rate immediately following drug infusion. This effect was evident at all drug doses tested and was not apparently dose dependent. Similar effects have been noted in several additional studies utilizing different drug doses and test species. While this effect is relatively short lived, rapidly developed and easily monitored, future studies should closely monitor for any evidence of cardiotoxicity and/or changes in cardiac conduction.

17) **BRL 39123A: Toxicity in Cynomolgus Monkeys by Repeated Intravenous Administration for 4 Weeks (28 Days)**, Study ID BRL 1181/88674, GLP, Study Initiation: 10 Nov. '87, Study Site:
Compound Tested: BRL 39123A, batch GBD 16 (87.2% pure free acid [pfa]).

Doses Tested: 0, 5, 10, 25, and 50 mg/kg (pfa), b.i.d., drug was administered twice daily as an intravenous infusion, 6 hours between infusions (Cumulative daily doses were 0, 10, 20, 50 and 100 mg/kg)

Dose Volume and Route: 2.0 ml/kg, IV, 1 ml/min.

Solvent and Control: sterile saline

Species, Strain, Sex: 19 male and 19 female cynomolgus monkeys (Macaca fascicularis), estimated age 2-4 years, and weighing 2.1-4.0 kg.

Test conditions: Test animals were randomly assigned to the 5 test groups (3 animals/sex/group). An additional 2 animals/sex were added to the control and high dose groups and maintained 'off-dose' for 14 days prior to necropsy. Drug was administered intravenously twice a day, with 6 hours between doses. Drug dosing was continued for 28 days. Physical signs, body weight, food and water consumption, and mortality were monitored twice weekly during baseline and recovery phases of the study, and daily during drug administration. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at intervals throughout the study. Gross and microscopic pathology was assessed at the termination of the dosing or 'off-dose' interval.

One female animal in the 10 mg/kg b.i.d. was sacrificed moribund before termination of the study (day 26). Hypoactivity and huddled posture, loose/liquid feces, and progressive weight loss were the most frequently observed clinical signs occurring in this animal. Blood samples taken at termination were unsatisfactory due to severe hemolysis. Gross and histological assessments failed to reveal specific pathologies which could account for the morbidity observed.

In surviving animals, body weight gain was reduced sporadically among males and females from all dose groups during the 4 week drug administration. Four of 5 females from the 50 mg/kg b.i.d. group showed significant weight loss (mean of approximately 160 grams) during dosing. Weight gain in former high dose and control animals during the off-dose period was comparable. Food intake was reduced somewhat (10-25%) during the first 2 weeks of dosing among animals (male and female) in the high and high-intermediate dose groups (the differences were generally not statistically significant). Food intake was normal during the off-dose period. Water consumption was not obviously effected by drug treatment or cessation.

Administration of penciclovir (IV) induced a 10-20 beat/minute increase in heart rate immediately following drug infusion, particularly among animals in the high dose group. The changes in heart rate were relatively short lived and of rapid onset; there were no other changes in ECG parameters detected. There were no significant changes in ophthalmologic findings associated with drug administration.

Hematologic parameters were not altered by penciclovir treatment, except for an increase in lymphocytes among the high dose animals ($8.23 \times 10^3/\text{mm}^3$ versus $5.17 \times 10^3/\text{mm}^3$ [control]), and sporadic variations seen in animals from all dose groups. There was no evidence of a change in bone marrow cellularity at the time of necropsy. Changes in blood chemistry evident at the end of the dosing interval included increases in creatinine (25 and 50 mg/kg b.i.d. groups), urea nitrogen (50 mg/kg b.i.d. group), globulin and total protein (25 [trend] and 50 mg/kg b.i.d. groups), and bilirubin (50 mg/kg b.i.d. group). A significant decrease in inorganic phosphate was evident in animals from the high dose group, and was sporadically seen in animals from several additional groups (there was no evident dose dependency). All changes appeared to regress somewhat during the off-dose period. Additional differences in blood chemistry appeared randomly distributed among the treatment groups and unrelated to the onset and cessation of drug dosing.

Urine volume was significantly increased, while specific gravity and osmolality were decreased in all (10/10) animals from the 50 mg/kg b.i.d. group at the end of dosing. Additional animals from all dose groups showed similar effects (25 mg/kg - 2/6; 10 mg/kg - 4/6; 5 mg/kg - 2/6; control - 3/10). Urinary alkaline phosphatase levels were significantly increased among males and females from the 25 and 50 mg/kg b.i.d. groups versus the control. Interstitial nephritis, renal tubule inflammation with epithelial hyperplasia,

dilated and/or basophilic tubules, oedema, and mononuclear cell infiltrates, were evident at the termination of dosing. Hyperplasia or a prominent appearance of the collecting duct epithelium was evident in 4/4 former 50 mg/kg b.i.d. animals following the off-dose period. Urine volume, specific gravity, osmolality, and alkaline phosphatase levels showed recovery or regression to normal values during the off-dose period.

Thymus weight was significantly reduced (50% versus control) among animals in the 25 and 50 mg/kg b.i.d. groups. In addition, covariate analyses (adjusting for terminal differences in body weight) showed significant increases in kidney (50 mg/kg b.i.d. group) and adrenal weights (25 and 50 mg/kg b.i.d. groups). At the end of the off-dose period, kidney weights remained elevated among penciclovir treated animals.

Comments: 1) The kidney appears to be the primary site of toxicity following the intravenous administration of penciclovir as demonstrated in multiple species (monkey, dog, rat). Changes in clinical chemistry and urinalysis, along with histologic data, suggest that significant toxic effects are evident within 1-4 weeks of the initiation of repetitive drug administration. Further, the toxic effects on the kidney are not clearly dose related, but are in part apparently idiosyncratic (as evidenced by the distribution of effected animals and the severity of the histologic and urinalysis changes). Gradual partial recovery following drug cessation is also evident.

2) As discussed in several previous studies (using multiple species), the intravenous administration of penciclovir appears to increase heart rate immediately following drug infusion. This effect is evident at most drug doses tested and is not apparently dose dependent. While not specifically evaluated, the rate of drug infusion may effect the heart rate changes observed, as evidenced by the somewhat smaller changes seen in the present study (infusion of 2 ml/kg over 2 min.) versus those seen in previous studies (infusion of 2 ml/kg over 6 seconds). The mechanism of the rate enhancing effect of penciclovir, whether direct (receptor interaction) or indirect (sympathetic agonist release or changes in ion levels), is not defined at this time. While the effect is relatively short lived, rapidly developed and easily monitored, future studies should closely monitor for cardiotoxicity and/or changes in cardiac conduction.

18 and 19) BRL 42810: A 14 Day Intravenous Range Finding Repeat Dose Study in Cynomolgus Monkeys (Incorporating BRL 39123A and BRL 29906), Study ID BRL 1179/88479, and BRL 42810: A 14 Day Intravenous Range Finding Repeat Dose Study in Cynomolgus Monkeys (Incorporating BRL 39123A and BRL 29906). Histopathology of Injection Sites, Study ID BRL 1179/88479/Addendum, (Submission 000, Vol. 12, page 217).

Status: non-GLP

Study Initiation: 3 Nov., '87

Study Site:

Compound Tested: BRL 39123A, batch GBD 15

BRL 42810, batch CT14245

BRL 29906, batch CT13962

Doses Tested: BRL 39123A, 100 mg/kg/day (IV)

BRL 29906, 100 mg/kg/day (IV)

BRL 42810, 120, 200 and 280 mg/kg/day (IV)

(* 360 mg/kg/day for days 1-4)

Drug was administered once each day as an intravenous infusion

Dose Volume and Route: 2.0 ml/kg, IV, 1 ml/min.

Solvent and Control: sterile saline

Species, Strain, Sex: 5 male and 5 female cynomolgus monkeys (Macaca fascicularis), estimated age 2-3 years, and weighing 2.3-2.9 kg.

Test conditions: Test animals were randomly assigned to the 5 test groups (1 animal/sex/group). Drug was administered (IV) once a day for 14 days. Physical signs, body weight, food and water consumption, and mortality were monitored daily during drug administration. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at intervals throughout the study. Gross and microscopic pathology was assessed at the termination of the dosing or 'off-dose' interval. Physical signs, body weight, food and water consumption, and mortality were monitored twice weekly during baseline and recovery phases of the study, and daily during drug administration. Blood chemistry, hematology, urinalyses, ophthalmoscopy and electrocardiograms were performed at intervals throughout the study. Gross and microscopic pathology (kidney only) was assessed at the termination of dosing.

All animals survived the dosing interval. BRL 39123A and BRL 29906 when administered at 100 mg/kg produced no clinical signs or obvious changes in behavior, except for a single incidence (female, day 14) of vomiting approximately 20 minutes following administration of BRL 39123A. In contrast, BRL 42810 at 200-360 mg/kg/day induced gasping, panting and labored breathing, salivation, vomiting, pupil dilation, limb tremors and muscle rigidity (high dose), collapse (high dose), and lethargy. The signs generally began prior to completion of the 2 minute infusion and lasted for 3-15 minutes. Reduced activity was seen on one occasion in the male receiving 120 mg/kg. Bruising at the site of drug injection was seen in the majority of animals, while a single animal (BRL 42810) showed oedema of the injected limb.

Both animals receiving BRL 29906, along with the female receiving BRL 39123A and the female receiving BRL 42810 (200 mg/kg/day) showed gradual declines in body weight (approximately 200-300 grams) during the 14 days of drug administration. Food and water consumption were not consistently effected by drug treatment, except for a decrease in food consumption seen in the female animal dosed with BRL 42810 at 200 mg/kg/day.

Ventricular bigeminy and tachycardia was evident in a single female immediately following administration of BRL 42810 (Day 1) at a dose of 360 mg/kg. No other ECG changes were seen in any animal at any dose tested. There were no changes in ophthalmologic findings during the course of the study.

All four monkeys receiving BRL 29906 and BRL 39123A, showed elevated platelet counts (range 600-860 x 10³ cells/mm³) on day 14 of drug administration. Hemoglobin, hematocrit and RBC counts were somewhat reduced in 2 animals receiving BRL 42810, although the values were within the baseline range for the group. On day 14, inorganic phosphorus was reduced (1.5 mEq/l) in the female animal receiving BRL 42810 at 200 mg/kg. BUN was elevated in all 4 monkeys receiving BRL 29906 and BRL 39123A, while creatinine was elevated in one animal (BRL 39123A). Remaining hematologic, plasma chemistry and urinalysis values were not clearly effected by the drug treatment.

Renal tubule inflammation with epithelial hyperplasia and basophilia, dilated tubules, and cellular casts were evident in the majority of animals. Birefringent crystalline material was evident in the kidneys of the majority of animals tested particularly at the higher doses. Slight to moderate perivascular hemorrhage at the site of drug infusion was noted in the majority of animals, with the incidence and severity appearing dose related (BRL 42810). Dark raised nodules were noted in the caecum and colon of several animals from all dose groups. Plural adhesions and subendocardial dark foci were also noted in several animals. No clear effects on organ weights were evident, although kidney weights were somewhat higher and thymus weight reduced in one animal receiving BRL 42810 (200 mg/kg) and the animals receiving BRL 29906 and BRL 39123A.

Comment: Failure to include a control group in this study makes identification and interpretation of any drug induced effects difficult if not impossible.

SUMMARY OF DERMAL IRRITANCY/TOXICITY STUDIES:

Multiple toxicity studies were conducted to evaluate the dermal, ocular and intravaginal irritancy potential, and contact sensitization effects of penciclovir cream. In summary, rats, rabbits and/or guinea pigs were exposed to one or more applications of penciclovir cream (of various formulations) to shaved regions (abraded and unabraded) of the dorsum, into the vagina or, to the eye/conjunctiva. In general, the application of any of the penciclovir containing creams caused minimal to mild dermal irritation responses with occasional edema at the site of application. However, only slightly less severe and/or similar reactions were evident in most test systems following the application of any of the placebo/vehicle "drug" formulations. Intravaginal application of penciclovir was found to be non-irritating when tested in the rabbit. In contrast, application of the test compound to the conjunctivae of the rabbit eye, produced mild-moderate redness and chemosis (inflammation) in all animals at 1 and 24 hours post-exposure with resolution of these effects by 48-72 hours.

COMPILATION OF EFFECTS AND DISCUSSION:

Multiple toxicity studies were conducted in rats and rabbits to evaluate the dermal/ocular and vaginal irritancy potential of penciclovir and/or the penciclovir cream (multiple cream formulations including the proposed "market" formulation were tested). The results of these studies are outlined in the following paragraphs.

In studies to assess the potential for dermal/eye and vaginal irritancy, rats and rabbits were exposed to one or more applications of a penciclovir formulation (formulations included: a) propylene glycol with cetomacrogol, b. aqueous cream, c) propylene glycol with DMSO, and d) water) either to shaved regions (abraded and unabraded) of the skin, the eye/conjunctiva and the vagina. Penciclovir cream, when applied once daily for 28 days to rats and rabbits (2 g cream/kg, nominal dose of 100 mg penciclovir/kg/day) resulted in very slight to minimal signs of irritancy in rats and occasional slight erythema in rabbits. Similar, or marginally reduced reactions were generally also seen with the vehicle creams. Microscopic examination of tissues from the application sites revealed minimal to moderate epidermal thickening in the majority of the repeat dose exposed animals. No evidence of vaginal irritation was noted in female rabbits following the administration of penciclovir cream. In contrast, application of the test compound to the conjunctivae of the rabbit eye, produced mild-moderate redness and chemosis (inflammation) in all animals at 1 and 24 hours post-exposure with resolution of these effects by 72 hours.

Overall, the results of the dermal and vaginal irritancy studies indicate that penciclovir cream is non-irritating or a very slight irritant to cutaneous tissues. Lastly, the application of penciclovir to the conjunctiva of the eye resulted in mild-moderate inflammatory responses, therefore the compound may be classified as a mild irritant to the conjunctivae of the eye.

A review of the individual studies is contained on the following pages.

Toxicity Studies Summary:**Dermal and Intravaginal Irritation****A) Studies Conducted Using the Proposed Market Formulation:**

- 1) BRL 39123: A 4 Week Dermal Repeat Dose Study in Rats, Study ID T91324/39123/R/DERM/RDS/28D. GLP, Beecham Phar., The Frythe, Welwyn, U.K., Study Initiation: 18 Sept., '91, BRL 39123, batch CT91 W046.
- 2) BRL 39123: A 9-Day Dermal Repeat Dose Study In Male Rabbits, Study ID T91207/39123/RAB/DERM/RFS/9D (Summary). non-GLP, Beecham Phar., The Frythe, Welwyn, U.K., Study Initiation: 1 July 1991, BRL 39123, batches W91044-W91046.
- 3) BRL 39123: A 4 Week Dermal Repeat Dose Study in Rabbits, Study ID 91322/39123/RAB/DERM/RDS/28D. GLP, Beecham Phar., The Frythe, Welwyn, U.K., Study Initiation: 29 Aug. '91, BRL 39123, batches: cream - CT91 W049; bulk drug - GBD40.

B) Studies Conducted Using an Aqueous Cream Formulation:

- 4) BRL 39123: A 14 Day Topical Range Finding Repeat Dose Study in Rats, Study ID T85626/39123/R/TOP/RFS/14D (Summary). non-GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 9 Dec., '85, BRL 39123, batch CT 18047.
- 5) BRL 39123: A 28 Day Topical Repeat Dose Study in Rats Followed by a 14 Day Off Dose Period, Study IDs T86307/39123 /R/TOP/28D/M and T86307/39123/R/TOP/28D/F. GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 16 Mar., '86, BRL 39123, batch CT 18215.
- 6) BRL 39123: Primary Dermal Irritation Study in Rabbits, Study ID DA/85/32. GLP, Beecham Phar., Great Burgh, Nr. Epsom, Surrey, U.K., Study Initiation: 16 Dec. '85, BRL 39123, batch CT 18066.
- 7) BRL 39123: A 14 Day Dermal/Intravaginal Range Finding Repeat Dose Study in Rabbits, Study ID T85625/39123/RAB/DERM /RFS/14D. non-GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 9 Dec., '85, BRL 39123, batch CT 18049.
- 8) BRL 39123: A 28 Day Topical Repeat Dose Study in Rabbits With a 14 Day Off Dose Period, Study ID 046/860207TG/39123 /RAB/TOP/RDS. GLP, Study Initiation: 14 Apr., '86, BRL 39123, batch CT 18164.
- 9) BRL 39123: A 28 Day Repeat Dose Study in Rabbits by the Intravaginal Route Followed by a 14 Day Off Dose Period, Study ID 86321/39123/RAB/IVG/RDS/28D. GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 2 Jun., '86, BRL 39123, batch CT 18215.

C) Studies Conducted Using a Propylene Glycol Cream Formulation:

- 10) BRL 39123: A 28 Day Dermal Repeat Dose Study in Rats, Study ID 89325/39123/R/DERM/RDS/28D. GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 6 Nov. '89, BRL 39123, batches: cream - CT 14959 and 14997; bulk drug - GBD32, 99.2% pure.
- 11) BRL 39123: Primary Dermal Irritancy Study in Rabbits Using BRL 39123 In A 40% Propylene Glycol Vehicle, Study ID 455/39123/RAB/PDI. GLP, Study Initiation: 8 Jan. '90, BRL 39123, batch CT 14997.
- 12) BRL 39123: Primary Dermal Irritancy Study in Rabbits Using BRL 39123 In A 40% Propylene Glycol Vehicle and 2.5% Decylmethyl Sulphoxide Vehicle, Study ID 456/39123/RAB/PDI. GLP, Study Initiation: 8 Jan. '90, BRL 39123, batch CT 14959.
- 13) BRL 39123: A 28 Day Dermal Repeat Dose Study in Rabbits, Study ID 89324/39123/RAB/DERM/RDS/28D. GLP, Beecham Phar., Stock, Essex, U.K., Study Initiation: 1 Nov. '89, BRL 39123, batches: cream - CT 14959 and

APPENDIX B: DERMAL IRRITANCY/TOXICITY STUDIES

14997; bulk drug - GBD32.

D) Miscellaneous Formulation:

14) BRL 39123: Skin Irritation Test In Rabbits. Study ID 375/39123/RAB/SKIN. GLP, Study Initiation: 15 Jan. 1990, BRL 39123, batch GBD34.

Eye Irritation

15) BRL 39123: Eye Irritation Test in Rabbits. Study ID 376/42810/RAB/EYE, Non-GLP, Test Site: Study Initiation: 22 Jan., 1990, BRL 39123, Batch: GBD34, purity not specified.

Toxicity Studies Reviews:

Dermal and Intravaginal Irritation

A) Studies Conducted Using the Proposed Market Formulation:

1) BRL 39123: A 4 Week Dermal Repeat Dose Study in Rats, Study ID T91324/39123/R/DERM/RDS/28D.

Status: GLP

Study Initiation: 18 Sept., '91

Study Site: Beecham Phar., The Frythe, Welwyn, U.K.

Compound Tested: BRL 39123 (5% w/w) in 40% propylene glycol with 0.9% cetomacrogol 1000, batch CT91 W046.

Doses Tested: 0 (vehicle) and 100 mg/kg

Dose Volume and Route: 2.0 ml/kg, topical.

Solvent and Control: cream base without BRL 39123

Species, Strain, Sex: Male and female Sprague Dawley rats (CPL: CD SD Br), age 4-5 weeks, weight 101-125 grams, 5 animals/sex /group, there were 3 treatment groups; BRL39123, vehicle treated and untreated controls..

Test conditions: Animals were divided into groups as follows: BRL 39123 treated (100 mg/kg), vehicle treated, and untreated controls. BRL 39123 (or vehicle) was applied topically to a shaved area (25 cm²) of the dorsal cervical region of the test rats once daily for a period of 28 days. Body weight and physical signs were assessed periodically during the study.

Macroscopic and histologic assessments were performed at study termination.

There were no premature deaths or changes in general physical signs during the study. Group mean body weight gain was slightly reduced (range of 8-20%) among the vehicle and drug treated animals (male and female) although the effect did not achieve statistical significance.

Microscopic examination of the application sites revealed minimal thickening of the epidermal layers among BRL 39123 and vehicle treated animals. There were no macroscopic indications of dermal irritancy produced by either the active or control creams during the 28 days of treatment.

Comments: 1) BRL 39123 (5% w/w) when administered (100 mg/kg) topically in a cream base containing 40% propylene glycol and 0.9% cetomacrogol appears to have minimal irritancy potential for the intact skin of the rat.

2) When compared with effects noted in several other dermal irritancy studies, the current results suggest that the cetomacrogol cream base containing BRL 39123 may have less irritancy potential than BRL 39123 in DMSO, SDS or carbomer 940

containing creams.

2) **BRL 39123: A 9-Day Dermal Repeat Dose Study In Male Rabbits**, Study ID T91207/39123/RAB/DERM/RFS/9D.

Status: non-GLP, Summary Report.

Study Initiation: 1 July 1991

Study Site: Beecham Phar., The Frythe, Welwyn, U.K.

Compound Tested: BRL 39123, batches;

W91045 - 5% BRL in 40% PG with Cetomacrogol 1000

W91046 - 5% BRL in 40% PG with SDS and Carbomer 940

W91044 - 5% BRL in 'Zovirax' cream base (40% PG)

D7574 - 5% Acyclovir cream (Zovirax)

Dose Volume and Route: 0.125 g cream/kg, topical

Control: Zovirax cream, batch D7574

Species, Strain, Sex: 5 male New Zealand White rabbits, age 4-5 months, initial weight 3-3.5 kg.

Test Conditions: Animals were housed singly. The BRL 39123 and Acyclovir containing creams were applied 1x/day for 9 consecutive days. Cream was applied to 1 of 4 non-abraded skin regions on the dorsum of each animal followed by a 6 hour occlusion. Test sites were monitored for the development of irritancy reactions before cream application, immediately after removal of the occlusive bandages, and 24 hours following each application. Histologic examinations were performed on tissues obtained from each test site 24 hours following the final cream application.

Multiple occurrences of slight - well defined erythema were noted in all animals at the application sites of the following creams: Zovirax (Acyclovir) cream, BRL 39123 in 'Zovirax' cream base, and BRL 39123 in 40% PG with SDS and Carbomer 940. A slight erythema was noted in 2/4 animals (1-2 occasions each) at the application site of BRL 39123 in 40% PG with Cetomacrogol 1000. Microscopic examination revealed minimal to mild epidermal thickening at the application site of all test creams, except for the BRL -Cetomacrogol 1000 formulation which resulted in negligible to very minimal effects. No other adverse effects were reported.

Comment: 5% BRL 39123 in 40% PG with Cetomacrogol 1000 (a non-ionic surfactant) appears to have slightly less dermal irritancy potential (as compared to 5% BRL 39123 in 40% PG with SDS and Carbomer 940 [an ionic surfactant], or a commercial acyclovir cream [Zovirax]) when applied to unabraded rabbit skin.

3) **BRL 39123: A 4 Week Dermal Repeat Dose Study in Rabbits**, Study ID 91322/39123/RAB/DERM/RDS/28D.

Status: GLP

Study Initiation: 29 Aug. 1991

Study Site: Beecham Phar., The Frythe, Welwyn, U.K.

Compound Tested: BRL 39123, bulk drug GBD40, 98.9% pure cream - CT91 W049, 5% w/w BRL 39123, 40% PG, 0.9% cetomacrogol 1000.

Dose Volume and Route: 2 g cream/kg/day, topical
(nominal dosage = 100 mg/kg/day)

Control: cream base without BRL 39123

Species, Strain, Sex: male and female New Zealand White rabbits, age 3-4 months, initial weight 2.5-3 kg, 3 animals/sex /treatment group, there were 3 treatment groups; BRL39123, vehicle treated and untreated controls.

Test Conditions: Animals were housed singly. BRL 39123 or control vehicle creams were applied 1x/day for 28 consecutive days. Cream was applied to a 10 x 10 cm region of abraded skin and covered by an occlusive bandage for 6 hours. Test sites were monitored for the development of irritancy reactions before cream application, immediately after removal of the occlusive bandages, and 24 hours following each application. Body weight and physical signs were

monitored periodically. Gross and histologic examinations of the test sites were performed on tissues obtained 24 hours following the final cream application.

There were no deaths during the study. Body weight gain was slightly reduced among BRL 39123 and vehicle cream treated female animals versus the untreated controls. Physical signs observed included occasional minimal to slight erythema at the site of application of the BRL 39123 or control creams. The majority of cream treated animals displayed erythema on 1-4 days during the 28 day treatment period. Scabbing was noted among 2/6 and 1/6 vehicle and BRL 39123 treated animals, respectively. Histologic examination revealed minimal to moderate epidermal thickening at the application site of either cream formulation (active or control), with inflammatory cell infiltration of the scabbed areas. There were no others adverse effects reported.

Comment: 1) The study results suggest that BRL 39123 (5% w/w) in a 40% propylene glycol and 0.9% cetomacrogol 1000 cream base had minimal to slight irritancy potential for abraded and occluded rabbit skin.

2) When compared with effects noted in several other dermal irritancy studies, the current results suggest that the cetomacrogol cream base containing BRL 39123 may have less irritancy potential than BRL 39123 in DMSO, SDS or carbomer 940 containing creams.

B) Studies Conducted Using an Aqueous Cream Formulation:

4) **BRL 39123: A 14 Day Topical Range Finding Repeat Dose Study in Rats, Study ID T85626/39123/R/TOP/RFS/14D.**

Status: non-GLP, Summary Report

Study Initiation: 9 Dec., '85

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batch CT 18047, 5% w/w cream

Doses Tested: 0 (vehicle) and 100 mg/kg

Dose Volume and Route: 2.0 ml/kg, topical

Solvent and Control: placebo cream (unidentified content), batches CT 18048 and CT 18050

Species, Strain, Sex: 6 male and 6 female Sprague Dawley rats (CRL: COBS[®] CD[®] SD Br rats), age and weight unknown.

Test conditions: Animals were divided into 3 groups (3 animals/sex/group) as follows: BRL 39123 treated, vehicle treated, and untreated controls. BRL 39123 (or vehicle) was applied topically (2 ml/kg of body weight; drug concentration 49 mg/g) to a shaved area of the dorsal cervical region of the test rats once daily for a period of 14 days.

Body weight gain was slightly reduced among treated male and female rats as compared with the untreated controls. Body weight gain among males was also reduced when compared with the vehicle treated controls, while that for treated females was slightly increased. Water intake was not effected by treatment, while food intake among treated females was slightly increased.

Occasional in-treatment signs included very slight to slight erythema (well defined borders) at the site of cream application among male animals. Similar signs were observed in the vehicle control group. No other macroscopic findings were reported for male or female animals.

Comment: 1) BRL 39123 when administered (100 mg/kg) topically in a cream base (5% w/w) appears to have only a slight irritancy potential for the intact skin.

2) The 5% w/w formulation of BRL 39123 was considered the highest practicable dose level which could be achieved and was therefore selected as the maximum dose for the subsequent 28 day repeat-dose study.

5) **BRL 39123: A 28 Day Topical Repeat Dose Study in Rats Followed by a 14 Day Off Dose Period**, Study IDs T86307/39123/R/TOP/28D/M and T86307/39123/R/TOP/28D/F.

Status: GLP

Study Initiation: 18 Mar., '86

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batch CT 18215, 5% w/w cream

Doses Tested: 0 (vehicle), 25, 50 and 100 mg/kg

Dose Volume and Route: 2.0 ml/kg (control and high dose groups), 0.5 and 1.0 ml/kg for the low and intermediate dose groups, respectively; topical.

Solvent and Control: placebo cream (unidentified content), batch CT 18216

Species, Strain, Sex: 50 male and 50 female Sprague Dawley rats (CRL: COBS^(R) CD^(R) SD Br rats), age 6-8 weeks, weight 135-205 grams. An additional 15 male and 15 female rats were treated concurrently and then allowed an off-treatment recovery period prior to sacrifice.

Test conditions: Animals were divided into 5 groups (10 animals /sex/group) as follows: BRL 39123 treated (100, 50 or 25 mg/kg), vehicle treated, and untreated controls. BRL 39123 (or vehicle) was applied topically (drug concentration 50 mg/g) to a shaved area (25 cm²) of the dorsal cervical region of the test rats once daily for a period of 28 days. Five additional male and female animals were assigned to each of the untreated, placebo cream and high dose groups, and were allowed a 14 day recovery period following treatment. Body weight, food and water consumption, hematology, clinical chemistries and urinalysis were assessed periodically. Macroscopic and histologic assessments were performed at study termination.

There was an approximate 2-3x increase in the incidence of dermal irritation among males and females in the drug treated groups as compared with the vehicle controls. Although there was substantial group overlap, the incidence of very slight to slight erythema (well defined borders) at the site of cream application generally increased with the administered dose. The incidence of very slight irritation was approximately 37 and 12 %, while slight to moderate irritation was approximately 5 and 0.5% among males and females respectively (24 hours post dosing). No other macroscopic findings were reported for male or female animals. Histologic assessment revealed minimal to moderate epidermal hyperplasia (increased number of nucleated cells and keratin) at the treatment site among animals from all treatment and control groups. No other histologic effects were detected and this effect was deemed unrelated to the treatment.

Body weight gain was slightly (10%) reduced among treated males (all dose groups) and females (intermediate dose only) as compared with the untreated controls. Body weight gain of males in the vehicle control group was also reduced somewhat when compared with the untreated controls. During the post-treatment recovery period, the body weight gain of males from the high dose group remained somewhat reduced as compared with the undosed and vehicle controls. All other groups (male and female) displayed similar weight gains as the control animals. Absolute and relative organ weights were unaffected by drug treatment, except for increases in pituitary and uterine weights among females from the high dose group (as compared with the untreated or vehicle controls) following the recovery phase of the study.

Food intake was not effected by drug treatment at any dose tested. Water consumption was slightly increased (approximate increase of 5 ml/day) in females from the high and intermediate dose groups during week 1 of testing and in females from all drug treatment groups during week 3. Intake of water

remained somewhat elevated during the recovery phase of the study among females from the 2 high dose groups.

There was a slight decrease in mean corpuscular hemoglobin (19.1 versus 19.4 µg [control]) and mean corpuscular hemoglobin concentration (314 versus 324 g/l [control]) of males in the high dose condition after 29 days of treatment. Females from the high dose group displayed only a decrease in MCHC (319 versus 324 g/l [control]). There was a small decrease in blood calcium levels among males from the intermediate and high dose groups after 29 days of drug application. Differences in MCH, MCHC and Ca were transient and receded during the 14 day recovery phase. All remaining hematologic, clinical chemistry, urinalysis and ophthalmologic parameters were within normal background variations.

Comment: At doses between 25 and 100 mg/kg, BRL 39123 has only slight irritancy effects on intact skin when administered topically in a cream base (5% w/w). Most, if not all of the observed effects appeared to recede during the post-dosing recovery phase of the study.

6) BRL 39123: Primary Dermal Irritation Study in Rabbits, Study ID DA/85/32.

Status: GLP

Study Initiation: 16 Dec. '85

Study Site: Beecham Phar., Great Burgh, Nr. Epsom, Surrey, U.K.

Compound Tested: BRL 39123, batch CT 16066, 5% w/w cream

Doses Tested: 0 (vehicle) and 50 mg/test site, topical

Dose Volume and Route: 1.0 ml/5 cm², topical

Solvent and Control:

aqueous cream BP, batch CT 18067

aqueous cream BP w/wo chlorocresol (FDD, Great Burgh), batches LNB 8898-95 and LNB 8898-94, respectively

aqueous cream BP with 2-phenoxyethanol (Evans), batch T0905GA

Species, Strain, Sex: 5 male New Zealand White rabbits, age not specified, initial weight approximately 2.5 kg.

Test Conditions: Animals were allocated into 2 groups and treated with either BRL 39123 cream (3 animals) or the vehicle (2 animals). An additional 3 rabbits were used to compare potential effects associated with the vehicle cream. Cream was applied topically (1.0 ml/5 cm², conc. = 50 mg/ml) to multiple shaved areas (with and without abrasion on opposite flank areas) of the left and right flanks of each animal. The treatment sites were covered by a gauze and tape occlusive dressing for 24 hours following application of the test article. Skin reactions were assessed 30 minutes, 7, 24 and 48 hours following the removal of the dressings. All animals and an additional vehicle formulation control group were tested 4 weeks following the initial study.

Application of the aqueous cream BP, both with and without preservatives, was associated with a barely discernable to defined erythematous (and sometimes edematous) reaction at the test sites. There were no apparent differences in the reaction of intact and abraded skin. Addition of BRL 39123 to the cream formulation did not increase the frequency or severity of the erythematous response. Erythema and oedema (if present) was typically evident only during the first 24 hours following removal of the occlusive dressings.

Comment: BRL 39123 appears to have minimal irritancy potential when applied to intact or abraded skin (rabbit) in a 5% w/w cream. The majority of the dermal irritancy noted appeared to be associated with the vehicle cream.

7) BRL 39123: A 14 Day Dermal/Intravaginal Range Finding Repeat Dose Study in Rabbits, Study ID T85625/39123/RAB/DERM/RFS/14D.

Status: non-GLP

Study Initiation: 9 Dec., '85

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batch CT 18049, 5% w/w cream

Doses Tested: 0 (vehicle) and 100 mg/kg, topical
0 (vehicle) and 25 mg/kg, intravaginal

Dose Volume and Route: 2.0 ml/kg, topical
0.5 ml/kg, intravaginal

Solvent and Control: placebo cream (unidentified content), batch CT 18050

Species, Strain, Sex: 4 male and 4 female New Zealand White rabbits, age not specified, initial weight 2-3 kg.

Test Conditions: Animals were randomly allocated into 2 groups (2 animals/sex/group) as follows: BRL 39123 treated and vehicle treated. BRL 39123 (or vehicle) was applied topically (2 ml/kg of body weight; nominal concentration of 50 mg/ml) to a shaved and abraded area (16 x 16 cm) of the dorsal cervical region of the test rabbits once each day for 14 days. The treatment sites were covered by a gauze and foil patch for 6 hours following each application of the test article. In addition, the test or placebo cream was administered intravaginally to female rabbits at a dose volume of 0.5 ml/kg of body weight (25 mg/kg).

Clinical signs and behavior, body weight, food and water consumption, hematology, clinical chemistries and urinalysis were assessed periodically. Detailed macroscopic assessments of the dermal and vaginal application sites were performed at study termination.

Body weight was not effected by treatment with BRL 39123. However, food and water consumption were slightly reduced (5-10%) in treated females, as was water consumption in treated males. Blood chemistry and urinalysis parameters were not effected by BRL 39123 treatment. A slight reduction in hemoglobin, hematocrit, RBC, lymphocyte and platelet counts was evident in 1 male animal treated with BRL 39123.

Physical signs included very slight to slight erythema (well defined borders) at the site of cream application among BRL 39123 treated animals. Similar, although less prominent, signs were observed in the vehicle control treated groups. Staining of the fur around the urogenital and anal regions was also observed in treated and control animals. No other macroscopic findings were reported for male or female animals.

Comment: 1) BRL 39123 when administered (100 mg/kg) topically in a cream base (5% w/w) appeared to have only a slight irritancy potential for abraded skin.

2) The 5% w/w formulation of BRL 39123 was considered the highest practicable concentration which could be achieved and was therefore selected for the subsequent 28 day repeat-dose study. Doses to be used in the follow-up trials were; topical, 25, 50 and 100 mg/kg; and, intravaginal, 50 mg/rabbit.

BRL 39123: A 28 Day Topical Repeat Dose Study in Rabbits With a 14 Day Off Dose Period, Study ID 046/860207TG/39123/RAB/TOP /RDS.

Status: GLP

Study Initiation: 14 Apr., '86

Study Site:

Compound Tested: BRL 39123, batch CT 18164, 5% aqueous cream

Doses Tested: 0 (vehicle), 25, 50 and 100 mg/kg

Dose Volume and Route: 2.0 ml/kg, topical

Solvent and Control: placebo cream (unidentified content), batch CT 18165

Species, Strain, Sex: 25 male and 25 female New Zealand White rabbits, age not specified, initial weight 2-2.8 kg.

Test conditions: Animals were randomly assigned to 1 of 5 treatment groups

(5 animals/sex/group) as follows: BRL 39123 treated (25, 50 or 100 mg/kg), vehicle treated, and untreated controls. BRL 39123 (or vehicle) was applied topically (2 ml/kg of body weight; conc. of 50 mg/ml) to a shaved and abraded area (100 cm²) of the dorsal cervical region once/day for a period of 28 days. The treatment site was covered by a gauze and foil patch for 6 hours after application of the test article. Two additional male and female animals were assigned to each of the untreated, placebo cream and high dose groups, and were allowed a 14 day recovery period following treatment. Clinical signs and behavior, body weight, food and water consumption, hematology, clinical chemistries and urinalysis were assessed periodically. Detailed macroscopic and histologic assessments were performed at study termination.

There were no treatment related deaths (one male animal from the untreated control group died on day 7 of the off-treatment phase of the study [numerous pleural and pericardial adhesions were detected upon necropsy, along with yellow-white patches on the liver]). Body weight gain, food and water consumption, clinical signs and ophthalmoscopy were not effected by the application of BRL 39123 cream. There were no significant changes in clinical chemistries or hematologic parameters during the study, except for an increase in mean corpuscular hemoglobin and corpuscular volume which was evident in a single high dose male after 22 days of drug administration. There were no between group differences evident during the 14 day recovery phase of the study.

Macroscopic signs observed at termination of dosing included slight and diffuse erythema at the site of cream application among BRL 39123 and vehicle treated animals. Histologic examination of tissues from the application site revealed a minimal to slight inflammatory reaction within the stratum papillare of the dermis (incidence: control = 6/10; low = 7/10; intermediate = 9/10; and high dose = 10/10). Granulocytic infiltrates were also observed in some hair follicles taken from 2 of 10 high dose animals. All other gross and microscopic signs observed at necropsy appeared to be randomly distributed and unrelated to drug treatment. There were no observed differences between the previously treated and control animals at the end of the 2 week 'off-dose' recovery period.

Comment: BRL 39123 when administered topically (5% w/w cream) at doses between 25 and 100 mg/kg appeared to have only a slight irritancy potential for abraded skin. Slight erythema and inflammation with associated granulocytic infiltration was evident following 28 days of drug administration, but receded during a 14 day recovery period.

9: BRL 39123: A 28 Day Repeat Dose Study in Rabbits by the Intravaginal Route Followed by a 14 Day Off Dose Period, Study ID 86321/39123/RAB/IVG/RDS/28D.

Status: GLP

Study Initiation: 2 Jun., '86

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batch CT 18215, 5% w/w cream

Doses Tested: 0 (vehicle) and 50 mg/rabbit/day

Dose Volume and Route: 1.0 ml/rabbit, intravaginal

Solvent and Control: placebo cream (unidentified content), batch CT 18216

Species, Strain, Sex: 21 female New Zealand White rabbits, age not specified, initial weight 2-3 kg.

Test Conditions: Animals were randomly allocated into 3 groups (7 animals/group) as follows: BRL 39123 treated, vehicle treated, and untreated control. BRL 39123 (or vehicle) was administered intravaginally (1 ml/rabbit; nominal concentration of 50 mg/ml) to the female rabbits for 28 consecutive days. At termination of drug dosing 5 animals from each treatment group were euthanized, while the remaining 2 animals/group were maintained for an additional 2 week 'recovery' period prior to euthanasia. Clinical signs,

behavior, and body weight were assessed periodically. Macro- and microscopic assessments of the vaginal application site were performed at study termination.

There were no premature deaths during the study. Body weight gain was slightly reduced (13% decrease versus untreated controls) during treatment with BRL 39123; however, weight gain among BRL 39123 treated animals was increased during the subsequent recovery phase of the study. Physical signs included staining of the fur around the urogenital and anal regions in treated and control animals. No other clinical signs were noted during the study.

Reddening (erythema) of the vaginal wall was observed in all test groups and was deemed unrelated to treatment with BRL 39123. There were no other macroscopic findings either at the end of treatment or following the 14 day recovery period. There was no evidence of histologic damage due to the administration of BRL 39123 or the placebo cream.

Comment: BRL 39123 when administered intravaginally (50 mg/rabbit) in the rabbit was without irritant effects.

C) Studies Conducted Using a Propylene Glycol Cream Formulation:

10) **BRL 39123: A 28 Day Dermal Repeat Dose Study in Rats**, Study ID 89325/39123/R/DERM/RDS/28D.

Status: GLP

Study Initiation: 6 Nov. 1989

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batches: cream -

CT 14959 - 5% (w/w) BRL 39123 in 40% propylene glycol and 2.5% DMSO

CT 14997 - 5% (w/w) BRL 39123 in 40% propylene glycol; bulk drug - GBD32, 99.2% pure.

Dose Volume and Route: 2 g cream/kg/day, topical (nominal dosage = 100 mg/kg/day)

Control: corresponding creams without BRL 39123

Species, Strain, Sex: male and female Sprague Dawley rats Crl:SD(CD)BR, age 4-5 weeks, initial weight range 76-100 g, 10 animals/sex/treatment group.

Test Conditions: Animals were housed singly. BRL 39123 or control vehicle creams were applied 1x/day for 28 consecutive days. Cream was applied to a 25 cm² area (shaved, unabrased) of the dorso-cervical region of each animal. The test sites were neither occluded or washed during the day. Test sites were monitored for the development of irritancy reactions before cream application, and periodically following each application. Body weight, food and water intake and ophthalmic status were monitored periodically during the treatment period. Hematologic and clinical or urine chemistries were performed at baseline and preterminal. Gross and histologic examinations were performed on tissues obtained 24 hours following the final cream application.

Slight to well defined erythema with occassional slight oedema was seen at the drug application site in all animals receiving the 40% propylene glycol with 2.5% DMSO cream formulation regardless of the presence of BRL 39123. Drying and cracking of the skin with the production of a flaky appearance was noted in animals treated with cream containing DMSO. Animals administered the 40% PG formulation (regardless of drug content) showed a somewhat lower incidence of slight erythema (without oedema or drying of the skin). For all study groups, the incidence and severity of the dermal reactions appeared to be more prominent during weeks 3 and 4 of testing, and at 3 versus 24 hours following drug application. Minimal epidermal thickening was noted at the application site in the majority of drug treated animals, regardless of the drug content of the applied cream.

Bodyweight gain was reduced among male (10-30%) and female (10-20%) animals dosed with the DMSO containing cream (regardless of drug content), and among males (30%) dosed with the 40% PG cream containing BRL 39123. Food consumption was also reduced 5-10% among the affected males, but not among the female test animals. Similarly, the efficiency of food utilization (i.e., bodyweight gain/food consumed) was slightly reduced among the DMSO treated animals (regardless of the drug content of the test cream). Water consumption was increased 5-25% among all male animals (versus the untreated controls), and was increased 5-15% among female animals treated with the DMSO-containing test creams. At terminal examination, liver (15-25%), heart (15%), thymus (25%), lung (10-15%) and pituitary (20-30%) weights were reduced among the DMSO treated and occasionally among the 40% PG with BRL 39123 treated male animals. Pituitary weight was reduced 15-22% among cream treated female animals, while adrenal weights were increased 10-15% among the DMSO treated females (regardless of the drug content of the cream).

Except for a slight increase in the number of circulating monocytes in association with the administration of any of the test cream formulations, there were no other clearly treatment related changes in any hematologic parameter. Marginal to slight decreases in total protein, serum globulins and sodium were evident among the male animals treated with the DMSO containing cream formulations, whereas only plasma sodium levels were reduced among the DMSO treated female animals. Marginal reductions in serum phosphate levels were seen among females treated with the 40% PG creams and males treated with the 2.5% DMSO containing creams. Urine volume was decreased and osmolality increased among all cream treated female animals and among the DMSO treated male animals. Male animals treated with the 40% PG cream formulations showed only a slight increase in urine osmolality.

- Comments:
- 1) The study results suggest that the propylene glycol cream base has minimal irritancy potential for the intact skin of the rat, but that this effect may be increased by the addition of the active agent (BRL 39123) and/or DMSO. Animals administered the 40% PG formulation (regardless of drug content) showed a somewhat lower incidence of slight erythema (without oedema or drying of the skin). For all study groups, the incidence and severity of the dermal reactions appeared to be more prominent during weeks 3 and 4 of testing, and at 3 versus 24 hours following drug application.
 - 2) An explanation for the multiple differences in group mean organ weights is not readily apparent although the overall reduction in bodyweight gain as seen among the test-cream treated animals likely accounts for most-all of these effects. However, increases in liver weight may be associated with enzyme induction following from drug metabolism, while the reductions in thymus weight may be due to an immunotoxic effect.
 - 3) Changes in urine volume and osmolality were likely due to reduced water intake (as is common with reduced food consumption and weight gain), although this was not measured in the study.
 - 4) Because histopathologic evaluations were not performed on all tissues from the study animals, it is not possible based on the available data to draw clear conclusions regarding the nature and/or potential impact of several of the toxic responses noted and discussed above.
 - 5) While dermal irritation and epidermal thickening were noted following the application of all cream formulations, the resultant degree of irritation does not appear to preclude development and/or use of these formulations.

11) **BRL 39123: Primary Dermal Irritancy Study in Rabbits Using BRL 39123 In A 40% Propylene Glycol Vehicle, Study ID 455/39123 /RAB/PDI.**

Status: GLP

Study Initiation: 8 Jan. '90

Study Site:

Compound Tested: BRL 39123, batch CT 14997, 5% w/w cream

Doses Tested: 0 (vehicle) and 25 mg/test site, topical

Dose Volume and Route: 0.5 ml/2.5 cm², topical

Solvent and Control: 40% propylene glycol cream base

Species, Strain, Sex: 3 male New Zealand White rabbits, age 12-13 weeks, initial weight approximately 2.5-2.7 kg.

Test Conditions: Cream (vehicle w/wo BRL 39123) was applied topically to multiple shaved areas (with and without abrasion) of the left and right flanks of each animal. The treatment sites were covered by a gauze and tape occlusive dressing for 24 hours following application of the test article. Skin reactions were assessed 30 minutes, 24, 48 and 72 hours following the removal of the dressings.

Application of the 40% propylene glycol cream was associated with a defined erythematous (and sometimes a slight edema) reaction at the test sites. Addition of BRL 39123 to the cream formulation did not increase the frequency or severity of the erythematous response but did slightly increase the degree of edema. There were no apparent differences in the reaction of intact or abraded skin. Erythema remained evident at 24 hours following removal of the occlusive dressings, but was not present at the final observation.

Comments: 1) Dermal irritancy reactions were noted in all animals and appeared to be primarily associated with the propylene glycol cream (although very slightly more severe reactions were evident at the site of BRL 39123 application). There were no apparent differences in the reaction of intact or abraded skin.

2) BRL 39123 (5% w/w) in a 40% propylene glycol cream was defined as a mild irritant to the intact or abraded skin of the rabbit.

12) **BRL 39123: Primary Dermal Irritancy Study in Rabbits Using BRL 39123 In A 40% Propylene Glycol Vehicle and 2.5% Decylmethyl Sulphoxide Vehicle, Study ID 456/39123/RAB/PDI.**

Status: GLP

Study Initiation: 8 Jan. '90

Study Site:

Compound Tested: BRL 39123, batch CT 14959, 5% w/w cream

Doses Tested: 0 (vehicle) and 25 mg/test site, topical

Dose Volume and Route: 0.5 ml/2.5 cm², topical

Solvent and Control: 40% propylene glycol w/ 2.5% Dec-MSO cream base (batch CT 14958)

Species, Strain, Sex: 3 male New Zealand White rabbits, age 12-13 weeks, initial weight approximately 2.5-2.7 kg.

Test Conditions: Cream (vehicle w/wo BRL 39123) was applied topically to multiple shaved areas (with and without abrasion) of the left and right flanks of each animal. The treatment sites were covered by a gauze and tape occlusive dressing for 24 hours following application of the test article. Skin reactions were assessed 30 minutes, 24, 48 and 72 hours following the removal of the dressings.

Application of the propylene glycol/Dec-MSO cream (w/wo BRL 39123) was associated with well defined erythematous reactions at the test sites of intact and abraded skin. Erythema remained evident at 24-48 hours following removal of the dressings, but was not present at 72 hours post-exposure.

Comments: 1) Dermal irritancy reactions were noted in all animals and

appeared to be associated with the propylene glycol Dec-MSO cream. There were no apparent differences in the reaction of intact or abraded skin.

2) BRL 39123 (5% w/w) in a 40% propylene glycol/2.5% Dec-MSO cream was defined as a mild irritant to the intact or abraded skin of the rabbit.

13) **BRL 39123: A 28 Day Dermal Repeat Dose Study in Rabbits**, Study ID 89324/39123/RAB/DERM/RDS/28D.

Status: GLP

Study Initiation: 1 Nov. 1989

Study Site: Beecham Phar., Stock, Essex, U.K.

Compound Tested: BRL 39123, batches: cream -

CT 14959 - 5% (w/w) BRL 39123 in 40% propylene glycol and 2.5% DMSO

CT 14997 - 5% (w/w) BRL 39123 in 40% propylene glycol; bulk drug - GBD32, 99.2% pure.

Dose Volume and Route: 2 g cream/kg/day, topical
(nominal dosage = 100 mg/kg/day)

Control: corresponding creams without BRL 39123

Species, Strain, Sex: male and female New Zealand White rabbits, age 4-5 months, initial weight 2-3 kg, 5 animals/sex /treatment group.

Test Conditions: Animals were housed singly. BRL 39123 or control vehicle creams were applied 1x/day for 28 consecutive days. Cream was applied to a 10 x 10 cm region of abraded skin on the dorsum of each animal. The test sites were covered by an occlusive bandage for 6 hours/day beginning immediately after application of the test compound. Test sites were monitored for the development of irritancy reactions before cream application, immediately after removal of the occlusive bandages, and 24 hours following each application. Body weight, food and water intake and ophthalmic status were monitored periodically during the treatment period. Hematologic and clinical or urine chemistries were performed at baseline and preterminal. Gross and histologic examinations were performed on tissues obtained 24 hours following the final cream application.

Slight to well defined erythema and edema were seen at the drug application site in all animals, regardless of vehicle formulation or drug content. However, slightly higher incidence of moderate erythema, hardening of the skin and production of flaky appearance, were noted in animals treated with cream containing DMSO. Reactions tended to be more pronounced during weeks 1 and 2 of treatment, and to regress during the 18 hour interval between treatments. Minimal epidermal thickening was noted at the application site in the majority of drug treated animals (versus the vehicle controls).

Moderate to marked proteinuria was noted in 2 animals from each of the active treatment groups. Slight increases in serum calcium levels were noted in 2 animals treated with BRL 39123 cream without DMSO and in 1 animal treated with BRL 39123 cream with DMSO. Organ weight changes included: increased heart weight among drug treated animals and those treated with the DMSO containing vehicle; increased kidney weight among males treated with BRL 39123 cream without DMSO; moderate to marked increases in liver weight among drug and vehicle treated male animals; increased lung weight among males treated with BRL 39123 and the DMSO containing vehicle, while reductions in lung weight were seen in females treated with BRL 39123 containing and the corresponding vehicle cream without DMSO; and, markedly reduced spleen weight among drug treated female animals (and to a lesser extent among vehicle treated animals), while male animals treated with the active or vehicle cream without DMSO showed increased spleen weight. Slight reductions in thymus and ovarian weights were evident in treated male and female animals respectively, while males showed slight increases in group mean prostate weight. Lastly, all

treatment groups showed slight reductions in thymus weight. There were no apparent treatment related effects on body weight, food and water consumption, food utilization, hematologic or ophthalmoscopic status.

Comments: 1) The study results suggest that the propylene glycol cream base has minimal to moderate irritancy potential for abraded skin, and that this effect may be enhanced by the addition of the active agent (BRL 39123) and DMSO. While irritation was noted following the application of all cream formulations, the resultant degree of irritation does not appear to preclude continued testing and development.

2) An explanation for the multiple differences in group mean organ weights is not readily apparent from the study results or previous reports. However, increases in liver weight may be associated with enzyme induction following from drug metabolism, while increased kidney weight may be related to renal interstitial or tubular inflammation (not noted in the histopathology report) which has been frequently seen with other nucleoside analogues.

Proteinuria among several of the drug treated animals is likely associated with renal glomerular damage and interstitial inflammation, as has been noted with several of the nucleoside analogues.

3) Changes in group mean thymus and spleen weights while not consistent between sexes, do appear to be drug related. The organ weight changes noted may be indicative of lymphoid tissue depletion and/or splenic congestion with cellular debris. Immunologic toxicities have been noted with several other nucleosides, and it is therefore recommended that penciclovir be evaluated for immunotoxic effects.

4) Because histopathologic evaluations were not performed on all tissues from the study animals, it is not possible based on the available data to draw clear conclusions regarding the nature and/or potential impact of several of the toxic responses noted and discussed above. It is recommended that in the future, the sponsor conduct complete histopathologic evaluations of all standard tissues from animals used in toxicity studies of penciclovir.

D) Miscellaneous Formulations:

14) BRL 39123: Skin Irritation Test In Rabbits, Study ID 375/39123/RAB/SKIN.

Status: GLP

Study Initiation: 15 Jan. '90

Study Site:

Compound Tested: BRL 39123, batch GBD34

Doses Tested: 0 (vehicle) and 5 g/test site

Dose Volume and Route: 0.5 ml water/2.5 cm², topical

Solvent and Control: distilled water

Species, Strain, Sex: 3 male New Zealand White rabbits, age 12-14 weeks, initial weight approximately 3.0-3.5 kg.

Test Conditions: BRL 39123 was applied topically to a shaved area (without abrasion) of the dorsal-cervical region of each animal. The treatment site was covered by a gauze and tape occlusive dressing for 4 hours following application of the test article. Skin reactions were assessed 30 minutes, 24, 48 and 72 hours following the removal of the dressings.

Application of BRL 39123 (in water) to the intact skin of the rabbit was

APPENDIX B: DERMAL IRRITANCY/TOXICITY STUDIES

without irritant effect during the 4 day observation period.

Comment: No dermal irritancy reactions were noted. BRL 39123 was classified as a non-irritant to the intact skin of the rabbit.

Eye Irritation

15) BRL 39123: Eye Irritation Test in Rabbits, Study ID 376/42810/RAB/EYE.
Status: Non-GLP

Test Site:

Study Initiation: 22 Jan., 1990

Doses Tested: 0.1 ml of unspecified concentration

Compound: BRL 39123, Batch: GBD34, purity not specified.

Solvent: distilled H₂O, 37°C, Negative Control

Test Animal: 4 male New Zealand White Rabbits, 3.0-3.5 kg and approx. 12-14 weeks of age.

Test Procedure: Twenty-four hours before testing the eyes of all animals were examined for evidence of ocular irritation or defect. On the day of testing 0.1 ml of the experimental compound (95 mg of BRL 39123 in distilled H₂O) was placed in the right eye of each rabbit. The contralateral eye remained untreated and was used as a control. Assessments of damage and/or irritation were made 1, 24, 48, and 72 hours following treatment.

Results: Mild-moderate redness and inflammation of the conjunctivae were evident in all animals at 1 and 24 hours postexposure, with resolution of these effects by 72 hours. Discharge was evident in 3/3 animals at 1 hour post-exposure, with discharge evident in 1 animal through 48 hours post-exposure. There was no evidence of concurrent damage to the cornea or iris of the eye in any animal.

Comments: 1) The compound was classified as a mild irritant to the conjunctivae of the rabbit eye.

2) The study report does not include information regarding the concentration/purity of the test solution. This omission of critical information makes the study results nearly uninterpretable.

2) The sponsor has tested the compound at only one concentration (unspecified) and duration of exposure. Thus, it is impossible to determine if more significant irritation, tissue erosion or scarring would occur with increased concentration, duration of exposure, or repeat exposure.

APPENDIX C: IMMUNOTOXICOLOGY STUDIES

SUMMARY:

Penciclovir was tested in multiple in vivo and in vitro assays for immunotoxic and/or immunostimulant/hypersensitization effects. In the rat, despite a slight degree of splenomegaly and thymic atrophy evident after 28 days of IV dosing, the study results did not indicate that BRL 39123A caused any immune system suppression. In the guinea pig, the repeated administration of penciclovir cream (proposed market formulation and multiple investigatory pre-market formulations) failed to elicit a dermal hyper-sensitization response. Also in the guinea pig and rat, 'sensitized' animals showed no evidence of anaphylaxis when challenged with either penciclovir or famciclovir. In vitro studies of penciclovir and/or famciclovir showed only minimal interaction/covalent binding between either compound and human serum proteins or albumin.

COMPILATION OF EFFECTS AND DISCUSSION:

Penciclovir and/or famciclovir were tested in multiple in vivo and in vitro assays for immunotoxic and/or immunostimulant/hypersensitization effects. Multiple studies were conducted in mice, rats, guinea pigs and human derived tissues using the proposed market formulation, various pre-market investigatory product formulations, and several miscellaneous preparations. The results of these studies are outlined in the following paragraphs.

In male rats, despite a slight but statistically significant degree of splenomegaly and thymic atrophy, the study results did not indicate that the administration of BRL 39123A (80 [pfa] mg/kg/day, IV) for 28 days resulted in immune system suppression. In several studies conducted with multiple product cream formulations (formulations included: a) propylene glycol with cetomacrogol, b) aqueous cream, c) propylene glycol with DMSO, and d) water), repeated administration of penciclovir (concentrations up to 5% w/w in cream; 0.5 ml or g/animal) failed to elicit any dermal hyper-sensitization responses in guinea pigs. When tested in the mouse and guinea 'passive' and/or 'active' cutaneous anaphylaxis models, both penciclovir and famciclovir were without activity. In addition, neither compound produced detectable levels of IgG1a or IgE/IgG1b antibodies. Lastly, in vitro studies conducted with penciclovir and famciclovir at concentrations up to 200 times the peak plasma concentrations achieved in humans following oral dosing with famciclovir, showed only minimal interaction/covalent binding between either compound and human serum proteins or albumin.

Overall, the results of the immunotoxicology and immunostimulant studies conducted with penciclovir and/or famciclovir suggest that it is without immunotoxic effects.

A review of the individual studies is contained on the following pages.

Toxicity Studies Summary:**In Vivo Studies****Immunotoxicity**

1) **BRL 39123A/Acyclovir: A 28 Day Immunotoxicological Repeat Dose Study In Male Rats By The Intravenous Route**, Study ID T89307/39123A/29906/R /IV/RDS/28D, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 6 Feb. 1989, Compound: BRL 39123A, Batch: GBD18, 85.5% pure free acid.

Contact and Delayed Hypersensitivity Studies**A) Studies Conducted Using the Proposed Market Formulation:**

2) **BRL 39123: A Study To Determine The Potential Of A Topical Formulation Of BRL 39123 To Cause Delayed Contact Hypersensitivity In The Guinea Pig**, Study ID T91905/39123/DNCB/GP/TOP/BUEHLER. GLP, Beecham Phar., The Frythe, Welwyn U.K., Study Initiation: 7 Oct., '91, BRL 39123, batch WA1064.

B) Studies Conducted Using an Aqueous Cream Formulation:

3) **BRL 39123: Contact Sensitivity in Guinea Pigs**, Study ID DA/85/31. GLP, Beecham Phar., Great Burgh, Nr. Epsom, Surrey, U.K., Study Initiation: 6 Jan., '86, BRL 39123, batch CT 18066.

C) Studies Conducted Using a Propylene Glycol Cream Formulation:

4) **BRL 39123: Delayed Dermal Sensitization Test in the Guinea Pig**, Study ID A/B/12261. GLP, Study Initiation: 16 Sept. '88, BRL 39123, batch CT 14568.

5) **BRL 39123: Bushler Contact Sensitization Study in the Guinea Pig**, Study ID 4/13, GLP, Test Site: Safepharm Lab. Limited, Derby, UK, Study Initiation: 6 Feb., 1990, Compound: BRL 39123, Batch: GBD34, purity 99.2%.

D) Miscellaneous Formulations:

6) **BRL 42810 and BRL 39123A: Assessment of Antigenicity in the Mouse Passive Cutaneous Anaphylaxis Test**, Study Report ID TF-1012/BRL-42810/2 and BRL 1332/942965. GLP,

Study Initiation: 21 June 1994, BRL 42810 and BRL 39123A, batches WPK 9617 and WPB 2001, 100% and 85% (as BRL 39123) pure, respectively.

7) **BRL 42810 and BRL 39123A: Assessment of Antigenicity in the Guinea-Pig Active Anaphylaxis and Passive Cutaneous Anaphylaxis Tests**, Study Report ID TF-1011/BRL-42810/2 and BRL 1333/942968. GLP,

Study Initiation: 21 June 1994, BRL 42810 and BRL 39123A, batches WPK 9617 and WPB 2001, 100% and 85% (as BRL 39123) pure, respectively.

In Vitro Studies

8) **The In Vitro Protein Reactivity of BRL 39123A**, Study ID BF-1009/BRL-039123/1. GLP, SmithKline Beecham Pharmaceuticals, Harlow, UK, Study Initiation: 19 June 1990, BRL 39123A, batch GBD 18.

9) **¹⁴C-BRL 39123A: The Measurement of the Extent of the Covalent Binding to Human Serum Albumin In Vitro**, Study ID TF-1010/BRL-039123/1. GLP,

Initiation: 31 Jan. 1994, ¹⁴C-BRL 39123A, batch 32634-153.

Study

Toxicity Studies Reviews:

In Vivo Studies

Immunotoxicity

1) **BRL 39123A/Acyclovir: A 28 Day Immunotoxicological Repeat Dose Study In Male Rats By The Intravenous Route, Study ID T89307/39123A/29906/R/IV/RDS/28D, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 6 Feb. 1989, Compound: BRL 39123A, Batch: GBD18, 85.5% pure free acid.**

Doses Tested: 0 and 80 (pfa) mg/kg/day (BRL 39123A & Acyclovir)

Dose Volume and Route: IV, 10 ml/kg, 3 ml/min.

Vehicle or Control: sterile saline

Species, Strain, Sex, Age, WT: Male Sprague-Dawley rats (Crl: CD(SD)Br), age 4-5 weeks, 8 animals/dose group.

Test Conditions: Animals were dosed with BRL 39123A, acyclovir or saline (IV) once per day for 28 consecutive days. Clinical signs, body weight and food consumption were monitored. A post-mortem examination was made of each study animal, with full macro and microscopic evaluation of tissues from all animals. Spleen and peritoneal lavage specimens were collected under aseptic conditions for the in vitro assessment of immune cell function.

Mortalities: There were no premature deaths.

Clinical Signs, Body Weight and Food Consumption: Clinical signs were limited to occasional bruising and/or irritation at the injection site in all groups. Body weight gain was slightly reduced among the drug treated animals (BRL 39123A & acyclovir), the effect achieving marginal statistical significance early in the treatment interval. A slight decrease in food intake and efficiency of food utilization was evident among drug treated animals during the first week of dosing.

Macro- and Microscopic Pathology: Slight increases in adrenal and spleen weight and, a decrease in thymus weight, were seen in the drug treated animals. No histopathological changes were noted in these tissues to correspond with the gross organ weight variation.

Immunologic Assessments: Spleen cells derived from animals treated either with BRL 39123A or acyclovir displayed greater non-specific proliferation in tissue culture medium ($p < 0.05$), when compared with cells derived from the untreated control animals. In addition, splenic cells derived from the drug treated animals showed significantly increased proliferative responses to several mitogenic compounds, including; Concanavalin A, Pokeweed mitogen and lipopolysaccharide. There were no differences in the splenic cell responses of the drug treated groups. Lastly, the ability of peritoneal derived macrophages to phagocytize sheep red blood cells, with or without the presence of opsonizing anti-sera, did not vary between the treatment and control groups.

Comments: 1) Despite a slight but significant degree of splenomegaly and thymic atrophy, the study results do not indicate that the administration of BRL 39123A or acyclovir at a dose of 80 (pfa) mg/kg/day (IV for 28 days) resulted in immune system suppression in the male rat.

2) Differences in the proliferative response of spleen cells derived from the treated animals may be related to an overall increase in the rate of lymphocyte production in the drug treated animals or, to a change in the lymphoid cell population present in the spleen during drug treatment. However, histologic evaluation of splenic and other lymphoid organs from the drug and control

treatment animals did not reveal any difference in the distribution of T cell sub-populations.

Contact and Delayed Hypersensitivity Studies

A) Studies Conducted Using the Proposed Market Formulation:

2) **BRL 39123: A Study To Determine The Potential Of A Topical Formulation Of BRL 39123 To Cause Delayed Contact Hypersensitivity In The Guinea Pig, Study ID T91905/39123/DNCB/G/TOP/BUEHLER.**

Status: GLP

Study Initiation: 7 Oct. 1991

Study Site: Beecham Phar., The Frythe, Welwyn U.K.

Compound Tested: BRL 39123 (5% w/w) in 40% propylene glycol with 0.9% cetomacrogol 1000, batch WA1064.

Doses Tested: 0 (vehicle) and 100 mg/kg, topical

Dose Volume and Route: 0.4 ml, topical

Solvent and Control: cream base without BRL 39123

Positive Control: 1-chloro-2,4-dinitrobenzene (DNCB; Sigma Lot # 100H0738), 0.4 ml of an ethanol solution (Hayman Lot SIN1170)

Species, Strain, Sex: female Dunkin-Hartley guinea pigs, age 8-12 weeks, initial weight 320-430 g, 10 animals/group.

Test Conditions: Animals were allocated into groups and sensitized with either BRL 39123 cream, the vehicle cream, DNCB vehicle control, or DNCB. On days 1, 8 and 15, 0.4 gm of the test compound (vehicle, or positive control) was applied with an occlusive dressing. Dressings were removed after six hours and the application sites washed clean. Thirteen days after the last induction treatment a region of the contralateral flank of each animal was shaved and the test compound applied under an occlusive bandage for 6 hours. Skin reactions at the challenge site were recorded 24 hours following application.

During the induction phase of the study, 2 animals from the BRL 39123 treatment group displayed individual instances of slight to mild erythema, while the majority of animals in the DNCB test group displayed mild to moderate erythema (progressing in severity with each induction exposure). Minimal to slight erythema was noted in 2 animals 24-48 hours following the application of the placebo cream (challenge phase), although no reactions were observed at the applications sites for BRL 39123 containing cream. As expected, challenge with DNCB resulted in mild to moderate erythema in animals previously induced with DNCB.

Exposure to BRL 39123 cream had no effect on body weight gain during the induction phase of the study, whereas exposure to DNCB resulted in reductions in weight gain which achieved statistical significance by week 3 of the induction phase of the study. No other physical signs were reported to be associated with any study treatment.

Comments: 1) Under the conditions of this test, the BRL 39123 cream formulation (40% PG and 0.9% cetomacrogol 1000) being evaluated showed little or no potential for the induction of contact hypersensitivity in the guinea pig.

2) The positive challenge responses noted with DNCB are consistent with historical records and demonstrate the sensitivity of the test system.

B) Studies Conducted Using an Aqueous Cream Formulation:

1) **BRL 39123: Contact Sensitivity in Guinea Pigs, Study ID DA/85/31.**

Status: GLP

Study Initiation: 6 Jan., '86

Study Site: Beecham Phar., Great Burgh, Nr. Epsom, Surrey, U.K.

Compound Tested: BRL 39123, batch CT 18056, 5% w/w cream,
and micronised BRL 39123 (batch CT 18134, GBD 15) as a 5%
suspension in petroleum jelly (batch GB 1335)

Doses Tested: 0 (vehicle) and 100 mg/kg, topical

Dose Volume and Route: 0.2 ml, topical

Solvent and Control: aqueous cream BP, batch CT 18067

Adjuvant: Freund's complete (Difco), 0.2 ml of 50% emulsion
(FCA)

Positive Control: 1-chloro-2,4-dinitrobenzene (DNCB; Sigma Lot # 44F-0565),
0.2 ml of a 0.3-0.02% solution in propylene glycol (Sigma Lot # 93F-0238)

Species, Strain, Sex: 35 male Dunkin-Hartley guinea pigs, age not specified,
initial weight 250-300 g

Test Conditions: Animals were allocated into 3 groups and sensitized with
either BRL 39123 cream (20 animals), the vehicle cream (10 animals), or DNCB
(5 animals). On day -1, the dorsal cervical region of each animal was shaved
and a 0.2 ml solution of 10% sodium lauryl sulphate (SLS) was applied on an
occlusive bandage. On day 0, the SLS was removed and 0.2 ml of the test
compound (see groups listed above) was applied with an occlusive dressing. On
days 1, 3 and 6, 0.2 ml of the test compound was reapplied. On day 3, 0.2 ml
of FCA was injected intradermal at the test drug application site. All
dressings were removed on day 8.

Multiple areas of each flank were shaved on day 21, and 0.2 ml of BRL 39123
(in petroleum jelly) or 0.2% DNCB applied to the left flank of each animal,
while placebo cream or 0.02% DNCB was applied to the right flank. Assessment
of dermal irritation and/or hypersensitivity reactions were made on days 22-
24, following discontinuation of drug exposure on day 22. Animals from the
original BRL 39123 cream and vehicle control groups were challenged with BRL
39123 (cream and jelly) and the control cream on day 34 of the study. Dermal
irritation was subsequently assessed on days 35-37.

Several animals from each test group displayed significant skin ulceration at
the contact site for the tapes used to hold the occlusive bandages. As a
result several animals were sacrificed following the day 21 challenge test. As
stated, these reactions were evident in all treatment groups and appeared
unrelated to the test compound.

Animals challenged with the placebo cream on days 21 and 34, whether initially
sensitized with the placebo or BRL 39123 containing cream, displayed only
faint erythematous reactions at the application site which disappeared in 24-
48 hours following bandage removal. Following challenge with BRL 39123 (in
petroleum jelly) on days 21 and 34, 2/10 and 1/9 animals originally sensitized
with the placebo cream showed faint erythematous reactions (2 animals showed
reactions which dissipated within 24 hours while the response in the 3rd
animal developed after 72 hours).

On days 21 and 34, 2/20 and 2/14 animals originally sensitized with BRL 39123
cream displayed faint erythematous reactions to the challenge with BRL 39123
in petroleum jelly. All responses disappeared within 48 hours. Three of 9
animals sensitized with the vehicle cream displayed slight erythematous
reactions on day 34 when challenged with the BRL 39123 cream. Reactions in 2
animals resolved within 48 hours of the challenge. Four of 14 animals
originally sensitized with BRL 39123 cream displayed faint erythematous
reactions on day 34 when challenged with the BRL 39123 cream. These reactions
resolved within 48 hours. Two additional animals displayed mild responses at
the 48 hour follow-up. All responses were resolved by 72 hours post-challenge.

Comments: 1) BRL 39123 when administered topically (5% w/w) to guinea pigs
in an aqueous cream or petroleum jelly does not appear to induce
allergic contact hypersensitivity.

2) The mild erythematous reactions observed in this study were probably associated with the cream vehicle, which had been previously demonstrated to be a mild dermal irritant in the rabbit. All reactions induced by the BRL 39123 cream were faint to slight in intensity and resolved in 24-48 hours after application.

3) The responses of animals sensitized and challenged with DNCB were moderate to severe in intensity as expected.

C) Studies Conducted Using a Propylene Glycol Cream Formulation:

4) BRL 39123: Delayed Dermal Sensitization Test in the Guinea Pig, Study ID A/B/12261.

Status: GLP

Study Initiation: 16 Sept. '88

Study Site:

Compound Tested: BRL 39123, batch CT 14568, 5% w/w cream

Dose Volume and Route: 0.5 ml, topical

Solvent and Control: cream, batch CT 14569

Species, Strain, Sex: female Dunkin-Hartley guinea pigs, age not specified, initial weight 339-460 g.

Test Conditions: Animals were allocated into 3 groups and sensitized with either BRL 39123 cream, the vehicle cream or, left untreated. On days 1, 8 and 15, the dorsal cervical region of each animal was shaved and a 0.5 ml aliquot of cream was applied for 6 hours on an occlusive bandage. Subsequently, on test day 28, the flanks of all test animals were shaved free of fur. On day 29, each animal received a challenge exposure (6 hrs) to the BRL 39123 cream (at conc. of 5% and 2.5%) and the placebo cream. On day 39 of testing, all drug treated and untreated control animals were exposed (topical application) to the BRL 39123 cream, while the former placebo treated controls were dosed with placebo cream.

Assessment of dermal irritation and/or hypersensitivity reactions were made on each 'challenge' test day, following discontinuation of drug exposure. After the conclusion of the initial challenge drug application, 4 treated and 6 placebo treated animals showed dermal irritation responses (severity scores of 0.35 and 0.9, respectively). Similarly, following the second drug 'challenge', 6 test animals, 4 treated control animals and no untreated control animals showed dermal irritation responses to the application of BRL 39123 or the placebo. All reactions, including those to the control cream, were of magnitude 2 (moderate erythema) or less. The reactions generally decreased in severity or resolved within 48 hours.

Comments: 1) BRL 39123 or the placebo cream when administered topically to guinea pigs caused mild to moderate erythematous reactions. These reactions, since they occurred in all treatment groups, were likely associated with the cream vehicle. All reactions induced by the BRL 39123 or placebo creams were mild to moderate in intensity and resolved in 24-48 hours after application.

3) During the second drug challenge, the responses of animals previously sensitized (6/10) with BRL 39123 were more frequent than among other test groups. The test laboratory concluded that BRL 39123 may have induced some degree of contact sensitization in the test animals.

5) BRL 39123: Buehler Contact Sensitization Study in the Guinea Pig, Study ID 4/13.

Status: GLP

Test Site:

Study Initiation: 6 Feb., 1990

Compound: BRL 39123, Batch: GBD34, purity 99.2%.

Dose: 5% (w/w) BRL 39123 in cream base

Solvent: 40% propylene glycol, w/wo 2.5% Dec-MSO

Controls: 40% propylene glycol, w/wo 2.5% Dec-MSO

Test Animal: female albino Dunkin-Hartley guinea pigs, 360-450 grams, 8-12 weeks of age at start of study.

Test Procedure: Two phases were conducted in the Buehler test a) induction of response and b) challenge of response. During induction, the test agent (5% w/w BRL 39123 in 40% propylene glycol (w/wo 2.5% Dec-MSO) was applied to a shaved area of skin on the left flank (15 x 35 mm area) of each animal (20 animals/group). The treatment area was then covered with absorbent lint and tinfoil, and held in place with surgical tape. The dressing was kept in place for 6 hours during each induction period, with exposure being repeated on days 0 (1st exposure), 7 and 14. An identical 'induction' procedure was conducted on the control animals (20 animals/group) using the vehicle alone. The challenge phase (post-exposure day 28) of the procedure used identical procedures except that the test agent was applied to an area of the right flank of each animal.

Measurements: Dermal irritation (erythema and inflammation) was evaluated 24 hours following each of the induction trials and at 24 and 48 hours following the challenge trial.

Results: Mild to moderate erythema with occasional edema was noted following each induction trial with either of the BRL 39123 creams or the respective placebo creams. Edema appeared to be less frequent and/or severe with the Dec-MSO containing cream. The irritation responses decreased in severity with repeat exposure. No dermal responses to the test material were noted for experimental or control animals during the 'challenge' test session. Bodyweight gain among treated and control animals was similar over the approximately 30 day duration of the study.

Comment: 1) BRL 39123 appears to be slightly irritating, but non-sensitizing in the guinea pig skin test.

2) Concurrent positive controls were apparently not conducted.

D) Miscellaneous Formulations:

6) **BRL 42810 and BRL 39123A: Assessment of Antigenicity in the Mouse Passive Cutaneous Anaphylaxis Test**, Study Report ID TF-1012/BRL-42810/2 and RRI 1332/942965. GLP,

Study Initiation: 21 June 1994, BRL 42810 and BRL 39123A, batches WPK 9617 and WPB 2001, 100% and 85% (as BRL 39123) pure, respectively.

Doses Tested: 0, 10 and 100 mg/kg

Dose Volume and Route: 10 ml/kg, ip or po.

Positive Control: HSA, 0.2 mg/kg, ip.

Vehicle: 0.9% saline or sterile water

Species, Strain, Sex: 50 male ICR CD-1 mice, age approximately 6 weeks, weight range 27-34 grams; 24 male Wistar rats, age approximately 8 weeks, weight range 221-256 grams.

Test Procedure: Groups of male mice received vehicle, HSA, BRL 42810 or BRL 39123A, 1-3 times/week for 3-5 weeks. Measurement of IgE antibodies in the serum of the drug sensitized mice was measured by the PCA test in male rats.

Results and Summary: BRL 39123A and BRL 42810, whether administered orally or by ip injection, did not induced IgE antibodies in mouse serum (induced no antigenic response as measured in the rat PCA test).

Comment: The doses of BRL 39123 used in the study were approximately 1 and 10 times the human therapeutic dose of BRL 39123.

7) **BRL 42810 and BRL 39123A: Assessment of Antigenicity in the Guinea-Pig Active Anaphylaxis and Passive Cutaneous Anaphylaxis Tests**, Study Report ID

TF-1011/BRL-42810/2 and BRL 1333/942968. GLP,
Study Initiation: 21 June 1994, BRL 42810
and BRL 39123A, batches WPK 9617 and WPB 2001, 100% and 85% (as BRL 39123)
pure, respectively.

Doses Tested: 0, 10 and 100 mg/kg

Dose Volume and Route: 2 mL/kg, sc or po.

Positive Control: HSA, 5 mg/kg, sc.

Vehicle: 0.9% saline or sterile water

Species, Strain, Sex: 105 male Dunkin-Hartley Guinea-Pigs, age approximately
6 weeks, weight range 409-596 grams.

Test Procedure: Groups of male Guinea-Pigs received vehicle, HSA, BRL 42810
or BRL 39123A (with or without Freund's Complete Adjuvant), 1-3 times/week for
3-4 weeks. The antigenic potential of the drugs were measured using systemic
anaphylaxis (ASA) and passive cutaneous anaphylaxis (PCA) tests.

Results and Summary: BRL 39123A and BRL 42810, whether administered orally or
by sc injection, did not induced measurable levels of IgG₁ or IgE/IgG_{1b}
antibodies, nor induce a systemic anaphylactoid response in sensitized animals
when challenged by IV injection.

Comment: BRL 39123 and BRL 42810 appear to be non-antigenic when
administered to Guinea-Pigs under the conditions of this study.

In Vitro Studies

8) The In Vitro Protein Reactivity of BRL 39123A, Study ID BF-1009/BRL-
039123/1. GLP, SmithKline Beecham Pharmaceuticals, Harlow, UK, Study
Initiation: 19 June 1990, BRL 39123A, batch GBD 18.

Conc. Tested: 5:1 molar ratio with HSA

Positive Controls: dinitrofluorobenzene, iodoacetamide

Vehicle: 0.2 M sodium phosphate buffer at pH 7

Test Procedure: BRL 39123A was incubated for up to 24 hours with human serum
albumin or L-cysteine (molar ratio of 5:1), to determine the level of
reactivity with primary amines and free thiol groups.

Results and Summary: Following incubation for 24 hours, the level of covalent
binding of BRL 39123 with either the free amino or thiol groups of HSA or
cysteine, ranged from approximately 1-3% of the available reaction groups. The
positive control compounds showed reactivity at levels of approximately 50-
85%.

Comment: The concentration of BRL 39123 used in the incubation solution was
approximately 200 times the maximal concentration (C_{max}) of drug
achieved in plasma following therapeutic administration in man. It
appears unlikely that the level of protein reactivity noted in
this study represents a significant toxicologic risk in man.

9) ¹⁴C-BRL 39123A: The Measurement of the Extent of the Covalent Binding to
Human Serum Albumin In Vitro, Study ID TF-1010/BRL-039123/1. GLP,

Study Initiation: 31

Jan. 1994, ¹⁴C-BRL 39123A, batch 32634-153.

Conc. Tested: 6 and 60 µg/ml

Incubation Solution: HSA (4.5% w/v) in PBS

Test Procedure: BRL 39123A was incubated for 6-24 hours with human serum
albumin to determine the level of covalent binding to human serum albumin.

Results and Summary: Following incubation for 6-24 hours, the level of
irreversible binding of BRL 39123 to HSA ranged from approximately 0.03-0.14%
of the initial drug concentration. The level of binding to HSA increased as a
function of longer incubation and an increased concentration of drug in the
reaction mixture. The maximum level of covalent binding (3.04 nM/g protein
[203 µM/M protein]) was noted in the 60 µg/ml reaction mixture when incubated
for 24 hrs.

Comment: The concentration of BRL 39123 used in the incubation solution was approximately 1 and 10 times the maximal concentration (C_{max}) of drug achieved in plasma following the therapeutic administration of BRL 39123 in man. It appears unlikely that the level of protein reactivity noted in this study represents a significant toxicologic risk in man.

APPENDIX D: REPRODUCTIVE TOXICITY STUDIES

SUMMARY:

The sponsor has conducted standard Segment I-III reproductive toxicology studies in the rat and rabbit. Results of the studies revealed minimal or no effects of intravenous penciclovir on fetal abnormalities and viability, multigenerational development and reproduction, reproductive performance of treated animals and, fertility of treated females. In contrast, chronic dosing studies conducted with famciclovir (see NDA 20-363, review date June 1994) showed that long-term exposure resulted in significant adverse effects on the male reproductive organs (including testicular atrophy and seminiferous tubule degeneration), and induced profound adverse effects on sperm production and morphology. Furthermore, with long-term famciclovir exposure fertility among treated animals was significantly decreased and pre-implantation losses were increased. The recovery of sperm production appeared related to the time off-dose, with approximately equal numbers of animals showing full, partial or no recovery of sperm production after multiple (approx. 13 cycles) spermatogenic cycles. Similar testicular and hypospermatogenic effects have been noted in animals with other members of this chemical class, however, these effects have not been demonstrated in human patients.

COMPILATION OF EFFECTS AND DISCUSSION:

The sponsor has conducted multiple studies in the rat and rabbit to assess the potential for reproductive toxicity produced by exposure penciclovir. (Similar studies were previously reported for famciclovir, and were reviewed as part of NDA 20-363, June 1994.) General reproductive performance and fertility studies (Segment I) were conducted in male and female rats, while peri- postnatal exposure studies (Segment III) were conducted in female rats. Teratology studies were conducted in the rat and rabbit. A summary of the study findings is presented in the following paragraphs.

General reproductive performance and fertility studies were conducted in male and female rats at intravenous doses of 0, 30, 50 and 80 mg/kg/day. Results of studies in the rat indicated that BRL 39123 had no effects on mating performance (fraction mating and pre-coital interval), fertility (conception rate, litter size and sex ratio) or, fetal survival (in utero and post-partum viability). The high dose used in these studies of BRL 39123 was previously demonstrated as causing mild-moderate nephrotoxicity (as demonstrated by renal tubule injury, interstitial nephritis/nephrosis, increased urine volume with decreased osmolality, and mild changes in BUN and creatinine). Among male and female animals dosed at the intermediate and high dose levels, there were marked dose-related increases in water consumption in the intermediate and high dose groups. Overall, penciclovir failed to demonstrate any adverse effects on the reproductive performance and/or fertility of male and female rats.

In contrast, famciclovir profoundly reduced the fertility of male rats when administered for extended intervals (12 weeks or more) prior to mating (see NDA 20-363, June 1994). The results of multiple studies suggested that the adverse effects of BRL 42810 on the male reproductive system were both dose and time dependent (i.e., progressive with increasing drug dose and duration of drug exposure). Furthermore, the effects appeared to progress among some animals even after dose cessation. Significant adverse effects on sperm production, sperm counts and morphology, and on the morphology of the male reproductive organs were evident when famciclovir was administered for extended intervals in multiple species (mouse, rat, dog). Fertility among male animals treated at 500 mg famciclovir/kg/day for 10 weeks (earliest assessment interval) or longer was significantly decreased. A significant proportion of the high dose treated animals became sterile within 10 weeks of the start of

drug administration and, remained sterile 26-30 weeks following drug withdrawal. Pre-implantation loss was increased among affected males, although there did not appear to be any adverse effects on the fetus due to paternal exposure. While the recovery of sperm production appeared to recover following the cessation of dosing with famciclovir, assessments performed at 6-30 weeks off-dose showed nearly equal numbers (ratio of 1:1:1) of animals with normal, subnormal or no sperm production¹.

Teratology studies with penciclovir were conducted in the rat and rabbit at doses (IV) of 0, 30, 50 and 80, and 0, 15, 30 and 60 mg/kg/day, respectively. In both the rat and rabbit, famciclovir was administered intravenously at a single dose level (360 and 120 mg/kg/day, respectively) as a comparison for penciclovir. Results of these studies indicated that the IV administration of BRL 39123 at the dose levels selected was minimally maternally toxic, as demonstrated by transient reductions in weight gain among high dose treated rats, and static body weights among high dose treated rabbits. IN the rat, the intravenous administration of penciclovir did not adversely effect the course and outcome of pregnancy. Litter size and weight, implantation rates, incidence of fetal resorptions (early, middle, or late), the number of viable pups, mean foetal body weight and placental weight, and the ratio of male to female foetuses was comparable among all groups. However, in the rat the administration of BRL 42810 (80 mg/kg) increased the incidence of ovoid eyes or lenses (4.3% vs. 1.7% [control]), and was associated with 2 cases of an enlarged superior intercostal veins (versus 0 controls and BRL 39123A treated animals)².

In the rabbit teratology study, pre-implantation loss was higher among drug treated animals than among the concurrent control group, although all values were within the historical control range. Live litter size was reduced and post-implantation loss increased in animals receiving BRL 39123 at 30 and 60 mg (pfa)/kg. However, as with pre-implantation effects, the effects of BRL 39123A on litter size and late fetal loss were within the historical control range. In the surviving progeny, mean body and placental weights were unaffected by drug treatment at any of the doses tested. Major malformations and visceral anomalies were evident in all groups and did not appear to be related to treatment with the test compound. Overall, the teratology studies conducted in the rat and rabbit indicate that BRL 39123 at doses up to 60-80 mg/kg/day had no effect on the course and outcome of pregnancy and, suggest that it is not likely to be a teratogen.

Lastly, the potential adverse effects of BRL 39123 were examined in a multi-generational study of development and reproduction in the rat. The study results showed no significant adverse effects of BRL 42810 on the fertility of females from the F₂ generation and, the survival and growth of the F₁ generation males and females. There were no demonstrable adverse drug effects noted in the reproductive performance of the F₁ generation or on the developmental milestones of the F₂ generation. However, it should be noted that penciclovir was administered intravenously at doses of 0, 30, 50 and 80 mg/kg/day to the female rats, and even at the highest dose tested there was

¹ Sterility has previously been reported in animals treated with acyclovir and ganciclovir. While the data regarding adverse testicular and/or spermatogenic effects in humans treated with either of these drugs are not as clear, it appears that ganciclovir may induce sterility in humans while acyclovir has not demonstrated such an effect.

² The slight increase of ovoid eyes/lenses noted in BRL 42810 exposed foetuses may reflect a recoverable developmental delay.

minimal demonstration of maternal toxicity (gestation weight gain was very slightly [statistically significant] lower than control, while water intake was slightly increased; food intake, gestation length, lactation weight change and terminal kidney weights were unaffected). Thus, the doses tested may not have been adequate to demonstrate the potential adverse effects of penciclovir on the exposed offspring.

As discussed in the NDA review for famciclovir (FAMVIR®; NDA 20-363, June 1994), the administration of [¹⁴C]famciclovir to pregnant rats and rabbits showed that placental passage and fetal exposure to drug related material occurred at levels nearly comparable to maternal exposure. In both species, penciclovir was the predominant metabolite of BRL 42810 found in the maternal circulation and in the fetuses (BRL 42359 [the 6 deoxy precursor of penciclovir] accounted for the majority of the remaining [¹⁴C] radiolabeled material in the rat, whereas in the rabbit, BRL 42359 and BRL 48959 were identified as the major metabolites in addition to penciclovir). Penciclovir was rapidly secreted into the milk of lactating rats following an oral dose of [¹⁴C] famciclovir (40 mg/kg). Milk concentrations were considerably higher than those observed in plasma.

Individual study reviews are contained in the following pages.

Toxicity Studies Summary:**Segment I Testing:**

- 1) **BRL 39123A: A Study To Determine The Effects Of Intravenous Administration On The Fertility And General Reproductive Performance Of The Male Rat**, Study ID T91503/39123A/R/IV/F+GRP (Male), GLP, Test Site: Beecham Pharm., The Frythe, Welwyn, UK, Study Initiation: 30 July 1991, BRL 39123A, Batch: WPB 2001, purity >86.1% pfa.
- 2) **BRL 39123: A Study to Determine the Effects of Intravenous Administration on the Fertility and General Reproductive Performance of the Female Rat**, Study ID T91504/39123A/R/IV/F+GRF[Female]), GLP, SmithKline Beecham Pharm., The Frythe, Welwyn, Herts, UK, Study Initiation: 3 Sept. 1991, BRL 39123A, Batch: WPD 2001.

Segment II Testing:

- 3) **BRL 39123A, BRL 42810: A Study to Determine The Effects of Intravenous Administration of BRL 39123A and BRL 42810 on the Course and Outcome of Pregnancy in Rats**, Study ID T87504/39123A/42810/R/IV /TG, GLP, Test Site: Beecham Pharm, Stock, Essex, UK, Study Initiation: 17 Feb., '87, Compound: BRL 39123A, batch GBD 10, and BRL 42810, batch GBD 14.
- 4) **BRL 39123A/BRL 42810: A Study to Determine the Maximum Tolerated Dose of BRL 39123A by Intravenous Administration and of BRL 42810 by Both Oral and Intravenous Administration in Non-Pregnant New Zealand White Rabbits**, Study ID T87500/39123A/42810/RAB/PO-IV/MTD. GLP, Test Site: Beecham Pharm, Stock, Essex, UK, Study Initiation: 26 Jan., '87, Compound: BRL 39123A, batch GBD11, BRL 42810, batches GBD13 and GBD14.
- 5) **BRL 39123A, BRL 42810: A Study to Determine The Effects of Intravenous Administration of BRL 39123A and BRL 42810 on the Course and Outcome of Pregnancy in New Zealand White Rabbits**, Study ID T87501/39123A/42810/RAB/IV/TG, GLP, Test Site: Beecham Pharm, Stock, Essex, UK, Study Initiation: 24 Mar., '87, Compound: BRL 39123A, batch GBD 10, and BRL 42810, batch GBD 15.

Segment III Testing:

- 6) **BRL 39123A: A Study to Determine the Effects of Intravenous Administration on the Peri- and Post-Natal Development of the Rat**, Study ID T92501/39123A/R/IV/PPN, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 7 Jan., 1992, Compound: BRL 39123, Batch: WPD 2001, purity 86.1 g (pfa)/g.

Toxicity Studies Reviews:**Segment I Testing:**

- 1) **BRL 39123A: A Study To Determine The Effects Of Intravenous Administration On The Fertility And General Reproductive Performance Of The Male Rat**, Study ID T91503/39123A/R/IV/F+GRP (Male).
Status: GLP
Study Site: Beecham Pharm., The Frythe, Welwyn, UK
Study Initiation: 30 July 1991
Compound Tested: BRL 39123A, Batch: WPB 2001, purity >86.1% pfa

Doses Tested: 0, 30, 50 and 80 mg/kg/day

Dose Volume and Route: 10 ml/kg, 3 ml/min, IV

Vehicle or Control: sterile physiological saline

Species, Strain, Sex, Age, WT: male Sprague Dawley rats (CRL: CD(SD)Br rats), age 5-6 weeks, weight range 191-234 grams at study initiation, 24 male animals/test group. Untreated female animals were bred with the treated male animals at approximately 11-12 weeks of age, weight range of 224-302 grams.

Test Conditions: BRL 39123A was administered once/day by intravenous infusion in a lateral tail vein for 10 weeks prior to mating. Male animals were maintained on drug treatment until experiment Day 106-110, following successful completion of the female breeding/gestation phase of the study. At dose cessation all surviving male animals were sacrificed for assessment of sperm pathology.

Female animals were sacrificed at 20 days post-coitus for the evaluation of uterine contents. All fetuses/neonates were weighed, sexed, and examined for abnormalities. Mortality, physical signs, body weight and food intake were measured regularly throughout the study. Fertility and standard litter parameters were measured.

Dose Preparations: All dosing solutions were assayed and found to be within 7% of the nominal concentration.

Mortality, Clinical Signs, Body Weight, Food and Water Consumption of the Drug

Treated Males: A total of 3 drug-treated male animals (1 animal from each of the control, low and high dose groups) died or was sacrificed in moribund condition during the period of drug treatment. The high dose animal was sacrificed on day 45 due to excessive local irritation/necrosis at the site of drug infusion. The low dose treated animal was found dead on day 67 of treatment with hemorrhagic areas of the thymus, while the control animal died under anaesthesia on day 100 (i.e., post-breeding). There were no other premature deaths during the drug treatment phase of the study.

Clinical signs among the drug treated animals were restricted to evidence of local irritation, sometimes with necrosis, at the site of drug infusion. This effect was most evident among the high dose treated male animals (7/24). Body weight and food consumption of the drug treated animals was not consistently effected by the administration of BRL 39123A. Water consumption was increased in a dose related manner among males from the intermediate (approx. mean increase of 15%) and high dose groups (approx. mean increase of 80%), beginning immediately following dose initiation and escalating to maximal effects after 5-6 weeks of treatment.

Hematology, Clinical Chemistry and Urinalysis: Among the high dose treated animals there were progressive and statistically significant reductions (5-10%) in hemoglobin, hematocrit, and red blood cell counts. Slight increases in platelet counts (approx. 10%) were evident among these same. Increases in BUN (up to 60% at the high dose), total protein (10%) and globulins (20%), potassium (10%) and calcium (<5%) were evident among the high and intermediate dosed animals. Serum AST levels, but not ALT, were slightly elevated among animals from the high dose group.

Urine volume was increased (approx. 50-200% over the control) and osmolality decreased (approx. 50%) among the high dose treated male animals during the 0-6 hours and 6-24 hours post-dosing intervals. Somewhat smaller changes in urine volume and osmolality were seen among the intermediate dose animals during the 6-24 hour post-dosing interval. Urinary creatinine and creatinine clearance were slightly increased among the high dose treated animals.

Gross and Microscopic Pathology (Drug Treated Males): Absolute testicular weights were reduced slightly among the intermediate and high dose treated (significant) animals. However, relative testicular weights were not effected

by treatment with BRL 39123A at any dose tested. In addition, absolute and relative epididymal weights were sporadically reduced among all drug treatment groups when compared to the concurrent control. However, it should be noted that the absolute organ weights for the drug treated animals remained within the historical control range for untreated animals in this laboratory, and may therefore not reflect true drug effects. Microscopic examination of the testes failed to reveal any clearly drug related pathologies.

Among the intermediate and high dose treated animals, both absolute and relative weights of the kidneys were increased (approx. 15-30%) in a dose related manner. Two intermediate and 4 high dose treated animals showed evidence of slight-marked renal pelvic dilatation. Microscopic examination of the kidneys revealed a dose-related increase in the incidence and severity of tubular nephropathy (4/23, 20/24 and 23/23) among the drug treated animals versus the concurrent controls (0/23). In addition, an increase in the incidence of interstitial nephritis was evident among the high dose treated animals (10/23) when compared with the controls (3/23). Unlike the results of several previous studies, at the time of necropsy no crystalline structures were detected in the renal tubules of the drug treated animals.

There were no other macroscopic or microscopic abnormalities noted among the drug treated animals.

Sperm Evaluation: Sperm motility, concentration and morphology were not clearly effected by 10 weeks of daily drug exposure to BRL 39123A at any dose tested.

Fertility and General Reproductive Performance of Drug Treated Males: The fertility and general reproductive performance of the drug treated animals was comparable to the concurrent controls.

Litter Parameters (Caesarian Section): There were no clearly drug-related effects on live litter size, pre- and post-implantation losses, foetal and placental weights, and sex ratios of the offspring.

Litter Abnormalities (Caesarian Section): A slight increase in the number of skeletal anomalies was noted among the offspring of the high dose treated males, including: reduced/irregular ossification of the bones of the skull and caudal vertebral centra, presence of rudimentary 14th ribs, asymmetric pelvic girdle or pelvic shift, and variant pubis(es). A slightly increased incidence of darkened adrenal was also evident among the offspring of the high dose treated male animals. However, because of the low incidence of these effects and their falling within the historical range for abnormalities in untreated animals (in this laboratory), a direct relationship to drug treatment can not be determined.

Physical Signs and Body Weight Gain Among Females: There were no differences in body weight gain or clinical signs among female animals mated to the male animals of any drug treatment group.

Comments: 1) As noted in several previous studies, the repeat administration of BRL 39123A had significant adverse effects on the morphology of and functioning of the kidneys. Furthermore, similar adverse renal effects have been noted in the rat following the repeat oral administration of BRL 42810 (famciclovir; see NDA 20-460). Under the conditions of the present study, the estimated NOEL dose for adverse renal effects in the rat of IV penciclovir is 30 mg/kg/day.

2) At paternally toxic (as demonstrated by renal and hematologic effects) doses of up 80 mg/kg/day (IV), BRL 39123A was without

significant adverse effects on the reproductive functioning of male rats.

3) Based on the study results, the estimated NOEL dose for adverse testicular effects in the SD rat is 50 mg BRL 39123A (IV)/kg/day. It should be noted that these results are in close correspondence with the results of studies conducted with BRL 42810 (famciclovir; the diacetylated precursor of penciclovir).

4) There did not appear to be any adverse effects on the fetus due to paternal exposure to BRL 39123A.

2) BRL 39123: A Study to Determine the Effects of Intravenous Administration on the Fertility and General Reproductive Performance of the Female Rat, Study ID T91504/39123A/R/IV/F+GRP[Female]

Status: GLP

Test Site: SmithKline Beecham Pharm., The Frythe, Welwyn, Herts, UK

Study Initiation: 3 Sept. 1991

Compound: BRL 39123A, Batch: WPD 2001, 0.861 g pfa/g of bulk.

Test Procedure: A total of 112 female Sprague-Dawley rats (Charles River UK Ltd.; 10-11 weeks of age; 195-237 grams) were randomly assigned to one of the 4 treatment groups (28 animals/dose). BRL 39123 was administered once per day by intravenous infusion (lateral tail vein; dose volume 10 ml/kg at 3 ml/min) in doses of 0 (saline vehicle control), 30, 50 or 80 mg/kg. Dosing was initiated 2 weeks prior to mating and was continued until termination of the F₀ generation (day 20 of gestation or following weaning of offspring). Females were mated one-to-one with untreated males (approximate age 11-12 weeks; 275-3500 grams) of the same strain obtained from the same supplier.

Morbidity/mortality and bodyweight were recorded daily during the study. Food and water consumption were measured prior to pairing in the F₀ generation. Oestrous cycles were determined by vaginal washing. Fourteen animals in each treatment group were sacrificed on day 20 of gestation, while the remainder were allowed to litter naturally and were then terminated on day 25 post-partum (at weaning). A detailed macroscopic examination of all F₀ dams was performed at the time of sacrifice. Fetuses from gravid females were weighed, sexed and examined externally for abnormalities. Alternate foetuses were preserved for soft tissue examination or, were examined internally, eviscerated and preserved for skeletal examination.

All offspring of the F₀ generation were evaluated for growth, survival, behavior (activity, motor coordination (rota-rod), and passive avoidance), and developmental milestones (tooth eruption, surface and mid-air righting reflexes, eyelid and pinnae separation, testes descent, vaginal opening, pupillary response). Selected offspring from the F₀ generation were used to form the undosed second generation (F₁; 24 male and 24 female offspring from each of the F₀ generation treatment conditions). All non-selected offspring from the F₀-F₁ generation were sacrificed and necropsied as described above.

At approximately 10 weeks of age, males and females of the F₁ generation (F₁-F₁ offspring) were mated on a one-to-one pairing within the previous treatment groups. On day 20 of gestation, one half of the females from each group were necropsied and the fetuses examined. The remaining F₁ females were allowed to litter naturally and rear their young to weaning, at which time the animals were sacrificed and examined. All observations and samples from the F₁ generation and litters were the same as for the F₀ generation.

RESULTS:

Dose Preparations: All dosing solutions were assayed and found to be within 5%

of the nominal concentration.

Mortality (F₀): One low dose treated animal was sacrificed in moribund condition on day 4 of treatment. Signs observed beginning on day 2 included, hypoactivity, hunched posture, pallor and emaciation, coat staining, distended abdomen and orbital discharge. Post-mortem examination revealed blood stained fluid in the peritoneal and cranial cavities, enlargement and hemorrhages of the liver and spleen, and thymic congestion. The exact cause of death could not be determined. There were no other deaths among the drug treated or control animals of the F₀ generation.

F₀ Body Weight, Food and Water Consumption: A transient (significant) reduction (5%) in body weight was noted among the high dose treated animals on day 2 of drug dosing. However, normal body weight and weight gain were reestablished by day 4 of treatment and were comparable for all groups thereafter. Similar to the reduction in body weight, the high dose treated female animals displayed an approximately 6% reduction in food intake during the initial 4 days of drug treatment. Food consumption was generally comparable for all study groups beginning at day 4 of treatment.

Water intake was significantly increased by the IV administration of BRL 39123A among animals from all treatment groups. While the effects were small among the low and intermediate dose treated animals (approx. 5-20% increase), the increase in water intake ranged between 25-75% among the high dose treated animals. Increased water intake was maintained among the treated animals throughout the period of observation.

F₀ Reproductive Parameters and Fertility. Timing of oestrous cycles, pre-ovulatory interval and gestation length, were unaffected by drug treatment. There were no apparent drug related effects on the fertility or conception rate of the F₀ females.

F₀ Post-Mortem Examination: Both absolute and relative weight of the kidneys were increased (5-10%) among the high dose treated animals terminated on day 20 of gestation. Among the F₀ animals allocated to litter, renal weight (absolute and relative to body weight) remained elevated (5-11%) at the time of weaning. One low dose treated animal was found to have a mammary adenocarcinoma at termination on day 20 of gestation. There were no other macroscopic abnormalities noted among the drug treated F₀ generation.

F₀ Litter Parameters (F₁-F₂ animals): There was a slight reduction in the number of corpora lutea produced among the high dose treated female animals, although this effect was not statistically significant nor was it outside the historical control range. However, there were no apparent drug related effects on mean litter size, pre- and post-implantation loss, number of implantation sites or the ratio of male to female offspring. Mean litter, foetal and placental weights were comparable for all study groups. Litter size (day 1 post partum) and viability of offspring (survival to weaning) were equivalent for all groups. Body weight gain, physical, reflex and behavioral development of offspring were unaffected by BRL 39123A administration.

The incidence of major malformations was not apparently effected by treatment with BRL 39123A at any dose tested. The only anomaly noted was the presence of a cervical rib in one animal from each of the control, low and intermediate dose groups and three animals from the high dose group. Although this anomaly increased with dose, the number of affected animals (group or study) did not exceed the historical control range. Minor visceral anomalies observed among animals from the high dose group included slight increases in the incidence of immature lenses, undescended thymus lobes, shortening of the brachycephalic trunk and intrahepatic hemorrhages. The incidence of mottled appearing livers, and enlargement of the renal vein was also noted among several high dose

offspring. Skeletal anomalies seen among fetuses from the high dose group included a slight increase in the incidence of delayed ossification of facial structures and hyoid bone, and increased numbers of variant sternbrae (>2) in the 1-4 region.

F₁-F₁ Generation Findings During Lactation and at Weaning: A slight increase in the incidence of renal pelvic dilatation (within the historical control range) was noted among the offspring (those not selected to form the F₁ generation) of the drug treated animals at the time of weaning. There were no other apparent drug related macroscopic pathologies noted among offspring which died during the period of lactation or which were sacrificed at the time of weaning.

Mortality: F₁: One low dose animal was sacrificed in moribund condition on day 7 post-partum. Necropsy revealed uterine dilatation and hemorrhage at several former implantation sites. Microscopic examination of mammary tissues revealed normal appearing secretory activity. Since this death was observed only in one animal from the low dose group, and was not observed in any animal from the intermediate or high dose groups, it appears unlikely to have been related to drug treatment.

One additional female animal, the offspring of an intermediate dose treated animal, was sacrificed on day 29 of the F₁ generation following a cage related mechanical injury of one forepaw. No other abnormalities were noted.

F₁ Body Weight: A transient reduction (5%) in body weight was noted among the high and intermediate dose female animals on day 8 following selection of the F₁ generation. However, at the time of selection of the F₁ generation, and at the conclusion of the 14 day pre-mating observation period, the mean body weights for all treated female animals were comparable to the controls. No differences in body weight or weight gain were evident among the male animals of the F₁ generation. Post-natal and lactational increases in body weight were comparable among females from all treatment groups.

F₁ Reproductive Parameters and Fertility: Oestrous cycles were comparable among females of the F₁ generation. Mating, conception rate and fertility were unaffected by treatment of the preceding generation. Gestation length (20.5-22 days) was comparable for all treatment groups.

F₁ Litter Parameters (F₁-F₁ animals): The number of corpora lutea, pre- and post-implantation losses, number of implantation sites and litter size were comparable for all study groups. The ratio of male to female offspring was similar in all groups. Mean litter, foetal and placental weights were comparable for all study groups. Litter size (day 1 post partum) and viability of offspring (survival to weaning) were equivalent for all groups. Body weight gain and physical signs noted among the offspring were unaffected by BRL 39123A treatment of the F₁ generation.

There were no treatment related macroscopic findings among offspring that died prior to weaning. Terminal examinations performed at the time of weaning, revealed a slight increase in the incidence of renal pelvic dilatation among animals from the drug treatment groups versus the concurrent controls. However, since this effect was within the historical control range it was considered potentially random and unrelated to drug treatment of the F₁ generation. An increased incidence of undescended testes was evident among males from all study groups (including controls; as compared with historical control values), but was most pronounced among the offspring of drug treated animals. No concurrent abnormalities of the testes or abdominal wall in any affected animal was noted. No evaluation of malformations or visceral/skeletal variations was performed on the F₁-F₁ animals, since no apparent drug related effects had been identified in the F₀-F₁ animals.

F. Post-Mortem Examination: A slight increase in the incidence of renal pelvic dilatation was noted among male animals from the high dose group (i.e., offspring of high dose treated F₀ females). All remaining pathologies appeared randomly distributed among animals from the treated and control groups, and appear unlikely to have been related to drug treatment. Both absolute and relative weight of the kidneys were unaffected by treatment.

- Comments:
- 1) The IV administration of BRL 39123A induced some minor signs of toxicity in females of the F₀ generation. Observed effects included treatment induced transient changes in body weight and food consumption, increases in water intake, and alterations in the absolute/relative weight of the kidneys.
 - 2) There were no apparent effects of the administration of BRL 39123A on the reproductive performance and fertility of females from the F₀ generation. Survival and development/growth of the F₀-F₁ generation were not significantly altered (except transient abnormalities in weight gain) by exposure to BRL 39123A.
 - 3) There were no apparent effects of the administration of BRL 39123A on the reproductive performance and fertility of females from the F₁ generation. Survival and development/growth of the F₁-F₂ generation were not significantly altered by BRL 39123A dosing of the F₀ generation.
 - 4) Males of the F₀ generation from all treatment dose groups displayed significant increases in the incidence of undescended testicles. However, this finding may be attributable to abnormally high values seen among all study groups, including the concurrent controls which were outside of the historical control range.

Segment II Testing:

3) BRL 39123A, BRL 42810: A Study to Determine The Effects of Intravenous Administration of BRL 39123A and BRL 42810 on the Course and Outcome of Pregnancy in Rats. Study ID T87504/39123A /42810/R/IV/TG, GLP, Test Site: Beecham Pharm., Stock, Essex, U.K., Study Initiation: 17 Feb., '87.

Compound Tested: BRL 39123A, batch GBD 10, 85.6% pfa
BRL 42810, batch GBD 14, bulk

Doses Tested: BRL 39123A, 0, 30, 50, and 80 mg (pfa)/kg
BRL 42810, 0, 360 mg/kg

Dose Volume and Route: 10 ml/kg, 3.0 ml/min., IV

Solvent and Control: sterile saline

Species, Strain, Sex: female and male Sprague Dawley rats (CRL: CD(SD) Br rats), Female: age 8-9 weeks, weight 180-200 grams; male: age 8-9 weeks, weight 230-250 grams. There were 26 female animals in each test group. Females were paired one-to-one with males during breeding.

Test Conditions: Animals were randomly allocated into 5 groups (26 females/group). Test compound was administered by intravenous infusion once each day between days 6 and 15 of gestation (post-coitus). Mortality, physical signs, body weight and food/water consumption were measured. On day 20 (post-coitus), the dams were sacrificed and given a detailed macroscopic external and internal examination. All fetuses were weighed, sexed, and examined externally for abnormalities. Alternate foetuses were retained for evaluation of soft-tissue anomalies, with the remaining foetuses being used for evaluation of skeletal and internal organ anomalies (high dose and control groups only).

Administration of BRL 42810 was associated with 2 premature deaths (1 animal on each of days 6 and 7). The deaths occurred during or shortly after drug

administration and were accompanied by tremors and convulsions. Necropsy failed to show a specific cause of the premature deaths although congestion of the lungs and thymus was noted in both animals. Three additional animals treated with BRL 42810 displayed single episodes of muscle tremors, abnormal respiration (gasping and/or labored breathing) spastic movements and convulsions. There were no deaths associated with the administration of BRL 39123A. Body weight gain, food and water consumption were not effected by BRL 39123A or BRL 42810 at the doses tested.

Pregnancy rate, litter size, and litter weight, were not effected by either test compound. At the doses tested, neither drug altered implantation rates, increased the incidence of fetal resorptions (early, middle, or late), nor effected the number of viable pups, their mean body weight and placental weight. The ratio of male to female foetuses was comparable among all groups.

Individual instances of major malformations were noted in 5 fetuses; however, the distribution of effects (2 control, 1 each in the low and intermediate BRL 39123A groups, and 1 foetus in the BRL 42810 treatment group) appeared unrelated to the test compounds. Administration of BRL 42810 and BRL 39123A (80 mg/kg), was associated with a slight increase in the incidence of congested adrenal medullae (2-3% vs. 1% [control]), renal pelvis cavitation (1.5% vs. 0% [control]), and kinked/dilated ureters (23% vs. 17% [control]). Blood was noted in the abdominal cavity of 3 foetuses (as compared with 0 controls) from females treated with BRL 39123A at 80 mg/kg. BRL 42810 increased the incidence of ovoid eyes and/or lenses (4.3% vs. 1.7% [control]), and was associated with 2 cases of an enlarged superior intercostal vein (versus 0 controls and BRL 39123A treated animals) in exposed animals.

Comments: Doses used in this study were based, in part, on the results of 2 previous studies with BRL 39123A (T85617/39123A/R/IV/RFS/10D and T86304/39123A/Acyclovir/R/IV/RDS/10D), which showed kidney toxicity associated with the test compound at doses in excess of 30 mg/kg.

BRL 42810, when administered intravenously at a dose of 360 mg/kg induced immediate maternal toxicities including seizures, convulsions and death.

The results suggest that BRL 39123A and BRL 42810 have minimal or no teratogenic potential, and no effects on the course or outcome of pregnancy in the rat. The slightly increased incidence of ovoid eyes/lenses noted in BRL 42810 exposed foetuses may reflect a developmental delay, which should be monitored in the follow-up segment III study.

4 BRL 39123A/BRL 42810: A Study to Determine the Maximum Tolerated Dose of BRL 39123A by Intravenous Administration and of BRL 42810 by Both Oral and Intravenous Administration in Non-Pregnant New Zealand White Rabbits, Study ID T87500/39123A/42810/RAB/PO-IV/MTD, GLP - incomplete audit report, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 26 Jan., '87.

Compound Tested: BRL 39123A, GBD11, 86.3% pure free acid (pfa)
BRL 42810, batches GBD13 and GBD14, purity unspecified

Doses Tested: doses are the pure free acid (pfa) of BRL 39123A
BRL 39123A, 15-240 mg/kg (IV)

BRL 42810, 30-360 mg/kg (IV), 150-1000 mg/kg (PO)

Dose Volume and Route: BRL 39123A, 2-3 ml/kg (IV)

BRL 42810, 2-3 ml/kg (IV), 4-6 ml/kg (PO)

Solvent: sterile saline for IV, distilled H₂O for PO

Species, Strain, Sex: 34 female New Zealand White rabbits, weight 2.5-3.5 kg, age unspecified. Animals were randomly assigned to test groups with 4-5

animals/group.

Test conditions: BRL 39123A (IV) and BRL 42810 (IV and PO) were administered once each day using a sequential doubling dose procedure. Dosing was continued for 14 days or until the MTD had been reached. Animals from the control groups were subsequently dosed at the suspected MTD for 14 days. Physical signs, body weight, food and water consumption were recorded daily. A macroscopic post mortem examination was performed at the conclusion of testing with kidney tissue being preserved.

Body weight, food and water consumption were all reduced somewhat following doses of BRL 39123A (IV) > 120 mg/kg. Pronounced physical/behavioral changes were observed in some animals shortly after administration of BRL 39123A at doses > 90 mg/kg. Signs included abnormal respiration with prostration and convulsions (3/5 animals dosed at 120 mg/kg for 5 days). There were no premature deaths following BRL 39123A administration and recovery from abnormal signs was typically evident in 1-3 hours.

Escalating daily oral doses of BRL 42810 had no effect on body weight, food consumption or mortality. A slight decrease in water intake was evident following administration of 1,000 mg/kg. Abnormal respiration was evident following oral doses of 600 and 1,000 mg/kg of BRL 42810. The intravenous administration of BRL 42810 resulted in the death of 1 animal at 240 mg/kg, and 1 animal at 360 mg/kg (only 1 additional animal was dosed at 360 mg/kg). An additional animal died prematurely following 4 days of dosing at 180 mg/kg (IV). At doses of 180 mg/kg or greater, signs of ataxia, lethargy, prostration and abnormal/increased respiration were evident in the majority of animals. Body weight and food consumption was reduced at doses of 180 mg/kg or greater, while water consumption was not consistently changed.

Pale striations and/or depressed regions on the surface of the kidneys, along with striations extending into the cortex and/or medullary regions, were observed in multiple animals at necropsy. Several animals receiving intravenous BRL 39123A and BRL 42810 also showed scattered petechia and hemorrhage of the lungs.

Comments: AS in several other species, the kidney appears to be a target organ for toxic effect of BRL 39123. Petechia and hemorrhage of lung tissues have not been reported in other species but warrant close follow-up.

The toxic manifestations of BRL 39123 do not appear to be closely related to the dose administered. Instead, the threshold for adverse responses appears to be somewhat idiosyncratic, which may make monitoring and clinical use of this drug more difficult.

Based on the results of this study the sponsor selected the following doses for rabbit teratology studies:

BRL 39123A - 15, 30 and 60 mg/kg (pfa), IV
BRL 42810 - 60, 250 and 1,000 mg/kg, PO
BRL 42810 - 120 mg/kg, IV

5) BRL 39123A, BRL 42810: A Study to Determine The Effects of Intravenous Administration of BRL 39123A and BRL 42810 on the Course and Outcome of Pregnancy in New Zealand White Rabbits, Study ID T87501/39123A/42810/RAB/IV/TG, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 24 Mar., 1987.

Compound Tested: BRL 39123A, batch GBD 10, 85.6% pfa
BRL 42810, batch GBD 15, bulk

Doses Tested: BRL 39123A, 0, 15, 30, and 60 mg (pfa)/kg
BRL 42810, 0, 120 mg/kg

Dose Volume and Route: 2 ml/kg, 3.0 ml/min., IV

Solvent and Control: sterile saline

Species, Strain, Sex: female New Zealand White rabbits, weight 2.5-3.5 Kg. There were 16 female animals in each test group.

Test Conditions: Animals were randomly allocated into 5 groups (16 females/group). Females were paired one-to-one with males during breeding. Test compound was administered by intravenous infusion once each day between days 6 and 18 of gestation (post-coitus). Mortality, physical signs, body weight and food/water consumption were measured. On day 28 (post-coitus), the dams were sacrificed and given a detailed macroscopic external and internal examination. All fetuses were weighed, sexed, and examined externally and internally for abnormalities. All foetuses were processed for skeletal anomalies. The head from alternate foetuses were retained for serial sectioning.

There were no premature deaths in the study. One animal dosed with BRL 39123A at 60 mg (pfa)/kg displayed an increased respiratory rate and muscle tremors on day 16 (day 10 of dosing) approximately 15 minutes following the drug infusion. One animal receiving BRL 42810 (120 mg/kg, day 8) collapsed unconscious immediately after being dosed, and showed signs of distress (with vocalizations) and increased respiration after regaining consciousness. Two additional animals displayed increases in respiratory rate shortly after (15 minutes) administration of BRL 42810. Generally, all physical signs receded within 2-4 hours.

High dose BRL 39123A induced a small loss (approximately 30 g) in weight between days 6 and 8, normal weight gain between days 8 and 14, and the maintenance of body weight between days 14 and 19. Normal body weight gain was re-established following the cessation of drug administration. Administration of lower doses of BRL 39123A (15 and 30 mg (pfa)/kg) and BRL 42810 (120 mg/kg) had no effect on absolute body weight or weight gain. Food and water intake were not affected by administration of BRL 42810 or BRL 39123A, except in the high dose BRL 39123A group where food consumption mirrored the change in body weight.

Pre-implantation loss was higher among the drug treated animals than the controls, although the values were within the historical control range. There were no gross pathologies evident in the dams, and no group differences in kidney weights at the time of necropsy. Live litter size was reduced and post-implantation loss increased in animals receiving BRL a at 30 and 60 mg (pfa)/kg. One animal dosed at 30 mg (pfa)/kg showed total litter death with 3 early resorptions. However, as with body weight, the effects on litter size and fetal loss seen with BRL 39123A were within the historical control range. Litter size and the incidence of fetal resorption were not effected by BRL 42810.

Mean foetal body weight and placental weight were not effected by treatment with BRL 39123A and BRL 42810. The ratio of male to female foetuses was comparable among all groups. Major malformations and visceral anomalies were evident in all groups and did not appear to be related to treatment with either test compound. Visceral anomalies included several instances of lenticular opacities (BRL 39123A: 1 foetus at 30 mg/kg; BRL 42810: 3 foetuses at 120 mg/kg), ovoid lenses (BRL 39123A: 1 foetus at 60 mg/kg), and increased cavitation of the renal pelvis (BRL 39123A: 1 and 3 foetuses treated 15 and 60 mg/kg, respectively).

Comments: BRL 39123A, at doses which induced potentially lethal toxicities in the dam, had only slight effects in reducing the rate of implantation (all effects were within the historical control range).

While, there was no clear evidence of any drug induced effects on

the incidence of visceral or skeletal anomalies, several minor anomalies (abnormalities of the eye and/or kidney) were seen which correspond to observations from the previous rodent teratology study.

Segment III Testing:

6) **BRL 39123A: A Study to Determine the Effects of Intravenous Administration on the Peri- and Post-Natal Development of the Rat**, Study ID T92501/39123A/R/IV/PPN, GLP, Test Site: Beecham Pharm., Stock, Essex, UK, Study Initiation: 7 Jan., 1992, Compound: BRL 391213A, Batch: WPB 2001, purity 86.1 g (pfa)/g.

Ninety-six female Sprague-Dawley rats (CrI:CD(SD) BR) (weight range 297-416 grams at initiation of drug dosing; age 12-14 weeks) were used in the study. Following evidence of successful mating with untreated male animals, the female animals were randomly allocated to the 4 treatment groups. Treatment with BRL 39123 was begun on day 15 post-coitus, and continued until necropsy on day 25 post-partum. BRL 39123 (batch WPB 2001) was prepared in sterile saline and administered IV infusion (3 ml/min, volume of 10 ml/kg) in the following doses: 0 (saline), 30, 50 or 80 mg/kg/day. Females were dosed once each day.

Assessment of general physical signs and mortality was performed on a daily basis throughout the study. Body weights were measured on days 0, 3, 6, 9, and 12 post coitus, and daily during drug treatment. Food and water consumption were measured on days 0-3, 3-6, 6-9, 9-12, 12-15 and 18-20 post coitus. All animals were allowed to litter naturally and rear their young until weaning. Physical signs and mortality, along with measures of development (surface and mid-air righting reflexes, penetration of incisors, separation of eyelids and visual function) were performed on all offspring during the pre-weaning period.

RESULTS:

Dose Preparations: All dosing solutions were assayed and found to be within 10% of the nominal concentration.

Mortality FC: One high dose female was sacrificed in moribund condition on day 25 post-coitus (day 7 of drug treatment). Signs preceding death included significant weight loss (39g during 2 days), hypoactivity, hunched posture, pallor, coat staining, vaginal and orbital discharges, and labored breathing. Post-mortem examination revealed compacted fecal pellets in the colon, bilateral renal pelvic dilatation, blood in one uterine horn and a red/brown vaginal discharge. The exact cause of death could not be determined. There were no other premature deaths among the drug treated or control animals.

FC Clinical Signs, Body Weight, Food and Water Consumption: Several drug treated animals showed transient (1-5 days) signs of irritation and/or scabbing at the site of drug infusion. Among the high dose treated animals there was evidence of a reduction (approx. 5%) in weight gain (significant beginning at day 18) during the period of gestation, with variable effects during the period of lactation. Food consumption was unaffected by the administration of BRL 39123, while water intake was increased approximately 15% among these animals. There were no consistent drug related effects on body weight, food or water intake among animals from the low and intermediate dose groups.

FC Reproductive Parameters: The length of gestation was 21.0-22.0 days (except one high-dose animal which littered on day 22.5), with all values being within

the historical control range for the laboratory. Post-implantation survival and the numbers of live births were unaffected by treatment with BRL 39123.

F- Litter Parameters (F₂-F₁ animals): There was a slight reduction in litter size among the high dose treated females, apparently due to an increase in the incidence of pre-implantation fetal losses. However, since implantation in the rat occurs at approximately day 6 post-coitus, and drug treatment was not initiated until day 15 post-coitus, it appears that the reduction in litter size among the high dose animals was due to a random event. The mean litter weights for animals from the low and high dose treatment groups were slightly higher than that of the concurrent controls. Post-gestational weight gain was comparable for all study groups, although absolute body weight remained slightly elevated among the high and low dose offspring at the time of weaning (i.e., maintaining the slight group differentials evident at birth). There were no other differences in litter size, offspring viability or other group parameters among the various treatment groups.

F-F₁ Physical and Reflexological Development: There were no apparent differences in the physical, neurologic or behavioral development of the offspring of animals treated with BRL 39123.

F- and Offspring Post-Mortem Examination: Post mortem examination of the adult females revealed no apparent drug related effects, and only 2 nipples with reduced secretory activity in the mammary tissue of one control animal that lost its litter. Examination of the offspring revealed no apparent drug related abnormalities. Unlike in several preceding studies, there was no apparent drug related increase in absolute or relative weights of the kidneys among the drug treated animals.

- Comments:
1. The IV administration of BRL 39123 produced modest toxic effects in the parent females, including: increased water intake and reduced body weight gain during gestation.
 2. Unlike in several preceding studies, there was no apparent drug related increase in absolute or relative weights of the kidneys among the drug treated animals. Since the same drug doses were used in this study as had demonstrated renal effects in previous studies, it can only be assumed that the lack of effect was due to the shorter period of drug administration used in the current study.
 3. Reduced litter size and an increased incidence of pre-implantation fetal losses as seen among the high dose treated animals were likely unrelated to drug treatment, as pre-implantation events preceded the initiation of drug dosing in this study.
 4. There was no evidence of any significant adverse effect on the offspring of BRL 39123 treated animals, at dose which showed modest toxicity for the dams.

APPENDIX E: ONCOGENIC POTENTIAL

SUMMARY:

Penciclovir, either as the topical or intravenous product has not been evaluated for carcinogenic potential. However, famciclovir (the oral pro-drug form of penciclovir) has been tested in rodents and found to increase the incidence of mammary adenocarcinomas in female rats (a common tumor in aging female rats), without significantly altering other tumor frequencies. Drug exposure in the female rat was approximately 1.5x human exposure for the oral product when used in accordance with the product label (recommended human dose of 500 mg t.i.d.; inter-species dose comparisons based on 24 hr AUC). Given the short duration and low level of exposure to penciclovir following topical application of the cream product (4 days dosing; estimated total applied dose of 0.05 mg/kg/day), it appears unlikely that the use of penciclovir topical cream (DENA VIR®) poses a significant risk of increased tumor incidence in man. In accordance with 21 CFR 201.56 (d) (3) and 21 CFR 201.57 (f) (6), it is recommended that the 'systemic carcinogenicity' data for famciclovir not be included in the product label for topical penciclovir (DENA VIR®), since no absorption of penciclovir following topical dosing has been demonstrated.

COMPILATION OF EFFECTS AND DISCUSSION:

The carcinogenic potential of topical and/or intravenous penciclovir has not been tested. However, carcinogenicity studies have previously been conducted with famciclovir (FAM VIR®; NDA 20-363, June 1994), the orally available pro-drug of penciclovir. A summary of the study results obtained with famciclovir is presented below (for a full review of the rodent carcinogenicity studies conducted with famciclovir, the reader is referred to NDA 20-363, supplements 000 and 009).

Results for Famciclovir: Famciclovir was evaluated for oncogenic potential in two separate multi-year (2 year duration) bioassays conducted in the Sprague-Dawley rat and the CD-1 mouse¹. In both studies, famciclovir was administered in standard rodent chow at concentrations intended to produce the following daily exposure levels: a) rat - male: 0, 50, 120, 300 (240)²; female: 0, 50, 200, 750 (600) and, b) in the mouse (male and female) 0, 50, 200, 750 (600) mg/kg/day. Drug doses in the mouse and rat oncogenicity studies were selected based on preceding toxicologic studies of 13 weeks (mouse) and 6 months (rat) duration. Results of the studies are summarized in the following paragraphs.

¹ **Oncogenicity Studies for Famciclovir:**

^{1a} **BRL 42810: A Study Of Tumorigenic Potential In The Mouse By Prolonged Administration In The Diet**, Study ID T88405/42810/M/DI/CARC/102W, GLP, Beecham Pharm., Stock, Essex, UK., Study Initiation: 18 July 1988, BRL 42810, batch GBD 27.

^{1b} **BRL 42810: A Study Of Tumorigenic Potential In The Rat By Prolonged Administration In The Diet**, Study ID T88404, GLP, Beecham Pharm., Stock, Essex, UK., Study Initiation: 23 Aug. 1988, BRL 42810, batches GBD 25, 30, 32 and 39.

² As a result of reductions in bodyweight and weight gain seen among high dose treated animals in both studies, these doses were reduced after treatment weeks 29 and 34 in the rat and mouse studies, respectively. The reduced doses were maintained for the duration of each 2-year study and are presented in parentheses.

In both studies reductions in body weight (absolute and/or weight gain; approximate differences of 10-30%) were evident following 2-6 months of drug administration, with progression of effects with continued duration of exposure. As a result of the reduced weight gain, the high dose treatments in the mouse and rat studies were reduced after weeks 29 and 34 of dosing. Despite the reductions in famciclovir dose, weight gain among the high dose animals generally lagged behind that of the control animals during the remainder of the study. Changes in food consumption appeared unrelated to drug treatment in both studies.

No clearly treatment-related effects on premature mortality were evident in either study. However, a significant increase in pre-mature mortality was evident among the intermediate dose females (with a similar trend among the high dose female animals) in the mouse study. In neither study was premature mortality among the drug treated animals associated with a consistent pattern of toxicologic effects. In both studies, there appeared to be no treatment-related effects on differential white blood cell counts, red or white cell morphology, or clinical signs.

Non-neoplastic lesions seen among the treated mice included increases in lung/bronchial adenomatosis, kidney nephropathy (high dose males) and, cystitis (males) of the urinary bladder, testicular atrophy and hypospermatogenesis (a dose-related 2-7x increase in the incidence of seminiferous tubule atrophy) and, among female animals (high and intermediate dose) an increased incidence of ovarian cysts and uterine endometrial polyps. Among famciclovir treated rats, several non-neoplastic changes seen included hepatocellular acidophilic alterations and necrosis, hyperplasia of mammary tissues, ovarian cysts and pancreatic exocrine cell alterations. Further, in the rat the testes remained a major site of toxicity following exposure to BRL 42810 for 2 years. The relationship of several of these lesions (renal nephropathy in mice, ovarian cysts in mice and rats and, pancreatic exocrine alterations and testicular degeneration in rats) to the administration of famciclovir is not clear as these lesions spontaneously appear in ageing animals of these strains.

There was no evidence of any drug related increase in tumors among mice treated at any dose level (cross species dose comparisons up to 0.4x the human exposure based on 24 hour AUC). However, in the rat oncogenicity study there was an increase in the incidence of mammary adenocarcinomas among the high dose treated female animals (approximately 1.5x the human systemic exposure following the recommended human dose of 500 mg tid; comparison based on the 24 hr AUC value for penciclovir). Further, the increase in adenocarcinomas among high dose treated females did not increase the incidence of animals with mammary tumors of all type, nor was it associated with an increase in the size or multiplicity of these tumors (i.e., tumor burden). No evidence of a reduced time-to-tumor onset was evident among the high dose treated female animals. There were no other significant effects of the administration of famciclovir on the incidence of other tumors in mice or male rats. The sponsor contends that because of the lack of in vitro and in vivo genotoxic activity (except for clastogenicity with BRL 42810 and the lack of evidence of DNA adduct formation), the increased incidence of mammary tumors seen in female rats likely represents an epigenetic effect not directly mediated by the test compound.

Inter-Species Dose Comparisons for the Oral Drug Product: For FAMVIR® (oral famciclovir) dose comparisons were based on 24 hr AUC values as determined for the test animals and human patients (using the highest recommended daily dose of 500 mg tid; all dose comparisons are based on the circulating levels of penciclovir). Following the maximum recommended dose of 500 mg t.i.d. in man, the average AUC for male and female subjects was 27.9 and 33.2 µg.hr/ml. The sponsor agreed to the use of the larger value in the computation of all cross-

species dose comparisons.

In the rat oncogenicity study, female animals were dosed at levels up to 600 mg/kg/day (approx. 1.5x the 24 hr human exposure based on the AUC for penciclovir), with no observable increase in tumors being seen at the intermediate dose level of 300 mg/kg/day (approx. 0.6x the 24 hr human exposure based AUC). Male rats were dosed at levels up to 240 mg/kg/day, which approximates (0.9x) the human systemic exposure to penciclovir. In the mouse oncogenicity study, male and female animals were dosed at levels up to 600 mg/kg/day, achieving systemic drug exposure levels of approximately 0.4x the human daily exposure (based on AUC values for penciclovir).

Inter-Species Dose Comparisons for the Topical Drug Product: For DENAVIR® (topical penciclovir) estimated dose comparisons can not be based on measured AUC values, since no measurable drug was found in the plasma or urine of the subjects in the clinical trials. Therefore the following inter-species dose comparisons are based on "nominal" and "body surface area" adjusted doses. For the following dose comparisons it is assumed that all of the topically applied drug product undergoes absorption and systemic distribution, achieving a maximum daily exposure level of 0.05 mg/kg.

"Nominal Dose" Comparisons: Based on the administered mg/kg doses, comparisons for male and female mice range from 1000-12000x the human exposure (based on the tested doses of 50-600 mg/kg/day). For male rats, the exposure range was 1000-4800x the human exposure. The exposure range of female rats ranged from 1000-12000x the human exposure, with no observable adverse effects being evident at exposure levels up to 4000x the human exposure.

"Body Surface Area Adjusted Dose" Comparisons: Based on surface area dose adjustments, the daily human drug exposure from topical penciclovir is roughly equivalent to doses of 0.35 mg/kg and 0.6 mg/kg in the rat and mouse, respectively. Therefore, comparisons for the doses administered to male and female mice range from 83-1000x the human exposure (based on 'No-Effect' doses of 50-600 mg/kg/day). For male rats, the exposure range was 143-686x the human exposure. Lastly, the exposure range of female rats ranged from 143-1000x the human exposure, with no observable tumorigenic effects being evident at exposure levels up to 571x the human exposure.

Conclusions and Comments: Exact systemic exposure following the application of penciclovir topical cream can not be defined since all assay results found that drug levels (if present) were below the limit of detection of the test assay (0.1 ng/ml). While it appears logical that some drug exposure occurs, the level of absorption and systemic distribution appears to be exceeding low. It should be noted that in the preceding inter-species dose comparisons, it was assumed that all of the applied drug product was absorbed and underwent systemic distribution. However, it appears likely that in human patients the actual level of systemic drug exposure from the topical drug product is lower than the estimate used in the preceding calculations. Thus it is likely that the dose comparisons presented above represent the minimum inter-species dose differences and that the actual inter-species dose differences are greater than those presented.

CONCLUSIONS: Although an increased progression to mammary adenocarcinomas was seen among the high dose treated female rats, the pattern of the response did not suggest that a genotoxic mechanism was implicated in the development of these tumors, since no overall increase in mammary tumors was evident. In addition, 'No-Effect' doses were evident in both species and sexes evaluated. Together with the short duration of treatment and the low level of exposure to penciclovir following topical application of the cream product (4 days dosing; estimated total applied dose of 0.05 mg/kg/day), it appears unlikely that the use of penciclovir topical cream (DENAVIR®) poses a significant risk of

increased tumor incidence in man. Therefore, it is recommended that in accordance with 21 CFR 201.56 (d)(3) and 21 CFR 201.57 (f)(6) the 'systemic carcinogenicity' data for famciclovir not be included in the product label for topical penciclovir (DENA VIR®), since no absorption of penciclovir following topical dosing has been demonstrated.

APPENDIX F: GENOTOXICITY STUDIES

SUMMARY:

Penciclovir was evaluated for potential genotoxic effects in a standard battery of assays, which included: a) assays for unscheduled DNA synthesis in HeLa cells in vitro, b) bacterial and mammalian cell gene mutation assays in vitro and, c) in vitro and in vivo assays for chromosomal aberrations. The results of several assays suggest that penciclovir has some clastogenic activity, although these effects were generally coincident with signs of cellular toxicity and thus suggest that the responses might be due to non-specific cellular effects and not direct DNA-drug interaction. Similarly, the increased mutation rate noted in the mouse lymphoma assay occurred at relatively high drug concentrations which inhibited cell replication and growth.

COMPILATION OF EFFECTS AND DISCUSSION:

Penciclovir (sodium salt and/or the free base) was tested in a standard battery of in vitro and in vivo genotoxicity assays. Testing included assays for unscheduled DNA synthesis in HeLa cells in vitro, bacterial and mammalian cell line gene mutation assays in vitro and, in vitro and in vivo assays for chromosomal aberrations. The results of the genetic toxicity tests conducted with penciclovir are as follows:

- a) no increase in unscheduled DNA damage-repair in HeLa S3 cells at doses up to 5,000 $\mu\text{g/mL}$ (\pm S9 activation),
- b) no increase in mutations in the Ames agar assay with Salmonella typhimurium (TA97, TA98, TA100, TA1535 and TA1538) or Escherichia coli (CM891) at doses up to 2,000 $\mu\text{g/plate}$ (\pm S9 activation),
- c) an increase in mutations in the L5178Y mouse lymphoma assay at concentrations $\geq 1,000 \mu\text{g/mL}$ (\pm S9 activation),
- d) an increase in the incidence of chromosomal breaks and rearrangements in cultured human lymphocytes incubated with penciclovir at doses $\geq 250 \mu\text{g/mL}$ without activation (the compound being negative at doses up to 600 $\mu\text{g/mL}$ when incubated with an S9 activation fraction),
- e) an increase in the incidence of micronuclei in the bone marrow of mice after a single dose of $\geq 500 \text{ mg/kg}$. The threshold for activity was approx. 300 mg/kg , and
- f) no increase in the incidence of micronuclei in the bone marrow of mice given a single dose of up to 4,800 mg/kg .

In general, penciclovir was typically found to be inactive in the induction of unscheduled DNA damage-repair and gene mutation. Exceptions to this included the mouse lymphoma and human lymphocyte assays, in which penciclovir increased the incidence of mutations and/or chromosomal abnormalities following exposure to drug at concentrations $\geq 1,000$ and 250 $\mu\text{g/ml}$, respectively. In the later assay, the increased incidence of chromosomal aberrations was noted only in the absence of the S9 rat liver metabolic activation fraction. This is similar to the effects noted with famciclovir, and suggests that the addition of the S9 fraction resulted in the extracellular conversion of penciclovir to a form which was incapable of penetrating the cell or nuclear membrane. Lastly, in the mouse micronucleus assay the incidence of chromosomal abnormalities was increased following the IV administration of penciclovir but not BRL 39123 (the free base form of penciclovir) at doses ≥ 500 and $\leq 4,800 \text{ mg/kg}$, respectively. Signs of significant cytotoxicity were seen in the bone marrow of affected animals following the administration of penciclovir at and below those doses resulting in genotoxic effects.

Similar to penciclovir, famciclovir (the diacetylated orally available pro-drug of penciclovir; see NDA 20-363, June 1994) was without activity in the

standard gene mutation, DNA damage/repair and chromosomal aberration assays. The only exception to this, was an increase in the incidence of polyploidy in human peripheral lymphocytes incubated with high concentrations of famciclovir in vitro. However, the polyploidy was concurrent with signs of cytotoxicity, suggesting that the increased endoreduplication was likely a non-specific effect of cellular inhibition/toxicity. The addition of a rat liver microsomal activation fraction reduced the polyploidy seen. Further, it should be noted that no genotoxic effects were seen in the micronucleus and dominant lethal assays (although bone marrow suppression and other toxic effects were evident in these studies). This pattern of effects suggests that extracellular metabolism and lack of cell penetration might best explain the results seen in the peripheral lymphocyte assay. Lastly, while polyploidy is a genetic abnormality, it is generally not considered a genotoxic response since it occurs naturally in some cells types and in increased frequency among cells exposed to cytotoxic agents.

In summary, while clastogenic effects were seen in several assays following the administration of penciclovir (and famciclovir; see NDA 20-363, June 1994), these effects were generally coincident with signs of significant cellular toxicity, suggesting that the responses might be due to nonspecific cellular effects and not direct DNA-drug interaction. Similarly, the increased rate of mutation seen in the mouse lymphoma test with penciclovir occurred following exposure to relatively high concentrations of drug which clearly inhibited cell replication and growth. Lastly, it should be noted that similar or more potent clastogenic and mutagenic effects have been reported for other of the synthetic nucleoside analogues.

Reviews of the individual studies are contained on the following pages.

Toxicity Studies Summary:

- 1) BRL 39123: Report Of Microbial Mutagenicity Tests In Ames Agar Plate Assays With Salmonella typhimurium TA97, TA98 And TA100 (Duplicate Tests), GLP, Study ID T85/737/39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 18 Apr. '85, Compound: BRL 39123, Batch: GBD6.
- 2) BRL 39123: Report Of Microbial Mutagenicity Tests In Ames Agar Plate Assays With Salmonella typhimurium TA1535 And TA1538 (Replicate Tests), GLP, Study ID 278/39123/ Test Initiation: 17 Jul. '89, Compound: BRL 39123, Batch: GBD32, 99.5% pure free acid.
- 3) BRL 39123: Report Of Microbial Mutagenicity Tests In Agar Plate Assays With Escherichia coli CM891 (Replicate Tests), GLP, Study ID 281/39123/E.COLI, Test Site: Initiation: 13 Jul. '89, Compound: BRL 39123, Batch: GBD32, 99.5% pure free acid.
- 4) BRL 39123A: Report Of Unscheduled DNA Synthesis (DNA Repair) In HeLa Cell Cultures, GLP, Study ID T89/712/39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 9 Nov. '89, Compound: BRL 39123A, Batch: GBD18, 86% pure free acid.
- 5) BRL 39123: Report Of Mutation Tests With L5178Y Mouse Lymphoma Cells At The TK Locus, GLP, Study ID T86/754/39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 11 Mar. '89, Compound: BRL 39123, Batch: CT 18189.
- 6) BRL 39123: Report Of In Vitro Metaphase Analysis With Human Lymphocytes In Culture, GLP, Study ID T86/752/39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 24 Feb. '86, Compound: BRL 39123, Batch: GBD15, 99.8% pure.
- 7) BRL 39123: Report Of A Micronucleus Test In The Mouse, GLP, Study ID T85/741/39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 24 Jun. '85, Compound: BRL 39123, Batch: GBD7, 99.0% pure.
- 8) BRL 39123A: Report Of Micronucleus Test In CD-1 Mice By The Intravenous Route, GLP, Study ID T88/797/39123A, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 9 Dec. '88, Compound: BRL 39123A, Batch: GBD18, 85.5% pure free acid.
- 9) BRL 39123A: Report Of Micronucleus Threshold Test After Intravenous Injection In The Mouse, GLP, Study ID T89/711 /39123, Test Site: Beecham Pharm., Stock, Essex, UK, Initiation: 20 Nov. '89, Compound: BRL 39123A, Batch: GBD18, 85.5% pure free acid.
- 10) Comparative Micronucleus Tests with Penciclovir, Acyclovir, Ganciclovir and Caffeine in the Mouse by the Intravenous Route (Multi-Dose Study), Studies: T92/769/39123A and T92/770/ACV/GCV/Caffeine, Beecham Pharm., The Frythe, Welwyn, UK, 16 Dec., 1992, Compound: 39123A, Batch: WPB 2001, 0.861 g (pfa)/g.

Toxicity Studies Reviews:

- 1) BRL 39123: Report of microbial mutagenicity tests in Ames agar plate assays with Salmonella typhimurium TA97, TA98 and TA100 (duplicate tests), Report: T85/737/39123, Beecham Pharm., Stock, Essex, UK, Aug. 1985, Batch GBD 6.

Penciclovir was tested in an agar plate assay against Salmonella typhimurium strains TA97, TA98 and TA100 at doses of 0 [vehicle (dimethyl sulfoxide)], 125, 250, 500, 1,000 or 2,000 µg per plate in the presence and absence of an Aroclor 1254 stimulated S9 fraction from rat liver. The test was carried out in duplicate on two different occasions. An appropriate positive control was used in the presence of microsomal activation but none in the absence.

Under the conditions of the test, penciclovir was negative for the induction

of reverse mutations in Salmonella typhimurium in the presence or absence of microsomal activation.

2) BRL 39123: Report of microbial mutagenicity tests in Ames agar plate assays with Salmonella typhimurium TA1535 and TA1538 (replicate tests), Report: 278/39123 March 1981, Batch GBD 32.

Penciclovir was tested in an agar plate assay against Salmonella typhimurium strains TA1535 and TA 1538 at doses of 0 [vehicle (dimethyl sulfoxide)], 125, 250, 500, 1,000 or 2,000 µg per plate in the presence and absence of an Aroclor 1254 stimulated S9 fraction from rat liver. The test was carried out in duplicate on two different occasions. Appropriate positive controls were used in the presence and absence of microsomal activation.

Under the conditions of the test, penciclovir was negative for the induction of reverse mutations in Salmonella typhimurium in the presence or absence of microsomal activation.

3) BRL 39123: Report of microbial mutagenicity tests in agar plate assays with Escherichia coli CM891 (replicate tests), Report: 281/39123/E.coli, March 1981, Batch GBD 32.

Penciclovir was tested in an agar plate assay against Escherichia coli CM891 at doses of 0 [vehicle (dimethyl sulfoxide)], 125, 250, 500, 1,000 or 2,000 µg per plate in the presence and absence of an Aroclor 1254 stimulated S9 fraction from rat liver. The test was carried out in duplicate on two different occasions. Appropriate positive controls were carried out in the presence and absence of activation.

Under the conditions of the test, no significant increases versus control in the colony numbers (tryptophan reversions) were noted at any dose of BRL 42810 tested (with or without microsomal activation).

4) BRL 39123A: Report of unscheduled DNA synthesis (DNA repair) in HeLa cell cultures. Report: T89/712/39123, Beecham Pharm., Stock, Essex, UK, Aug. 1990, Batch GBD 18.

Penciclovir was tested at doses of 0 [vehicle (water)], 8, 40, 200, 1,000 or 5,000 µg/mL to assess its ability to induce unscheduled DNA synthesis into HeLa S3 cells in culture as measured by the incorporation of [³H]-labeled thymidine in the presence and absence of activation with liver S9 from rats treated with Aroclor 1254. The test was carried out in duplicate on two different occasions. Appropriate positive controls were carried out in the presence and absence of activation.

Under the conditions of the test, no significant increases in unscheduled DNA synthesis were detected.

5) BRL 39123: Report of mutation tests with L5178Y mouse lymphoma cells at the TK locus. Report: T86/754/39123, Beecham Pharm., Stock, Essex, UK, Oct. 1986, Batch CT 18189.

Penciclovir was tested at doses of 0 [vehicle (dimethyl sulfoxide)], 250, 500, 1,000 or 2,000 µg/mL to assess its ability to induce mutations at the thymidine kinase (TK) locus in mouse lymphoma L5178Y cells in the presence and absence of activation with liver S9 from rats treated with Aroclor 1254. The test was carried out in duplicate on two different occasions. Appropriate positive controls were carried out in the presence and absence of activation.

Under the conditions of the test, penciclovir produced significant dose-related mutational events at concentrations of 1,000 and 2,000 µg/mL in mouse

lymphoma L5178Y cells in the presence or absence of activation.

6) **BRL 39123: Report of in vitro metaphase analysis with human lymphocytes in culture.** Report: T86/752/39123, Beecham Pharm., Stock, Essex, UK, Feb. 1987, Batch CT 18163.

Penciclovir at doses of 0 [vehicle (dimethyl sulfoxide)], 150, 300 and 600 µg/mL (in the presence of S9) and 125, 250 or 500 µg per mL (in the absence of S9) was evaluated for genetic activity by metaphase analysis of human peripheral lymphocytes cultured in vitro. The cells were collected from a single healthy male volunteer. Activity was tested in the presence and absence of activation with liver S9 from rats treated with Aroclor 1254. Appropriate positive controls were carried out in the presence and absence of activation. For each treatment, single cultures were grown for 48 hours. At that time, the cultures were treated for four hours (with activation) and for 25 hours (without activation). The cells were washed and grown for an additional 73 hours. Three hours prior to harvesting, the cells were treated with colcemid to arrest cells at metaphase. The cells were fixed and examined for chromosomal affects.

Although doses over 400 µg/mL were not tested for toxicity, the number and quality of metaphases were reported to be suitable for analysis. In the absence of metabolic activation, penciclovir induced significant increases in chromosomal breaks and rearrangements at 250 µg/mL and above, with significant numerical changes at the high dose. In the presence of activation, there were no significant increases in chromosomal anomalies at any dose. It is concluded that under the conditions of the test, penciclovir induces chromosomal breaks and rearrangements in the absence of exogenous metabolic activation in human lymphocytes in vitro.

7) **BRL 39123: Report of a micronucleus test in the mouse,** Report: T85/741/39123, Beecham Pharm., Stock, Essex, UK, Dec. 1985, Batch GBD 7.

Five male and five female CD-1, Charles River mice per time point per dosage group were administered penciclovir by gavage at doses of 0 [vehicle (1% methyl cellulose)], 2,400 or 4,800 mg/kg. At 24 or 48 hours, femoral bone marrow samples were taken and smears were prepared and examined for increases in micronucleated polychromatic erythrocytes. Where possible, at least 1,000 cells were scored for each animal. Cyclophosphamide (examined after 24 hours only) was used as a positive control.

Penciclovir showed no genetic activity under the conditions of the assay.

8) **BRL 39123A: Report of micronucleus test in CD-1 mice by the intravenous route.** Report: T88/797/39123A, Beecham Pharm., Stock, Essex, UK, Oct. 1990, Batch GBD 18.

Five male and five female Crl: CD-1 (ICR) BR), Charles River mice per time point were administered BRL 39123A intravenously at doses of 0 [vehicle (saline)], 600 (males only) or 750 mg/kg (females only). At 24, 48 or 72 hours, the animals were killed. Femoral bone marrow samples were taken and smears were prepared and examined for increases in micronucleated polychromatic erythrocytes (MPE). Where possible, at least 1,000 cells were scored for each animal. Cyclophosphamide (examined after 24 hours only) was used as a positive control. The above was designated the main test. A supplementary test was carried out on an additional five males and five females dosed at 0 [vehicle (saline)] or 100 mg/kg and five females dosed at 750 mg/kg. An additional five male and female positive control animals were dosed with cyclophosphamide. All the animals in the supplemental group were killed at 48 hours and treated as above.

BRL 39123A at all doses tested, was toxic to the bone marrow erythrocytes as shown by the reduced percentages of polychromatic cells at 48 and 72 hours postdose. In the main test, a slight increase in MPEs was seen in female mice 48 hours after administration of BRL 39123A at 750 mg/kg. In the supplemental test, a slight increase in MPEs was seen in males and females 48 hours (the only timepoint tested) after administration of BRL 39123A at 500 mg/kg and a significant increase in females (the only animals tested) after administration of 750 mg/kg. Thus, under the conditions of the test BRL 39123A was positive in the mouse micronucleus test at doses of 500 mg/kg and above.

9) **BRL 39123A: Report of micronucleus threshold test after intravenous injection in the mouse**, Report: T89/711/39123, Beecham Pharm., Stock, Essex, UK, Sept. 1990, Batch GBD 18.

Five male Crl: CD-1 (ICR) BR, Charles River mice per time point were administered BRL 39123A intravenously at doses of 0 [vehicle (saline)], 148, 222, 333, 500 or 750 mg/kg. At 48 hours, the animals were killed. Femoral bone marrow samples were taken and smears were prepared and examined for increases in micronucleated polychromatic erythrocytes (MPE). Where possible, at least 2,000 cells were scored for each animal.

The results of the test are shown in Table 1.

Table 1
Polychromatic Cell Data in Mouse Micronucleus Test
After Treatment With BRL 39123A

Treatment mg/kg	Cells Scored	% With MPE	% Polychromatic Cells
Saline	10,000	0.01	33.25
148	10,000	0.00	19.99
222	10,000	0.02	20.87
333	10,000	0.03	16.48
500	10,000	0.11	7.54
750	10,000	0.23	13.43

From these data, one can conclude that BRL is toxic to marrow erythrocytes and that the threshold in the test falls at approximately 300 mg/kg. Above that dose, BRL 39123A induces micronuclei in polychromatic erythrocytes in mice.

10) **Comparative Micronucleus Tests with Penciclovir, Acyclovir, Ganciclovir and Caffeine in the Mouse by the Intravenous Route (Multi-Dose Study)**, Studies: T92/769/39123A and T92/770/ACV/GCV/Caffeine, Beecham Pharm., The Frythe, Welwyn, UK, 16 Dec., 1992, Compound: 39123A, Batch: WPB 2001, 0.861 g (pfa)/g.

Groups of four or six male mice (Crl: CD-1 (ICR) BR; Charles River) were administered BRL 39123A (0, 103.6, 155.4, 233.6, 349.8, 524.7 or 787.1 mg [bulk drug]/kg), acyclovir or ganciclovir (0, 40, 60, 90, 135, 203 or 304 mg/kg; both drugs) or caffeine (0, 56, 75 or 94.5 mg/kg), by intravenous infusion on two consecutive days. Cyclophosphamide (75 mg/kg; PO) was administered to a separate group of animals as a positive control. All study animals were terminated 24 hours following the second drug infusion. Femoral

bone marrow samples were taken and smears were prepared and examined for increases in micronucleated polychromatic erythrocytes (MPE). Where possible, at least 1,000 cells were scored for each animal. The above was designated the main test.

Two animals administered caffeine at a dose of 94.5 mg/kg died immediately after dosing and were replaced with substitute animals. There were no premature deaths among animals dosed with penciclovir, acyclovir or ganciclovir. Bruising of the tail at the site of study drug infusion was noted in all study animals dosed with penciclovir or acyclovir, and animals given ganciclovir at doses other than 40 and 203 mg/kg.

BRL 39123A caused minimal-moderate bone marrow toxicity at doses \geq 155.4 mg/kg as evidenced by the decrease in the percentage of polychromatic cells. Mild-moderate cytotoxic effects on the bone marrow were observed at all doses of acyclovir and ganciclovir tested. No adverse effects on the bone marrow were noted following the administration of caffeine.

Significant increases in the incidence of micronucleated polychromatic erythrocytes were noted for all study compounds, beginning at 349.8 mg/kg for penciclovir, 135 mg/kg for acyclovir, 90 mg/kg for ganciclovir and 75 mg/kg for caffeine. The threshold for increased %MNPCE was estimated for each of the compounds by regression analyses with the following results: penciclovir, 314 mg/kg; acyclovir, 112 mg/kg; ganciclovir, 40 mg/kg; and caffeine, 67 mg/kg.

The study results are shown in Table 2 on the following page.

- Comments:
- 1) Under the conditions of the present study, all of the test compounds (penciclovir, acyclovir, ganciclovir and caffeine) demonstrated minimal-moderate in vivo genotoxic potential (as measured by increases in the percent of micronucleated polychromatic erythrocytes in the mouse bone marrow 24 hrs following drug administration).
 - 2) In the mouse micronucleus assay, the apparent in vivo potency of the nucleoside analogues to cause genotoxic effects appears to be GCV > ACV > PCV (based on the estimated no-effect thresholds for the compounds). Whether a similar threshold for adverse genotoxic effects associated with the use of nucleoside analogues is evident in the human is not known.

Table 2
Polychromatic Cell Data in Mouse Micronucleus Test
After Treatment With BRL 39123A, Acyclovir, Ganciclovir or Caffeine

Treatment	Dose mg/kg	%MNPCE	I from Control	%MNNE	%PCE
Saline	---	0.13	---	0.04	40.18
Cyclophos.	75	3.65	121.7	0.11	35.74
Penciclovir	103.6	0.10	---	0.05	42.98
	155.4	0.08	---	0.06	30.45
	233.6	0.10	---	0.09	19.77
	349.8	0.23	1.8	0.08	26.58
	524.7	0.28	2.2	0.11	16.57
	787.1	0.73	5.6	0.09	22.05
Acyclovir	40	0.05	---	0.02	28.80
	60	0.23	1.8	0.05	25.98
	90	0.18	1.4	0.09	21.11
	135	0.25	1.9	0.13	24.09
	203	0.53	4.1	0.14	29.08
	304	0.68	5.2	0.15	21.80
Ganciclovir	40	0.18	1.4	0.06	23.42
	60	0.10	---	0.10	24.51
	90	0.25	1.9	0.06	23.98
	135	0.60	4.6	0.10	26.00
	203	0.85	6.5	0.11	17.27
	304	2.30	17.7	0.17	16.06

Saline	---	0.03	---	0.15	35.98
Caffeine	56	0.05	1.7	0.03	41.80
	75	0.23	7.7	0.11	38.21
	94.5	0.23	7.7	0.13	40.59

 All doses expressed as bulk compound. MN - micronucleated, PCE - polychromatic erythrocyte, NCE - normochromatic erythrocyte, %PCE - % polychromatic of total erythrocyte count (PCE+NCE)

APPENDIX G: GENERAL PHARMACOLOGY STUDIES

SUMMARY:

Penciclovir was tested in a battery of standard in vivo and in vitro bioassays to determine the general pharmacologic effects of the compound. Study data indicate that penciclovir was relatively inactive in the production of central or peripheral nervous system effects, hematologic or metabolic effects except at high dose levels. Primary responses noted following the bolus intravenous administration of penciclovir were generally confined to transient cardiovascular effects (prolongation of the P-R and Q-T intervals) and, renal tubular injuries caused by insolubility.

COMPILATION OF EFFECTS AND DISCUSSION:

Penciclovir was tested in a standard battery of in vivo and in vitro bioassays to determine the general and/or "secondary" pharmacologic effects of the compound. An outline of the study findings follows.

Penciclovir was administered (IV) in ascending doses up to 300 (pfa) mg/kg in the cardiovascular/respiratory toxicity dog test system. In most other whole-animal studies conducted with penciclovir it was administered intravenously at doses only up to 160 mg/kg. The in vitro studies were generally tested at concentrations of up to $3 \times 10^{-4}M$. Test doses were selected based on the results of previously conducted toxicity trials.

In the anaesthetized dog, systolic blood pressure was increased slightly following the intravenous administration of penciclovir at 3-10 (pfa) mg/kg, while at doses ≥ 30 mg/kg both mean and systolic blood pressure were reduced in a dose related manner. Compensatory increases in heart rate (up to 80 beats/min.), along with a dose related decrease in the amplitude of the R wave of the ECG were noted at doses of BRL 39123 ≥ 30 mg/kg. Cardiovascular and respiratory responses to all reference compounds were slightly to markedly blunted (dose related) following the administration of BRL 39123 at doses of 30-300 mg/kg (but not at the lower doses tested). Also, at high concentrations, penciclovir showed a slight positive chronotropic effect in isolated guinea pig atrium. Despite the alteration in cardio-respiratory responses induced by the reference agents, the overall pattern of effects suggests that there was not a selective interaction of penciclovir with either the sympathetic or parasympathetic autonomic nervous systems.

In the rat, the IV administration of penciclovir (160 [pfa] mg/kg; as noted above this was generally the highest dose tested) caused significant changes in renal function including diuresis, increased urinary excretion of sodium and chloride, reduced excretion of potassium and reduced PSP clearance. These changes were likely associated with physical trauma to the renal ductules due to product insolubility in the urinary environment and the resultant formation of drug crystals in the renal lumen. These findings appear to correlate with the signs of nephrotoxicity noted in several of the repeat dose studies conducted penciclovir.

Penciclovir was evaluated in a battery of behavioral assays for interaction with either the central or peripheral nervous systems. In the Irwin assay, penciclovir (IV doses of 15-160 [pfa] mg/kg, but not 5 mg/kg) administration resulted in slight-moderate decreases in locomotor activity. However, no significant effects on coordinated motor activity (inclined plane and wire-grasping) or on a separate measure of spontaneous locomotor activity were noted in mice given penciclovir (IV; up to 160 [pfa] mg/kg). Penciclovir showed no evidence of anesthetic activity on several tests. Gastric acid secretion and gastric motility were slightly reduced following IV penciclovir administration, while intestinal motility (as measured by charcoal transit

time) was unaffected. Overall, these results suggest that penciclovir has no significant effects on the central or peripheral nervous systems.

Penciclovir demonstrated no anti-inflammatory or antigenic activity, or effects on blood coagulation/platelet aggregation at any dose/concentration tested. Further, penciclovir failed to induce hemolysis when added in vitro in increasing concentrations to buffered and suspended erythrocytes (penciclovir did however cause pronounced hemolysis when added to unbuffered cells at a concentration of 1%, apparently due to changes in pH). No significant evidence of hemolysis was noted in any of the repeat dose toxicity studies with IV penciclovir. When tested in vitro, penciclovir demonstrated minimal covalent binding to the free amino or thiol groups of HSA. Lastly, serum glucose, free fatty acids and triglycerides and, hepatic function (MEOS) were unaltered following short term treatment with penciclovir.

In summary, penciclovir was relatively inactive in the production of peripheral or central nervous system effects, hematologic, antigenic and metabolic effects except at high dose levels. Primary responses noted were generally confined to cardiovascular and/or renal effects following rapid IV administration of high doses of drug.

A compilation of the individual study reviews is contained on the following pages.

Pharmacology Studies Summary:**Effects on the Nervous System:****A) Assessments in Whole Animals:**

- 1) Naive Behaviour (Irwin Profile) in Mice Employing the Intravenous Route, Study ID PA-1004/BRL-39123/1. non-GLP,
Study Initiation: 17 April 1989, BRL 39123A, batch GBD 18.
- 2) Effect on the Motor Coordination (Inclined Plane) in Mice Employing the Intravenous Route, Study ID PA-1005/BRL-039123/1. non-GLP,
Study Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.
- 3) Effect on the Motor Coordination (Traction Test) in Mice Employing the Intravenous Route, Study ID PA-1007/BRL-039123/1. non-GLP,
Study Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.
- 4) Effect on Spontaneous Motor Activity in Mice Employing the Intravenous Route, Study ID PA-1006/BRL-039123/1. non-GLP,
Study Initiation: 13 March 1989, BRL 39123A, batch GBD 18.
- 5) Anti-Tremorine Activity in Mice Employing the Intravenous Route, Study ID PA-1010/BRL-039123/1. GLP,
Study Initiation: 26 June 1989, BRL 39123A, batch GBD 18.
- 6) An Hypnotic Potentiation Study in Mice Employing the Intravenous Route, Study ID PA-1001/BRL-039123/1. non-GLP,
Study Initiation: 6 Feb. 1989, BRL 39123A, batch GBD 18.
- 7) Effect on PMT Evoked Convulsions in Mice Employing the Intravenous Route, Study ID PA-1002/BRL-039123/1. non-GLP,
Study Initiation: 15 Feb. 1989, BRL 39123A, batch GBD 18.
- 8) A Body Temperature Study in Mice Employing the Intravenous Route, Study ID PA-1003/BRL-039123/1. non-GLP,
Study Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.
- 9) Local Anaesthetic Effects on Corneal Reflex of the Guinea Pig, Study ID PA-1011/BRL-039123/1. GLP,
Initiation: 21 Sept. 1989, BRL 39123A, batch GBD 18.

B) Assessments in Isolated Animal Tissues:

- 10) Effects on the Stimulated Phrenic Nerve Diaphragm Preparation of the Rat, Study ID PA-1012/BRL-39123/1. non-GLP,
Study Initiation: 13 April 1989, BRL 39123A, batch GBD 18.
- 11) Effects on the Isolated Vas Deferens and on its Response to Noradrenaline in Rats, Study ID PA-1016/BRL-039123/1. non-GLP,
Study Initiation: 3 Nov. 1988, BRL 39123A, batch GBD 18.
- 12) Effects on the Spontaneous Motility of Non-Pregnant Rat Isolated Uterus, Study ID PA-1027/BRL-039123/1. GLP,
Study Initiation: 2 Jan. 1990, BRL 39123A, batch GBD 18.
- 13) Effects on the Response of the Guinea Pig Isolated Trachea to Histamine, Study ID PA-1015/BRL-039123/1. GLP,
Study Initiation: 1 Oct. 1990, BRL 39123A, batch GBD 18.
- 14) Effects on the Spontaneous Motility of the Isolated Ileum of the Rabbit, Study ID PA-1019/BRL-039123/1. non-GLP,
Study Initiation: 15 Nov. 1988, BRL 39123A, batch GBD 18.
- 15) Effect on the Response of Guinea Pig Isolated Ileum to Various Agonists, Study ID PA-1014/BRL-039123/1. non-GLP,
Study Initiation: 15 Dec. 1988, BRL 39123A, batch GBD 18.

Effects on Autonomic or Contractile Tissues:

A) Assessments in Whole Animals:

- 16) Effect on the Charcoal Transit in Mice Employing the Intravenous Route, Study ID PA-1017/BRL-039123/1. non-GLP,
Study Initiation: 28 Nov. 1988, BRL 39123A, batch GBD 18.
- 17) Effect on Gastric Secretion in Rats Employing the Intravenous Route, Study ID PA-1018/BRL-039123/1. GLP,
Study Initiation: 28 Nov. 1989, BRL 39123A, batch GBD 18.
- 18) Effects on the Spontaneous Motility of the Rabbit Stomach In Situ, Study ID PA-1020/BRL-039123/1. GLP,
Study Initiation: 15 Oct. 1990, BRL 39123A, batch GBD 18.

B) Assessments in Isolated Animal Tissues:

- 19) Effects on the Response of the Isolated Right and Left Atria of the Guinea Pig, Study PA-1013/BRL-39123/1. non-GLP
Study Initiation: 29 June 1989, BRL 39123A, batch GBD 18.

Antinociceptive Activity:

- 20) Antinociceptive Activity in the Writhing Test in Mice Employing the Intravenous Route, Study ID PA-1008/BRL-039123/1. GLP,
Study Initiation: 5 Feb. 1990, BRL 39123A, batch GBD 18.
- 21) Antinociceptive Activity in the Tail-Flick Test in Mice Employing the Intravenous Route, Study ID PA-1009/BRL-039123/1. GLP,
Study Initiation: 2 July 1990, BRL 39123A, batch GBD 18.

Cardiovascular and Haematologic Interactions:

A) Assessments in Whole Animals:

- 22) A Cardiovascular/Respiratory Toxicity Study in Anaesthetised Beagle Dogs Employing the Intravenous Route, Study Code: T86001/39123A/D/IV/ CVS. GLP, Beecham Pharmaceuticals Res. Div., Stock, UK, Study Initiation: 3 Feb. 1986, BRL 39123A, batch GBD 6.

B) Assessments in Isolated Animal Tissues:

- 23) To Assess the In Vitro Ability of BRL 39123A to Promote the Adsorption of Plasma Proteins onto Human Red Blood Cells, Study Code: CP/AG/90/2. GLP, SmithKline Beecham Res. Develop., The Frythe, UK, Study Initiation: 14 June 1990, BRL 39123A, batch GBD 18.
- 24) Effects on the Excretion of ICG in Rats Employing the Intravenous Route, Study ID PA-1023/BRL-039123/1. GLP
Study Initiation: 8 Sept. 1989, BRL 39123A, batch GBD 18.
- 25) An In Vitro Study of Haemolysis on Rabbit Red Cells by BRL 39123A, Study ID TF-1005/BRL-039123/1. GLP, SmithKline Beecham Res. Develop., The Frythe, UK, Study Initiation: 13 Aug. 1992, BRL 39123A, batch WPB 2001.
- 26) Effect on Blood Coagulation in Rats Employing the Intravenous Route, Study ID PA-1026/BRL-039123/1. non-GLP,
Study Initiation: 23 Jan. 1989, BRL 39123A, batch GBD 18.
- 27) A Platelet Aggregation Study on Guinea Pig Plasma. Study ID PA-1025/BRL-039123/1. GLP, Study
Initiation: 20 Sept. 1990, BRL 39123A, batch GBD 18.

Renal Effects:

28) **Effects on the Renal Excretion in Rats, Employing the Intravenous Route, Study ID PA-1021/BRL-039123/1. non-GLP.**

Study Initiation: 7 Nov. 1988, BRL 39123A, batch GBD 18.

29) **Effect on the PSP Excretion in Rats Employing the Intravenous Route, Study ID PA-1022/BRL-039123/1. non-GLP.**

Study Initiation: 12 Dec. 1988, BRL 39123A, batch GBD 18.

Anti-Inflammatory Activity:

30) **An Anti-Inflammatory Study in Rats Employing the Carrageenan Paw Inflammation Model, Study ID PA-1028/BRL-039123/1. GLP**

Study Initiation: 11 Oct. 1990, BRL 39123A, batch GBD 18.

Miscellaneous Effects:

31) **Effects on Blood Sugar, Free Fatty Acids and Triglycerides in Rats Employing the Intravenous Route, Study ID PA-1024/BRL-039123/1. GLP,**

Study Initiation: 17 Sept. 1990, BRL 39123A, batch GBD 18.

Pharmacology Study Reviews:**Effects on the Nervous System:****A) Assessments in Whole Animals:**

1) **Naive Behaviour (Irwin Profile) in Mice Employing the Intravenous Route, Study ID PA-1004/BRL-39123/1. non-GLP.** Study Initiation: 17 April 1989, BRL 39123A, batch GBD 18.

Doses Tested: 5, 15, 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline

Vehicle: distilled water

Species, Strain Sex: 40 male Crl:COBS CD-1(ICR)BR mice, age not reported, 20-24 g.

Results and Summary: Intravenous doses of 15 or 160 mg/kg BRL 39123A induced reactivity and defecation, decreased muscle tone and locomotor activity, and increased passivity. Effects were seen at up to 2 hours after treatment. No effects were observed with intravenous administration of 5 mg/kg BRL 39123A.

2) **Effect on the Motor Coordination (Inclined Plane) in Mice Employing the Intravenous Route, Study PA-1005/BRL-039123/1. non-GLP.**

Study Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive control, diazepam 8 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(ICR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A produced no effect on the ability of mice to stand on an inclined plane.

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

3) **Effect on the Motor Coordination (Traction Test) in Mice Employing the Intravenous Route**, Study ID PA-1007/BRL-039123/1. non-GLP,

Study Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive control, diazepam 8 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(ICR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A had no effect on the ability of mice to grasp a wire with the fore paws (at up to 3 hours after treatment).

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

4) **Effect on Spontaneous Motor Activity in Mice Employing the Intravenous Route**, Study ID PA-1006/BRL-039123/1. non-GLP,

Study Initiation: 13 March 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive controls, amphetamine 10 mg/kg, p.o., chlorpromazine 8 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 60 male Crl:COBS CD-1(CR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A produced no change in spontaneous motor activity at up to 2 hours after treatment.

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

5) **Anti-Tremorine Activity in Mice Employing the Intravenous Route**, Study ID PA-1010/BRL-039123/1. GLP, Study Initiation:

26 June 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: 0.5% aq. methylcellulose, p.o.; positive control, atropine 4 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(CR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A did not modify the tremorgenic or other effects of 10 mg/kg tremorine given i.p.

Comment: Controls were shared with another study in which the test compound was administered p.o.

6) **An Hypnotic Potentiation Study in Mice Employing the Intravenous Route**, Study ID PA-1001/BRL-039123/1. non-GLP Study

Initiation: 6 Feb. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive control, chlorpromazine 8 mg/kg, p.o.

Vehicle: distilled water

Species, Sex, Strain: 30 male Crl:COBS CD-1(CR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A did not prolong thiopentone-induced sleep time (evaluated as loss of righting reflex).

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

7) **Effect on PMT Evoked Convulsions in Mice Employing the Intravenous Route,** Study ID PA-1002/BRL-039123/1. non-GLP, Study

Initiation: 15 Feb. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive control, diazepam 8 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(ICR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A had no modifying effects on tonic convulsions induced by pentylenetetrazole (150 mg/kg administered s.c. 1 hour post-dose).

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

8) **A Body Temperature Study in Mice Employing the Intravenous Route,** Study ID PA-1003/BRL-039123/1. non-GLP, Study

Initiation: 30 Jan. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: saline; positive control, chlorpromazine 8 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(CR)BR mice, age not reported, 20-24 g.

Results and Summary: A statistically significant decrease in body temperature was observed at 60 minutes post-dose, but this effect was not observed at other time-points up to 3 hours after treatment and was not considered to be biologically significant.

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

9) **Local Anaesthetic Effects on Corneal Reflex of the Guinea Pig,** Study ID PA-1011/BRL-039123/1. GLP, Study Initiation: 21

Sept. 1989, BRL 39123A, batch GBD 18.

Doses Tested: 0.3, 1, 3%

Dose Volume and Route: 0.1 mL, instillation into conjunctival sac

Control: vehicle; positive control, lidocaine 1%

Vehicle: distilled water

Species, Strain, Sex: male Dunkin-Hartley guinea pigs, number and age not reported, 350-400 g.

Results and Summary: BRL 39123A had no effect on corneal reflex at $\leq 3\%$.

B) Assessments in Isolated Animal Tissues:

10) **Effects on the Stimulated Phrenic Nerve Diaphragm Preparation of the Rat,** Study ID PA-1012/BRL-39123/1. non-GLP, Study

Initiation: 13 April 1989, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 10^{-2} , 3×10^{-3} M

Dose Volume and Route: 0.25 or 0.75 mL, organ bath

Control: vehicle (?)

Vehicle: distilled water

Species, Strain, Sex: 7 male Crl:COBS CD (SD)BR rats, age not reported, 150-175 g.

Results and Summary: BRL 39123A produced no significant modification of the response of the phrenic nerve-diaphragm preparation to electric stimulation.

11) **Effects on the Isolated Vas Deferens and on its Response to Noradrenaline in Rats,** Study ID PA-1016/BRL-039123/1. non-GLP.

Study Initiation: 3 Nov. 1988, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-6} , 10^{-5} , 10^{-4} , 5×10^{-4} , 10^{-3} , 3×10^{-3} M

Dose Volume and Route: 0.1 or 0.3 mL, organ bath

Control: vehicle (?)

Vehicle: distilled water

Species, Strain, Sex: 5 male Crl:COBS CD (SD)BR rats, age not reported, 150-175 g.

Results and Summary: BRL 39123A had no effect on the isolated rat vas deferens.

12) **Effects on the Spontaneous Motility of Non-Pregnant Rat Isolated Uterus,** Study ID PA-1027/BRL-039123/1. GLP, Study

Initiation: 2 Jan. 1990, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} M

Dose Volume and Route: 0.1 mL, organ bath

Control: vehicle

Vehicle: distilled water

Species, Strain, Sex: 7 female Crl:CD (SD)BR rats, age not reported, 151-175 g.

Results and Summary: Non-significant decreases in height of contractions (~20%) and increased frequency of contractions were seen at 10^{-3} M. The no effect level was 10^{-4} M.

13) **Effects on the Response of the Guinea Pig Isolated Trachea to Histamine,** Study ID PA-1015/BRL-039123/1. GLP, Study

Initiation: 1 Oct. 1990, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-6} , 10^{-5} , 10^{-4} , 5×10^{-4} , 10^{-3} , 3×10^{-3} M

Dose Volume and Route: 0.1 or 0.3 mL, organ bath

Control: vehicle

Vehicle: distilled water

Species, Strain, Sex: 6 male Dunkin-Hartley guinea pigs, age not reported, 350-400 g.

Results and Summary: Decreases in duration of histamine-induced contractions were observed at $\geq 10^{-3}$ M. The no effect level was 5×10^{-4} M.

14) **Effects on the Spontaneous Motility of the Isolated Ileum of the Rabbit,** Study ID PA-1019/BRL-039123/1. non-GLP, Study

Initiation: 15 Nov. 1988, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 3×10^{-3} M

Dose Volume and Route: 0.09 to 0.2 mL, organ bath

Control: vehicle (?)

Vehicle: distilled water

Species, Strain, Sex: 6 male New Zealand white rabbits, age not reported, 2300-2500 g.

Results and Summary: Statistically significant increases in heights of spontaneous contractions (maximum - 26%) were observed at concentrations $\geq 10^{-4}$ M. The no effect level was 10^{-4} M.

15) **Effect on the Response of Guinea Pig Isolated Ileum to Various Agonists,** Study ID PA-1014/BRL-039123/1. non-GLP, Study

Initiation: 15 Dec. 1988, BRL 39123A, batch GBD 18.

Doses Tested: 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 3×10^{-3} M

Dose Volume and Route: 0.1 or 0.3 mL, organ bath

Control: acetylcholine, histamine, serotonin, barium chloride

Vehicle: distilled water

Species, Strain, Sex: 12 male Dunkin-Hartley guinea pigs, age not reported, 250-300 g.

Results and Summary: BRL 39123A increased the heights of contractions induced by serotonin (100 - 200 ng/mL) and barium chloride (50 - 100 μ g/mL), with calculated EC_{50} values of 1.94×10^{-3} and 3.14×10^{-3} M, respectively. The no effect level for modification of acetylcholine- or histamine-induced contractions was 3×10^{-3} M, and the no effect level for modification of serotonin- or barium chloride-induced contractions was 10^{-4} M.

Effects on Autonomic or Contractile Tissues:

A) Assessments in Whole Animals:

16) **Effect on the Charcoal Transit in Mice Employing the Intravenous Route,** Study ID PA-1017/BRL-039123/1. non-GLP Study
Initiation: 28 Nov. 1988, BRL 39123A, batch GBD 18.
Dose Tested: 160 mg/kg
Dose Volume and Route: 5 mL/kg, i.v.
Control: saline; positive control, atropine 160 mg/kg, p.o.
Vehicle: distilled water
Species, Strain, Sex: 30 male Crl:COBS CD-1 (ICR)BR mice, age not reported, 20-24 g.
Results and Summary: BRL 39123A had no effect on charcoal transit time.

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

17) **Effect on Gastric Secretion in Rats Employing the Intravenous Route,** Study ID PA-1018/BRL-039123/1. GLP, Study
Initiation: 28 Nov. 1989, BRL 39123A, batch GBD 18.
Doses Tested: 5, 15, 160 mg/kg
Dose Volume and Route: 2 mL/kg, i.v.
Control: vehicle; positive control, atropine 1 mg/kg, intraduodenal
Vehicle: 0.9% saline
Species, Strain, Sex: 35 male Crl:CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL 39123A, 160 mg/kg, produced statistically significant decreases in gastric juice volume and total acidity and an increase in gastric juice pH. The no effect level was 15 mg/kg.

18) **Effects on the Spontaneous Motility of the Rabbit Stomach In Situ,** Study ID PA-1020/BRL-039123/1. GLP, Study
Initiation: 15 Oct. 1990, BRL 39123A, batch GBD 18.
Doses Tested: 5, 15, 160 mg/kg
Dose Volume and Route: 2 mL/kg, i.v.
Control: vehicle
Vehicle: 0.9% saline
Species, Strain, Sex: 12 male New Zealand white rabbits, age not reported, 2.5-3.0 kg.
Results and Summary: BRL 39123A, 160 mg/kg, inhibited spontaneous stomach motility in 2 of 3 rabbits for up to 3 hours after treatment. The no effect level was 15 mg/kg.

B) Assessments in Isolated Animals Tissues:

19) **Effects on the Response of the Isolated Right and Left Atria of the Guinea Pig,** Study PA-1013/BRL-39123/1. non-GLP
Study Initiation: 29 June 1989, BRL 39123A, batch GBD 18.
Doses Tested: 10^{-7} , 10^{-6} , 10^{-5} , 10^{-4} , 10^{-3} , 3×10^{-3} M
Dose Volume and Route: 0.08 to 0.1 mL, organ bath
Control: vehicle
Vehicle: Krebs buffer
Species, Strain, Sex: 8 male Dunkin-Hartley guinea pigs, age not reported, 350-400 g.
Results and Summary: Chronotropic activity was observed on the right atrium from 10^{-7} to 3×10^{-3} M. No isotropic activity on the left atrium was observed at any concentration used. The no effect level was 10^{-4} M.

Antinociceptive Activity:

20) **Antinociceptive Activity in the Writhing Test in Mice Employing the Intravenous Route**, Study ID PA-1008/BRL-039123/1. GLP

Study Initiation: 5 Feb. 1990, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: vehicle; positive control, aspirin 150 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(CR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A did not modify p-phenylquinone-induced writhing in mice.

Comment: Although positive controls were reported to have been shared with another study in which the test compound was administered p.o., it should be noted that this companion study (apparently study ID TA-1008/BRL- 042810/1) appears not to have been conducted contemporaneously.

21) **Antinociceptive Activity in the Tail-Flick Test in Mice Employing the Intravenous Route**, Study ID PA-1009/BRL-039123/1. GLP

Study Initiation: 2 July 1990, BRL 39123A, batch GBD 18.

Dose tested: 160 mg/kg

Dose Volume and Route: 5 mL/kg, i.v.

Control: vehicle; positive control, morphine 10 mg/kg, p.o.

Vehicle: distilled water

Species, Strain, Sex: 30 male Crl:COBS CD-1(ICR)BR mice, age not reported, 20-24 g.

Results and Summary: BRL 39123A had no effect on evoked tail-flick response in mice.

Comment: Although positive controls were reported to have been shared with another study in which the test compound was administered p.o., it should be noted that this companion study (apparently study ID TA-1009/BRL- 042810/1) appears not to have been conducted contemporaneously. Also, it should be noted that the summary sheet reports the dose volume for BRL 39123A to have been 10 mL/kg, whereas the description of the study design reports the dose volume to be 5 mL/kg, which is probably correct.

Cardiovascular and Haematologic Interactions:

A) Assessments in Whole Animals:

22) **A Cardiovascular/Respiratory Toxicity Study in Anaesthetised Beagle Dogs Employing the Intravenous Route**, Study Code: T86001/39123A/D/IV/CVS. GLP

Beecham Pharmaceuticals Res. Div., Stock, UK, Study Initiation: 3 Feb. 1986, BRL 39123A, batch GBD 6.

Doses Tested: 3, 10, 30, 100, 300 mg/kg

Dose Volume and Route: 2 mL/kg, i.v.

Control: vehicle (?); positive controls, acetylcholine, histamine, isoprenaline, noradrenaline, 1,1-dimethyl-4-phenyl-piperazinium, i.v.; also, bilateral carotid occlusion

Vehicle: sterile water

Species, Strain, Sex: 1 male and 1 female beagle dogs, - 11 months old, 12.2 kg (♂) 10.8 kg (♀).

Results and Summary: Slight increases in pulse pressure and slightly variable heart rates were observed at 3 and 10 mg/kg. Slight to marked, dose-related reductions in blood pressures and pulse pressures and prolonged increases in heart rates were seen at 30, 100, and 300 mg/kg. Slightly increased respiratory rate was seen at 100 mg/kg (♀) and slightly reduced peak respiratory air flow was seen at 300 mg/kg (♂). Sustained dose-related

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reductions in R wave amplitude were seen in both dogs at ≥ 30 mg/kg. Slightly enlarged T wave amplitude was seen at 100 mg/kg (♀) and reduced T wave amplitude and ST depression were seen at 300 mg/kg (♀). Clinical chemistry changes did not appear to be significant: slight and somewhat erratic decreases in inorganic phosphate, potassium, and calcium were observed. Crystalluria was seen in the female after doses of 100 and 300 mg/kg. Reduced responses to reference stimuli were dose related at ≥ 30 mg/kg, becoming markedly suppressed at 100 and 300 mg/kg. Histopathology of the kidneys demonstrated tubular necrosis associated with crystalline deposits in the cortex, medulla, and papilla. The no effect level in this study was < 3 mg/kg.

B) Assessments in Isolated Animal Tissues:

23) **To Assess the In Vitro Ability of BRL 39123A to Promote the Adsorption of Plasma Proteins onto Human Red Blood Cells**, Study Code: CP/AG/90/2. GLP, SmithKline Beecham Res. Develop., The Frythe, UK, Study Initiation: 14 June 1990, BRL 39123A, batch GBD 18.

Doses Tested: serial dilutions from 0.1 M to 8×10^{-4} M

Dose Volume and Route: 0.25 mL, in vitro

Control: phosphate-buffered saline (PBS); positive control, cephalothin serially diluted in PBS from 0.4 M to 8×10^{-4} M

Vehicle: PBS

Species, Strain, Sex: human red blood cells obtained from a healthy male volunteer

Results and Summary: BRL 39123A demonstrated no ability to promote adsorption of plasma proteins to red blood cells. High concentrations of BRL 39123A (0.4 M and 0.2 M) produced gross haemolysis, reportedly due to high pH (~11). Cephalothin (0.05 M and 0.1 M) promoted adsorption of polyspecific and IgG-specific immunoglobulin sera and anti-albumin antiserum, respectively, to red blood cells.

24) **Effects on the Excretion of ICG in Rats Employing the Intravenous Route**, Study ID PA-1023/BRL-039123/1. GLP, Zambelletti Res. Lab., Milan, IT, Study Initiation: 8 Sept. 1989, BRL 39123A, batch GED 18.

Dose Tested: 160 mg/kg

Dose Volume and Route: 2 mL/kg, i.v.

Control: vehicle

Vehicle: 0.9% saline

Species, Strain, Sex: 20 male Crl:CD (SD)BR rats, age not reported, 151-175 g.

Results and Summary: BRL 39123A had no effect on hepatic function evaluated as ICG clearance.

25) **An In Vitro Study of Haemolysis on Rabbit Red Cells by BRL 39123A**, Study ID TF-1005/BRL-039123/1. GLP, SmithKline Beecham Res. Develop., The Frythe, UK, Study Initiation: 13 Aug. 1992, BRL 39123A, batch WPB 2001.

Doses Tested: 1, 0.1, 0.01, 0.003%

Dose Volume and Route: 2.5 mL, in vitro

Control: vehicles; positive control, distilled water

Vehicles: 0.9% saline and PBS

Species, Strain, Sex: red blood cells obtained from 3 New Zealand white rabbits, age and weight not reported

Results and Summary: BRL 39123A demonstrated concentration-dependent hemolytic activity at $\geq 0.01\%$ when diluted in saline, and at 1% when diluted in PBS. Denaturation of hemoglobin was observed at 1% in saline. The no effect level (in PBS) was 0.1%.

26) **Effect on Blood Coagulation in Rats Employing the Intravenous Route**, Study ID PA-1026/BRL-039123/1. non-GLP, Study Initiation: 23 Jan. 1989, BRL 39123A, batch GBD 18.

Dose Tested: 160 mg/kg
Dose Volume and Route: 5 mL/kg, i.v.
Control: saline
Vehicle: distilled water
Species, Strain, Sex: 20 male Crl:COBS CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL 39123A had no effect on the coagulation parameters PT and APTT.

27) A Platelet Aggregation Study on Guinea Pig Plasma, Study ID PA-1025/BRL-039123/1. GLP, Study Initiation: 20 Sept.
1990, BRL 39123A, batch GBD 18.
Doses Tested: 0.5, 1, 2 mM
Dose Volume and Route: 9-18 μ L, in vitro
Control: vehicle
Vehicle: 0.9% saline
Species, Strain, Sex: 10 male Dunkin-Hartley guinea pigs, age not reported, 350-400 g.
Results and Summary: BRL 39123A had no effect on platelet aggregation in vitro.

Renal Effects:

28) Effects on the Renal Excretion in Rats, Employing the Intravenous Route, Study ID PA-1021/BRL-039123/1. non-GLP, Study
Initiation: 7 Nov. 1988, BRL 39123A, batch GBD 18.
Doses Tested: 5, 15, 160 mg/kg
Dose Volume and Route: 5 or 2 mL/kg, i.v.
Control: saline
Vehicle: distilled water
Species, Strain, Sex: 50 male Crl:COBS CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL 39123A, 160 mg/kg, produced marked increases in urine sodium, chloride, and total volume, and decreases in urine potassium and osmolality, for up to 6 hours after treatment. Compared to saline controls, statistically significant decreases in urine sodium and chloride were seen in the 5 mg/kg group, but not in the 15 mg/kg group. Given that the control animals received intravenous saline and the treated animals received drug dissolved in water, the biological significance of the results in the low dose group is questionable. The sponsor states that the no effect level in this study was 15 mg/kg.

29) Effect on the PSP Excretion in Rats Employing the Intravenous Route, Study ID PA-1022/BRL-039123/1. non-GLP, Study
Initiation: 12 Dec. 1989, BRL 39123A, batch GBD 18.
Doses Tested: 5, 15, 160 mg/kg
Dose Volume and Route: 5 or 2 mL/kg, i.v.
Control: saline
Vehicle: distilled water
Species, Strain, Sex: 50 male Crl:COBS CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL 39123A, 160 mg/kg, produced a statistically significant increase in serum PSP concentration, indicating a significant impairment of renal clearance. The no effect level was 15 mg/kg.

Anti-Inflammatory Activity:

30) An Anti-Inflammatory Study in Rats Employing the Carrageenan Paw Inflammation Model, Study ID PA-1028/BRL-039123/1. GLP,
Study Initiation: 11 Oct. 1990, BRL 39123A, batch GBD 18.
Dose Tested: 160 mg/kg

Dose Volume and Route: 2 mL/kg, i.v.
Control: vehicle; positive control, aspirin 138 mg/kg, p.o.
Vehicle: 0.9% saline
Species, Strain, Sex: 30 male Cr1:COBS CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL39123A demonstrated no anti-inflammatory activity in this model.

Comment: Positive controls were shared with another study in which the test compound was administered p.o.

Miscellaneous Effects:

31) Effects on Blood Sugar, Free Fatty Acids and Triglycerides in Rats
Employing the Intravenous Route, Study ID PA-1024/BRL-039123/1. GLP,
Study Initiation: 17 Sept. 1990, BRL 39123A,

batch GBD 18.

Dose Tested: 160 mg/kg
Dose Volume and Route: 2 mL/kg, i.v.
Control: vehicle
Vehicle: 0.9% saline
Species, Strain, Sex: 20 male Cr1:COBS CD (SD)BR rats, age not reported, 151-175 g.
Results and Summary: BRL 39123A, given for 10 consecutive days, had no significant effect on serum concentrations of glucose, free fatty acids, or triglycerides.

APPENDIX H: PHARMACOKINETICS AND ADME STUDIES

SUMMARY:

Multiple pharmacokinetic and ADME studies conducted with penciclovir were submitted in support of the NDA. In summary, the pharmacokinetic and metabolic characteristics of penciclovir in rats, dogs and man, suggest strong similarities between the species and support the use of these species in estimating the safety profile of penciclovir when administered to humans.

COMPILATION OF EFFECTS AND DISCUSSION:

The sponsor has conducted multiple pharmacokinetic and ADME (absorption, distribution, metabolism and excretion) studies with penciclovir in support of the NDA. Similar studies were previously reported for famciclovir (BRL 42810: the oral pro-drug form of penciclovir) and have been reviewed in NDA 20-363, June 1994. Following from the chemical similarity of the compounds and the extensive co-development/testing of these drugs, the following PK/ADME summary includes information for both compounds. A brief summary of the findings from these studies is included in the following paragraphs.

General Background: BRL 39123 (Penciclovir) is a substituted nucleoside analogue of guanine. It is suggested that the drug is selectively phosphorylated intracellularly to the mono-, di- and triphosphate, by virally and cellularly encoded thymidine kinases. The initial phosphorylation reaction is purportedly accomplished only by the viral induced thymidine kinase of herpes infected cells. BRL 39123 tri-phosphate, it is suggested, exerts its antiviral activity through incorporation in the herpes virus genome and/or through interference with viral DNA synthesis. Penciclovir is inactive prior to conversion to BRL 39123 tri-phosphate.

Percutaneous Absorption: Multiple studies of the percutaneous absorption of penciclovir (some using the market formulation for the topical cream) were conducted in rats, rabbits and in vitro. These studies included both intact and abraded skin systems. In rodents, when [¹⁴C] penciclovir cream was applied to intact skin, the majority of the administered radioactivity (approx. 88% (±1.6)) was retained at the application site at the end of the 24 hour exposure period. Urine, faecal and carcass levels of radioactivity were all below the limit of reliable detection (< 0.01% of the administered dose). Total recovery of radioactivity was approximately 92%. In contrast, when applied (6 hours) to the abraded skin of rabbits there was an average 3-5% percutaneous absorption. However, as with intact skin, the majority of the drug product was retained on/in the skin of the application site and on the dressing materials. Small, but measurable, amounts of radio-activity (and by inference - BRL 39123) were retained in the skin of the application site and were still being excreted in the urine and faeces of each rabbit at the end of the 96 hour follow-up period.

In an in vitro test system using 'intact' or tape-stripped human skin, the study results suggested that transport of BRL 39123 was increased approx. 3-fold by the removal of the stratum-corneum layer of the skin. Further, the transport and incorporation of radioactivity across the tissue specimens increased in extent during the 24 hour assessment period, for both intact and tape-stripped tissues. Similar to the results obtained in whole animals, the transport of BRL 39123 across the component layers of the skin was low (3-5%), although not as that noted in vitro (0.1% vs. 1-5%). Overall, regardless of the test system used, the percutaneous absorption of penciclovir appears to be relatively low (5% or less of the administered dose).

Oral Administration: Absorption of [¹⁴C]famciclovir following oral dosing in the mouse, rat, dog and rabbit was generally between 50-100% (doses up to 4000, 250 and 1000 mg/kg in the rat, dog and rabbit), with peak plasma levels (C_{max}) occurring approximately 1 hour after dosing in all species. By comparison, the oral availability of penciclovir was somewhat lower at approximately 20-50% of the administered dose. BRL 39123 (penciclovir) was the primary metabolite found in the circulation of all species following the oral administration of famciclovir. The data clearly indicate that famciclovir undergoes substantial first pass metabolism in the liver, and is almost undetectable in the systemic circulation. In the monkey, there was some evidence of the metabolism of penciclovir to the 8-hydroxy derivative (BRL 44072). Overall, the pharmacokinetic profile for famciclovir is actually that for penciclovir, which will be discussed almost exclusively throughout the subsequent paragraphs.

Intravenous Administration: In both the rat and dog, the intravenous administration of 10 mg/kg of BRL 39123 resulted in peak drug levels immediately post-infusion with blood concentrations in the range of 10.9-16.1 μ g/ml (mean of 13.8 μ g/ml). In the rat, blood drug levels declined in a monophasic manner with a half-life of approximately 20-40 minutes, while in the dog drug levels declined in a biphasic manner with an initial half-life of 25 minutes and terminal half-life of 2.9 hrs. In the dog, $AUC_{(infinity)}$ values ranged from 29-40.4 μ g.hr/ml following a 10 mg/kg IV dose of BRL 39123, with a clearance rate of 3.6-4.2 L/hr., and volume of distribution of 11-15 liters (approximately equal to body weight). The concentration of drug in whole blood was approx. 50% of that in plasma (due to volume dilution), and suggested only limited association between radioactivity and red blood cells (generally 10% or less). No evidence of accumulation of drug related material was observed during repeat dose studies. Peak plasma (C_{max}) levels and systemic exposure (AUC) to penciclovir and its precursors could not be reliably measured during dietary intake of the test compound by rats.

Tissue Distribution: Following the oral administration of [¹⁴C]famciclovir (40 mg/kg) to rats, the tissues with relatively high concentrations of drug related product included the GI tract, liver, kidneys, thyroid/parathyroid, seminal vesicles, aorta and skin. Relatively low concentrations were observed in neural tissue. Concentrations of radioactivity declined rapidly following the acute or daily administration of [¹⁴C]famciclovir, with little evidence of drug accumulation. A somewhat slower decline in radioactivity was noted in the testes of the rat.

Tissue Distribution-Fetal Drug Exposure: Administration of [¹⁴C]famciclovir to pregnant rats and rabbits demonstrated that placental passage and fetal exposure to drug related material occurred at levels nearly comparable to maternal exposure. In the rat fetus, penciclovir and BRL 42359 (the 6 deoxy precursor of penciclovir) accounted for the majority of the [¹⁴C] radiolabeled material, whereas in the rabbit, BRL 42359 and BRL 48959 were identified as the major metabolites in addition to penciclovir. Penciclovir was rapidly secreted in the milk of lactating rats after an oral dose of [¹⁴C]famciclovir (40 mg/kg). Milk concentrations were considerably higher than those observed in plasma.

Excretion: Following the oral administration of [¹⁴C]BRL 42810 or the intravenous administration of [¹⁴C]BRL 39123A to rats, mice, and dogs, nearly all of the administered radioactivity was recovered in urine and faeces (65-85% and 10-20%, respectively), during the subsequent 72-96 hours. Drug elimination was quite rapid with over 90% of the excreted radioactivity being eliminated within the first 6 or 24 hours. The terminal plasma half-life of penciclovir was estimated at less than 1 hour in the rat and at about 2 hours in the dog. In all species studied, TLC and HPLC analyses performed on urine specimens indicated that >>5% of the urinary radioactivity co-eluted with a

BRL 39123 control sample. All other radioactive peaks accounted for a small fraction of the urinary radioactivity.

Miscellaneous: Ex vivo studies revealed only low level binding of penciclovir (and its precursors) to plasma proteins from either the rat (11-24%) or dog (12-22%) when tested at concentrations of 2-20 mg/ml. The distribution of drug related material in rat blood was approximately even between the plasma and cells, whereas in the dog there was evidence of nearly complete exclusion of the drug from blood cells (i.e., the drug product was found almost exclusively in the plasma of the dog). In both species, the volume of distribution for drug related materials was estimated at approximately 1 L/kg.

Comparison of Pharmacokinetic and ADME Data in Animals and Man:

In man, as in all species tested, the bioavailability of famciclovir was high (approx. 75%) with oral administration at doses up to 750 mg (approx. 15 mg/kg). Rapid conversion of famciclovir to penciclovir (the predominant drug form detected in the systemic circulation) and ERL 42359 was evident in man, as it had been in the mouse, rat, dog and monkey. This metabolic conversion appears to be mediated by a cytosolic aldehyde oxidase, and is independent of the P450 system. As seen in preclinical evaluations, the drug kinetics in human subjects were dose independent. Renal excretion of penciclovir was the primary route of drug elimination in man, as had been demonstrated in the preclinical animal studies. Plasma clearance of penciclovir was estimated at 0.37 L/h/kg following a 5 mg/kg IV dose, which was similar to that observed in the dog (0.33 L/h/kg) after a 25 mg/kg IV dose and was 1/3-1/7 the rate noted in the rat (0.9-1.7 L/h/kg) following an IV dose of 40 mg/kg. Active tubular secretion of drug related material is suggested in all species evaluated. In man, as in the dog, the estimated plasma elimination half-life of penciclovir was approximately 2 hours. The volume of drug distribution in man was estimated at 1 L/kg (similar to the rat and dog), with plasma protein binding of approximately 6-16%.

In conclusion, when evaluated in multiple test systems, the percutaneous absorption of penciclovir appears to be relatively low (5% or less of the administered dose). Further, the pharmacokinetic and metabolic profiles for penciclovir (and for famciclovir) as determined in multiple animal species suggests strong similarities to the data obtained in man. Together, these findings support the use of the animal toxicology studies conducted with penciclovir and famciclovir for estimating the safety profile of penciclovir when administered to humans.

Reviews of the individual studies are contained on the following pages.

Pharmacokinetics/ADME Studies Summary:

- 1) The Percutaneous Absorption of Radioactivity Following a Single Topical Application of a Cream, Containing 5% w/w [¹⁴C]BRL 39123A, to the Shaved, Unabraded, Unoccluded, Dorsal Skin of the Male Rat at a Nominal Dose Level of 1 ml of Cream/kg (Approx. 45 mg BRL 39123/kg), Study ID D86612/39123 /14, GLP, Beecham Phar., Harlow, Essex, U.K., Study Initiation: 18 Mar., '86, [¹⁴C]BRL 39123A, batch 8475-69.
- 2) The Percutaneous Absorption of Radioactivity Following a Single Topical Application of [¹⁴C]BRL 39123 Formulated as a 5% w/w Cream to the Shaved, Abraded, Dorsal Skin of Male Rabbits at a Nominal Dose Level of 0.5 ml/kg (Approx. 23 mg of BRL 39123/kg) With Occlusion for 6 Hours, Study ID D86618/39123/15, GLP, Beecham Phar., Harlow, Essex, U.K., Study Initiation: 17 Mar., '86, [¹⁴C]BRL 39123A, batch 8475-57.
- 3) Percutaneous Absorption of Drug-Related Material Following Topical Administration of [¹⁴C]BRL 39123 as a 1% Cream Formulation (2 g Cream /kg) To The Male Rat, Study ID 802/175-1011, GLP, Study Initiation: 11 Nov., 1994, [¹⁴C]BRL 39123A, batch 50457-065.
- 4) A study to investigate plasma concentrations and urinary excretion of BRL 39123 following a single intravenous administration of BRL 39123A to male and female rats at a nominal dose level of 40 mg/kg (Study BF-1002/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990)
- 5) The elimination and blood concentrations of radioactive material following single intravenous administration of [¹⁴C]BRL 39123A to rats at a nominal dose level of 10 mg of BRL 39123/kg (Study D85670/39123/4, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1985)
- 6) Blood concentrations, plasma concentrations and elimination of drug-related material following a single intravenous administration of [¹⁴C]BRL 39123A to female rats at a nominal dose level of 40 mg BRL 39123/kg (Study D89642/39123/48, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1989)
- 7) Tissue Concentrations of Radioactivity and Tissue Radiation Doses Following a Single Intravenous Administration of [¹⁴C]BRL 39123A to Male Pigmented Rats at a Nominal Dose Level of 10 mg of BRL 39123/kg, Study D88627/39123/37, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, Study Initiation: 15 Nov., 1988, [¹⁴C]BRL 39123A, batch 8475-79.
- 8) [¹⁴C]BRL 42810: Whole-Body Autoradiography Following Either Single Or Repeated Oral Administration (40 mg/kg) to the Male Rat, Study 802/103-1011, GLP, Study Site: Study Initiation: 1 Nov., 1993, [¹⁴C]BRL 42810, batch 32634-172, >99% pure.
- 9) Blood and plasma concentrations and elimination of radioactive material following a single intravenous administration of [¹⁴C]BRL 39123A to dogs at a nominal dose level of 10 mg of BRL 39123/kg (Study D85671/39123/5, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1985)
- 10) A study to investigate plasma concentrations and urinary excretion of BRL 39123 following a single intravenous administration of BRL 39123A to male and female dogs at a nominal dose level of 50 mg/kg (Study BF-1001/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990)
- 11) Metabolic and pharmacokinetic studies in female dogs following intravenous administration of [¹⁴C]BRL 39123A at a nominal dose level of 25 mg BRL 39123/kg (Study BF-0001/BRL 039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990)
- 12) The elimination of compound-related material following single intravenous administration of [¹⁴C]BRL 39123A to cynomolgus monkeys at a nominal dose level of 10 mg of BRL 39123/kg (Study D87635/39123/34, GLP,

Study Site: Beecham Pharm., Harlow, UK, 1987)

13) The pattern of radiometabolites in the urine of male rats dosed once intravenously with [¹⁴C] BRL 39123A at a dose level of 10 mg of BRL 39123/kg (Study D86640/39123/24, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1986)

14) Plasma concentrations of radioactivity and of BRL 39123, and the pattern of radiometabolites in urine following a single intravenous administration of [¹⁴C]BRL 39123A to male dogs at a dose level of 10 mg of BRL 39123/kg (Study D86643/39123/25, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1986)

15) Metabolic studies following a single intravenous administration of [¹⁴C]BRL 39123A to male dogs and healthy male human subjects at nominal doses of 25 and 5 mg of BRL 39123/kg, respectively (Study BF-1004/BRL-039123/1, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990)

16) Comparative Metabolic Pattern Studies Following an Intravenous Administration of [¹⁴C]BRL 39123A to Rat and Man at Nominal Doses of 40 and 5 mg of BRL 39123/kg, respectively, Study BF-1010/BRL-039123/1, GLP, Beecham Pharm., The Frythe, Welwyn, Herts, U.K., Study Initiation: 4 Feb., 1990, [¹⁴C]BRL 39123A, >84% pure.

17) The in vitro protein binding of BRL 39123 in human, dog and rat plasma (Study BF-1003/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990-1991)

18) In vitro cell partitioning of [¹⁴C]BRL 39123A (penciclovir) in male rat, dog and human blood (Study BF-1008/BRL-039123/1, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1992)

19) In Vitro Percutaneous Absorption and Cutaneous Distribution of [¹⁴C]BRL 39123 Across Intact and Tape-Stripped Human Skin, Study ID M95-002, GLP, Study Initiation: 19 Dec., 1994, [¹⁴C]BRL 39123A, 98.3% pure.

20) Effect of BRL 42810 (famciclovir), BRL 39123 (penciclovir) and BRL 42359 on testosterone 6-beta hydroxylation in human liver microsomes (Study D91609/42810/79, GLP, Study Site: Beecham Pharm., The Frythe, UK, 1991)

21) Factors Affecting Famciclovir and BRL 42359 Metabolism by Mammalian Molybdenum Hydroxylases and the Potential for Metabolism Drug-Drug Interactions With Famciclovir and Penciclovir, Study BF-1035/BRL-042810/2 (DR95832), non-GLP, Study Site: Beecham Pharm., The Frythe, UK, Study Initiation: not specified, BRL 42810 and 42359, batch numbers not specified.

22) The Effect of BRL 39123 on the Hepatic Levels of Cytochrome P450 and Related Parameters in Sprague dawley Rats After Intravenous Administration at 0, 10, 30 and 80 mg/kg/day for 13 Weeks, Study D802/104-1011, GLP, Study Initiation: 30 Sept., 1993.

Pharmacokinetics/ADME Study Reviews:

1) The Percutaneous Absorption of Radioactivity Following a Single Topical Application of a Cream, Containing 5% w/w [¹⁴C]BRL 39123A, to the Shaved, Unabraded, Unoccluded, Dorsal Skin of the Male Rat at a Nominal Dose Level of 1 ml of Cream/kg (Approx. 45 mg BRL 39123/kg), Study ID D86612/39123/14.

Status: GLP

Study Initiation: 18 Mar., '86

Study Site: Beecham Phar., Harlow, Essex, U.K.

Compound Tested: [¹⁴C]BRL 39123A, batch 8475-69

Doses Tested: 45 mg/kg

Dose Volume and Route: 1 ml/kg, topical

Solvent: cream base, batch GB 1330

Species, Strain, Sex: Rat, Crl:CD, male, weight range 300-350 grams, 3

animals.

Test conditions: Animals received a single application of BRL 39123. Urine and faecal samples were obtained at intervals up to 24 hours post-application. At the conclusion of the 24 hour exposure period, specimens of urine, faeces, skin (application site) and carcass were assayed for their content of radioactive BRL 39123.

A mean of 87.7% (± 1.6) of the administered radioactivity was retained at the application site at the end of the 24 hour exposure period. The urine, faecal and carcass concentrations of radioactivity were all below the limit of reliable detection ($< 0.01\%$ of the administered dose). The mean total recovery of radioactivity was approximately 92%, with some radioactivity (0.5-12%) being found on the bars of the restraint cages at the termination of the study.

Comment: The results suggest that BRL 39123 undergoes negligible percutaneous absorption when applied to unabraded skin for a period of 24 hours.

2) The Percutaneous Absorption of Radioactivity Following a Single Topical Application of [14 C]BRL 39123 Formulated as a 5% w/w Cream to the Shaved, Abraded, Dorsal Skin of Male Rabbits at a Nominal Dose Level of 0.5 ml/kg (Approx. 23 mg of BRL 39123/kg) With Occlusion for 6 Hours, Study ID D86618/39123/15.

Status: GLP

Study Initiation: 17 Mar., '85

Study Site: Beecham Phar., Harlow, Essex, U.K.

Compound Tested: [14 C]BRL 39123A, batch 8475-57

Doses Tested: 45 mg

Dose Volume and Route: 0.5 ml/kg, topical

Solvent: cream base, batch GR 1330

Species, Strain, Sex: Three New Zealand White rabbits, male, weight range 2.43-2.50 kg.

Test conditions: Animals received a single 6 hour application of BRL 39123 to a shaved and abraded area of the dorsal cervical region. The occlusive dressing was removed and the application site cleansed at the end of the 6 hour exposure period. Urine and faecal samples were obtained at intervals up to 96 hours post-application. At the conclusion of the study, specimens of urine, faeces, skin (application site) and carcass were assayed for their content of radioactive BRL 39123.

A mean of 70.3% (± 2.3) of the administered radioactivity was retained on the occlusive dressing at the end of the application period. An additional 18.6% (± 3.2) of the administered dose was measured at the application site (skin) at the end of the 96 hour follow-up period. Urine and faecal recovery of radioactivity amounted to 1.0% (± 0.1) and 1.9% (± 0.3) of the administered dose, respectively. Radioactivity retained in the carcass (exclusive of the application site) was measured at 2% (limit of detection). Mean total recovery of radioactivity was approximately 92.2% (± 0.9) of the administered dose, with some radioactivity (0.4%) being found on the cage and collar materials.

Comment: The results suggest that BRL 39123 undergoes some (3-5%) percutaneous absorption when applied to abraded skin for a period of 6 hours. However, the majority of the drug is retained in the skin of the application site and on any dressing materials.

Small, but measurable, amounts of radioactivity (and by inference - BRL 39123) was retained in the skin of the application site and was still being excreted in the urine and faeces of each rabbit at the end of the 96 hour follow-up period. The sponsor should determine whether drug which is retained in the skin of the

application site may act as a 'pool' or 'reservoir', which may maintain low levels of systemic drug exposure over prolonged post-application intervals.

3) Percutaneous Absorption of Drug-Related Material Following Topical Administration of [¹⁴C]BRL 39123 as a 1% Cream Formulation (2 g Cream/kg) To The Male Rat, Study ID 802/175-1011.

Status: GLP

Study Initiation: 11 Nov. 1994

Study Site

Compound Tested: [¹⁴C]BRL 39123A, batch 50457-065, Radio- and chemical purity of 99.6% and 98.3%, and BRL 39123, batch LRS-8, 99.4% pure.

Doses Tested: 40 mg/kg

Dose Volume and Route: 2 g/kg, topical (approx. 0.6 mg/cm²)

Solvent: cream base (40% propylene glycol and 0.9% cetamacrogol 1000)

Species, Strain, Sex: Rat, Crl:CD(SD)BR, male, weight range 281-310 grams, 7-8 weeks of age, 3 animals/group.

Test conditions: Animals received a single application of BRL 39123 to abraded/occluded or non-abraded/intact skin. Urine and faecal samples were obtained at intervals up to 24 hours post-application. At the conclusion of the 24 hour exposure period, specimens of urine, faeces, skin (application site) and carcass were assayed for their content of radioactive BRL 39123.

During the initial 24 hours following topical application to abraded skin, approximately 7% of the administered dose of radioactivity was recovered in the urine (6.6%) and faeces (0.4%) of the test animals. In contrast, after application to intact skin less than 0.2% of the administered dose was recovered in the excreta. Under both study conditions (abraded or intact skin) approximately 5-7% of the administered dose was retained at the site of application following washing. The majority of the radioactivity (82-85.5%) was recovered from the application site at the conclusion of the 24 hour test period in the residual cream and bandages. The total recovery of radioactivity was approximately 95% for both study treatments.

Comment: Following topical application of a 1% [¹⁴C]BRL 39123 in propylene glycol/cetomacrogol cream to abraded and occluded skin, systemic absorption of radioactivity (as demonstrated by elimination in the excreta) was approx. 7% of the administered dose. An additional 5-7% of the administered radioactivity was retained at the site of application following washing. The study results suggest that BRL 39123 undergoes negligible percutaneous absorption when applied to unabraded skin for a period of 24 hours.

4) A study to investigate plasma concentrations and urinary excretion of BRL 39123 following a single intravenous administration of BRL 39123A to male and female rats at a nominal dose level of 40 mg/kg (Study BF-1002/BRL-039123/2, GLP, Study Site: Eechem Pharm., Harlow, Essex, UK, 1990, Batch: unspecified)

Groups of five male and five female Sprague Dawley CD rats were administered a single intravenous dose of 40 mg/kg BRL 39123A (the sodium salt of BRL 39123) in saline. Blood was sampled for 6 hours from three males and three females. Two males and three females from the blood sampling study (plus an additional male) were again administered a single dose of 40 mg/kg BRL 39123A and urine was collected for 48 hours.

Clearance is primarily renal. The small number of animals used precludes conclusions of differences based on sex.

Table. Pharmacokinetic values and urinary excretion of BRL 39123 in rats administered a single intravenous 40 mg/kg dose of BRL 39123A

Male		Female
V (L)	0.35, 0.46	0.19-0.29
(L/kg)	1.01, 1.33	0.83-1.26
t _{1/2} (h)	0.41	0.42-0.56
AUC _{0-∞} (μg·h/ml)	18.6, 19.3	26.3-43.2
Urinary recovery (%)	70.9, 91.4	83.5-90.7
CL _r (L/h)	0.59, 0.61	0.22-0.30
CL _r (L/h/kg)	1.56, 1.67	0.94-1.21
CL (L/h)	0.58, 0.78	0.24-0.39
CL (L/h/kg)	1.67, 2.25	1.06-1.70

5) The elimination and blood concentrations of radioactive material following single intravenous administration of [¹⁴C]BRL 39123A to rats at a nominal dose level of 10 mg of BRL 39123/kg (Study D85670/39123/4, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1985, Batches: Radiolabeled - 8475-35, 79.9% pure, Non-radiolabeled - GBD 5, 86.6% pure)

Three 200-250 g male Sprague-Dawley CD rats were administered a single intravenous dose of 10 mg/kg [¹⁴C]BRL 39123A in 0.9% sterile sodium chloride. Blood, urine, and feces were collected for 72 hours. Expired air was collected for 48 hours. All samples were analyzed for radioactivity and urine and plasma were analyzed for BRL 42810 and its known metabolites.

The highest concentrations of radioactivity were measured between 3 minutes after dosing, with a C_{max} of 10.9-16.1 μg/ml of BRL 39123 equivalents. BRL 39123 could be detected in plasma for 4-6 hours. About 68% of the radioactive dose was excreted in the urine by 72 hours (61% in the first 6 hours). Approximately 19% of the dose was excreted in the feces and <0.1% was found in expired air.

Table. Elimination of radioactivity in rats after a single intravenous dose of 10 mg/kg of [¹⁴C]BRL 39123A

Percentage of dose	Male	Female
Urine 0-6 h	51-79	52-59
6-24 h	3-5	3-10
24-48 h	1	1-3
Feces 0-24 h	4-17	14-23
24-48 h	2-6	2-7
GI tract 72 h	0.4	0.4
Carcass 72 h	0.6	0.6
Total recovery	90-92	83-90

Blood radioactivity

t _{max} (min)	3	3
C _{max} (μg 39123 equiv/ml)	13-16	11-15

Comment: What are the 20% impurities in the study material? Their presence may confound interpretation of the study.

6) Blood concentrations, plasma concentrations and elimination of drug-related material following a single intravenous administration of [¹⁴C]BRL 39123A to

female rats at a nominal dose level of 40 mg BRL 39123/kg (Study DB89642/39123/48, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1989, Batches: Radiolabeled - 11283-77, 86.6% pure, Non-radiolabeled - GBD 18, 86% pure)

Eighteen 200-230 g female Sprague-Dawley CD rats were administered a single intravenous dose of 40 mg/kg [¹⁴C]BRL 39123A in 0.9% sterile sodium chloride. Blood, urine, and feces were collected for 72 hours from groups of three rats. Twelve animals were killed in groups of three and plasma from 10 and 30 minutes, and 1 and 3 hours after dosing were collected. All samples were analyzed for radioactivity and urine and plasma were analyzed for BRL 39123 and possible metabolites.

The highest concentrations of radioactivity were measured 3 minutes after dosing, with a C_{max} of 57-60 µg/ml of BRL 39123 equivalents. BRL 39123 could not be detected in plasma by 6 hours. The 8-hydroxy derivative of BRL 39123 (BRL 44072) was detected at 10 minutes and accounted for <1% of plasma radioactivity. About 85% of the radioactivity was excreted in the urine and 11% was excreted in the feces. BRL 39123 was the only radioactive component detected in the urine and accounted for 96-99% of radioactivity in the urine (82% of the dose).

Table. Elimination of radioactivity in female rats after a single intravenous dose of 40 mg/kg of [¹⁴C]BRL 39123A

	Percentage of dose
Urine 0-6 h	84
6-24 h	1.2
24-48 h	0.4
Feces 0-24 h	7.6
24-48 h	3.0
Cage washings 72 h	0.6
GI tract 72 h	<0.2
Carcass 72 h	0.4
Total recovery	97.6
Blood radioactivity	
t _{max} (min)	3
C _{max} (µg 42810 equiv/ml)	57-60

7) Tissue Concentrations of Radioactivity and Tissue Radiation Doses Following a Single Intravenous Administration of [¹⁴C]BRL 39123A to Male Pigmented Rats at a Nominal Dose Level of 10 mg of BRL 39123/kg, Study D88627/39123/37.

Status: GLP

Study Initiation: 15 Nov., 1988

Study Site: Beecham Pharm., Harlow, Essex, UK

Compound Tested: [¹⁴C]BRL 39123A, batch 8475-79, Radio- and chemical purity of 77.1% and 99.1%, and BRL 39123, batch GBD-18, 85.5% pure.

Doses Tested: 10 mg/kg

Dose Volume and Route: 3 ml/kg, IV

Solvent: sterile saline

Species, Strain, Sex: Lister Hooded rat, OLA Strain, male, weight range 182-205 grams, 3 animals/group.

Test conditions: Animals received a single intravenous dose [¹⁴C]BRL 39123 and were sacrificed at various time-points between 10 min. and 120 hours post-dosing. At the conclusion of the exposure period, the blood, plasma, urine, feces and most major organs of each animal were assayed for the content of

radioactivity. Whole body autoradiographs were also performed on a limited number of specimens.

Peak concentrations of radioactivity were measured 10 minutes after dosing distributed to nearly all tissues, although the highest concentrations were in association with the kidneys, urinary bladder contents and the GI tract (predominantly the small intestine). Tissue levels of radioactivity declined rapidly during the 24 hours post-dosing, such that at the end of this interval radioactivity could only be detected in the GI tract, liver, skin and eyes. By 120 hours after dosing, no radioactivity could be detected in any organ by whole body autoradiography. During the 120 hour follow-up period, a total of 85.1% and 6.6% of the administered radioactivity was recovered in the urine and faeces, respectively, of the test animals. The majority of the radioactivity was recovered in the urine during the initial 24 hour post-dosing interval. The mean concentrations of radioactivity in several major body tissues at multiple time-points following dosing are presented in the following table.

Table. Tissue radioactivity in male pigmented rats after a single intravenous dose of 10 mg/kg [¹⁴C]BRL 39123A

Time after Dose	µg of BRL 39123 equiv./g or ml			
	10min.	1.5hr	24 hr	48hr
Blood	11.8	0.385	0.006	<0.004
Adrenal	10.3	1.00	0.126	0.054
Eyes	2.33	0.245	0.062	0.041
Heart	10.4	0.382	0.016	0.007
Kidney	146	4.16	0.112	0.050
Liver	14.9	1.71	0.359	0.187
Lung	13.1	0.761	0.175	0.115
Spleen	10.2	0.679	0.076	0.038
Testes	3.55	1.93	0.039	0.019

8) [¹⁴C]BRL 42810: Whole-Body Autoradiography Following Either Single Or Repeated Oral Administration (40 mg/kg) to the Male Rat, Study 802/103-1011.

Status: GLP

Study Initiation: 1 Nov., 1993

Study Site:

Compound Tested: [¹⁴C]BRL 39123A, batch 32634-172, Radio- and chemical purity of 99% and 99.5%, and BRL 39123, batch BN5, 99.5% pure.

Doses Tested: 40 mg/kg

Dose Volume and Route: 3 ml/kg, oral

Solvent: aqueous methyl cellulose

Species, Strain, Sex: male Sprague-Dawley (CrI:CD(SD)BR) rats, age approx. 8 weeks, weight range 182-205 grams, 5 animals/group.

Test conditions: Animals received single or multiple (7) oral doses of [¹⁴C]BRL 42810 and were sacrificed at various time-points between 15 min. and 72 hours post-dosing. At the conclusion of the exposure period whole body autoradiographs were performed on animals from each treatment condition.

Following single or repeated oral dosing with [¹⁴C]BRL 42810, peak concentrations of radioactivity of 6.73 and 9.00 µg equivalent/g were measured in the plasma at 1 hour post-administration. Similarly, the highest concentrations of radioactivity in the tissues were recovered at this time-point, particularly in association with the gastro-intestinal tract and the kidneys and bladder contents. Low levels of radioactivity were distributed throughout the remaining tissues of the body, with the tissue levels being slightly higher following repeated oral dosing than seen after a single oral dose. Under both conditions of dosing, the radioactivity was generally cleared from the plasma

and tissues by 4 hours post-administration. The only deviations from this pattern were the retention of low levels of radioactivity in the liver, muscle tissue, and skin of animals multiply dosed with [¹⁴C]BRL 42810. In the latter group of animals, retained skin radioactivity was found in association with the fur and hair follicles.

- Comments:
- 1) The retention of radioactivity in the skin of animals dosed with [¹⁴C]BRL 42810 appears to be predominantly in/on the fur and in association with the hair follicles. Accumulation and/or escalation of the tissue concentration of radioactivity was evident in animals dosed for 7 days versus those dosed only once. No information was provided as to whether the retained radioactivity was incorporated into the elongating hair shafts or was present in follicular sebaceous excretions.
 - 2) As demonstrated in multiple previous studies conducted with BRL 39123 or BRL 42810, radioactivity was rapidly and widely distributed to nearly all body tissues following oral administration with subsequent rapid clearance via renal elimination. The primary tissues in which high levels of radioactivity were evident were those associated with drug absorption (i.e., the gastro-intestinal tract) and elimination (i.e., the kidneys and bladder).

9) **Blood and plasma concentrations and elimination of radioactive material following a single intravenous administration of [¹⁴C]BRL 39123A to dogs at a nominal dose level of 10 mg of BRL 39123/kg (Study D85671/39123/5, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1985, Batches: Radiolabeled - 8475-35, 79.5% pure, Non-radiolabeled - GBD 5, 86.6% pure)**

Three male beagle dogs (12.7-14.6 kg) were administered a single intravenous dose of 10 mg/kg [¹⁴C]BFL 39123A (the sodium salt of BRL 39123) in saline. Blood was sampled and urine and feces were collected for 96 hours after dosing.

Elimination of radioactivity was predominantly renal and was essentially complete after 12 hours. About 86% of the radioactivity was eliminated in the urine and 10% in the feces. A maximum of 16% of the radioactivity was associated with erythrocytes (at 12 hours).

10) **A study to investigate plasma concentrations and urinary excretion of BRL 39123 following a single intravenous administration of BRL 39123A to male and female dogs at a nominal dose level of 50 mg/kg (Study BF-1001/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990, Batches: Radiolabeled - 11283, 86.6% pure, Non-radiolabeled - GBD 18, 86% pure)**

Three male (14-16.8 kg) and three female beagle dogs were administered a single intravenous dose of 50 mg/kg BRL 39123A (the sodium salt of BRL 39123) in saline. Blood was sampled for 24 hours after dosing and urine was collected for 48 hours after dosing.

Elimination of BRL 39123 was predominantly renal and was essentially complete after 24 hours. Plasma clearance and volume of distribution appeared to be lower for females than males, but the small number of animals precludes definitive conclusions about differences between the sexes. Comparison of AUC values in this study (at 50 mg/kg) with the study conducted at 10 mg/kg suggests dose proportionality.

Table. Pharmacokinetic values and urinary excretion of BRL 39123 in dogs administered a single intravenous 50 mg/kg dose of BRL 39123A

	Male	Female
V (L)	12-15	5.6-11.4
(L/kg)	0.73-1.1	0.51-0.93
t _{1/2} (h)	2.3-2.6	2.1-2.6
AUC _{0-24h} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	165-178	181-353
AUC _{0-∞} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	178-194	199-415
Urinary recovery (% dose)	63-77	75-98.5
CL (L/h)	3.4-4.0	1.5-3.8
CL (L/h/kg)	0.20-0.28	0.14-0.31
CL _R (L/h)	2.3-2.6	1.2-3.4
CL _R (L/h/kg)	0.16	0.12-0.27

11) **Metabolic and pharmacokinetic studies in female dogs following intravenous administration of [¹⁴C]BRL 39123A at a nominal dose level of 25 mg BRL 39123/kg (Study BF-0001/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990, Batches: Radiolabeled - 11283-77, 86.6% pure, Non-radiolabeled - GBD 18, 86% pure)**

Three female beagle dogs were administered a single intravenous dose of 25 mg/kg [¹⁴C]BRL 39123A (the sodium salt of BRL 39123) in saline. The same female dogs were dosed a second time 2 weeks later. After the first dosing, blood, urine and feces were collected for 96 hours after dosing. In the second phase blood was collected for 3 hours and the metabolite profile was determined.

Elimination of radioactivity was predominantly renal and was essentially complete after 12 hours. BRL 39123 was the only radioactive compound detected in the urine and it represented 97%-99% of the total urinary radioactivity. A maximum of 23% of the radioactivity was associated with erythrocytes (at 12 hours). These results are similar to those obtained at 10 mg/kg.

Table. Pharmacokinetic values and urinary excretion of BRL 39123 in female dogs administered a single intravenous 25 mg/kg dose of [¹⁴C]BRL 39123A

C _{max} ($\mu\text{g}/\text{ml}$)	42.9
T _{max} (h)	0.06
V _{ss} (L)	9.25
(L/kg)	0.89
t _{1/2} (h)	1.99
AUC _{0-12h} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	78.6
AUC _{0-∞} ($\mu\text{g}\cdot\text{h}/\text{ml}$)	79.9
Urinary recovery (% dose)	91.9
feces (% dose)	3.3
CL (L/h)	3.22
CL (L/h/kg)	0.31
CL _R (L/h)	2.96
CL _R (L/h/kg)	0.28

12) **The elimination of compound-related material following single intravenous administration of [¹⁴C]BRL 39123A to cynomolgus monkeys at a nominal dose level of 10 mg of BRL 39123/kg (Study D87635/39123/34, GLP, Study Site:**

Beecham Pharm., Harlow, Essex, UK, 1987, Batches: Radiolabeled - 8475-79, 77.1% pure, Non-radiolabeled - GBD 11, 85.9% pure)

One male and two female cynomolgus monkeys (2.3-2.5 kg) were administered a single intravenous dose of 10 mg/kg [¹⁴C]BRL 39123A. Urine, feces, and cage debris were collected for 48 hours. Urine samples were analyzed for radiometabolites by HPLC.

The high percentage of the dose in the cage debris may have been due to contamination by urine. Of the administered dose, 65% was excreted in urine and 5% was eliminated in the feces. The major component in the urine was BRL 39123. In contrast to rats, dogs, and humans, BRL 44072 (the 8-hydroxy derivative of BRL 39123) accounted for 24% of the urinary radioactivity and 15% of the dose in the cynomolgus monkeys.

Table. Elimination of radioactivity in male monkeys after a single intravenous dose of 10 mg/kg of [¹⁴C]BRL 39123A

Percentage of dose	
Urine 0-24 h	64.9
24-48 h	0.3
Feces 0-24 h	<2.1
24-48 h	2.9
Cage debris 0-24 h	11.3
24-48 h	0.4
Total recovery	81.8
Urine (% of urinary radioactivity)	
	0-24 h
BRL 39123	72.8
BRL 44072	23.5
total	96.3

13) The pattern of radiometabolites in the urine of male rats dosed once intravenously with [¹⁴C] BRL 39123A at a dose level of 10 mg of BRL 39123/kg (Study D86640/39123/24, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1986, Batch: 8475-79, 86.5% pure)

Three 197-238 g male Sprague-Dawley CD rats were administered a single intravenous dose of 40 mg/kg [¹⁴C]BRL 39123A in 0.9% sterile sodium chloride. Urine was collected for 48 hours. All samples were analyzed for radioactivity.

Of the administered dose, 87% was excreted in 24 hours and 89% was excreted in 48 hours. BRL 39123 accounted for 91-99% of the urinary radioactivity.

14) Plasma concentrations of radioactivity and of BRL 39123, and the pattern of radiometabolites in urine following a single intravenous administration of [¹⁴C]BRL 39123A to male dogs at a dose level of 10 mg of BRL 39123/kg (Study D86643/39123/25, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1986, Batches: Radiolabeled - 8475-79, 86.5% pure, Non-radiolabeled - GBD 8, 85.3% pure)

Three male beagle dogs were administered a single intravenous dose of 10 mg/kg BRL 39123A (the sodium salt of BRL 39123) in saline. Blood and urine were sampled for 48 hours after dosing.

Clearance is primarily renal. BRL 39123 accounted for 96-98% of the urinary radioactivity and 85% of the dose.

Table. Pharmacokinetic values and urinary excretion of BRL 39123 in male dogs administered a single intravenous 10 mg/kg dose of [¹⁴C]BRL 39123A

V (L)	11-14.8
t _{1/2} (h)	1.8-2.8
AUC _{0-∞} (μg*h/ml)	29-40
Urinary recovery (% of dose)	85
BRL 39123 (% of urinary radioactivity)	96-98
CL (L/h)	3.6-4.2
C _{max} (μg/ml)	14.8-18.5
T _{max} (min)	3-11

15) **Metabolic studies following a single intravenous administration of [¹⁴C]BRL 39123A to male dogs and healthy male human subjects at nominal doses of 25 and 5 mg of BRL 39123/kg, respectively (Study BF-1004/BRL-039123/1. GLP. Study Site: Beecham Pharm., Harlow, Essex, UK, 1990, Batch: 11283-77, 84.8% pure)**

Three male beagle dogs (11.1-15.3 kg) were administered a single intravenous dose of 25 mg/kg [¹⁴C]BRL 39123A in saline on two occasions, 2 weeks apart. After dosing, urine and feces were collected for 72 hours and blood was sampled for 72 hours and analyzed for radioactivity and radiolabeled compounds. Human data are from study 39123A/005/BP/001/prince.

BRL 39123 was the only radiolabeled compound detected in dog plasma or urine. In addition, urine of humans contained an unidentified metabolite (approximately 3% of dose). BRL 44072, the 8-hydroxy derivative of BRL 39123 and a major urinary metabolite of 39123 in cynomolgus monkeys, was not detected in the urine of humans or dogs.

Table. Pharmacokinetic data for BRL 39123 for dogs administered a single intravenous dose of 25 mg/kg of [¹⁴C]BRL 39123A

C ₀ (μg/ml)	52
C _{max} (μg/ml)	43
t _{1/2} (h)	2.1
AUC _{0-∞} (μg*h/ml)	79.4
Cl _R (L/h)	3.7
CL (L/h)	4.4
V _d (L)	13.4
radioactivity	
urine (% of dose)	92.7
feces (% of dose)	3.8

For comparison, the C_{max} and AUC_{0-∞} values for BRL 39123 for humans given a 5 mg intravenous infusion of [¹⁴C]BRL 39123A were 5.7 μg/ml and 13 μg*h/ml, with a t_{1/2} of 2 hours.

16) **Comparative Metabolic Pattern Studies Following an Intravenous Administration of [¹⁴C]BRL 39123A to Rat and Man at Nominal Doses of 40 and 5 mg of BRL 39123/kg, Respectively, Study BF-1010/BRL-039123/1.**

Status: GLP

Study Initiation: 4 Feb., 1990

Study Site: Beecham Pharm., The Frythe, Welwyn, Herts, U.K.
 Compound Tested: [¹⁴C]BRL 39123A, batches 11283-77 and 13274-51, >84% pure free acid, and BRL 39123, batch GBD 18, >85% pure free acid.
 Doses Tested: 40 and 5 mg/kg
 Dose Volume and Route: Rat, 10 ml/kg; Man, 1 hr infusion, IV
 Solvent: sterile saline

Species, Strain, Sex: male Sprague-Dawley (CrI:CD(SD)BR) rats, age approx. 9 weeks, weight range 220-254 grams, 3 animals/group or time point.

Test conditions: Animals and human subjects received a single intravenous dose of [¹⁴C]BRL 39123A following which blood, urine and faecal (rats only) samples were collected at various time-points between 10 min. and 72 hours post-dosing. Human data are from study 39123A/005/BP/001/prince.

BRL 39123 was the predominant radiolabeled compound detected in rat plasma or urine. In addition, urine of humans contained an unidentified metabolite (approximately 3% of dose). BRL 44072, the 8-hydroxy derivative of BRL 39123 and a major urinary metabolite of 39123 in cynomolgus monkeys, was not detected in the urine of humans or rats.

Table. Pharmacokinetic data for BRL 39123 for rats and man administered a single intravenous dose of 40 or 5 mg/kg of [¹⁴C]BRL 39123A

	Rat	Human
C _{max} (µg/ml)	27.9	4.1-5.7
AUC _{0-∞} (µg*h/ml)	23.4	8.3-12.9
Recovery of radioactivity		
urine (% dose) 0-24 hrs	81.5	
0-72 hrs	82.6	89
feces (% dose) 0-24 hrs	7.4	
0-72 hrs	9.8	
Total Recovery	93	95

Comments: 1) In both rats and human subjects, BRL 39123 appears to be excreted unmetabolized in the urine following intravenous administration at doses of 40 and 5 mg/kg, respectively. In man, a minor metabolite accounting for approx. 3% of the administered dose was eluted immediately before unmetabolized BRL 39123 on HPLC analysis (the exact structure of the metabolite was not identified in this study).

2) Excretion in the urine appears to be the predominant route of elimination for intravenously administered BRL 39123A in both rats and male humans, accounting for 80-90% of the eliminated radioactivity in both species.

17) The *in vitro* protein binding of BRL 39123 in human, dog and rat plasma (Study BF-1003/BRL-039123/2, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1990)

In vitro plasma protein binding of BRL 39123 was determined in human, dog, and rat plasma by ultrafiltration.

The percentage of BRL 39123 protein bound was <20% and was not dose related for rats, dogs, and humans between 2 µg/ml and 20 µg/ml. Freezing and thawing of the samples had a minimal effect.

18) *In vitro* cell partitioning of [¹⁴C]BRL 39123A (penciclovir) in male rat, dog and human blood (Study BF-1008/BRL-039123/1, GLP, Study Site: Beecham Pharm., Harlow, Essex, UK, 1992, Batch: 13274-51)

Blood samples from rats, dogs, and humans were incubated with [¹⁴C]BRL 39123A for 30 minutes in the concentration range 1-300 µg/ml for rats and dogs and 0.3-30 µg/ml for humans.

For rats and humans the ratio of blood to plasma radioactivity was approximately 1, whereas for dogs the ratio was 0.5. In dogs <10% of the radioactivity was found in erythrocytes.

19) **In Vitro Percutaneous Absorption and Cutaneous Distribution of [¹⁴C]BRL 39123 Across Intact and Tape-Stripped Human Skin, Study ID M95-002.**

Status: GLP

Study Initiation: 19 Dec., 1994

Study Site:

Compound Tested: [¹⁴C]BRL 39123A, batch 50457-065, Radio- and chemical purity of 99.6% and 98.3%.

Doses Tested: 1 mg

Dose Volume and Route: 0.1 g, topical

Solvent: "commercial formulation" cream base

Test System: cryopreserved human abdominal skin, dermatomed to 200-250 µm thickness, with and without tape-stripping (to remove the stratum corneum).

Test conditions: Tissue plugs were placed in a Bronaugh flow-through tissue apparatus surrounded by Hanks' balanced salt solution. The test article was applied to the epidermal surface of each skin sample, and the receptor fluid collected at 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after application. At the end of the 24 hour test session all skin specimens were separated into component layers (dermis, epidermis and stratum corneum) and assayed for the content of radioactivity.

During the 24 hours following application, approximately 0.02% and 0.31% of the applied radioactivity were recovered from the receptor fluid of "intact" and "tape-stripped" skin specimens. The transport rate of radioactivity across the tissue plugs generally increased during the 24 hour assessment interval, although it remained well below (approx. 1/3) the rate of transport of [³H] H₂O at all time points. Similar to the transport rate, the epi-dermal and dermal concentrations of radioactivity increased over time to maximal levels of 67 (+37) and 22 (+15) or, 391 (+349) and 217 (+333) µg equivalents/gram of tissue, in intact and tape-stripped skin, respectively. The majority of the radioactivity was recovered from the application site at the conclusion of the 24 hour test period in the residual cream. The total recovery of radioactivity was approximately 99% for both study treatments.

Comments: 1) The study results suggest that transport of BRL 39123 was increased approximately 3-fold by the removal of the stratum-corneum layer of the skin. Further, the transport and incorporation of radioactivity across the tissue specimens increased in extent during the 24 hour assessment period, for both intact and tape-stripped tissues.

2) Similar to the findings in several studies conducted in whole animals, the transport of BRL 39123 across the component layers of the skin was low. However in the present study, the level of transdermal transport was approx. an order of magnitude lower (0.1% vs. 1-5%) than that observed in intact animals.

3) Lastly, a significant question exists regarding the comparability of the "tape-stripping" produced dermal lesions versus the lesions-in-depth of the skin which are associated with herpetic lesions.

20) **Effect of BRL 42810 (fanciclovir), BRL 39123 (penciclovir) and BRL 42359 on testosterone 6-beta hydroxylation in human liver microsomes (Study**

D91609/42810/79, GLP, Study Site: Beecham Pharm., The Frythe, UK, 1991, Batches: BRL 42810, GBD 59, 99.2% pure; BRL 39123A, CT 30232, 85.4% pure; and BRL 42359, GBD 2, 99.4% pure)

BRL 42810, BRL 42359, and BRL 39123 did not inhibit testosterone 6 β -hydroxylation in two human liver microsome preparations (cytochrome P4503A4) after incubations of up to 30 minutes at 150 μ M. Conversion of BRL 42359 to BRL 39123 was negligible after incubation with human liver microsomes. Incubation with the human liver cytosol, however, resulted in extensive conversion after 30 minutes (59% with NADPH and 54% without NADPH).

21) **Factors Affecting Famciclovir and BRL 42359 Metabolism by Mammalian Molybdenum Hydroxylases and the Potential for Metabolism Drug-Drug Interactions With Famciclovir and Penciclovir**, Study BF-1035/BRL-042810/2 (DR95832), non-GLP, Study Site: Beecham Pharm., The Frythe, UK, Study Initiation: not specified, BRL 42810 and 42359, batch numbers and purity not specified.

BRL 42810 and BRL 42359 were incubated in vitro with liver enzyme extracts or tissue slices from rats, guinea pigs, rabbits and humans. The study results indicate that the 6-oxidation reaction of BRL 42810 and BRL 42359 occurs primarily by the aldehyde oxidase enzyme, although oxidation by xanthine oxidase is possible in the absence of high concentrations/activity of aldehyde oxidase. When incubated with human tissue extracts, the oxidation of BRL 42810 and BRL 42359 was reduced by the addition of cimetidine, chlorpro-mazine, isovanillin, quinine, quinidine, methotrexate, hydralazine, the phenothiazines, amacrine, β -estradiol and menadione (inhibitors or substrates for aldehyde oxidase). The effects of famciclovir on the metabolism of 6-mercaptopurine and methotrexate were slight. Famciclovir, penciclovir and BRL 42359 did not alter the metabolism of 5-FU when incubated in vitro with rat or human liver fractions.

- Comments:
- 1) The study results suggest that in humans, the oxidative metabolism of BRL 42810 is carried out by liver aldehyde oxidase and is unlikely to be significantly inhibited by the presence of allopurinol (an inhibitor of xanthine oxidase activity).
 - 2) The study results suggest that famciclovir and its metabolites are unlikely to alter the metabolism of 6-mercaptopurine and methotrexate in humans.
 - 3) The study results suggest that famciclovir and its metabolites are unlikely to interact with the metabolism (elimination) of 5FU in humans.

22) **The Effect of BRL 39123 on the Hepatic Levels of Cytochrome P450 and Related Parameters in Sprague dawley Rats After Intravenous Administration at 0, 10, 30 and 80 mg/kg/day for 13 Weeks**, Study 802/104-1011.

Status: GLP

Study Initiation: 30 Sept., 1993

Study Site:

Compound Tested: BRL 39123A, batch W93045

Doses Tested: 0, 10, 30 and 80 mg/kg/day

Dose Volume and Route: 10 ml/kg, IV

Solvent: sterile saline

Species, Strain, Sex: male and female Sprague-Dawley (CrI:CD(SD)BR) rats, age approximately 7 weeks (at dose initiation), weight range: male, 168-234 g; female, 146-199 g.

Test conditions: Animals received once daily intravenous infusions of BRL 39123A. At the conclusion of the 13 weeks of drug treatment, specimens of liver were obtained from 3 animals/sex/dose group, quick frozen, and retained

for the analysis of liver microsomal enzyme content.

The study results suggest that following 13 weeks of daily dosing, BRL 39123 did not significantly alter the liver microsomal contents of total protein, cytochrome P450 specific content, ethoxyresorufin O-dealkylase (CYP1A1), testosterone 16 β -hydroxylase (CYP2B), testosterone 6 β -hydroxylase (CYP3A) and lauric acid 12-hydroxylase (CYP4A) activities, at any dose level tested.

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing and Controls Section

NDA #: 20-629

CHEMISTRY REVIEW #: 1

REVIEW COMPLETED: August 29, 1996

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	October 16, 1995	October 16, 1995	October 19, 1995
NC (MV sample list)	March 27, 1996	April 1, 1996	April 5, 1996
NC (MV sample list)	April 19, 1996	April 24, 1996	May 5, 1996
BC (stability update)	June 13, 1996	June 14, 1996	June 20, 1996
BC (EA response)	June 14, 1996	June 17, 1996	June 21, 1996
BC (CMC response)	June 25, 1996	June 27, 1996	July 11, 1996
NC (EA response)	July 2, 1996	July 3, 1996	July 11, 1996
BC (CMC response)	July 23, 1996	July 24, 1996	August 8, 1996
BC (MV documentation)	July 26, 1996	July 27, 1996	August 11, 1996
BC (CMC commitments)	August 1, 1996	August 2, 1996	August 16, 1996
NC (CMC commitments)	August 8, 1996	August 9, 1996	-
BL (Tube/carton labels)	August 8, 1996	August 9, 1996	August 16, 1996

NAME/ADDRESS OF APPLICANT:

SmithKline Beecham Pharmaceuticals
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101-7929

DRUG PRODUCT NAME:

Proprietary:

DENAVIR™

Nonproprietary:

Penciclovir cream

CHEM. TYPE/THER. CLASS:

1S

DRUG CLASS:

7030110

PHARMACOLOGICAL CATEGORY:

Antiviral

INDICATION:

Treatment of herpes labialis (cold sores)

DOSAGE FORM/STRENGTH:

1% Cream; 2- and 5-g tubes

ROUTE OF ADMINISTRATION:

Topical

CHEMICAL NAME/STRUCTURAL FORMULA:

USAN, BAN, INN: Penciclovir

Laboratory Code: BRL 39123 (free acid)

Uninverted USAN: 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine.

CAS: 6H-purin-6-one, 2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-.

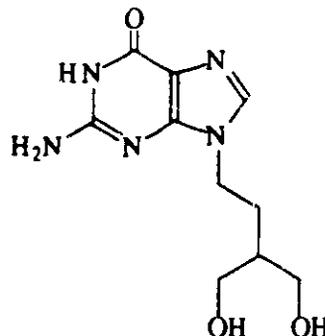
Other names used in application: 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine;

2-amino-1,9-dihydro-9-[4-hydroxy-3-(hydroxymethyl)butyl]-6H-purin-6-one.

CAS Registry: 39809-25-1

Molecular Formula: C₁₀H₁₃N₅O₃

Molecular Weight: 253.262



SUPPORTING DOCUMENTS:

NDA 20-363 Famciclovir tablets (Famvir[®]) for the treatment of herpes zoster infection (shingles); approved June 29, 1994
NDA 20-363/SCM-001 Manufacture of famciclovir and precursor at Carrigline, Cork, Ireland; approved December 27, 1996
NDA 20-363/SE1-004 Famciclovir tablets (Famvir[®]) for the treatment of recurrent genital herpes infections; approved December 11, 1995

at

RELATED DOCUMENTS:

NDA 20-363 Chemistry Review #1
NDA 20-363/SCM-001 Chemistry Review #1
NDA 20-363/SCM-006 Chemistry Review #1

MEMORANDA OF TELEPHONE FACSIMILES:

See Appendix 8.

MEMORANDA OF TELEPHONE CONVERSATIONS:

See Appendix 9.

CONSULT REVIEWS:

Environmental Assessment (N. Sager, HFD-357). See Section D.
Trademark (CDER Labeling and Nomenclature Committee). See Section F.

REMARKS/COMMENTS:

Penciclovir is a purine nucleoside analogue with antiviral activity against herpes viruses. It is structurally related to acyclovir and ganciclovir. Penciclovir cream is a topical formulation for the treatment of recurrent herpes labialis (cold sores). Famciclovir, a pro-drug of penciclovir, is available as an oral tablet for the treatment of herpes zoster infection (shingles) and recurrent genital herpes infections.

DRUG SUBSTANCE

documented.

The overall drug substance synthesis is adequately

Drug substance stability has been evaluated under a variety of conditions. The only reported degradation product of penciclovir, 9-(4-hydroxy-3-hydroxymethylbutyl)xanthine (BRL 42817), is observed only after prolonged exposure to acid and base at elevated temperatures. Penciclovir is otherwise stable at elevated temperatures. No significant changes are observed after 24 months at 40°C. Penciclovir is not affected by light or oxygen. The proposed retest interval of 24 months when stored at or below 30°C is supported by the available stability data and is acceptable.

DRUG PRODUCT

Except for an identity test and microbial limits tests, the release tests may be performed on bulk cream. However, if the bulk is stored for longer than 1 month, the applicant will perform full analytical testing on the filled tubes. If the bulk is stored for more than 6 months, the applicant has reluctantly agreed to perform additional testing on the bulk in addition to full testing on filled tubes.

The stability of the drug product has been evaluated under normal and accelerated conditions, including temperature cycling. Data were obtained on production scale batches of cream packaged in 2-g tubes, and on pilot-scale (clinical) batches of cream packaged in 10-g tubes. No stability problems were observed in samples stored at 30°C for 24 - 36 months, or at 40°C for 6 months. The only incidence of physical instability (i.e., collapse of cream) was observed in temperature cycling studies when the upper temperature slightly exceeded 45°C. Stability data on bulk cream have been provided. However, the results are of limited use because test samples were only collected from the surface.

The first 3 commercial/validation batches of each size package will be placed on stability. Tubes will be stored at 25°C/60% RH and 40°C/75% RH. Attributes to be monitored include description (revised to include microscopic evaluation of consistency in emulsion structure and phase separation), assay, propylene glycol content, water content, degradation products, rheology and microscopic examination (particle size). The applicant has agreed to perform limited preservative effectiveness testing and in-vitro release testing. In addition, the applicant has agreed to perform temperature cycling (25°C/40°C) on the first 3 batches. Annually thereafter, one batch of each size will be placed on stability at 25°C/60% RH. Testing will be limited to description, assay, propylene glycol content, and degradation products.

The applicant has requested a 24 month expiry for drug product stored "at or below 30°C". The proposed expiry is supported by the available stability data and is acceptable.

LABELING

From the chemist's perspective, the most problematic issue related to the labeling involved the applicant's choice of a suitable trademark. It had been their desire to use FAMVIR™, the trademark for famciclovir tablets, as common family name for famciclovir and penciclovir products. This proposal was totally unacceptable to the Division. Subsequently, VECTAVIR (the trademark for penciclovir in the UK), and then DIMINIVIR, were proposed as trademarks. Although found acceptable by the CDER L&NC, these names were withdrawn from consideration by the applicant. DENAVIR™ was only recently proposed. Primarily because of the similarity to indinavir (Crixivan, an HIV-protease inhibitor), the L&NC recommended that this trademark not be used. Although advised of these concerns, the applicant has chosen to retain DENAVIR™. No further objections were raised within the Division.

The applicant has agreed to make several minor changes to the package insert, and to the tube and carton labeling. See Section F.

ESTABLISHMENT EVALUATION

The EER for the SmithKline Beecham facilities in the UK was submitted on January 2, 1996.

The drug substance manufacturing facilities at _____ were found to be acceptable based on profile class. The Irvine facility was inspected during March 1996, and was found to be acceptable. Inspection of the drug product manufacturing facility at Crawley, UK, was completed in April 1996, and was also found to be acceptable. Notification of the acceptability of the applicant's cGMP Compliance Status was received from the Office of Compliance on June 24, 1996. See Appendix 7 for a copy of the completed EER.

METHODS VALIDATION

Methods validation samples and documentation were sent to the Northeast Regional Laboratory following the pre-approval inspection in April. The validation work there is nearing completion. It was recently learned that the second validation laboratory, Division of Drug Analysis, did not receive samples. Apparently only one set of samples was collected during the PAI. The applicant has been asked to submit the second set of samples directly to DDA.

ENVIRONMENTAL ASSESSMENT

The Environmental Assessment has been reviewed by CDER Environmental Assessment Team. Several minor deficiencies were noted during the review. The applicant's response has been reviewed and was found to be adequate. A FONSI was issued on July 9, 1996.

PATENT INFORMATION

Penciclovir and pharmaceutically acceptable salts thereof, specifically the sodium salt, are claimed in U.S. Patent No. 5,075,445, which expires December 24, 2008. At the time of submission, patent applications for formulations, compositions and/or methods of use were pending before the U.S. Patent and Trademark Office.

CONCLUSIONS & RECOMMENDATIONS:

Deficiencies in the original submission have been adequately addressed by SmithKline Beecham. As revised, the chemistry, manufacturing and controls are adequate to assure the identity, quality, purity and strength of the drug substance and the drug product. NDA 20-629, as amended, for penciclovir cream 1%, 2- and 5-g tubes, is therefore recommended for approval from the chemistry, manufacturing and controls perspective.


Mark R. Seggel, Review Chemist

Concurrence:

HFD-530/CChen *arc* 8/30/96

cc:

Orig NDA	HFD-530/JMahoney
HFD-530/Div. File	HFD-530/DMorse
HFD-830/ESheinin	HFD-530/GSherman
HFD-530/CChen	HFD-530/BDavit
HFD-530/GChikami	HFD-530/MSeggel

File: N20-629.cr1

ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT
FOR

NDA 20-629

BRL 39123

(penciclovir)

Topical

1% w/w Cream

FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF ANTI-VIRAL DRUG PRODUCTS
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-629

**BRL 39123 (penciclovir) Topical
1% w/w Cream**

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for BRL 39123 (penciclovir) Topical (1% w/w Cream), SmithKline Beecham Pharmaceuticals has prepared an abbreviated environmental assessment in accordance with 21 CFR 25.31a(b)(3) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Penciclovir is a synthetic drug intended for topical application in the treatment of recurrent herpes labialis caused by herpes simplex virus (HSV). The drug substance will be manufactured at SmithKline Beecham facilities in Scotland and the United Kingdom. The drug product will be manufactured at a SmithKline Beecham facility in the United Kingdom. The finished drug product will be used predominantly in homes.

Disposal may result from production waste such as out of specification lots, returned goods and user disposal of empty or partly used product and packaging. Pharmaceutical waste will be disposed of by the manufacturer at a licensed facility. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, although minimal quantities of unused drug may be disposed of in the sewer system.

Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release.

The Center for Drug Evaluation and Research has concluded that the product be manufactured, used and disposed of without any expected or environmental effects. Adverse effects are not anticipated endangered or threatened species or upon property in or eligible for listing in the National Register of Historic Places.

7/9/96
DATE

Nancy B. Sager

PREPARED BY
Nancy B. Sager
Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

7/9/96
DATE

Charles P. Hoiberg

CONCURRED
Charles P. Hoiberg
Division Director
Office of New Drug Chemistry-Division 1
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

SENSITIVE

REVIEW
OF
ENVIRONMENTAL ASSESSMENT
FOR

NDA 20-629

BRL 39123

(penciclovir)

1% Topical Cream

DIVISION OF ANTI-VIRAL DRUG PRODUCTS
(HFD-530)

CENTER FOR DRUG EVALUATION AND RESEARCH

DATE COMPLETED: APRIL 9, 1996

ENVIRONMENTAL ASSESSMENT

1. Date:

EA dated: 09/13/1995
Consult #1: 01/23/1996

CSO: John Mahoney

2. Name of applicant/petitioner:

SmithKline Beecham Pharmaceuticals

ADEQUATE

3. Address:

Four Falls Corporate Center
Route 23 and Woodmont Avenue
P.O. Box 1510
King of Prussia, PA 19406

ADEQUATE

4. Description of the proposed action:

a. Requested Approval:

The applicant is requesting approval of the product. A brief description of the product packaging is not provided. **DEFICIENT**

b. Need for Action:

Used in the treatment of herpes labialis.

ADEQUATE

c. Production Locations:

i. Proprietary Intermediate(s):

It is not clear whether input chemical is a proprietary intermediate or not. **DEFICIENT**

ii. Drug Substance:

Stages 1 and 2
SmithKline Beecham Pharmaceuticals
Shewalton Road
Irvine, Ayrshire, KA11 5AP
Scotland

Stage 1-4
SmithKline Beecham Pharmaceuticals
Clarendon Road
Worthing
West Sussex BN14 8QH
U.K.

ADEQUATE

iii. Finished Dosage Form:

SmithKline Beecham Pharmaceuticals Co.
Magpie Wood
Manor Royal
Crawley
West Sussex BN14 8QH
U.K.

ADEQUATE

Facility Description & Adjacent Environment:

A brief description has been provided for each of the facilities. ADEQUATE

d. Expected Locations of Use (Drug Product):

"...will be used in the United States of America with predominant use coinciding with areas of greatest population density" Based on the dosage form and use the product would be expected to be used predominantly in homes. ADEQUATE

e. Disposal Locations:

Returned goods will be destroyed by high temperature incineration. Two facilities are identified in the EA. The permitting information is provided. Two of the permits are expired. Updated information will be requested. DEFICIENT

5. Identification of chemical substances that are the subject of the proposed action:

Drug Substance: Penciclovir

Chemical Name: 9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine.

CAS #: 39809-25-1

Molecular Weight: 253.26 (as free acid)

Molecular Formula: C₁₀H₁₅N₅O₃

Structural Formula: Provided on page 10.

Physical Descrip.: White to pale yellow solid

Additives: The excipients used in the drug product are provided.

Impurities: Not provided.

DEFICIENT The impurities that may be in the drug substances are not identified. Identify any impurities that are likely to be found at levels > 1% and provide the CAS number, if available. If no impurities are likely, please state so.

6. Introduction of substances into the environment: For the site(s) of production:

a. Substances Expected to be emitted:

The substances expected to be emitted from each factory are described in confidential appendices. **ADEQUATE**

An input chemical from stage 1 is identified as

It is not clear whether this is considered a proprietary intermediate or not. If it is, manufacturing information would have to be provided regarding its production. **DEFICIENT**

b. Controls (Air, Liquid Effluent, Solid):

General controls are discussed. The controls used in drug substance production include using closed systems, pretreatment of aqueous waste prior to discharge, off-site disposal of some solvents and disposal of solid waste at licensed facilities. **ADEQUATE**

Only aqueous waste streams are discussed for the drug product production site. Given the type of operation air (particulate matter) and solid (pharmaceutical waste) would be expected. This should be discussed since a self-certification as described in the Industry Guidance, which could be submitted in lieu of this information, has not been provided. **DEFICIENT**

- c. **Compliance with Federal, State and Local Emission Requirements:**

Certifications of compliance are provided. **ADEQUATE**

- d. **Effect of Approval on Compliance with Current Emissions Requirements:**

Estimates are provided in the confidential appendices. **ADEQUATE**

- e. **Estimated Expected Emitted Concentration/Quantities:**

of the active ingredient is expected to be used which is equivalent to according to the companies calculations, by FDA calculations. **ADEQUATE**

7. **Fate of emitted substances in the environment:**
8. **Environmental effects of released substances:**
9. **Use of resources and energy:**
10. **Mitigation measures:**
11. **Alternatives to the proposed action:**

Format items 7-11 are not required for abbreviated EA's submitted pursuant to 21 CFR § 25.31a(b)(3).

12. **List of preparers, & their qualifications (expertise, experience, professional disciplines) and consultants:**

The preparers are identified along with their titles. **ADEQUATE**

13. **Certification:**

The certification is acceptable. **ADEQUATE**

14. **References:**

References are provided. **ADEQUATE**

15. **Appendices:**

Seven non-confidential and four confidential appendices are provided. **ADEQUATE.**

Confidentiality

The confidential and non-confidential information is clearly identified. **ADEQUATE**

DRAFT DEFICIENCY LETTER

This information may be provided in the form of confidential/non-confidential addendum to the EA dated September 13, 1996 rather than revising the entire EA to incorporate this information.

1. Format item 4:

- a. A brief description of the product packaging should be included.
- b. Please confirm that the disposal facilities identified in format item 4.6 have current permits. Only the new expiration date or statement that they have applied for a new permit and issuance is pending is necessary.

2. Format item 5:

Impurities that may be in the drug substances are not identified. Identify any impurities that are likely to be found at levels > 1% and provide the CAS number, if available. If no such impurities are likely, please state so.

3. Format item 6:

- a. Information regarding the production of Stage 1 input chemical and its production site location (format item 4) should be provided if it is considered a proprietary input chemical.
- b. Only aqueous waste streams are discussed for the drug product production site. Given the type of operation, air (particulate matter) and solid (pharmaceutical waste) emissions would be expected. Please discuss the controls used for these waste streams.

Endorsements:

HFD-357/NBSager

HFD-003/RLWilliams

Handwritten:
4/9/96
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MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA #: 20-629

REVIEWER : G. Sherman
CORRESPONDENCE DATE : 10/16/95
CDER RECEIPT DATE : 10/17/95
REVIEW ASSIGN DATE : 10/19/95
REVIEW COMPLETE DATE: 06/27/96

SPONSOR: SmithKline Beecham
One Franklin Plaza
P.O. Box 7929
Philadelphia, PA 19101-7929

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Antiviral

INDICATION: Herpes labialis (cold sores)

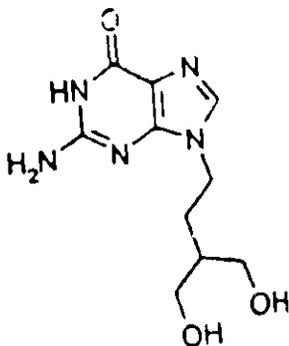
DOSAGE FORM: topical

PRODUCT NAMES:

- a. PROPRIETARY: Penciclovir
- b. NONPROPRIETARY:
- c. CHEMICAL: 9-[4-hydroxy-3-(hydroxymethyl) but-1-yl]guanine

STRUCTURAL FORMULA:

Mol. Formula: $C_{10}H_{15}N_5O_3$
Mol. Weight: 253.26



SUPPORTING DOCUMENTS: NDA 20-363
 NDA 20-363/S-001
 NDA 20-363/S-004

BACKGROUND:
Penciclovir

In this NDA, SmithKline Beecham is seeking approval of Penciclovir (PCV) for the treatment of herpes labialis. Penciclovir is the active metabolite of the prodrug Famciclovir, which has been approved for the treatment of herpes zoster. The sponsor has provided in vitro and in vivo data to in support of this new indication.

An acyclic nucleoside analogue of guanine, penciclovir is similar to acyclovir in its mechanism of action. In a number of studies, the sponsor has evaluated PCV against several herpesviruses, including HSV, VZV, EBV and CMV, although most studies have utilized HSV. As the proposed indication for the use of penciclovir is herpes labialis, this report will review those studies that have evaluated the antiviral activity of PCV against herpes simplex virus.

Herpes simplex Virus

Family Herpesviridae

As the prototype member of the family Herpesviridae, herpes simplex virus (HSV) is the most studied and consequently most completely characterized of the herpesviruses. The 80-odd (and still growing) members of this family of DNA-containing animal viruses vary widely in host range [mammals, birds, fish, amphibians] and biological properties but share several distinguishing features: i) a 162 capsomere capsid, ii) a cell-acquired lipid-containing envelope, iii) a large (MW = 108) linear

double-stranded genome, and iv) the ability to remain latent in the infected host.

As a human pathogen herpes simplex also belongs to a select group of 4, the human herpesviruses, which include Epstein-Barr virus (EBV), cytomegalovirus (CMV) and varicella-zoster (VZV). The clinical manifestation of HSV infection are characterized by spreading mucocutaneous lesions affecting either the oral cavity or the genitalia. Two distinct serotypes of the virus exist, designated type 1 and 2, which are correlated to the anatomical site of isolation, type 1 found in facial, type 2 in genital forms of the disease.

Pronouncements from Fundamentalists notwithstanding, the disease is relatively benign; in healthy adults it is normally self-limiting and resolved within two weeks. Occasionally the virus may produce severe systemic illnesses in weakened hosts, specifically newborns and patients with impaired immune functions.

Following primary infection, which in most cases does not produce clinically apparent disease, a latent phase ensues during which virus cannot be detected, but becomes associated with ganglia of the central nervous system. In response to appropriate stimuli and by mechanisms unknown, virus may be reactivated, resulting in the recurrence of lesions presumably by intraaxon transport of virus from ganglion to epithelium.

Augmenting the medical importance of the virus has been evidence of the transforming properties of HSV DNA in vitro. In addition, a link between HSV-2 and human cervical carcinoma has been established, but the failure to demonstrate that the link is more than merely associative has diminished the likelihood that HSV plays a significant role in human oncogenesis.

Virion structure

The mature, infectious virion, weighing approximately 10^{15} grams, consists of 4 structural components: an icosahedral protein shell, called the capsid which surrounds an electron-opaque core; an amorphous tegument which lies between the capsid and the outer membrane or envelope. The core contains the viral DNA, coiled very tightly in the form of an annulus or torus. Electron microscopic

studies have been unable to reveal the precise arrangement of DNA in the core, but do suggest stages in packaging, whereby the compact coil is preceded by a diffuse structure. The envelope is acquired during the egress of the capsid from the cell nucleus and seems to be derived from patches of cellular membrane into which the major glycoproteins coded by the virus have been inserted. The tegument describes the indistinct structures lying between the capsid and the envelope. It contains varying amounts of viral proteins, whose functions are largely unknown.

Capsids are approximately 100 nm in diameter, consisting of 162 capsomeres arranged in an icosahedron, with an inner core approximately 50 nm in diameter. Any preparation of virus contains several types of particles: capsids lacking cores (empty capsids), capsids with cores (nucleocapsids) as well as particles with and without envelopes.

The purified virion contains more than 20 structural proteins of 12,000 - 250,000 MW. which are distributed as follows: 1. there is a protein spindle of unknown complexity around which the DNA is wound in the core; 2. there are probably proteins along the DNA helix within the capsid; 3. there are proteins that comprise the capsid; 4 protein constituents of the tegument; and 5. at least 6 glycoproteins which are found anchored in the envelope.

The protein composition of capsids isolated from empty, full and enveloped particles are very similar, consisting of 4 structural proteins (VP5, VP19, VP23 and VP24). Nucleocapsids contain 2 additional proteins (VP21 and VP22a), which by virtue of their association with full capsids, may be involved in encapsidation and/or envelopment.

Genome structure and organization

The genome of HSV has several unusual physical properties. These are rooted in its sequence organization, the most complex of all the herpesviruses.

The genome is a linear duplex approximately 100×10^6 daltons in molecular weight and having a relatively high G and C content. Single-stranded HSV DNA is able to form "dumbbell"-shaped structures, evidence that sequences present at both ends are repeated in an inverted form internally. These repeat regions

flank unique regions which are of unequal size and appropriately designated U_l and U_s, respectively. The reiterated sequences flanking U_l designated ab and b'a' are each approximately 9 kbp, while those flanking U_s, designated a'c' and ca are each approximately 6.5 kbp. Each unique region, along with its flanking repeats constitute an "arm", either long or short, of the genome.

The "a" sequence is repeated internally as well as at the ends of the genome. Most HSV-1 strains contain only one "a" sequence at the terminus of the S component, but may contain a variable number at the L/S junction and at the end of the L component. Consequently, a more accurate description of HSV DNA structure would be indicated as

$$a_n b-U_l b' a' c'_m -U_s -ca$$

where n and m vary from 1 to greater than 10.

Sequence analysis has revealed that the structure of the "a" sequence is quite complex, consisting of both unique (U) and directly repeated (DR) sequences. "A" sequences vary with strain from 250 to 500 base pairs in length, the variability due to differences in copy number and sequence of the DR elements. As an example, the 501 bp "a" sequence from HSV-1, strain F, is represented by DR₁-U_lb-(DR₂)₁-(DR₄)₁-U_sc-DR₁.

The "a" sequences confer upon the HSV genome one of its most unusual properties: the ability of the L and S arms to invert relative to one another. Inversion appears to be accomplished by site-specific recombinations within the "a" region and is mediated, at least in part, by trans-acting viral gene products. Accordingly, this gives rise to four isomers of the molecule which differ in the orientation of the L and S arms with respect to one another. This mixture of isomers, all of which are infectious and present in equimolar amounts in virus preparations accounts for the pattern of molar and submolar restriction fragments observed in digests of purified virion DNA. Since there are two possible termini per L and S arm, the four possible L/S junctions per molecule, a theoretical maximum (assuming that the endonuclease cuts outside the repeat regions) of 8 submolar restriction fragments can be generated: four 0.5M terminal fragments and four 0.25M junction fragments.

Intracellular growth cycle

General

Studies have shown that both naked and enveloped particles are adsorbed and may be pinocytosed. The preferential mode of entry, however, is probably via fusion of the envelope with the cell membrane. After penetration, the virus nucleocapsid moves through the cytoplasm to the nuclear pore, where uncoating ensues and DNA is released into the nucleus.

Morphogenesis begins in the nucleus, which is the site for transcription and replication of viral DNA and the assembly of capsids. Empty capsids are the most likely precursors to full nucleocapsids, the formation of which is closely correlated with the maturation of progeny viral DNA. Nucleocapsids acquire envelopes as they pass through the inner nuclear membrane into the lumen of the endoplasmic reticulum, after which virus may remain cell-associated or move via the ER to the cell membrane where they exit either by budding or by entering adjoining cells by cell fusion. The replicative process is complete within 18 hours after infection.

Regulation of gene expression

Morphogenesis is a complex process and occurs within the context of the highly ordered and sequential expression of at least 70 gene products, many of which are involved in DNA synthesis and encapsidation.

Somewhat arbitrarily, gene expression is divided into 3 major phases: immediate early (IE) or alpha, delayed early (DE) or beta and late (L) or gamma. The first genes to be expressed are the 5 IE genes (IE 0, 4, 22, 27 and 47) which are transcribed by the host RNA polymerase II. IE proteins are synthesized in the cytoplasm during the first 4 hours of infection. After their synthesis they migrate to the nucleus where they are required to activate transcription of delayed early genes. These polypeptides are synthesized at maximal rates between 5 and 7 hours after infection. Among these proteins are enzymes important in DNA synthesis such as DNA polymerase and its accessory protein (UL42), the major DNA binding protein (ICP8), the viral helicase (UL5) and primase (UL52), and the viral origin binding protein (UL9). In addition,

delayed early polypeptides are involved in the shutoff of IE and host protein synthesis and in the induction of late genes.

The onset of DE gene expression precedes the onset of viral DNA replication which in turn delimits the third stage of infection, the synthesis of late gene products. These appear in maximal amounts at approximately 9 hours after infection and include most of the structural components of the virion. Some of these are basic DNA binding proteins which are involved in viral DNA folding and encapsidation, although at present the function of only a few of the structural proteins is known with any degree of certainty.

While this framework is convenient, it clearly is an oversimplification of a complex system of regulation. All genes do not fall neatly into the assigned groups and many overlap the existing categories. This situation has been remedied somewhat with the introduction of additional subgroups of the DE (beta 1 and beta 2) and L (gamma 1 and gamma 2), to reflect additional heterogeneity of viral gene expression. For instance, the two subgroups of the L class of polypeptides describe those proteins whose synthesis is independent of viral DNA synthesis and those whose synthesis is stringently dependent upon viral DNA synthesis. Synthesis of polypeptides in the first subclass, also known as "leaky late" may be reduced but are detectable in the absence of viral DNA replication, whereas those in the second subclass, or "true late" are not observed without viral DNA synthesis. The major capsid protein, VP5 (ICP5) and VP22a (ICP35) are members of the "leaky late" group of proteins, as are probably most, if not all of the structural proteins of the virus. Probably the best evidence in support of that supposition is the ability of DNA⁻ mutants to synthesize capsids at the NPT, which are identical in protein composition to those synthesized by wt virus.

DNA replication

HSV DNA is synthesized within the infected cell nucleus by the virus encoded DNA polymerase. Synthesis can be detected by about 3 hours post infection and continues for another 9 to 12 hours. While the DNA polymerase has been well characterized, the kinetics of DNA synthesis have been difficult to deduce and electron microscopic studies of replicating DNA have been little help in establishing a model of DNA replication. It is not surprising,

therefore, that our understanding of HSV DNA replication rests mainly on a relatively small amount of experimental data which can be summarized as follows:

1. On the basis of velocity sedimentation characteristics of replicating HSV DNA, viral DNA synthesis can be divided into 2 phases: (i) early after the onset of viral DNA synthesis, replicating DNA can be found sedimenting heterogeneously with S values up to twice that of parental HSV DNA; (ii) these are replaced later in infection by large tangled masses of DNA sedimenting up to 100 times faster than parental DNA. Velocity sedimentation analysis further indicates that these rapidly sedimenting structures can be chased into unit length DNA, thereby establishing a precursor-product relationship between the two.

2. Restriction endonuclease analysis indicates that sequences in pulse-labelled DNA are linked head-to-tail, as evidenced by the absence of terminal fragments and the increased relative molar concentrations of fragments containing L/S junctions.

The combination of high sedimentation rate (>200S) and lack of free termini supports the hypothesis that herpesvirus replicative intermediates are concatamers linked head-to-tail and generated by a rolling circle form of replication. The required circular template, according to this model, would be generated from parental DNA via annealing of exposed "a" sequences at the ends of the genome. Thereafter, tandem arrays of unit-length molecules would be generated by strand displacement from the template. Since this generates only a single isomer of the genome (per parental genome), additional sequence rearrangements are required to produce the three other isomers: intramolecular recombination must take place either on the concatamer, or on unit-length molecules cleaved from the concatamer. Progeny unit-length genomes, representing all 4 sequence arrangements, could serve substrate for immediate encapsidation or as templates for additional rounds of replication, generating additional tandem arrays of sequence isomers by the rolling-circle mechanism.

As previously mentioned, electron micrographs of replicating HSV DNA offer no direct confirmation of this model of replication. Typically, 200S DNA is a tangled mass, in which no discrete or predominant structure appears. Herpesvirus DNA extracted from the early phase of infection is more amenable to study but is very

heterogeneous in composition, containing 1) unit size DNA, either in, linear or circular form, 2) longer than unit-length molecules with and without replication loops, 3) Y-shaped molecules, either unit-length or longer, with branch sizes of variable length and 4) molecules with terminal loops or lariats. Although full circles and lariats with circles of unit length are compatible with a rolling circle model of replication, as are longer than unit length molecules, other structures are difficult to explain and are consistent with other modes of replication. Since the interpretation of electron microscopic data is complicated by the difficulty in discriminating artifacts and recombination forms from bona fide replication structures, any results should be viewed with caution. Nevertheless, preference for the rolling circle model of replication has become ingrained in the literature, due more to the persuasiveness of its proponents than to the weight of the evidence. It most certainly is not due to a lack of alternate replication possibilities, the most plausible and elegant of which argues that the replicative intermediates are circles, which are generated by a theta or sigmoid mode of replication from circular parental templates. This mechanism satisfies the requirement for endless molecules, linked head-to-tail, and simplifies the problem of isomer formation to a simple intramolecular recombination event between L/S junctions from a single infecting parental molecule. From the resulting 2 circular isomers, all 4 linear isomers can be generated, depending upon the L/S junction chosen for scission. Appropriate intramolecular recombination between repeat regions is accomplished far more readily and effectually within the limited choices of a circle than on a concatamer or between two separate molecules.

To account for the high sedimentation value of the intermediates, the model further proposes that progeny molecules remain topologically linked (a scenario with precedence in nature) as interlocking circles. Subsequent rounds of replication would lead to higher molecular weight structures composed of interlocking or catenated circles.

Alternatively, viral DNA replication could rely upon more than one mode of replication, whereby early synthesis generates unit length circular molecules, which in turn could serve as templates for the generation of concatamers via the rolling circle mechanism.

Unfortunately, it has been impossible to discriminate between these, or other modes of replication. Further progress in defining the mechanism of DNA synthesis seems dependent upon technical innovation, whereby the isolation and analysis of replicative intermediates can be performed under conditions which minimize damage to these inherently fragile structures.

This has not, however, been a deterrent to progress in identifying viral genes required for herpesviral DNA replication. A simple complementation assay has been developed in which combinations of cloned restriction fragments of HSV DNA provide the trans-acting functions required for in vivo replication of cotransfected plasmids containing an HSV origin of replication. A simple complementation assay has been developed in which combinations of cloned restriction fragments of HSV DNA provide the trans-acting functions required for in vivo replication of cotransfected plasmids containing an HSV origin of replication. This system is based upon the results from several laboratories that have shown that transfected plasmid DNAs containing either of the two origins of replication, ori_s or ori_L, are replicated in HSV-1 infected cells. The complementation assay has shown that a set of seven genes is essential for HSV origin-dependent DNA replication. Two of these genes code for known early protein: the major DNA binding protein (ICP8) (130Kd) and the DNA polymerase (140Kd). The remaining five (ORF sizes: 99Kd, 80Kd, 94Kd, 51Kd and 115Kd) include an origin-binding protein (UL9), a DNA polymerase processivity factor (UL42), and the three members of the primase-helicase complex (UL5, UL8, UL52). UL5 and UL52 have been shown to encode helicase and primase functions, respectively, while UL8 is required for efficient primer utilization by the HSV DNA polymerase.

DNA cleavage and packaging

The maturation of progeny genomes from replicative intermediates involves 2 major steps: a site-specific recognition and cleavage reaction, and the encapsidation of unit-length DNA. The details of these events are not known, but undoubtedly involve the activity of trans-acting virus-coded gene products acting on appropriate cis-acting sequences. Because the "a" sequence is present at the termini of the DNA it is not surprising that it plays a role in the generation of mature unit-length DNA molecules. As has been amply demonstrated using standard and defective viral

genomes, all packaged DNA is cleaved at an "a" sequence and an "a" sequence is necessary for propagation of defective genomes.

Studies using ts mutants of Pseudorabies virus indicate that DNA processing and encapsidation are closely connected, in which case the signals directing these functions would be expected to lie in close proximity; the mapping of cleavage-packaging signals to a 248 bp subregions of "a" of HSV (containing intact Uc, Ub and partial DR1 and DR4 elements) serves to confirm this supposition. Actual cleavage occurs adjacent to those sequences (asymmetrical within the DR1 repeat) suggesting a "measure and cut" process is involved.

Since the concatameric structure of replicative DNA is virtually a foregone conclusion, models for packaging require that 2 sequence-specific cleavages must take place a genome distance apart to generate unit-length DNA. The details are unknown, but may involve initiation of packaging from a specific sequence, measurement of unit-length genomes by a "headful" or "scanning" mechanism, and then cleavage at a second recognition site once a specific amount of DNA has been packaged. A problem inherent in this mechanism is the generation of termini lacking an "a" sequence, which occurs when cleavage takes place at junctions containing only 1 "a" sequence. Such termini have not been observed in total intracellular viral DNA, which is surprising since the predominant junctions in both standard and defective virus contain a single "a" sequence.

By comparison, the maturation of unit length genomes from a pool of covalently circular intermediates would be rather straightforward, involving the release of individual circles from the catenanes as a result of cutting at an L/S junction. Equimolar quantities of unit-length molecules would be generated, assuming equal chance of cutting one of two L/S junctions on both circular isomers.

Nucleocapsid assembly

Until recently, few biochemical studies have been performed on nucleocapsid assembly and although mutants blocked in assembly have been available for many years, they have been underutilized as tools for probing the events involved in this aspect of virion morphogenesis.

Predominant evidence, both from EM studies and ts mutants, indicates that empty capsids are assembled first and accumulate in the nucleus. Nucleocapsids are then generated as empty capsids acquire a genome equivalent of DNA. Accordingly, DNA must be processed and packaged but it is not known whether cleavage occurs prior to, during or after encapsidation. Nor is there firm information on the identity or role of gene products that accomplish these processes, although it seems likely that some of the nucleocapsid proteins play a role in the incorporation and condensation of viral DNA in the core. Interestingly, it has been shown that an internal capsid protein VP19C (ICP32) is a DNA-binding protein, and as such, may function in anchoring viral DNA in the capsid. It has also been shown that two late viral proteins of 21Kd and 22Kd that bind specifically to the "a" sequences of the HSV chromosome. It is possible that these proteins may be involved in the correct cleavage or encapsidation of replicative DNA, although it has also been demonstrated that the 35Kd product of UL15 also appears required for cleavage of genomic viral DNA. Studies have also indicated that correct processing of a transiently bound capsid protein VP22a (ICP35) is associated with DNA maturation and the appearance of nucleocapsids.

Mechanism of Action

Mechanistically, PCV is similar to ACV in inhibiting viral replication. In cells infected with HSV, PCV is phosphorylated to the monophosphate form by the viral thymidine kinase. Subsequent phosphorylation to the triphosphate form is performed by cellular enzymes. PCV-TP inhibits the HSV DNA polymerase by competitive inhibition with the natural substrate, GTP. In addition, the sponsor claims that incorporation of PCV-MP appears to inhibit chain elongation, resulting in inhibition of viral DNA synthesis. PCV is inactive against HSV strains which lack a functional TK gene, which supports the claim that phosphorylation is required for the activation of the compound.

Competitive Inhibition

Although PCV and ACV are similar in mechanism, qualitative differences between the two antiviral agents have been observed. The phosphate esters of PCV, unlike those of ACV, are chiral, allowing the possibility of forming (R) and (S) enantiomers of the triphosphate ester. The (S) enantiomer is the analog of the natural

nucleotide substrate, dGTP. In studies using purified HSV-1 and HSV-2 DNA polymerases, the K_i values for the S enantiomer(S) of PCV-TP with respect to the natural substrate were 8.5 μM and 5.8 μM respectively, whereas the K_i 's of ACV was 0.07 μM for both enzymes. A 90:10 R,S PCV racemate, which approximates the relative amounts of the enantiomers in HSV infected cells, had K_i values of 16.0 μM and 9.5 μM , respectively, against HSV polymerases 1 and 2. Thus, ACV-TP, is approximately 80 to 220-fold more active in competitively inhibiting the viral DNA polymerase.

Similar results were found using partially purified VZV DNA polymerase. An (R,S) PCV-TP racemate was less active than ACV-TP (IC_{50} 75 μM PCV vs. 0.88 μM ACV) due to lower affinity of PCV-TP for the viral polymerase (K_i 8.3 μM PCV vs 0.1 μM ACV). In studies using only the S enantiomer of PCV-TP, IC_{50} was 104 μM PCV vs. 0.3 μM for ACV.

In studies using radioactively labelled (S) PCV-TP, ACV-TP and dGTP, the incorporation of (S) PCV-TP was less than 1% that of dGTP (compared to 17% for ACV) indicating that PCV-TP is a poorer substrate for incorporation into DNA than ACV.

Comments: The substantially higher K_i values of PCV relative to ACV is an important concern, as competitive inhibition forms the basis of the antiviral activity of PCV. In subsequent studies, and in labelling information, the sponsor will emphasize that PCV is phosphorylated more readily than ACV, and that the concentration of PCV-TP is higher than that of ACV-TP in HSV-infected cells. It should be noted, however, that high PCV triphosphate levels can not overcome or compensate for the inherently lower affinity of PCV-TP for the viral DNA polymerase. It should be noted also that failure to achieve saturating levels of PCV-TP will result in less than maximal inhibition of the viral polymerase. This issue will be of significance in HSV infection of keratinocytes, where PCV-TP concentrations are far lower than in MRC-5 cells (see Penciclovir phosphate formation and stability).

There is also some discrepancy concerning the relative activities of the R and S enantiomers of PCV-TP. The sponsor states that the (S) enantiomer of PCV is predominately formed in HSV-1 infected cells (>95%), and that this enantiomeric form of PCV is the more active metabolite. However, in "Studies on the inhibition of VZV DNA polymerase by (S)-PCV-TP", the IC_{50} value for the (S) form was

higher than that for the (R,S, 90:10)racemate (104 μ M vs. 75 μ M). Thus, the R enantiomer appears to contribute significantly to the antiviral activity of PCV. As will be seen the following section (chain termination) an (R,S) enantiomeric mixture was more effective than the (S) alone in inhibiting DNA chain extension by HSV DNA polymerase.

Chain termination

The sponsor performed assays to determine the ability of PCV to terminate DNA elongation in chain extension assays using primed M13 (Fig. 3) In these assays, dGTP was absent, and the other dNTPs were present at a concentration of 50 μ M.

Comment: This study demonstrated inhibition of DNA synthesis by HSV-2 DNA polymerase in the presence of 50 μ M PCV-TP or ACV-TP, but that (S) PCV appears to allow some further synthesis beyond the initial incorporation of PCV-MP. Further synthesis was less pronounced using the (R,S) form of PCV-TP. Both the (R,S) and (S) forms of PCV-TP were less effective in terminating DNA extension than ACV. In these assay conditions, it was also shown that 50 μ M concentrations of ACV and PCV (both the (S) and (R,S) forms) were equally effective in terminating chains synthesized by the MRC-5 pol α as by the HSV-2 polymerase.

Lanes 2 and 7 in figure 3 demonstrate that additional incorporation of nucleoside monophosphates occurs beyond the initial incorporation of the (S) enantiomeric analogue of PCV. There appears to be significant termination at additional sites, which do not correspond to anticipated guanosine monophosphate residues. Termination at these sites would appear to contradict one of the proposed mechanisms of action (chain termination) of the drug.

In addition, figure 3 lacks information concerning the extension products in the control lanes (1 and 7). Since the photograph excludes the top of the gel, it can not be determined if the ladder of fragments in lanes 1 and 7 represent all of the extension products. If they do, the activities of both the MRC-5 DNA polymerase α and the HSV-2 DNA polymerase are low, suggesting that the assay conditions are suboptimal.

Fig 3.



Using "physiological conditions" (i.e. 300 μ M PCV-TP, (90:10 S:R) or 18 μ M ACV-TP) (Fig. 4), sponsor states that PCV was a more potent chain terminator than ACV. In these extension assays, the level of dGTP used was either 12 μ M or 1 μ M (based upon published values of dGTP found in ACV-treated HSV infected or uninfected human fibroblast cells). PCV-TP was used either at (i) a concentration of 300 μ M (90% (S) and 10% (R) isomer) to reflect values found in HSV-2 infected MRC-5 cells incubated with 10 μ M PCV for 4 hours or (ii) at 0.25 μ M to reflect the concentration found in uninfected MRC-5 cells. dATP, dCTP and dTTP were present in all assays at 50 μ M. Sponsor states that at 0.25 μ M inhibitor and 1 μ M dGTP, no difference in inhibition of DNA extension by MRC-5 DNA polymerase α or HSV-2 polymerase by PCV-TP or AVC-TP was observed, with only slight reduction in chain extension observed relative to the control lane. Sponsor claims that at 300 μ M PCV-TP and 12 μ M dGTP, PCV-TP appeared to be a more effective inhibitor of DNA elongation than ACV (18 μ M ACV-TP, 12 μ M dGTP).

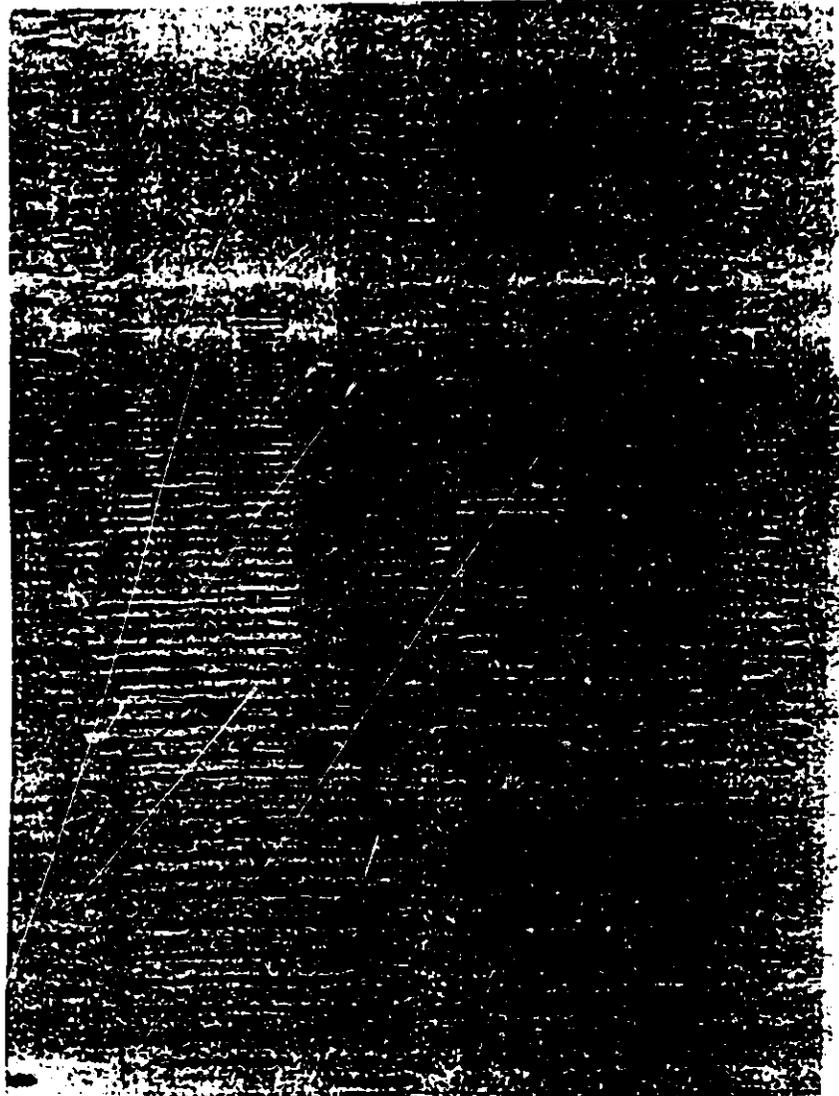
Comment: From the data, it appears that, under "physiological" conditions, there is very little difference between ACV-TP, PCV-TP and a positive control (dGTP) in inhibiting elongation by the viral polymerase. Evidence of chain termination at G residues is not detectable, and the extension products appear virtually identical to those found in a control lane (+ dGTP) containing no inhibitor. A comparison of PCV-TP and ACV-TP (300 μ M vs. 18 μ M) on MRC-5 DNA polymerase α indicates that DNA synthesis is inhibited more strongly by PCV than by ACV.

Evaluation of figure 4 is difficult due to lack of appropriate positive controls, excessive crowding of sample lanes on the autoradiogram, the absence of size markings to determine lengths of extension products and lack of lane numbering. Nevertheless, in this gel, the failure of PCV to terminate chains solely at expected G residues is apparent (see "45 μ M (S)-PCV-TP, 5 μ M(R)-PCV-TP), as at least 6 additional stop sites, some of which represent terminations at adenine and thymidine residues, can be seen. These data are difficult to reconcile with the proposed chain termination mechanism of action for this nucleoside analogue.

The mode of action of PCV, as a competitive inhibitor, appears to depend primarily upon the establishment of high levels of intracellular PCV-TP. Unlike ACV-TP, PCV-TP has low-affinity for the viral polymerase. The high levels of PCV-TP required (16.0 μ M

Figure 4
DNA Elongation assays under 'Physiological' nucleotide conditions

MRC-5 Polymerase α HSV-2 DNA Polymerase



Notes
Assays were performed as described in Methods, with the combinations of dGTP-inhibitor as shown under each lane. All assays contained 50 μ M dATP, dCTP and dTTP.

C Klenow
 A Dideoxy
 C Sequencing
 1
 50 μ M ACT-1P
 45 μ M (S)-PCV-1P, 5 μ M (R)-PCV-1P
 No addition
 50 μ M dGTP
 12 μ M dGTP, 18 μ M ACT-1P
 12 μ M dGTP, 270 μ M (S)-PCV-1P, 30 μ M (R)-PCV-1P
 12 μ M dGTP
 1 μ M dGTP, 0.25 μ M ACT-1P
 1 μ M dGTP, 0.275 μ M (S)-PCV-1P, 0.25 μ M (R)-PCV-1P
 1 μ M dGTP
 C Klenow
 A Dideoxy
 C Sequencing
 1
 50 μ M ACT-1P
 45 μ M (S)-PCV-1P, 5 μ M (R)-PCV-1P
 No addition
 50 μ M dGTP
 12 μ M dGTP, 18 μ M ACT-1P
 12 μ M dGTP, 270 μ M (S)-PCV-1P, 30 μ M (R)-PCV-1P
 12 μ M dGTP
 1 μ M dGTP, 0.25 μ M ACT-1P
 1 μ M dGTP, 0.275 μ M (S)-PCV-1P, 0.25 μ M (R)-PCV-1P
 1 μ M dGTP

for the R,S racemate and 8.5 μM for the S enantiomer) are of concern, as high concentrations of PCV-TP may not be achievable in cells that may be infected by HSV in the course of infection (i.e. keratinocytes).

The sponsor claims that a dose response of PCV-TP against the HSV-2 is demonstrated in figure 5, in which extension products of HSV-2 polymerase synthesized in the presence of increasing amounts of PCV-TP (100 μM , 200 μM , 300 μM) are analyzed on a sequencing gel. Reactions included 12 μM dGTP, 50 μM each of dCTP, dATP and dTTP.

A dose-response relationship between PCV-TP and HSV-2 extension products can not be seen in this figure as all the lanes containing PCV-TP are devoid of products. Furthermore, the photograph cuts off the lower part of the gel, and with it the primer start site. Therefore, it is not possible to determine if DNA synthesis terminated at the expected position.

Penciclovir phosphate formation and stability

The rates of phosphorylation and triphosphate stability of PCV and ACV were compared in a variety of human cells - (keratinocytes (NHEK), human fibroblasts (MRC-5, FLOW 4000), epithelial-like cells (RPMI 2650, WI-38VA 13) -and a monkey cell line (Vero) infected with HSV-2.

A wide range of PCV-TP concentrations were formed in different infected cell types, indicating that cell type and infecting virus type play an important role in determining the efficiency with which PCV is phosphorylated. After incubation with 10 μM PCV (added 16 hpi with HSV-2) PCV-TP concentrations in cells at 4 hours post drug addition ranged from 884 pmoles/ 10^6 MRC-5 cells (220 μM) to 10 pmoles/ 10^6 WISH cells (2.5 μM). Under the same conditions, ACV-TP ranged from 18.5 μM in MRC-5 cells to 0.5 μM in WISH cells. Thus, the sponsor concludes that PCV-TP is more rapidly synthesized than ACV-TP in HSV-infected cells.

The higher levels of PCV-TP than ACV-TP are presumably due to the higher affinity of PCV for thymidine kinase ($K_i=173$ μM for ACV vs. 1.5 μM for PCV) and to greater stability of PCV-TP relative to ACV-TP in HSV-1, HSV-2 and VZV-infected cell lines. Greater stability is reflected in the prolonged activity of PCV in cell culture and animal models.

Figure 5

HSV-2 DNA Polymerase



Notes

Assays were performed as described in Methods, with the combinations of dGTP-inhibitor as shown under each lane. All assays contained 50 μ M dATP, dCTP and dTTP.

12 μ M dGTP
12 μ M dGTP, 18 μ M dCV-TP
12 μ M dGTP, 270 μ M (S)-PCV-TP, 30 μ M (R)-PCV-TP
12 μ M dGTP, 180 μ M (S)-PCV-TP, 20 μ M (R)-PCV-TP
12 μ M dGTP, 90 μ M (S)-PCV-TP, 10 μ M (R)-PCV-TP
12 μ M dGTP, 45 μ M (S)-PCV-TP, 5 μ M (R)-PCV-TP
12 μ M dGTP, 18 μ M (S)-PCV-TP, 2 μ M (R)-PCV-TP
12 μ M dGTP, 18 μ M dCV-TP
5' xtenor
4' Diexor
3' Sequencing

When HSV-2-infected keratinocytes were incubated, at 20 hpi, for 4 hours with 10 μ M PCV or ACV, 23 pmoles/ 10^6 cells (6 μ M) of PCV-TP were detected vs. 3 pmol ACV-TP/ 10^6 cells (0.75 μ M). Levels of ACV-TP increased to 13 pmol/ 10^6 (3 μ M) cells after treatment of infected cells for 6 hours whereas the concentration of PCV-TP remained essentially the same at 22 pmol/ 10^6 cells (5.5 μ M). Uninfected cells were treated with PCV at 10 μ M for up to 6 hours with PCV, but there was no evidence of phosphorylation.

Stability of PCV-TP was addressed in experiments in which HSV-2 infected cells (MRC-5, RPMI 2650, WISH and Vero) were pulse-labelled (for 4 hours) with radiolabelled PCV. Cell monolayers were infected with 1 pfu/cell of HSV-2 MS for 1 hour and then incubated with medium containing either [4'- 3 H] penciclovir or [2'- 3 H] acyclovir to give a final compound concentration of 10 μ M. After the 4 hour incubation, extracellular drug was removed and replaced with fresh medium. PCV-TP concentrations were assayed by HPLC at 0, 1 and 4 hours post drug removal. Sponsor reports that PCV-TP stability is greater than that of ACV-TP, with PCV-TP exhibiting prolonged stability during the 4 hour incubation.

In a separate study, the sponsor also performed stability studies in human keratinocytes infected with HSV-2. Intracellular triphosphate levels of PCV were higher than those of ACV (0.75 μ M vs. < 0.25 μ M, respectively) and PCV-TP levels were constant over the 4 hour chase period.

Comment: PCV-TP levels is cell-type and virus-type dependent. For example, PCV-TP levels in HSV-2 infected keratinocytes after 4 hours was only 6 μ M (compared to 220 μ M in HSV-2 infected MRC-5 cells. PCV-TP stability, likewise also varies with cell type. For instance, PCV-TP levels in human keratinocytes and VERO cells at 4 hours of chase were identical to those at the end of the pulse, whereas in RPMI 2650 cells and WISH cells PCV-TP concentrations were 50% of the levels at the end of the pulse.

One drawback of these studies is that triphosphate stability is measured very early in infection (from 1 to 5 hours postinfection) whereas triphosphate formation is measured later in infection (20 to 24 hours postinfection). The choice of times may reflect optimal conditions for measurement but may not be truly indicative of conditions at relevant time points in a natural infection. For instance, it remains unclear whether PCV-TP is stable late in

infection, or whether phosphate formation at early times of infection, during viral DNA synthesis have reached inhibitory levels. For instance, to this reviewer, it is problematic that the concentration of PCV-TP in HSV-2 infected keratinocytes is only 3 μM at 1 hour postinfection and 6 μM at 16 hours post infection, particularly in light of the 9.5 μM K_i of PCV-TP against the HSV-2 polymerase.

In addition, the studies detailing intracellular phosphate levels and stability have not ascertained levels of PCV in uninfected cells. This information is important when considering the long-term effects of treatment of PCV in patients, since all the cells in the patient would be exposed. Although the sponsor states that in uninfected cells, the level of PCV-TP is barely detectable (0.5 μM), levels of unphosphorylated PCV in cells may nevertheless reach high levels.

Effect of PCV on host cellular DNA polymerases

The effect of PCV on host cellular polymerases α , β , γ and δ were determined by competitive inhibition studies. The K_i of (R,S)-PCV-TP against MRC-5 DNA polymerase α was 45 μM and the IC_{50} values of (R,S) PCV-TP against MRC-5 pol α/δ , β and γ was >100 μM , >100 μM and 51 μM , respectively.

Comments: The K_i value of the (R,S) racemate against the MRC-5 DNA polymerase of 45 μM is only approximately 3 fold higher than the K_i for HSV-1 DNA polymerase (16.0 μM). In terms of selective inhibition, this is a rather small difference in affinity. However, since significant levels of PCV-TP are not generated in uninfected cells, the rather poor selectivity of PCV-TP should not be a problem.

In vitro activity

The potency of penciclovir was assessed using different assays, cell lines and multiplicities of infection. The results of these studies are summarized in Table 1.

Virus/Strain (# isolates)	Assay	End-Point	Cell line	Conc. of PCV in (ug/ml)	Reference
HSV-1/Clin. (16) UK	PR	IC50	MRC-5	0.3	39123/1A
HSV-1/Clin. (3)	PR	IC50	MRC-5	0.7	39123/2-13
HSV-1/Clin. (5)	PR	IC50	Hs68	0.8	39123/2-13
HSV-1/Clin. (4)	PR	IC50	WI-38	1.8	39123/2-13
HSV-1/Clin. (2)	PR	IC50	HEp-2	0.2	39123/2-13
HSV-1/Clin. (2)	PR	IC50	RD	0.6	39123/2-13
HSV-1/Clin. (9)	PR	IC50	WISH	0.2	39123/2-13
HSV-1/Clin. (2)	PR	IC50	RK13	0.2	39123/2-13
HSV-1/Clin. (5)	PR	IC50	WI-38 VA13	0.2	39123/2-13
HSV-2/Clin. (13) UK	PR	IC50	MRC-5	1.52	39123/1A
HSV-2/Clin. (9)	PR	IC50	MRC-5	2.1	39123/2-13
HSV-2/Clin. (6)	PR	IC50	Hs68	0.8	39123/2-13
HSV-2/Clin. (4)	PR	IC50	WI-38	1.9	39123/2-13
HSV-2/Clin. (2)	PR	IC50	HEp-2	1.5	39123/2-13
HSV-2/Clin. (2)	PR	IC50	RD	1.9	39123/2-13
HSV-2/Clin. (13)	PR	IC50	WISH	0.3	39123/2-13
HSV-2/Clin. (2)	PR	IC50	RK13	0.5	39123/2-13
HSV-2/Clin. (5)	PR	IC50	WI-38 VA13	0.8	39123/2-13
VZV/Clin. (5) UK	PR	IC50	MRC-5	3.1	39123/1A
VZV/Clin. (11) US	PR	IC50	MRC-5	4.0	39123/2-16
VZV/Clin. (12) UK	PR	IC50	MRC-5	5.1	39123/1-01
VZV/Clin. (12) UK	PR	IC50	Hs68	0.9	39123/2-01
HSV-1/SC16	PR PR PR AI AI DS ¹ YR	IC50 IC50 IC50 IC50 IC50 IC50 IC99	MRC-5	0.8 0.2 0.3 0.6 2.0 0.01 0.6	39123/1-11 39123/2-04 39123/1A 39123/1-11 39123/1A 39123/1-11 39123/1-11
HSV-1/C-42	PR	IC50	GP1405	14	Larsson, A
HSV-1/HFEM	PR PR	IC50 IC50	SCC-13 PHK	0.13 0.19	39123/1-05 39123/1-05

HSV-2/MS	PR	IC50	SCC-13	0.21	39123/1-05
	PR	IC50	PHK	0.12	39123/1-05
HCMV/AD-169	PR	IC50	MRC-5	52	39123/1A
EBV	AI	IC50	P3HR-1	10	39123/1A
	DS ¹	IC50	P3HR-1	1.5	39123/7A
	DS ²	IC50	P3HR-1	8	39123/7A
	DS ²	IC50	Ramos	18	39123/7A
SVV	PR	IC50	Vero	>125	39123/1-03

AI Antigen inhibition

PR Plaque reduction

VDI Viral DNA inhibition

YR Yield reduction

¹ by hybridization

² by [³H]thymidine incorporation

Comments: A number of assays, using laboratory strains and clinical isolates, and employing a variety of cell types, were used. In general, PCV is most effective against herpesviruses in the following order: HSV, VZV, EBV, CMV. CMV showed only marginal susceptibility. The IC₅₀ values generally varied by 3- or 4-fold for each virus type, per assay, as cell line was varied. However, more than 2-fold differences were observed in replicate experiments using the same cell line. Thus, it is difficult to determine the role that cell type plays in antiviral activity of the drug. Nor is it possible to conclude that PCV is more active against clinical isolates than laboratory strains. In addition, the MOI used in each experiment influences potency of the drug. The sponsor has demonstrated that the activity of PCV in cell culture is inversely related to the MOI used in the assay.

Clinical isolates of HSV-1 ranged in sensitivity to PCV in IC₅₀s from 0.2 to 1.8 ug/ml. However, the sponsor has not defined the resistant phenotype in terms of IC₅₀ values. The sponsor should indicate the drug concentration which clearly demarcates drug-sensitive and drug-resistant isolates.

Therapeutic index: In general, there was no cytotoxic effect in vitro at 100 ug/ml PCV. Thus, the therapeutic index ranged from 500 to 55 for clinical isolates of HSV-1, 330 to 50 for clinical isolates of HSV-2, 110 to 20 for clinical isolates of VZV, between

12 and 6 for laboratory strains of EBV, and 2 for a laboratory strain of human CMV.

Activity of PCV against acyclovir-resistant strains

The activity of PCV against clinical and laboratory-produced acyclovir (ACV) resistant HSV-1 strains was evaluated. Laboratory-produced mutants were obtained by serial passage of the parental virus in the presence of ACV and clones were isolated from the resistant population. The clinical or laboratory-derived ACV-resistant variants contained mutations either in the thymidine kinase gene, resulting in a TK⁻ phenotype, or the viral DNA polymerase; mutations in the viral polymerase presumably altered the substrate specificity of the enzyme.

The frequency with which ACV-resistant virus appears, (10^{-4}), is similar to that reported for PCV-resistant mutants.

Plaque reduction assays were performed in Vero cells or MRC-5 cells. In the 3 TK deficient and 2 TK altered strains tested in Vero cells, cross resistance to PCV was detected. IC₅₀s of PCV were from 4 to 73-fold greater against TK-deficient and from 2 to 5-fold greater against TK-altered phenotypes than the IC₅₀s against wild type virus. Similar results were found when testing was performed in MRC-5 cells. The one TK deficient mutant tested was 258-fold more resistant and the TK altered mutant was 138-fold more resistant to PCV. Three of 4 mutants with altered DNA polymerase enzymes were resistant to PCV with IC₅₀s from 4 to 15-fold greater than that of wild-type virus, with greater resistance observed in MRC-5 cells.

Resistance to PCV

Experiments were performed to generate PCV resistant mutants of HSV in vivo and in vitro.

PCV-resistant virus was selected by exposing HSV-2 in cell culture to 30 ug/ml PCV. Dilutions of HSV-2 (MS) were inoculated onto confluent Vero cell monolayers, virus was allowed to adsorb for 1 hour, at which time the inoculum was removed and replaced with medium containing 0.25% agarose, 10% newborn calf serum and 30 ug/ml PCV. Plates were incubated at 37° C until plaques were

visible. Plaques were picked and characterized with respect to thymidine kinase and DNA polymerase activity. Of 4 PCV-resistant mutants isolated in this manner, 3 were TK deficient and one had altered TK substrate specificity. Altered DNA polymerase activity was suspected, but not directly demonstrated, in 2 of these 4 mutants. The IC_{50} values for all 4 mutants were greater than 100ug/ml, whereas the IC_{50} of the parental strain was 1.7 ug/ml

Mice were infected intravaginally with 10^5 pfu of HSV-2 and treated with 0.2 mg/ml FCV in drinking water for 5 days from time of infection. A vaginal swab was taken daily from each animal, virus titer was determined and sensitivity of selected virus samples (day 6) determined by plaque reduction assay. Sponsor reports that virus isolates taken 6 days after genital infection showed the same sensitivity to PCV as the parental virus (HSV-2 MS) or virus from placebo-treated animals.

The in vitro activity of PCV against 20 clinical isolates each of HSV-1 and HSV-2 are shown in figure 6. IC_{50} values were determined by both plaque reduction assay (PRA) and enzyme-linked immunoassay (EIA). The mean (+/- standard deviation) IC_{50} values against HSV-1 isolates for PCV was 0.6 +/- 0.4 ug/ml and 0.8 +/- 0.3 ug/ml, by PRA and EIA, respectively. The IC_{50} values against HSV-2 isolates were 2.4 +/- 2.5 ug/ml and 2.2 ug/ml +/- 2 ug/ml by PRA and EIA, respectively.

Three HSV-2 isolates had intermediate sensitivities to PCV (IC_{50} from 2.16 to 2.5 ug/ml) and 2 isolates were resistant to PCV (IC_{50} greater than 3.5 ug/ml).

Comments: Virus isolates resistant to PCV can readily be selected in vitro. Cross-resistance of ACV resistant variants to PCV has also been demonstrated. Thus, it appears likely that long-term treatment of patients with PCV will result in the appearance of PCV resistant mutants among which will be found ACV resistant variants. Attempts to select PCV resistant mutants from mice after only 5 days of treatment with FCV can not justify the conclusion that "variants of HSV-2 resistant to PCV do not readily arise in vivo". Longer term treatment of animals with the drug should be performed before making this conclusion.

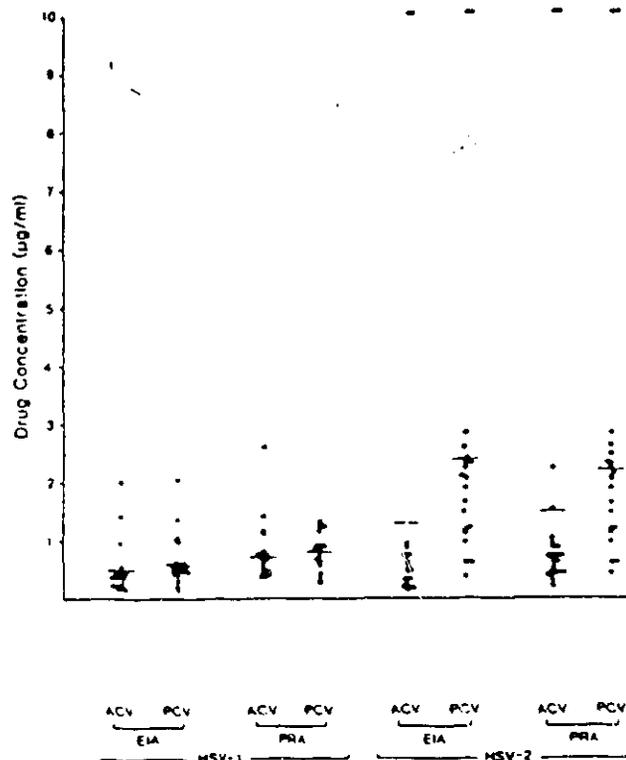


Fig. 6 Sensitivity of HSV clinical isolates to acyclovir (ACV) and penciclovir (PCV). Twenty clinical isolates each of HSV-1 and HSV-2 were tested by both EIA and PRA. The dots represent the IC_{50} of each HSV isolate. Horizontal bars represent means.

Effect of PCV on AZT, reverse transcriptase

Cell culture studies were carried out to investigate the effect of penciclovir on the inhibition of HIV-1 replication in M8166 cells. AZT inhibited HIV-1 replication with an approximate IC_{50} value of 0.1 ug/ml. This inhibitory activity was not markedly affected by the presence of PCV at a concentration of 100 or 10 ug/ml. In addition, PCV did not markedly affect the cytotoxicity of AZT in these cells.

In vitro studies were undertaken to determine the 50% inhibitory concentration (IC_{50}) of (R,S)-PCV-TP against HIV-1 reverse transcriptase. Using poly(rC).p(dG)₁₂₋₁₈ as template and

measuring the incorporation of [8-³H]dGTP, the IC₅₀ was 4 uM. In cells doubly infected with both HIV and HSV, the expected levels of PCV-TP would exceed the IC₅₀ value for HIV reverse transcriptase.

In vivo antiviral activity

PCV has been tested in mice infected with HSV-1 (SC16) either cutaneously, intranasally, intraperitoneally or intravaginally.

Cutaneous infection of mice (via the ear pinna) results in an infection that is usually self-limiting and has a low mortality. A dose-dependent reduction in lesion size was observed in cutaneously infected (10⁵ pfu) BALB/c mice which were treated systemically with a single daily subcutaneous dose commencing 5 hours after infection. Reduction in lesion size ranged from approximately 25% in 50 mg/kg/dose to approximately 40% in 100 mg/kg/dose to approximately 50% in 200 mg/kg/dose animals. No significant difference in rate of healing was seen between PCV-, ACV-treated and placebo-treated animals.

The effect of treatment by oral gavage with FCV or PCV cutaneously infected mice (4 X 10⁵ pfu) was also evaluated. Treatment with either 10 mg/kg/dose or 3 mg/kg/dose FCV or PCV was initiated 6 hpi and continued twice daily for 4 days. Mean lesion size after 4 days was identical for all treatment groups, including placebo. Subsequent lesion severity after discontinuation of therapy was reduced by 40% in animals treated with high doses of FCV. PCV at both doses, and FCV had negligible effect on lesion size.

The effect of PCV and FCV on BALB-c on HSV-1 infections in BALB-c mice infected intravaginally (10⁵ pfu) was evaluated. Animals were treated with either 0.2 mg/ml or 0.04 mg/ml PCV or FCV which was supplied in the drinking water for five days from time of infection. A vaginal swab was removed daily for 5 days from each animal and virus quantitated by plaque assay. PCV at 0.2 mg/ml and 0.04 mg/ml reduced virus titers by approximately 100-fold and 10-fold, respectively, during treatment. FCV at both doses reduced virus titers by about 10-fold compared to placebo-treated animals.

Using an intranasal route of infection in a murine model of encephalitis, Balb-c mice were inoculated with either 7 X 10⁴ pfu

HSV-1 (SC16) or 6×10^3 pfu HSV-2 (10). This infection normally results in rapid entry of virus into the central nervous system and is almost invariably fatal by 6 days postinfection. Animals were treated with PCV either subcutaneously from day of infection to 6 days post infection, or in drinking water from day of infection or day after, to 5 or 7 days after infection. 100 to 200 mg/kg PCV administered orally or subcutaneously was effective in reducing mortality and increasing mean survival time of mice infected by HSV-1. PCV did not reduce mortality of mice infected intranasally with HSV-2, however.

In similar studies involving intranasal inoculation of mice with HSV-1 (Goldthorpe, Boyd and Field, Antiviral Chem. and Chemotherapy (1992) 3(1), 37-47), treatment with PCV and FCV in drinking water resulted in rapid clearance of virus from all parts of the brain, but latent virus was detected in the peripheral and central nervous system of survivors 1 month post infection. Although PCV increased survival time and reduced mortality, there were fewer survivors in the groups receiving treatment from day 1 post infection than in those treated from the time of treatment (50% vs. 87%). Delaying the onset of treatment by more than one day post infection markedly reduced the ability of orally administered PCV and FCV to prevent death. The antiviral activities of PCV and FCV against HSV-2 infected mice were not evaluated in this study.

In the intraperitoneal (i.p.) model of infection, DBA/2 mice were infected with 5×10^5 pfu of HSV-1 (SC16). PCV was administered either orally, in drinking water, or subcutaneously at various times post infection. In intraperitoneally infected mice, virus replicates initially in visceral organs and then spreads to the central nervous system and causes death.

PCV administered subcutaneously as a single 50 mg/kg dose was effective in reducing titers of peritoneal washes when treatment commenced 5 hours after infection. Significant ($>10^2$) reductions in viral titer, relative to placebo-treated controls, were maintained until 72 hours post infection, after which virus titers rose to above untreated control levels. When treatment was delayed until 24 hours postinfection, reduction in viral titers was more transient; at 48 hours postinfection, viral titer was significantly lower ($>10^2$) but returned to above placebo-treatment levels by 72 hours postinfection.

Subcutaneous administration of PCV 24 hours after infection reduced viral peritoneal titers 48 hours post infection in a dose-dependent manner. A dose of 5.5 mg/kg reduced viral titer by 1000-fold, 1.8 mg/kg reduced viral titers nearly 100-fold, while 0.6 mg/kg reduced viral titer by approximately 10-fold.

In another experiment, PCV was administered in drinking water from time of infection to 43 hours after infection. Drug was present either at 1, 0.2 or 0.04 mg/ml concentrations. Peritoneal virus titers at 48 hours post infection were significantly reduced (approx. 10⁴-fold) in mice treated with 1 and 0.2 mg/ml concentrations of PCV, but no reduction in titer was observed in mice treated at the lowest concentration.

Intravenous administration of PCV 24 hours after infection was less effective than subcutaneous administration of PCV 24 hours post infection in reducing peritoneal viral titers at 48 hours post infection. A 10 mg/kg dose reduced viral titers approximately 100-fold, while 1 mg/kg was ineffective.

Significant reduction in mouse mortality was achieved by intraperitoneal and oral treatment of infected mice with PCV. For example, 20 mg/kg of PCV given by the i.p. route gave complete protection and oral treatment (1 mg/ml in drinking water) reduced mortality by greater than 90%.

In a comparison of PCV with FCV, mice infected intraperitoneally with HSV-1 were given a single oral dose of either drug (25 mg/kg) 24 hours after infection. Mean viral peritoneal titers (log₁₀ PFU/0.1 ml) at 48 hours postinfection were slightly higher for PCV (1.68 +/- 0.29) than for FCV (0.80 +/- 0.99).

Comment: Although the sponsor states that PCV is effective in reducing mortality and increasing mean survival time of mice infected intranasally with HSV-1, it should be noted that PCV was completely ineffective in this same model system against HSV-2.

The ineffectiveness of PCV in treating intranasally infected mice if treatment is commenced more than one day post infection is relevant to the clinical situation, as correct and accurate diagnosis of disease may not occur within this time frame.

Cutaneous HSV infections in guinea pigs

The antiviral activity of PCV was also evaluated against a cutaneous HSV-1 infection in guinea pigs. Virus replication occurs in the skin 1 to 7 days postinfection. Lesions are formed which crust over and heal during 6 to 12 days postinfection.

Animals were infected (by scarification) with HSV-1 (3×10^4 pfu) and treated topically, twice daily with 1% (w/v) PCV (or ACV) in aqueous cream or placebo from days 1 to 6 postinfection. Severity of lesions were assessed and scored. PCV effectively reduced (by approximately 50%) the severity of skin lesions. The effect of treatment with PCV was almost identical to that of ACV.

Significant reduction (approximately 35%) in lesion severity (compared to placebo-treated animals) was observed in animals treated with 5%, 1% or 0.75% PCV in a propylene glycol cream twice daily for 5 days commencing 72 hours postinfection with HSV-1.

In additional studies, the sponsor investigated the effect of topical treatment on virus loads within lesion sites. To do so, groups of 8 animals were treated with 1% PCV twice daily commencing 72 hours after infection. Placebo-treated animals acted as controls. Skin samples were taken from two animals immediately prior to start of treatment, and from two animals at 96, 104, 120 and 128 hours after infection. At these time points, animals were sacrificed, and the lesion sites excised and snap frozen. For processing, samples were weighed, disrupted in a blender, centrifuged to remove solid material. Supernatants were assayed for infectious virus by plaque assay on Vero cells.

At the start of therapy, mean lesion virus titer (\log_{10} pfu/gram of skin) was 5.85 ± 0.14 . On day 4 there was no significant difference between in mean lesion titers between and 1%PCV. On day 5, mean lesion titers in treated animals were reduced by 0.12 (\log_{10}).

Comment: Although reduction in lesion size is observed in cutaneous models of infection, PCV or FCV do not reduce significantly the overall time to healing of lesions. Although a statistically significant mean decrease in virus titer in lesions of treated animals was demonstrated, the small size of that mean

decrease (0.12 logs) reflects the poor activity antiviral activity of PCV in this model system.

The relative ineffectiveness of PCV in cutaneously infected guinea pigs may be reflective of the relatively low activity of the drug in HSV-infected guinea pig cells in vitro (IC₅₀ of 14 uM in GP 1405 cells vs. 0.1 uM in primary mouse cells).

Clinical trials

PCV resistant mutants

Although the sponsor monitored patients in clinical studies for virus shedding, and demonstrated that shedding ceased at least 25% sooner in PCV-treated individuals (vs. placebo), there was no attempt, in any of the clinical trials performed by the sponsor, to monitor clinical samples for the appearance of PCV-resistant variants of HSV.

Comment: By analogy with other nucleoside analogs that have been approved for clinical use, it is to be anticipated that variants of HSV will arise that are resistant to the drug. The mechanism of resistance would be either through mutation in the viral thymidine kinase, or the viral DNA polymerase.

The sponsor should conduct additional studies, in phase IV trials, to monitor clinical isolates for the appearance of drug-resistant mutants.

CONCLUSIONS: 1. Penciclovir is a nucleoside analogue which, like acyclovir, relies upon the viral thymidine kinase for initial phosphorylation to the monophosphate. Subsequent phosphorylation to the triphosphate form is performed by cellular kinases. Thus, the active form of the drug, PCV-TP accumulates only in virus-infected cells.

2. Although reminiscent of the modus operandi of acyclovir, penciclovir differs qualitatively in certain respects from ACV. PCV is phosphorylated more efficiently than acyclovir in infected cells, due to its higher affinity for the viral thymidine kinase.

The intracellular triphosphate of PCV is more stable than that of ACV. PCV-TP is not as active as ACV-TP in inhibiting viral DNA synthesis, however, due to lower affinity of PCV-TP for herpesvirus DNA polymerase. Higher intracellular triphosphate levels combined with a lower affinity for the viral DNA polymerase, nonetheless, appear to make PCV roughly comparable to ACV in antiviral activity.

3. From a mechanistic standpoint, PCV-TP is a competitive inhibitor of dGTP. However, definitive studies in support of its role as a chain terminator have not been presented in this NDA. The data that are provided indicate that DNA chains synthesized by HSV-2 polymerase are terminated at residues other than G under conditions where only dATP, dCTP, dTTP and PCV-TP are present. When nucleoside triphosphate and inhibitor are present at "physiological" concentrations, the effect on chain elongation appears minimal. Experiments which directly demonstrate incorporation of PCV into viral DNA have not been presented. Clearly, the data as supplied do not support the hypothesis that PCV acts as a chain terminator.

4. Cytotoxicity data indicate that uninfected cells accumulate barely detectable levels of PCV-TP; 100 ug/ml of PCV does not inhibit DNA synthesis in uninfected cells.

5. In vitro studies using a variety of cell lines and virus isolates indicates that PCV has activity against (in order of potency) HSV-1, HSV-2 and VZV. It has modest activity against Epstein-Barr virus and little activity against cytomegalovirus. The poor activity of PCV against CMV is probably attributable to the absence of thymidine kinase in this virus.

6. In vivo studies have assessed the activity of PCV in HSV infections in animals. Infection of mice was initiated either intravaginally, cutaneously, intranasally or intraperitoneally and treatment with either PCV or FCV was administered either orally, cutaneously, subcutaneously or orally, respectively. Depending upon treatments, PCV was administered from time of infection to 48 hours post infection. PCV and ACV exhibited very similar activities in most cases, although qualitative differences existed in short dosing experiments, presumably reflecting the prolonged intracellular stability of PCV-TP.

7. Resistance to PCV is to be anticipated, as mutations in either the TK gene or viral polymerase of HSV can lead to reduced sensitivity to the drug. PCV-resistant HSV mutants have been generated in cell culture. Of 4 mutants generated by exposure to high levels of PCV, all 4 affected viral thymidine kinase expression or function. Mutations in the viral DNA polymerase were suspected, but not directly demonstrated, in two of the four as well. Two acyclovir-resistant clinical isolates of HSV-1 were tested for sensitivity to PCV. One isolate, which was TK⁻, was resistant to PCV, while the other isolate, which was a DNA polymerase mutant, was sensitive to PCV. The frequency with which PCV-resistant HSV-1 mutants can be generated in cell culture (approximately 10⁻⁴) is similar to that observed with acyclovir.

RECOMMENDATIONS: The proposed use of Penciclovir topical cream for the treatment of herpes labialis is approved, provided the following microbiological concern is addressed by the sponsor:

1. As the development of resistance is a hallmark of nucleoside analog therapy, please evaluate representative clinical isolates for the emergence of PCV-resistant variants during the course of



 Microbiologist

CONCURRENCES:

HFD-530/Deputy Dir.		Signature	<u>7/12/96</u>	Date
HFD-530/SMicro	<u>N. Bival</u>	Signature	<u>6/28/96</u>	Date

for FR

cc:

- HFD-530/Original IND
- HFD-530/Division File
- HFD-530/Div Dir Reading file
- HFD-530/Pre-Clin Dep
- HFD-530/CO
- HFD-530/Pharm
- HFD-530/Chem
- HFD-530/SMicro
- HFD-530/Review Micro
- HFD-530/CSO