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NDA 020471

FIRM: ABBOTT LABS

1 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

Summary Basis of Approval
Cover Form

Appl #: 020471

Firm: ABBOTT LABS
Reviewing Div: 570
Trade Name: ZYFLO FILMTAB (ZILEUTON TABS) 400/600MG
Generic Name:

ZILEUTON

Approval Letter: Y

Statistician Review: Y

SBA Form: N

Bio/Dissolution Review: Y

Final Printed Labeling: N

Microbiologist Review: N

Medical Officer Review: Y

NAS/NRC Review: N

Chemist Review: Y

Pharmacologist Review: Y

Federal Register Notice: N

Completion Date: 13 FEB 97

204771

Approval Letter And Related Correspondence

Food and Drug Administration
Rockville MD 20857

NDA 20-471

Abbott Laboratories
Pharmaceutical Products Division
100 Abbott Park Road
Abbott Park, Illinois 60064-3500

Attention: James D. Steck
Director, PPD Regulatory Affairs

Dear Mr. Steck:

Please refer to your July 18, 1994, new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Zylflo (zileuton) 300 mg and 600 mg Tablets.

We acknowledge receipt of your amendments dated August 19, September 9 and 12, October 4 and 21, November 18 and 22, and December 14, 22, and 29, 1994, and January 5, 12, 17, 24, and 26, February 16 and 24, March 3, 6, 16, 28, 29, and 30, April 3 and 17, June 8 and 16, July 28, and August 15, 17, and 31, 1995, January 5, March 22, June 7, July 23 and 31, September 11, 26, and 30, October 4, 11, 23, 24, and 30, November 6, 7, 14, 18, and 19, 1996.

We have completed the review of this application, as amended, and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for the prophylaxis and chronic treatment of asthma in adults and children 12 years of age and older as recommended in the draft physician labeling submitted on November 19, 1996. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the draft physician labeling submitted on November 19, 1996, and the patient package insert submitted on November 18, 1996, and the November 19, 1996, mock-up carton and container labels. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit 20 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 "FPL for approved NDA 20-471." Approval of this submission by FDA is not required before the FPL may be used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of the labeling may be required.

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Send one copy to the Division of Pulmonary Drug Products and two copies of both the promotional material and the package inserts directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising, and
Communications, HFD-40
5600 Fishers Lane
Rockville, Maryland 20857

Please submit one market package of the drug product when it is available.

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

If you have any questions, please contact:

Ms. Betty Kuzmik
Project Manager
(301) 827-1051

Sincerely yours,

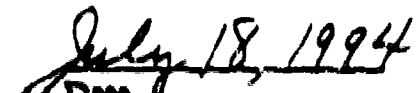
James Bilstad, M.D.
Director
Office of Drug Evaluation II
Center for Drug Evaluation and Research

DEBARMENT STATEMENT

In compliance with the Generic Drug Enforcement Act of 1992, Section 306(k)(1) of the act (21 USC 335a(k)(1)), we, Abbott Laboratories, certify the following with respect to this new drug application:

The applicant did not and will not use in any capacity the services of any person debarred under subsections (a) or (b) (sections 306(a) or (b) of the Federal Food, Drug, and Cosmetic Act), in connection with this application for approval of a drug product.


James D. Steck
Associate Director, Regulatory Affairs
Pharmaceutical Products Division
Abbott Laboratories
Abbott Park, Illinois


Date

Final Printed Labeling

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.

Medical Officers Review

NDA #20-471
SAFETY UPDATE III

DRUG NAME:

Zileuton

CHEMICAL NAME:

N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea

SPONSOR:

Abbott Laboratories

CATEGORY:

5-Lipoxygenase Inhibitor

INDICATION:

Maintenance Therapy for Asthma

DOSAGE FORM:

Film-coated Tablet

MEDICAL REVIEWER:

Raymond F. Anthracite, M.D.

SUBMISSIONS (VOLUMES) REVIEWED:

03/22/96 (22)

06/07/96 (51)

07/31/96 (1)

10/04/96 (1)

10/11/96 (1)

SAFETY UPDATE III

OVERVIEW

The original NDA was submitted in July 19, 1994 and summarized the results of 1,994 zileuton-treated patients in parallel or uncontrolled Phase II/III studies. The first safety update (SU, 11/18/94), was an integrated summary of 2573 zileuton patients from Phase II/III trials. The second safety update (SU2, 7/28/95) reported an additional 324 zileuton patients from three newly completed studies. Preliminary results from the long-term, open-label surveillance study, M94-199, were submitted 8/17/95.

Preclinical animal data revealed carcinomas in two rodent species and genotoxicity testing was positive. Therefore, zileuton was considered to be a genotoxic carcinogen and given a "not approvable" evaluation. The preclinical concerns have been addressed by the sponsor who submitted this third safety update (SU3, 6/7/96). It contained final results of a long-term safety surveillance study of close to 3000 asthma patients that was begun in September 1994 to compare usual care (UC) to zileuton 600 mg QID plus usual care (Z+UC) in trial M94-199, a 48-week, open-label study [2:27]. Specifically sought, was more information on the frequency, attack rate, time course and final outcome of liver test elevations or declines in white blood cell count.

Several facts have emerged about the effect of zileuton on liver enzymes, chiefly the ALT. In SU3, maximum post-baseline ALT's $\geq 3xULN$ were associated with zileuton more than with usual care alone in a ratio of 4 to 1. The first SU showed that patients with baseline ALT elevations were more likely to experience further elevations treated with zileuton than if treated with placebo. These observations suggest that normal screening ALT's prior to starting zileuton treatment should be required.

In SU3, zileuton patients with initial ALT elevations of 3-5xULN continued treatment and didn't fare very well. Over half withdrew prematurely, the majority because of further ALT elevations to $\geq 5xULN$. Some patients on zileuton who experienced elevated ALT's have gone on to develop symptoms or hyperbilirubinemia, and one case of clinical hepatitis has been reported. So far, virtually all patients who developed ALT elevations on zileuton and stopped the drug reverted to lower ALT values, most normalizing completely. These facts support regular monitoring of ALT's and discontinuing zileuton if elevations are noted during treatment.

Life-Table analysis from SU3 showed that zileuton-treated patients had the highest rate of initial ALT $\geq 3xULN$ during the first two months of treatment, 2% for

the first month and 1.3% for the second. Thereafter, from the third through the twelfth months, they had a mean rate of 0.34% per month, compared with 0.13% per month for usual care group. At least as germane as the more frequent ALT elevations in the zileuton group, was the more threatening outcomes, i.e. laboratory evidence of further hepatic dysfunction when the patients continued taking zileuton. Both the greater frequency of ALT's $\geq 3xULN$ in zileuton-treated patients and more severe consequences if treatment were continued, add weight to a proposal for periodic ALT monitoring past the second month of treatment. In fact, the life-table analysis shows a relatively constant monthly attack rate for zileuton-treated patients out to one year, hence there is no rationale for relaxing long-term ALT surveillance.

Zileuton-treated patients with ALT elevations who were prematurely terminated from studies were followed, per protocol. A "rebound" effect was seen in some of these, consisting of an initial reduction in ALT towards normal, but never to $< 1xULN$, followed by a second rise which was frequently to higher levels than the initial peak. This rebound began 20-40 days after zileuton had been discontinued in all four cases. A possibly related phenomenon was patients with normal ALT's who stopped taking zileuton and went on to have initial ALT elevations $\geq 3xULN$ within the next 1.5 months. These occurrences suggest that monitoring ALT's should be continued for 2 months after zileuton is discontinued when liver tests are normal, and should be continued until complete normalization of ALT $\leq 1xULN$, when this abnormality was the reason for drug withdrawal.

Other observations were that: 1) exposure duration of greater than three months was associated with greater time to resolution of abnormal ALT's; and, 2) abnormal ALT's increased with age in females. Hematologic variables showed some abnormalities, consisting primarily of low white blood cell counts. For the most part these were isolated values that were normal when repeated and/or of dubious clinical import.

NOTE TO READERS

Square brackets are used throughout this review as references to volumes and pages of the third safety update, FAX communications and separate submissions. The forms that these reference take are: [VOL:PAGE] for the third safety update; [FAX Date VOL:PAGE] for FAXes; and, [Submit. Date VOL:PAGE] for separate submissions. Several volumes and/or pages may be placed in one set of brackets with an organization like the following: [VOL:PAGE, PAGE-PAGE, VOL:PAGE-PAGE].

EXPOSURE

In the United States, 2947 asthma patients were enrolled at 233 sites with 2458 patients randomized to receive Z+UC and 489 to UC alone. An additional 224 patients at 14 sites in six Latin American countries were also studied and results of these will be submitted at a later date [2.27, 58, 67]. A tabular summary of cumulative treatment exposure is presented below. "Any exposure" was defined as at least one dose, in the Z+UC group, and at least one day, in the UC group [2.69].

SUMMARY OF DURATION OF DOSING IN U.S. TRIALS (2.69)		
Exposure Interval	Z+UC Number	UC Number
Any Exposure	2458	489
≥ 25 days (≥ 1 month)	2138	457
≥ 55 days (≥ 2 months)	1931	442
≥ 85 days (≥ 3 months)	1800	433
≥ 115 days (≥ 4 months)	1691	422
≥ 145 days (≥ 5 months)	1618	410
≥ 175 days (≥ 6 months)	1568	398
≥ 205 days (≥ 7 months)	1521	389
≥ 235 days (≥ 8 months)	1477	378
≥ 265 days (≥ 9 months)	1459	374
≥ 295 days (≥ 10 months)	1418	366
≥ 325 days (≥ 11 months)	1378	359
≥ 355 days (≥ 12 months)	241	93

The attrition rate for Z+UC was almost double that of UC at both six and eleven months. Attrition rate was defined as the number of patients in the following categories:

(Any Exposure - Exposed for ≥ 'n' Months)/Any Exposure
expressed as a percentage. For example, the attrition rates for Z+UC were:
 $100 (2458 - 1568)/2458 = 36.21\%$ at six months, and
 $100 (2458 - 1378)/2458 = 43.94\%$ at twelve months.

The corresponding attrition rates for UC alone were:

$100 (489 - 398)/489 = 18.61\%$ at six months, and
 $100 (489 - 359)/489 = 26.58\%$ at twelve months.

DEMOGRAPHICS

The baseline demographic profile showed no statistical differences between treatment groups for the variables Age, Height, Weight, Gender, Race, History Of Tobacco Use and History Of Allergic Rhinitis [4:101]. The mean age was about 43 years (range 15-86), with 8.4% patients being > 65 years of age. The average height was about 66.5 inches (range 48-87) and the average weight was about 170 lbs. (range 82-295). The distributions of some of the other variables are presented in the following table [2:67].

DEMOGRAPHIC CHARACTERISTICS BY TREATMENT GROUP [adapted from: Statistical Table 2]		
Variable	Z+UC (N = 2488) n (%)	UC (N = 475) n (%)
Gender		
Female	1490 (60.6)	308 (63.0)
Male	968 (39.4)	181 (37.0)
Race		
Caucasian	2236 (91.0)	449 (91.8)
Black	186 (7.5)	31 (6.3)
Other	37 (1.5)	9 (1.8)
History of Tobacco Use		
Yes	855 (34.8)	189 (38.7)
No	1589 (64.7)	299 (61.2)
Unknown	14 (0.6)	1 (0.2)
Allergic Rhinitis		
Yes	1742 (70.9)	343 (70.1)
No	708 (28.8)	144 (29.5)
Unknown	8 (0.3)	2 (0.4)

By comparison with the Integrated Safety Summary, including the first Safety Update, the asthmatic patients in this third safety update showed a slightly higher frequency of Caucasians (SU3 = 91%, ISS+SU = 85%), females (SU3 = 61%, ISS+SU = 55%) and participants in it averaged almost a decade older (SU3 = 43.2 years, ISS+SU = 34.5 years) [4:101]. None of these differences is thought to have an important effect on the safety profile.

OUTCOMES ADVERSE EVENTS

In this study, 93.0% of patients randomized to Z+UC and 86.9% of patients randomized to UC alone experienced at least one AE. The greatest differences in AE frequencies between treatment arms were found for "dyspepsia" and "nausea," which were more common in the Z+UC group, and "aggravation reaction" and "sinusitis," which were more frequent in the UC arm [2:72-3].

MOST FREQUENT AE'S BY COSTART TERM COMPARISON OF ASTHMA PATIENTS REPORTED IN THE 1st AND 3rd SAFETY UPDATES				
Adverse Event (COSTART TERM)	Third Safety Update		First Safety Update (PBO-Controlled)	
	Z+UC (N = 2458) n (%)	UC (N = 489) n (%)	Z (N = 847) n (%)	PBO (N = 491) n (%)
Aggravation Reaction	976 (39.7)	225 (46.0)	232 (27.4)	147 (29.8)
Dyspepsia	407 (16.6)	48 (9.8)	84 (7.9)	14 (2.9)
Nausea	291 (11.8)	28 (5.7)	39 (4.6)	18 (3.7)
Sinusitis	332 (13.5)	84 (19.2)	66 (6.6)	33 (6.7)

Placebo-controlled trials reported in the SU, also found "dyspepsia" to be much more frequent in the zileuton group (Z = 7.6%, PBO = 2.9%), but the other three AE's showed only small inter-group frequency differences [SU 1:145, 147-8]. AE's stratified by age, gender and race were analyzed for interaction with treatment group. Some interactions were found for age and gender, but they did not seem clinically meaningful [2:74-9]. AE's were also categorized by the duration of treatment, at first occurrence [2:79-81, 14:59-72].

FIRST OCCURRENCE OF ANY AE, STRATIFIED BY STUDY DURATION, LEE TABLE [Appendix B.1.6]				
Z+UC	0-3 Months N = 2458 n (%)	3-6 Months N = 1837 n (%)	6-9 Months N = 1593 n (%)	> 9 Months N = 1474 n (%)
		2152 (87.6)	95 (5.2)	19 (1.2)
UC	379 (77.8)	32 (7.3)	7 (1.7)	7 (1.8)
	0-3 Months N = 489 n (%)	3-6 Months N = 436 n (%)	6-9 Months N = 408 n (%)	> 9 Months N = 378 n (%)

This showed that the majority of AE's, for both treatment groups, were reported within the first three months of the study.

PREMATURE TERMINATIONS

A total of 1196 (49.6%) of patients prematurely withdrew from study M94-199. Compared with the UC group, total terminations were almost twice as frequent in the Z+UC group and early terminations because of an AE were almost ten times more frequent in the Z+UC group. Early withdrawals for reasons other than an AE were equally frequent in the two treatment groups. The following table shows the proportion of early terminations in each treatment group attributable to an AE or to a cause other than an AE and confirms this earlier finding in the interim analysis [2.70].

PREMATURE TERMINATIONS BY STUDY GROUP			
Treatment Group	Total Early Terminations n (% of Trt. Grp.)	Reason For Early Termination	
		AE n (% of Trt. Grp.)	Other than AE n (% of Trt. Grp.)
Z+UC (N = 2488)	1089 (43.8)	488 (19.8)	603 (23.7)
UC Alone (N = 489)	127 (26.0)	11 (2.2)	116 (23.7)

The three most frequent AE's leading to early termination in the Z+UC group were "aggravation reaction" (3.7%), "liver function tests abnormal" (2.7%) and "nausea" (2.4%) [2.81-4]. These findings are unchanged from the earlier SU2 report [8/17/95 1:18]. They also generally agree with the most frequent AE's causing premature termination in the zileuton arms of asthma studies reported in the ISS and first SU, which were "aggravation reaction" (5.3%) and "LFT's Abnormal," "AST Increased" or "ALT Increased" (1.0%) [SU 1:211, NDA Review P-140].

DEATHS

A total of five deaths, four in the U.S. (patients #1375, #2389, #3287 and #3558) and one in Latin America (patient #4007) have occurred in this study. Two patients died of status asthmaticus, two attendant to acute myocardial infarctions and one of blunt trauma suffered in an airplane accident. All five deaths occurred in patients from the Z+UC treatment group [2.89]. The 5:1 assignment frequency to the treatment groups, as well as CRF reviews indicating no common mechanism among those who died, point to a lack of evidence that zileuton played any part in the deaths.

HOSPITALIZATIONS

A total of 193 (6.56%) hospitalizations was reported for the 2947 patients in this surveillance study, 159 (6.47%) for the 2458 patients in the Z+UC group and 34 (6.95%) for the 489 patients in the UC arm. Ninety-four of the total of both treatment groups were attributed to asthma exacerbations, 73 (2.97%) in the Z+UC group and 21 (4.29%) in the UC arm [2.91]. Hospitalization was equally frequent in both

treatment groups but "aggravation reaction," as the cause, was more common in the UC arm.

MALIGNANCIES

Thirteen malignancies were reported, 10 (0.41%) in the Z+UC group and 3 (0.61%) in the UC arm. There were six cases of breast cancer, two of basal cell skin cancer, and one each of prostate, testicle, rectum, thyroid and lung cancer. The six breast cancer cases were distributed five in the Z+UC arm and one in the UC group, precisely the 5:1 assignment frequency of patients in the two study arms. The five breast cancers in the Z+UC group occurred after 7 months, 4 months, 3 months, 5 weeks and 2 weeks of zileuton exposure [2:91-7]. These data do not support a link between zileuton and malignancies.

OVERDOSES

There have been six overdoses with zileuton. One was an intentional overdose taken by a sixteen year old female (#3169) who ingested an undetermined amount of the drug, received emergent care and never developed any signs or symptoms. Four patients were reported to have accidentally ingested between two and three extra tablets with their regularly scheduled dose (#2337, #2354, #2722 and #3279). Only one of these four (#2337) developed AE's and they were "sleepiness" and "jitteriness." A single additional patient intentionally took three extra zileuton tablets with her morning dose because she was eager to resume treatment after a short lapse (#1336). Two hours after the dose she reported "dizziness," "blurred vision" and "sleepiness," all of which resolved within ninety minutes. There have also been three instances of children ingesting one tablet of a relative's zileuton supply and none experienced any untoward events [2:97-8].

PREGNANCIES

Despite protocol stipulations requiring contraception, there have been a total of 19 pregnancies in U.S. studies and four in Latin American trials. The pregnancies were detected within an estimated one-to-six weeks of gestation and all patients were prematurely terminated from the study. The outcomes of these 23 combined pregnancies are presented in the table below and the occurrence of congenital anomalies and spontaneous abortions in each of the treatment groups suggests that these may have been random occurrences [2:99-103].

SUMMARY OF PREGNANCY OUTCOMES (Table 18)								
Treatment Group	Premature Birth	Normal Birth	Spont. Abort.	Induced Abort.	Congen. Anomaly	Awalt Birth	Lost To F/U	TOTAL
Z+UC	0	4	3	8	1	1	2	17
UC Alone	1	0	2	1	1	1	0	6

CLINICAL LABORATORY DETERMINATIONS LIVER FUNCTION TESTS

Although mean laboratory values are not usually revealing, the average liver function test values at each visit provide a consistent picture. Baseline mean values showed no significant differences between Z+UC and UC groups. However all of the nine study visits showed higher mean ALT and AST values for the Z+UC arm than for the UC arm. These differences reached statistical significance at 4/9 visits for ALT and at 6/9 visits for AST [2:107].

Shift tables of baseline to maximum post-baseline values of these two enzymes were submitted. One prominent feature, probably attributable to the selection criteria, was the paucity of patient with abnormally high values at baseline. Although the numbers were small, there was a trend toward maximum post-baseline ALT elevations among those patients who were abnormally high at baseline in both Z+UC and UC groups. This was more prominent in the Z+UC arm (sum of lighter shaded cells/155 = 70.3%) than the UC arm (sum of lighter shaded cells/33 = 54.5%), which can be seen in the table below. [2:111]. The tendency for more patients with ALT levels elevated at baseline to have elevated levels during treatment and for zileuton to augment this phenomenon have both been noted before and were reaffirmed by the current study [SU 2:11, 34, NDA Review Pp 184-5, 188]. The sponsor specifically rejected this assertion by using a prospective inferential test in a retrospective-analysis [2:144].

NUMBER (%) OF PATIENTS IN EACH TREATMENT GROUP SHOWING CHANGES FROM BASELINE TO MAXIMUM POST-BASELINE ALT VALUES (Table 22)							
Group	Baseline	Maximum Post-Baseline ALT					Missing
		≤ ULN	1-3xULN	≥ 3-5xULN	≥ 5-8xULN	≥ 8xULN	
Z + UC	≤ ULN (N=2303)	1702 (73.9)	401 (17.4)	42 (1.8)	32 (1.4)	30 (1.3)	98 (4.2)
	> ULN (N=155)	42 (27.1)	104 (67.1)	3 (1.9)	1 (0.6)	1 (0.6)	4 (2.6)
	Total (N=2458)	1744 (71.0)	505 (20.5)	45 (1.8)	33 (1.3)	31 (1.3)	102 (4.1)

NUMBER (%) OF PATIENTS IN EACH TREATMENT GROUP SHOWING CHANGES FROM BASELINE TO MAXIMUM POST-BASELINE ALT VALUES (Table 22)							
Group	Baseline	Maximum Post-Baseline ALT					Missing
		< ULN	1-3xULN	> 3-5xULN	> 5-8xULN	> 8xULN	
UC	≤ ULN (N=455)	334 (73.4)	79 (17.4)	1 (0.2)	1 (0.2)	1 (0.2)	39 (8.6)
	> ULN (N=33)	11 (33.3)	16 (48.8)	1 (3.0)	1 (3.0)	0 (0.0)	4 (12.1)
	Total (N=488)	345 (70.7)	95 (19.6)	2 (0.4)	2 (0.4)	1 (0.2)	43 (8.8)

The sponsor has defined hepatic dysfunction as ALT $\geq 3xULN$. Analysis of eligible patients ('Total Baseline' - 'Total Missing') in the table above without regard to baseline ALT value, showed that the Z+UC group (darker shaded cells, n=109/2358, 4.62%) had over four times the frequency of hepatic dysfunction during treatment as did the UC group (darker shaded cells, n=5/445, 1.11%) [2:115].

The first interim analysis suggested that the frequency and magnitude of abnormal ALT's were both higher in women of older ages and this observation was confirmed by this final report. In females, the frequency of ALT $\geq 3xULN$ increased monotonically with age, by half-decade intervals [2:119]. The interim analysis also showed that ALT increases $\geq 3xULN$ were more common early in the course of treatment, as is demonstrated by the reviewer-synthesized table below. Two-thirds of the ALT elevations $\geq 3xULN$ occurred within 95 days of starting treatment and 61% occurred within the first two months [2:121, 123].

ZILEUTON PATIENTS WITH ALT ELEVATIONS $\geq 3xULN$ DISPLAYED BY EXPOSURE DURATION AT THE TIME OF THE FIRST OCCURRENCE (adapted from Table 27)			
< 95 Days		≥ 95 Days	
Total Eligible	n (%)	Total Eligible	n (%)
2358	72 (3.0)	1758	37 (2.1)
The 1758 patients in the ≥ 95 -days group is a subset of the 2358 patients in the < 95-days group			

A finding of the interim analysis was that some patients experienced initial elevations of ALT $\geq 3xULN$ between two and forty-six days after stopping the drug. This was not rare, having occurred in 8/109 (7.4%) patients who had an increased ALT of this magnitude, and half (4/8) had ALT's $\geq 5xULN$ [2:124-5]. Another finding was that the magnitude of the first ALT elevation was unrelated to the duration of exposure [2:125-6].

A key point in analyses of liver function test abnormalities was whether the risk decreased over time to a negligible value. Presumably, if this were the case,

laboratory surveillance could be reduced or eliminated. The interim report showed an initial two-month peak in the occurrence of abnormal ALT's, which then dropped down to about 0.5% and remained steady through 6-7 months of follow-up [8/17/95 1 39, NDA Review P-189]. This final report combined the data for drug exposures of greater than six months into three categories, 6-7 months, 8-10 months and 11-12 months. The most recent survival rates, calculated by standard methods and verified by teleconference and consultation with Dr. Gebert (FDA Statistician), is presented in the table below [2:121, Table 20, FAXes 9/23-4/96].

LIFE TABLE: PROBABILITY (PER MONTH) OF FIRST OCCURRENCE OF ALT $\geq$ 3xULN IN A SAMPLE OF PATIENTS WHO HAD YET TO DEMONSTRATE AN ELEVATION TO THIS CRITERION				
Visit (Interval)	Z+UC		UC	
	ALT $\geq$ 3xULN n / N	Prob. (1st Occurrence) per Month (%)	ALT $\geq$ 3xULN n / N	Prob. (1st Occurrence) per Month (%)
Days 2-35 (month 1)	43 / 2098	2.01	0 / 441	0.00
Days 36-65 (month 2)	24 / 1868	1.29	1 / 430	0.23
Days 66-95 (month 3)	6 / 1739	0.35	0 / 421	0.00
Days 96-125 (month 4)	6 / 1649	0.49	1 / 413	0.24
Days 126-155 (month 5)	6 / 1568	0.38	1 / 403	0.24
Days 156-215 (months 6-7)	10 / 1484	0.34	0 / 388	0.00
Days 216-305 (months 8-10)	8 / 1386	0.19	2 / 370	0.27
Days 306-365 (months 11-12)	4 / 707	0.28	0 / 203	0.00

The attack rate (probability of first occurrence of ALT $\geq$ 3xULN) in the Z+UC group was highest in the first two months then seemed to stabilize at about 1/3 of a percent per month through the twelfth month. A weak argument could be made for a very slight further reduction after the seventh month, but this could only be verified by more prolonged follow up. In contrast, the UC group showed an attack rate between none and one patient per month. The smaller denominator of the UC group resulted in unit/month numerator changes providing either 0.0% or 0.2% attack rates.

The outcome of these 109 patients was important in evaluating the utility of this laboratory marker of hepatic dysfunction and in developing a management strategy. The protocol for this trial specified that patients with ALT elevations 3-5xULN could continue in the study. They were to be withdrawn only for additional signs or symptoms, or for an increasingly abnormal ALT $\geq$ 5xULN. Sixty-one patients had initial ALT elevations 3-5xULN while taking the study drug and all were followed while continuing to take it. Thirty-two resolved on the study drug and three of these later withdrew for other reasons. Of the 29 who failed to resolve, one patient maintained an ALT in this range for the entire study and 28 patients were withdrawn.

21 because of further elevations in ALT to $\geq 5xULN$. The following table shows the fate of the 109 patients with ALT $\geq 3xULN$.

DISPOSITION OF PATIENTS WITH ALT $\geq 3xULN$						
1st Elevation On/Off Drug	OFF (n=8)		ON (n=101)			
	3-5xULN	5-8xULN	3-5xULN	5-8xULN	8-15xULN	$\geq 15xULN$
n	4	4	61	20	17	3
W/D (all causes)	N/A	N/A	31	20**	17**	3**
Resolved* on Drug	N/A	N/A	32	N/A	N/A	N/A
Mean Days (range)	N/A	N/A	25 (4-176)	N/A	N/A	N/A
Resolved* Drug D/Ced	2	3	28	19	17	2
Mean Days (range)	21 (6-36)	12 (7-16)	29 (1-104)	38 (1-111)	28 (7-80)	33 (26-38)
Unresolved	2	1	1	1	0	1

* "Resolved" was defined as ALT $< 2xULN$
 ** withdrawn per protocol, which specified patients with ALT $\geq 5xULN$ had to terminate prematurely

The magnitude of the initial ALT elevation was not correlated with the time to resolution. The strategy of allowing a patient to continue taking zileuton in the face of ALT of 3-5xULN led to premature withdrawal in 51% (31/61) and had a failure rate because of increasingly abnormal ALT's of 34% (21/61) [2:130-4].

The interim report of this study indicated that the duration of exposure was associated with longer times to resolution of increased ALT values and this final report confirmed it, as shown by the shaded summary cells of the table below. When the initial ALT elevation was $\geq 3xULN$ and occurred within three months of starting treatment, the mean time to resolution was about two and one half weeks. After three months of drug exposure, the mean time to resolution was close to eight weeks. [8/17/95 1:45, NDA Review P-190, 2:135].

TIME TO RESOLUTION OF ALT $\geq 3xULN$ AT DIFFERENT DRUG EXPOSURE DURATIONS AND DIFFERENT MAGNITUDES OF INITIAL ELEVATION [Table 32]				
Magnitude of 1st ALT Elevation	Duration of Drug Exposure at 1st ALT Elevation	N	Mean Time to Resolution (Days)	Time to Resolution Range (Days)
3-5xULN	< 95 Days	17	23.5	1-61
	≥ 95 Days	11	38.5	11-104
5-8xULN	< 95 Days	14	17.6	1-66
	≥ 95 Days	5	95.2	82-111
8-15xULN	< 95 Days	12	11.6	7-20
	≥ 95 Days	5	66.6	42-80

TIME TO RESOLUTION OF ALT $\geq$ 3xULN AT DIFFERENT DRUG EXPOSURE DURATIONS AND DIFFERENT MAGNITUDES OF INITIAL ELEVATION [Table 32]				
Magnitude of 1st ALT Elevation	Duration of Drug Exposure at 1st ALT Elevation	N	Mean Time to Resolution (Days)	Time to Resolution Range (Days)
$\geq$ 15xULN	< 95 Days	0	N/A	N/A
	$\geq$ 95 Days	2	33.0	28-38
ANY $\geq$ 3xULN	< 95 Days	43	18.5	1-81
	$\geq$ 95 Days	23	54.2	11-111

Once again, the magnitude of the initial elevation was not associated with time to resolution within either exposure duration category. However, the effect of duration of drug exposure on increasing time to resolution was present across all categories of magnitude of initial ALT elevation.

A "rebound" phenomenon was noted in three patients in the interim report. This consisted of abnormalities of ALT, sufficient to cause drug withdrawal, followed by declining ALT values which later reversed direction, climbing to new highs in the absence of continued drug ingestion. This final report added a fourth case to this group. Patient #2305 took zileuton for 140 days before elevations in ALT caused early withdrawal. Thirty days later a large rebound occurred with ALT's peaking at $>$ 20xULN. These elevations began returning to normal in twenty more days finally normalizing completely 100 days after premature withdrawal, and 70 days after the rebound began [5:189]. Of these four patients, three exhibited a magnitude of ALT elevation during rebound that was over twice that of the initial elevation. None of these patients completely normalized their ALT's $\leq$ 1xULN before rebounding [5:176, 189, 209, 238].

At our request, the sponsor gathered cases in which ALT $\geq$ 3xULN was associated with total bilirubin $>$ ULN, on the same day. The 8/17/95 interim report included narratives of six patients in this category and the current, final report added a seventh. The new patient was a 28 year-old female (#1453) who first developed AST and ALT elevations on the 290th treatment day that peaked on the 336th treatment day to 6.90 and 13.26xULN, respectively. The highest bilirubin (2.2 mg/dL) occurred on the 94th post-treatment day, at a time when all other liver enzymes were normal. This patient was correctly categorized as having hepatocellular injury and unrelated hyperbilirubinemia [2:128].

Of these seven cases, the maximum total bilirubin value was 2.5 mg/dL (#3212) and frank jaundice was not appreciated. Five of the seven had maximum ALT values $\leq$ 7xULN but the remaining two patients had peak ALT levels of 13 and 29xULN. Three of the seven had plausible alternate etiologies for the combined bilirubin and ALT abnormalities, one had cholelithiasis on ultrasound and two showed

patterns of bilirubin abnormality more consistent with Gilbert's Syndrome, although the ALT elevations were likely attributable to zileuton [2 126-130].

The patterns of liver injury were categorized according to criteria of an International Consensus Committee Meeting, previously referenced. Hepatocellular injury was defined as ALT > 2xULN or a ratio of ALT to alkaline phosphatase $\geq$ 5. Cholestatic injury was defined as alkaline phosphatase > 2xULN or a ratio of ALT to alkaline phosphatase < 2. A mixed pattern was classified when the ALT was > 2xULN and the alkaline phosphatase was > ULN and the ratio of ALT to alkaline phosphatase was between 2 and 5, not inclusive. Using this scheme, 82 patients were classified as hepatocellular (75.2%), 26 were mixed (23.9%) and one had a cholestatic pattern [2 127].

HEMATOLOGIC CELL COUNTS

This final report added only four patients to the 21 reported in the interim analysis who had at least one WBC $\leq$ $2.8 \times 10^9/L$. Of this total, 22 were in the Z+UC arm and 3 were in the UC arm, a few more from the Z+UC group than would be predicted by the 5:1 assignment ratio of the two treatments. Nineteen of the 22 Z+UC patients had one or two low values that subsequently normalized or reversed toward the normal range while on the study drug. Of the three patients who had not normalized or reversed, one (#2438) entered the study with low values that remained low, reversing toward normal only when the treatment drug was discontinued. Another patient (#1015) had an isolated low value at the final visit that subsequently normalized. The third (#1362) had a normal WBC at entry that dropped within the first month of treatment, normalizing when treatment was stopped and the patient withdrawn. Premature terminations for leukopenia, thrombocytopenia and anemia were unchanged since the interim report [2:180-8]. It appeared that effects on hematology parameters were not an important safety problem.

R. F. Anthracite, M.D. 10/23/96

Raymond F. Anthracite, M.D.
Medical Review Officer

cc:
NDA #20-471
HFD-570\Division Files
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HFD-570\CSO\Kuzmik

[Signature]
10/28/96

*Concise -
The data on hFT
monitoring generated from
this study will need to
be reflected in product
labeling.*
[Signature] 10/15/96

NDA #20-471

DRUG NAME:

Zileuton

PROPOSED TRADE NAME:

Leutrol

CHEMICAL NAME:

N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea

SPONSOR:

Abbott Laboratories

CATEGORY:

5-Lipoxygenase Inhibitor

INDICATION:

Maintenance Therapy for Asthma

DOSAGE FORM:

Film-coated Tablet

MEDICAL REVIEWER:

Raymond F. Anthracite, M.D.

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NOTE TO READERS

Square brackets are used throughout this review as references to original NDA volumes and pages, FAX communications and separate submissions. The forms that these reference take are: [VOL:PAGE] for the original NDA; [FAX Date VOL:PAGE] for FAXes; and, [Submit. Date VOL:PAGE] for separate submissions. Several volumes and/or pages may be placed in one set of brackets with an organization like the following: [VOL:PAGE, PAGE-PAGE, VOL:PAGE-PAGE].

CONDUCT OF THE REVIEW

Preliminary results of early protocols indicated that this drug was likely to show efficacy, but that disturbing liver function test elevations were increasingly being reported. Because of these early suggestions of possible safety concerns, this review began with the Integrated Safety Summary. Four months after the original NDA submission, the first Integrated Safety Update was submitted. This was incorporated into the integrated safety review.

The two pivotal trials M91-685 and M92-720 were next reviewed and these represent about one quarter of the entire review. Important corroborative studies were chosen, in consultation with the Group Leader, and a total of nine of these were reviewed. Nine additional pharmacokinetic studies were also reviewed. From these twenty studies, an Integrated Efficacy Summary was constructed. Background material in chemistry manufacturing and control, pre-clinical pharmacology and human pharmacokinetics was summarized as a preamble to the clinical section of this review.

The entire portfolio of information on each patient who terminated prematurely because of an adverse event (AE) in each of the two pivotal trials was reviewed. This included all case report forms, individual demographic data sheets, laboratory results, symptom scores, concomitant medications, peak flows, adverse events and all other patient diary entries of fifty-five patients occupying twenty volumes of the NDA report [1007-1026: all pages]. No important discrepancies from the summary reports were identified.

A total of seven deaths occurred during all of the protocols for this NDA. Six of these were detailed in the original NDA submission [968:all pages, 969:all pages] and the last was submitted with the first Safety Update [SU 26:1-117]. No important discrepancies from the summary reports were identified.

In order to verify that the data analyzed corresponded to the data found in the case report forms (CRF's), records of patients who had died or terminated prematurely due to an AE were scrutinized more thoroughly. A sample of data found in the CRF's was cross-checked against line listings that should have been faithful reproductions of the former. No discrepancies between data found in CRF's and data reproduced in line-listings was identified.

Following the Pulmonary Allergy Drug Advisory Committee meeting on 10 April 1995, the sponsor submitted a second, but non-integrated, safety update (SU2) on 28 July 1995. Preliminary results from a long-term safety surveillance study of over 1000 patients who had been exposed to

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zileuton for ≥ 6 months were reported 17 August 1995. Both the SU2 and preliminary results from the surveillance study were incorporated into this review following the Integrated Summary of Safety.

A retrospective review of the references provided for this NDA was performed to identify the major volumes from which the bulk of data for this report was drawn. This is an exhaustive list of 80 volumes cited, but does not represent every volume reviewed:

VOLUME CITATIONS IN THIS NDA	
Submission	Volumes
ORIGINAL NDA (12/19/94)	1-3, 136, 141, 143-6, 148-50, 162, 167, 176, 179, 180, 183-6, 193-5, 240, 241, 249, 250, 299, 302, 309, 320, 321, 388-91, 397, 405-7, 412, 968, 969, 1007-1026
SAFETY UPDATE 1 (11/18/94)	1, 2, 5, 14, 18, 23, 24, 26
AMENDMENT (11/22/94)	1
AMENDMENT (2/16/95)	1
FAX (3/3/95)	n/a
AMENDMENT (4/17/95)	1, 2
IND (3/24/95)	1
SAFETY UPDATE 2 (7/20/95)	1, 12
M94-199 Interim Report (8/17/95)	1, 2

An attempt was made to verify the data trail between the original laboratory values and the line listings provided in the NDA. Two investigators were chosen for each of the two pivotal trials based on the number of patients studied and the size of effect found at each site. Four patients were identified at each investigative site who had been randomized to the higher zileuton dose (600 mg QID). Longitudinal data for each patient at each site on FEV_{1,0}, WBC, ALT and GGT were tabulated and the DSI investigators were asked to verify these tabular results against original laboratory reports, on site. Pivotal trials, associated sites/investigators and patient numbers are presented below:

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STUDY	INVESTIGATOR/SITE	PATIENT NUMBERS
M91-685	Lazarus/San Francisco	#1305, #1306, #1308, #1312
	Pleskow/Encinita	#1061, #1064, #1176, #1628
M92-720	Fink/Milwaukee	#1149, #1322, #1324, #1327
	Seltzer/San Diego	#1044, #1606, #1610, #1611

Verified, selected laboratory values have not been received from any investigator/site. Thus far Fink/Milwaukee has been declared as "no action indicated" (NAI). Seltzer/San Diego and Pleskow/Encinita have been judged as "voluntary action indicated (VAI) no response required." Errors were noted at each site and the largest ones are scrutinized below.

Seltzer failed to report 6 very minor AE's in 5 patients to the sponsor. Review of case report forms and source documents at the Pleskow site showed 29 transcription errors made in the case report forms of 10 patients. Thirteen of 29 data points, derived from 5 of the 10 patients sampled, were presented in the DSI audit review. All were for the variables peak expiratory flow rate and FEV₁₀ and most were numerical inversions too small in magnitude to have effected overall results. Two of the 5 patients had taken zileuton 600 mg QID; 2 had taken zileuton and one had taken placebo. In 7/13 total instances, lower CRF values were recorded than were shown in the source document [195:161-2].

The DSI audit was never completed on investigator Lazarus, at the San Francisco site. The three audits that have been completed have identified only small errors. Moreover, these discrepancies were apparently not biased toward any of the study arms in terms of magnitude of the numbers and discrepancy directions that have been found. Based on the results of the DSI audits that have been completed as well as the findings by this reviewer of concordance between CRF's and line listing, we find data quality to be suitable for analysis. The fourth and last DSI audit will not be required and will be cancelled.

CHEMICAL MANUFACTURING AND CONTROL [1:109-14]

Chemical Name: N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea
Designations: Abbott-64077, ABT-077, Code 74975, USAN designated as zileuton
Molecular Formula: C₁₁H₁₂N₂O₂S
Molecular Weight: 236.29

Zileuton is manufactured at Abbott Laboratories' facilities in North Chicago, Illinois and at Laboratori Abbott facilities in Campoverde, Italy. Zileuton is a white to off-white crystalline material which is practically odorless. The drug substance is practically insoluble in water and hexane, slightly soluble in chloroform, sparingly soluble in acetonitrile. It is soluble in methanol

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and ethanol, and freely soluble in N,N-dimethylformamide. Zileuton is practically insoluble in acidic and neutral aqueous solutions. However, in basic aqueous solutions, the solubility is significantly increased to about 70 mg/mL at pH 12.

The drug is synthesized as a racemic mixture. No optical rotation has been detected in any bulk drug lots and all bulk drug lots had an R/S isomer ratio of 1.00 ± 0.01 . Zileuton can exist as two crystal forms. X-ray diffraction patterns have shown a mixture of anhydrous and monohydrated forms. Both forms have the same solubility in water. The monohydrate form can be converted to the anhydrous form by drying the sample and the moisture level of the bulk drug is controlled to produce predominantly the anhydrous form.

Zileuton is proposed for marketing as 300 mg, and 600 mg film-coated tablets. Tablets of all three strengths are of the same composition, differing only in compression weight and tablet tooling design. The tablets will be manufactured, packaged and labeled by Abbott Laboratories at the Abbott Park, Illinois or Barcelona, Puerto Rico facilities.

Based on the results of stability studies, a 24 month expiration date is proposed. The sponsor recommends tablet storage at 15-30 degrees Celsius in a well-closed container and protected from light.

PRECLINICAL PHARMACOLOGY/TOXICOLOGY [1:128-30]

Arachidonic acid, derived from membrane phospholipids, can lead to the biosynthesis of several oxygenated products called eicosanoids. These include prostaglandins and thromboxanes derived from cyclooxygenase and hydroxylated products 5-HETE, 12-HETE AND 15-HETE synthesized by distinct lipoxygenases. The enzymatic action of 5-lipoxygenase on arachidonic acid produces leukotrienes. Inhibition of 5-lipoxygenase prevents the biosynthesis of downstream products including 5-HPETE and the intermediate leukotriene A₄ (LTA₄). This latter compound is enzymatically hydrated to LTB₄, or conjugated with glutathione, to produce LTC₄. Subsequent cleavages convert LTC₄ to LTD₄ and LTE.

Zileuton is an inhibitor of 5-lipoxygenase. Inhibition appears to result from binding to the enzyme in addition to an interaction with active site iron by the n-hydroxyurea functionality within the molecule.

In cell lysates, zileuton inhibited the formation of 5-HETE in a time-dependent manner. Potency of inhibition was dependent on substrate concentration implying a competitive mechanism. When tested against cyclooxygenase, 12-lipoxygenase and 15-lipoxygenase, zileuton was weaker by two orders of magnitude than against 5-lipoxygenase. Zileuton was 40-100 fold more potent in inhibiting LTB₄ formation than in inhibiting 12-HETE or thromboxane B₂ (TXB₂).

Zileuton is a racemate with two enantiomers. In some models, the R(+) enantiomer was somewhat more potent than the S(-) enantiomer, but this difference was not statistically reproducible in all models. Oral dosing with both enantiomers produced similar degrees of leukotriene inhibition.

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Several *in vivo* and *in vitro* models showed anti-inflammatory activity. No protection against smooth muscle contraction, caused by several agonists, was seen suggesting that the activity of zileuton did not operate by the mechanism of non-specific bronchodilation. Some safety concerns were generated by preclinical studies. Prostatic and testicular atrophy was seen in young and mature dogs. Anti-androgenic properties of zileuton were evaluated for receptor binding affinity to prostatic androgen receptors from a variety of species. The zileuton racemate and its enantiomers bound with weak affinity *in vitro* to soluble androgen receptor from prostate glands of dogs, rats, monkeys and man. The highest affinity was found in dog prostate which was found to be many fold weaker in humans than in dogs.

Subacute toxicity was found in mice and rats. Increased liver weights and centrilobular hepatic cytomegaly in mice were considered to be secondary to hepatic enzyme induction. Hepatocellular vacuolation was also seen. Rats showed centrilobular hepatic cytomegaly, also considered to be secondary to liver enzyme induction. Elevations in alanine aminotransferase (ALT) and spontaneous renal disease with tubular epithelial cell proliferation were also seen in this species.

Evidence of chronic toxicity was found in rats and dogs. Rats were given up to 300 mg/Kg/day for six months by oral gavage. Increased liver weights and hepatocellular hypertrophy were seen at all dose levels. Proteinuria and increased incidence of more severe nephropathy were observed with the highest doses. Dogs were given up to 200 mg/Kg/day for six months producing plasma levels as high as 12 times the proposed clinical exposure. Glycosuria, ketonuria, increased LFT's (alkaline phosphatase, GGT), anemia, thrombocytopenia and neutropenia, renal tubular cellular changes, prostatic and testicular atrophy were all seen.

Embryotoxicity, peri- and post-natal toxicity, mutagenicity and carcinogenicity will be dealt with in depth by the pharmacologic reviewers. In brief, hepatic and kidney tubular adenomas and combined adenomas/carcinomas were seen in female mice. Female mice also showed an unusually high incidence of hemangiosarcomas, mostly ovarian in origin. Rats showed a significant and dose-related increased incidence benign testicular Leydig cell tumors. Increased tubular adenomas and adenocarcinomas were found in rats of both sexes.

HUMAN PHARMACOKINETIC (PK) AND BIOAVAILABILITY [1:216-20]

The pharmacokinetics of zileuton have been investigated in healthy male volunteers after administration of single and multiple doses of both capsule and tablet formulations. No abnormal accumulation of zileuton was observed after multiple dosing. The PK up to 2400 mg/day seemed linear. The estimated mean PK parameters of zileuton at steady state after administration of the recommended dose of 600 mg QID in healthy humans were taken from study M93-034 where 12 subjects supplied the following data.

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PARAMETER	MEAN $\pm$ SD
C_{Max} (mcg/mL)	6.0 $\pm$ 1.0
T_{Max} (hours)	2.3 $\pm$ 0.6
C_{Min} (mcg/mL)	1.8 $\pm$ 0.6
$AUC_{0-\infty}$ (mcg $\cdot$ hr/mL)	20.8 $\pm$ 3.4
CL/F (mL/min)	493 $\pm$ 79
$T_{1/2}$ (hours)	2.5 $\pm$ 0.3
Vd/F (L)	93 $\pm$ 19

Due to low aqueous solubility of zileuton, intravenous dosing could not be used to determine the absolute bioavailability in humans so an estimate was based on ^{14}C -zileuton study results. The fraction crossing the gastrointestinal barrier as unchanged drug was at least 85% and at least 40% of the dose was extracted and metabolized by the liver during the first pass.

Zileuton undergoes extensive hepatic metabolism. After single oral administration of ^{14}C -zileuton to healthy subjects, 94.5% of the radioactivity was recovered within 7 days in urine and only 2.2% was found in the feces. The major urinary metabolites in humans were approximately equal amounts of R(+) and S(-) zileuton glucuronides, designated G-1 and G-2, respectively. Unchanged zileuton and the N-dehydroxylated derivative of zileuton in the urine during clinical studies each accounted for less than 0.5% of the dose.

The presence of food after administration of a 600 mg zileuton tablet (final formulation) resulted in a significant (27%) increase in C_{Max} without significant changes in AUC or T_{Max} . The sponsor considers the effect of food on bioavailability to be negligible.

Special population studies showed that the PK of zileuton was essentially the same in males and females, healthy elderly (age > 65 years) subjects and younger (age 18-40 years) volunteers and was independent of race. Renal impairment did not effect the PK of zileuton, but the plasma clearance was reduced by half in subjects with hepatic impairment. The PK was found to be similar in healthy volunteers, asthmatics and patients with rheumatoid arthritis.

Interaction studies showed that the AUC of theophylline, propranolol and antipyrine approximately doubled following coadministration of zileuton, and that the prothrombin times during warfarin therapy increased after concomitant zileuton administration. Concomitant use of terfenadine and zileuton resulted in a significant increase in C_{Max} and AUC, but the QTc interval was relatively unchanged. Other interaction studies showed insignificant interactions between zileuton and sulfasalazine, naproxen, digoxin, phenytoin and prednisone.

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M91-685 "Phase III Study of the Effects of Zileuton and 600 mg QID and Placebo in Moderate Asthma"

OVERVIEW [184:26]

This was a pivotal Phase III study of the effects of zileuton, 600 mg, tablet QID or placebo in asthmatics with mild, moderate or severe airway obstruction. It was a randomized, double-blind, parallel-group, placebo-controlled, multi-center, 14-week trial. The study consisted of a 10-day, single-blind placebo lead-in period and a 13-week double-blind treatment period.

STUDY POPULATION [184:35]

Patients who met the 18-item study admission criteria for the single-blind period were enrolled in the 10-day lead-in period. After completing the single-blind period, those who met four additional selection criteria for the double-blind period were randomized.

SINGLE-BLIND PERIOD SELECTION CRITERIA [184:44]

1. Male or non-pregnant, non-lactating female, age 18 to 60 inclusive. Women must have been of non-childbearing potential, either post-menopausal or surgically sterile (including tubal ligation), or using oral contraceptives, barrier method contraceptive, intrauterine device or Norplant. Male participants must have agreed to use effective contraception throughout the study and for 90 days after discontinuing study drug. It was preferable that both partners use birth control. In addition, women who were less than two years post-menopausal, those with tubal ligations and those using IUD, barrier method, Norplant, or oral contraceptives were required to have a pregnancy test, the results of which must have been negative.
2. No clinically significant abnormalities detected on a 12-lead electrocardiogram.
3. Weight must have been within -20% to +30% of the values in the amended Metropolitan Life table for men and women.
4. FEV₁₀ of 40 to 80% of predicted when taken at least 48 hours after the last theophylline use and at least 8 hours after last beta-agonist use.
5. Reversibility of airway obstruction demonstrated by $\geq 15\%$ increase in FEV₁₀ from the best baseline effort when tested at least 30 minutes after two puffs of inhaled albuterol.
6. No hospitalization for asthma within the last month or more than once within the last 6 months.
7. Not taken an experimental drug within the preceding 30 days, or participated in a prior study with zileuton.
8. Patient did not take any medication on a chronic basis other than inhaled beta-agonists, and had not taken any of the following medications within the times specified before Study Day #1:
 - a. oral beta agonists and ipratropium bromide aerosol for ≥ 12 hours
 - b. anticholinergic eye drops for ≥ 24 hours

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- c. theophylline products and long-acting antihistamines $\geq$ 48 hours
 - d. hydroxyzine for $\geq$ 4 days
 - e. short-acting antihistamines for $\geq$ 48 hours before any Study Visit, otherwise allowed throughout the entire study
 - f. aspirin and other NSAID's for $\geq$ 1 weeks
 - g. systemic corticosteroids, inhaled cromolyn or inhaled corticosteroids for $\geq$ 4 weeks
 - h. astemizole for $\geq$ 3 months
 - i. coumadin or phenytoin for $\geq$ 2 weeks
 - j. alcohol intake limited to $\leq$ 2 ounces/day during the study
 - k. caffeine-ingestion to remain constant throughout the study; none allowed on Study Visit Days until the visit had been completed
9. No inhaled nasal steroids for $\geq$ 2 weeks, unless on a stable dose for $>$ 6 weeks which could be continued throughout the study.
 10. No history of allergy or adverse reaction to drugs similar to study medication.
 11. No psychiatric illness or active drug or alcohol abuse.
 12. No clinically significant findings or laboratory test abnormalities except those related to asthma (clinically significant CBC components defined as $<$ 90% of lower normal limit; liver tests defined as $>$ 1.5 of upper limit of normal for enrollment [186:32]).
 13. No history of cardiac, renal, hepatic, neurologic, endocrine or metabolic disease.
 14. Any immunotherapy $\geq$ 1 month, unless on stable dose for $>$ 3 months which could be continued throughout the study.
 15. Patient signed informed consent.
 16. Non-smoker for $>$ 1 year with total smoking history $<$ 10-pack-years; cigar, pipe and marijuana smoking prohibited.
 17. Able to withhold albuterol inhaler use for $\geq$ 8 hours before each Study Day Visit.
 18. Able to complete patient diaries and perform peak flow measurements as required.

ALLOWABLE CONCOMITANT THERAPY [184:27]

All medication use, including beta-agonist rescue medication, over-the-counter medication and other specifically allowed medication, were to be recorded in the patient diary.

1. oral contraceptives
2. estrogen replacement
3. thyroid replacement
4. inhaled nasal steroids, except as above
5. allergen immunotherapy, except as above
6. short-acting antihistamines, except as above
7. courses of systemic corticosteroids for exacerbations

SINGLE-BLIND, LEAD-IN PERIOD [184:35]

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Patients who met the 18 selection criteria listed above began taking their placebo lead-in medication and recording daily, nocturnal, and asthma symptoms, inhaled beta-agonist use, and PEF-R's in their diaries at the beginning of Study Day 1, the first day of the single-blind period.

DOUBLE-BLIND PERIOD SELECTION CRITERIA [184:47]

- a. complied with dosage regimen during 10-day, single-blind period by taking $\geq 75\%$ of medication (determined by tablet count) and having completed the diary in an acceptable manner
- b. best of three FEV₁₀ values at the end of the single-blind period was to be $\geq 40\%$ but $\leq 80\%$ of predicted
- c. total symptom score on the Daily Symptom Assessment Scale of at least 10 but not greater than 25 during the 10-day, single-blind period (Daily Symptom Scale is 0-3, so maximum, 10-day score is 30)
- d. used beta-agonist rescue medication inhaler on at least 20 separate occasions during the 10-day, single-blind period

DOUBLE-BLIND, TREATMENT PERIOD [184:35-6]

Patients who met the four additional selection criteria listed above were randomized to either zileuton, or 600 mg QID, or placebo. They were then administered the initial dose of the double-blind medication at the study site and remained there for at least six hours following the dose. The initial dose was given in the morning. Prior to dosing and at 30, 60, 120, 180, 300 and 360 minutes following the initial dose, the FEV₁₀ and FVC were taken in triplicate.

Study visits were scheduled on Study Day 18 (Double-Blind Day 8) and approximately every 14 days thereafter through Study Day 102 (Double-Blind Day 92). Pulmonary function tests were performed prior to morning dosing and serially over a six-hour period at Weeks 2, 6, 10 and 14 (Double-Blind Days 8, 36, 64 and 92). A beta-agonist inhalation test was performed at Week 12 (Double-Blind Day 78).

On Double-Blind Days 22 and 50, the first daily dose of study medication was taken by the patient before coming in for the visit. On these days pulmonary function testing was to be carried out before the second dose of the oral treatment was taken; i.e., within 360 minutes of the first dose of the treatment drug. Compliance with study medication was monitored by tablet counts and plasma concentrations of zileuton.

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STUDY SCHEMATIC - M91-685										
Study Day	Screen	1	11	18	32	46	60	74	88	102*
Double-Blind Day		-10	1	8	22	36	50	64	78	92*
Week			1	2	4	6	8	10	12	14*
* ...or date of last visit for premature terminations.										
1 Pulmonary function was done through 240 minutes post beta-agonist.										
2 Pulmonary function was done prior to and 30, 60, 120, 180, 240, 300 and 360 minutes post-dosing.										
3 Samples were collected from patients at selected sites for PK/PD testing.										

A subset of patients enrolled at selected centers underwent pharmacokinetic and pharmacodynamic (PK/PD) testing. These patients had PFT measurements and blood samples collected over a single dosing interval on Study Day 11 and Study Day 102 (Double-Blind Days 1 and 92). PFT's included FEV₁₀, FVC and PEFr and were completed immediately prior to and at 30, 60, 120, 180, 240, 300 and 360 minutes post-dosing. Additionally, blood samples were taken for the determination of plasma zileuton and whole blood LTB₄ levels, immediately prior to dosing and at 60, 120, 180, 240 and 360 minutes post-dosing. Samples collected for those determinations immediately prior to the first scheduled dose were collected within 10 minutes prior to that dose. The samples collected at the last designated time point were collected immediately prior to the administration of the second scheduled dose; i.e., immediately prior to the dose at 1300 hours.

Endocrine tests included FSH and LH levels for all patients. Testosterone levels were determined for all males and estradiol levels for all females [186:32].

Patients were given a diary at each visit at which study drug was dispensed. These diaries were used to record a variety of data [186:34]:

- a. time and amount of any medication ingestion, including regular, allowed, therapies and OTC remedies
- b. any unusual experiences (these will be reviewed by investigators at each visit to determine if they should be reported as AE's)
- c. AM (before first daily dose of study medication) and PM (3 hours after the third daily dose of study medication) domiciliary PEFr's [184:50]
- d. time of each rescue medicine (albuterol) use and the number of puffs
- e. Nocturnal Assessment scores (0-3), each morning [184:51, 186:53]
 - 0 = slept well; no asthma symptoms
 - 1 = spent restless and may have awakened because of asthma symptoms.; may have used inhaler
 - 2 = awakened more than once because of symptoms; used inhaler
 - 3 = awake all night because of symptoms; used inhaler

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- f. Daily Symptom Assessment score (0-3), each evening [184:51, 186:53]
- 0 = no symptoms at all; unrestricted activity
 - 1 = symptoms occurred, but with little or no discomfort; unrestricted activity
 - 2 = symptoms occurred, were sometimes annoying or affecting routine activity (e.g., dressing, walking)
 - 3 = symptoms severe -- very little improvement after inhaler use and requires the use of additional medication and/or doctor's visit
- g. Asthma Symptom Assessment -- evening assessment of four asthma symptoms, wheezing, shortness of breath, cough and nasal congestion, each on a three-point scale [184:51-2, 186:54]
- 0 = absent; no symptoms
 - 1 = symptom is frequently troublesome, but not sufficient to interfere with normal daily activity or sleep
 - 2 = symptom is sufficiently troublesome to interfere with normal daily activity or sleep
 - 3 = symptom is so severe as to prevent normal activity and/or sleep

COMMENT: Asthma Symptom Assessment required that the patient rate overall symptoms of wheezing, shortness of breath, cough and nasal congestion, for the past 24 hours. Interpreting changes in this index must address the confusing issue of nasal congestion as a valid asthma endpoint.

SAMPLE SIZE [186:44]

Calculations were based on a previous one-month study of zileuton and placebo in asthma, M90-460, where a standard deviation of 0.48 L. for the change from baseline in FEV₁₀ was found. It was estimated that approximately 100 patients per treatment group would be required to provide > 90% power at the two-sided 0.05 level for a pairwise comparison between a zileuton group and the placebo group, assuming that the expected difference between these two groups is 0.25 L. for the change from baseline in FEV₁₀.

This estimated sample size requirement assumes that the sample size is equally distributed across all sites, e.g., approximately 9 patients per 33 treatment sites. If more than half of the sites have an enrollment of 6, it is necessary to enroll a total sample size of approximately 360 in order to maintain 90% power.

COMMENT: This pivotal trial was "powered" for a previously discovered mean difference of zileuton from placebo, and for the variance of this difference. This begs the entire question of clinical significance and the degree of difference from placebo that zileuton should show to achieve this clinically meaningful effect.

STATISTICAL ANALYSES [186:40-3]

Primary Efficacy Variable:

FEV₁₀ at the scheduled study visits; more specifically, the percent change from baseline to the pre-dosing measurement at the final treatment visit

Secondary Efficacy Variable:

PEFR

Other Efficacy Variables:

- a. FVC
- b. average daily rescue medicine use (number of puffs and number of occasions)
- c. daily symptom assessment score
- d. nocturnal symptom assessment score
- e. asthma symptom assessment score
- f. response to beta-agonist inhalation
- g. frequency of acute asthma exacerbations

Baseline values were those determined at the end of the placebo lead-in phase for most variables. An average over the ten days of the lead-in period was used as the baseline for daily diary data; e.g., beta-agonist use and symptom scores. For pulmonary function measurements, changes from baseline to the pre-dosing determination at Weeks 2, 6, 10, 12 and 14 were calculated. Changes from baseline were to be compared among treatment groups using a two-way ANOVA model with effects for investigator, treatment and investigator-treatment interactions. The primary comparison was placebo vs. zileuton 600 mg QID and no adjustments for multiple comparisons between pairs of treatment groups was to be made.

Efficacy analyses for "all data" were based on all data: i.e., no exclusions (intent-to-treat). Additional efficacy analyses based on "evaluable data" were also performed, and are separately identified [184:62].

PATIENT ACCOUNTABILITY [184:76-7]

Four hundred and eighty-eight patients entered the study and 401 of these entered the double-blind treatment phase of the trial. These 401 were distributed as follows:

- 132 received 600 mg zileuton QID
- 134 received zileuton QID
- 135 received placebo

Eighty-one patients discontinued prematurely leaving 320 patients who completed the 13-week double-blind treatment phase of the study (79.8%).

BASELINE PARAMETERS [184:83-4]

There were no statistically significant differences for mean baseline airflow variables between either zileuton group, 600 mg QID QID, and the placebo group. No significant difference between groups was found for various symptom scores and measures of beta-agonist use.

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CHRONIC "TROUGH" CHANGE IN FEV_{1,0} [184:85-9]

On Double-Blind Days 8, 36, 64, 78 and 92 pulmonary function measurements were made before the morning dose of study medication. This corresponded to the "trough" plasma level of zileuton. The pre-dosing PFT's at each visit were performed after withholding the blinded study medication and any other medications; i.e., albuterol and short-acting antihistamines.

Each zileuton group demonstrated a greater mean improvement in trough FEV_{1,0} from baseline to each extended visit day than did the placebo group as can be seen in the graph below:

FIGURE 1 [184:86]

These between-group, differences from baseline only achieved statistical significance on Double-Blind Days 8, 92 and "final," and only for the 600 mg QID zileuton group compared with placebo. The QID zileuton group did not achieve statistically significant difference from placebo at any time point for this primary efficacy measure.

Mean percent changes from baseline in trough FEV_{1,0}, averaged over the five visits, were 11.5%, 9.2% and 5.2% for the zileuton 600 mg QID, the zileuton QID and placebo groups, respectively. The comparison between zileuton 600 mg QID and placebo was statistically significant for this average. The zileuton QID vs. placebo comparison did not achieve significance.

The mean values of FEV_{1,0} at baseline, at the final visit and the percent change, for each treatment group, are displayed in the table below. The average percent change over the five visits is also displayed [184:87].

TROUGH EFFECT BASED ON MEAN CHANGES AND MEAN PERCENT CHANGES FROM BASELINE IN FEV _{1,0} FOR ALL PATIENTS					
Visit	Treatment Group	Number	Mean Baseline (L)	Mean Change (L)	Mean Percent Change (%)
Final Visit	Z600	127	2.35	0.25	13.44
	Placebo	132	2.26	0.09	4.98
Average Of 5 Visits	Z600	127	2.35	n/a	11.49
	Placebo	132	2.26	n/a	5.24

The mean change for the zileuton 600 mg QID group was significantly different from placebo at the final visit. The zileuton dose did not achieve statistical significance from placebo for mean FEV_{1,0} change from baseline to the final visit. The mean percent change from baseline to final visit

and to the average of the five visits was also statistically significant for the zileuton 600 mg QID dose, relative to placebo. Again, the zileuton dose was not significantly different from placebo for the percent change from baseline to either final visit or average of the five visits.

COMMENT: The percent change calculated from the mean change and the mean baseline is not the same as the mean percent change. The latter is calculated by taking the mean of each individual percent change.

Subset analyses of chronic trough $FEV_{1,0}$ for patients with severe (<50% predicted $FEV_{1,0}$ at baseline), moderate (50% to 70%), and mild asthma at baseline (>70%) demonstrate that patients with more severe asthma had a greater magnitude of response, showing a 32.5% improvement over baseline to final visit for the zileuton 600 mg QID group, 29.3% improvement for the zileuton ID group and 11.5% for the placebo group. There was not a statistically significant interaction between subsets and treatments, however, both zileuton doses were significantly different from placebo in the "severe" baseline disease subset and only in that subset.

FIGURE 2 [184:89]

COMMENT: The primary efficacy variable was identified as the trough $FEV_{1,0}$ and this does, indeed, show a significant difference from placebo at some intervals for the 600 mg QID dose. This subset analysis, based on baseline disease severity, shows that virtually all of this effect on trough $FEV_{1,0}$ is in the patients who have "severe" asthma before entry into the active treatment period. Patients with "moderate" or "mild" disease appear to have trough $FEV_{1,0}$ values that are virtually indistinguishable from one treatment group to the next.

CHRONIC "PEAK" CHANGE IN $FEV_{1,0}$ [184:90-4]

The chronic effect of zileuton was also assessed by serial 6-hour pulmonary function measurements at Double-Blind Days 8, 36, 64 and 92. The "peak" improvement in pulmonary function was determined by the 2-4 hour post-dosing average of pulmonary function values and comparing this average to the pre-double-blind baseline.

By Double-Blind Day 8, patients receiving zileuton 600 mg QID demonstrated a 17.98% (0.37 L.) mean increase over pre-double-blind dosing for the 2-4 hour post-dose average. This peak mean increase was maintained at 13.66% or higher during the 14 weeks of treatment. Statistically significant differences between the zileuton, 600 mg QID, group and placebo in terms of peak post-dosing effect were seen on Days 8, 36, 92 and at the final visit. Only Day 64 did not reach statistical significance for the 600 mg QID zileuton group over placebo. At the final visit, the mean peak $FEV_{1,0}$ showed a mean absolute change from the pre-double-blind baseline of 0.32, 0.27 and 0.18 L for the zileuton 600 mg QID, zileuton QID and placebo groups, respectively. The

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zileuton. group failed to achieve significant difference from placebo for mean change or mean percent change at any of the study days.

FIGURE 3 [184:92]

PEAK EFFECT BASED ON MEAN CHANGES AND MEAN PERCENT CHANGES FROM BASELINE IN FEV _{1,0} FOR ALL PATIENTS AT THE FINAL VISIT					
Visit	Treatment Group	Number	Mean Baseline (L)	Mean Change (L)	Mean Percent Change (%)
Final Visit	Z600	126	2.35	0.32	16.44
	Placebo	129	2.26	0.18	8.87

COMMENT: Both the trough and peak mean FEV_{1,0} change were relative to very similar baselines, the pre-double-blind period, and the two can be compared. The zileuton 600 mg QID group trough FEV_{1,0} showed a mean baseline of 2.35 L and a mean change at the final visit of 0.25 L (sum=2.60 L). This compares with the mean peak baseline of 2.35 L and a mean change of 0.32 L at the final visit (sum=2.67 L). This places the mean change for the zileuton 600 mg QID dose from trough to peak at the final visit of about 0.07 L. Comparable values for the trough placebo mean baseline and mean change at the final visit are 2.26 L and 0.09 L (sum=2.35 L). The peak placebo mean baseline and mean change at final visit are 2.26 L and 0.18 L (sum=2.44 L). Therefore, the mean change for the placebo group from trough to peak at the final visit is about 0.09 L. This implies that chronic dosing with zileuton 600 mg QID had a salutary effect on the trough FEV_{1,0} that did not further improve 2-4 hours after the morning dose at the final visit, compared with placebo, although the mean peak FEV_{1,0} (baseline + change) at final visit was about 0.23 L higher in the zileuton 600 mg QID group than in the placebo group.

ACUTE BRONCHODILATORY (FIRST-DOSE) EFFECT [184:95-9]

A 6-hour series of pulmonary function measurements was obtained after the administration of the first dose of study medication on Double-Blind Day 1. The first dose of zileuton produced bronchodilation and reached its maximum effect between 2 and 4 hours after ingestion. Thirty minutes following administration of a single 600 mg dose, mean FEV_{1,0} values improved, compared to pre-dosing baseline, and remained increased through six hours of observation. The zileuton 600 mg dose achieved statistically significant difference from placebo for the 2nd through the 4th hours and for the 2-4 hour average over all 7 periods, both as mean change and as mean percent change.. From the following graph it can be seen that the maximum effect of the 600 mg dose is at 3 hours

and that the increase in FEV₁₀, in excess of placebo, was < 6% (Z600=13.36, P=7.51) and, in absolute terms, was 0.12 L. (Z600=0.28 L, P=0.16 L). The dose was not statistically different from placebo at any time during the serial first-dose PFT measurements, for any measure.

FIGURE 4 [184:96]

Subset analyses of the first dose effect for patients with severe (<50% predicted FEV₁₀), moderate (50% to 70%) and mild asthma (>70%) suggest that the difference between zileuton 600 mg QID and placebo was greatest for the severe subset and smallest for the mild subset for both percent and mean changes. However, no statistically significant differences between either zileuton dose and placebo was achieved in any subset.

FIGURE 5 [184:98]

PEAK EXPIRATORY FLOW RATES [184:99-103]

Another pulmonary function test shows evidence of zileuton efficacy over placebo. This is the morning (AM) and evening (PM) peak expiratory flow rate (PEFR) which was performed twice daily, in triplicate, and recorded in the patient diary. Analyses are based on bi-weekly averages.

The zileuton 600 mg QID group demonstrated statistically significantly greater mean improvements from baseline compared to the placebo group in the daily AM and PM PEFR's. These improvements were observed within the first 15 days (first interval) and were maintained throughout the 3 month trial.

The maximum improvement for the zileuton 600 mg QID group in AM PEFR occurred for visit Days 72-85 when the mean PEFR increased by 20.21 L/min over baseline, a 6.56% mean increase over baseline in peak flow. This was 15.99 L/min and 5.05% in excess of the mean change from baseline shown by the placebo group. Statistically significant differences from placebo were found at five of seven intervals for this largest zileuton dose. There was no statistically significant differences between the zileuton QID group and the placebo group for mean changes from baseline in AM PEFR's during any interval. The following line graph illustrates these points.

FIGURE 6 [184:100 top]

Statistically significant differences were seen in the mean changes from baseline in PM PEFR for all visit intervals, when the zileuton 600 mg QID group was compared to the placebo group. The maximum mean improvement from baseline in PM PEFR for this largest dose occurred at the 86-106 day biweekly interval. The PM PEFR was 21.53 L/min, a 5.93% mean increase over baseline peak flow. This represented 17.35 L/min and 4.4% increases in excess of placebo. The PM

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PEFR's for the QID zileuton group was statistically different from placebo only for the first biweekly interval (Visit Days 2-15), as is shown in the following graph.

FIGURE 7 [184:100 bottom]

Values for AM and PM PEFR, mean baseline and mean change averaged over all seven measurement intervals for all three treatment groups are presented in the table below.

AVERAGE OF ALL SEVEN MEASUREMENT INTERVALS OF AM AND PM PEFR's				
PEFR	Treatment Group	Number	Mean Baseline (L/min)	Mean Change (L/min)
AM	Z600	129	372.06	18.15
	Placebo	132	365.71	4.91
PM	Z600	129	418.23	18.71
	Placebo	132	412.25	2.64

RESPONSE TO BETA-AGONIST INHALATION TEST [184:103-4, 185:56-7, 193:215-6]

The improvement in FEV_{1.0} after administration of beta-agonist was assessed at Single-Blind Day 1 and Double-Blind Day 78. On Single-Blind Day 1, 30 minutes after beta-agonist administration, the mean percent change in FEV_{1.0} was 34.45%, 33.07% and 33.32% for the zileuton 600 mg QID group, QID group and for placebo, respectively. On Double-Blind Day 78, the mean response at 30 minutes post beta-agonist was 24.32%, 28.11% and 27.87% for the zileuton 600 mg QID group, QID group and the placebo group, respectively. A reviewer synthesized table follows.

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FEV _{1,0} RESPONSE TO INHALED BETA-AGONIST BASED ON PERCENT CHANGE FROM BASELINE TO POST-INHALATION, AT SEQUENTIAL SAMPLING TIMES							
Time Since Beta-Agonist Dose	Group	Single Blind Day 1			Double Blind Day 78		
		N	Baseline Mean	Mean % Change	N	Baseline Mean	Mean % Change
30 minutes	Z-600	110	2.30	34.45	110	2.59	24.32
	Placebo	104	2.36	33.32	103	2.53	27.87
60 minutes	Z-600	107	2.29	36.25	110	2.59	24.26
	Placebo	104	2.36	33.33	104	2.53	29.15
120 minutes	Z-600	109	2.29	32.14	109	2.59	20.09
	Placebo	104	2.36	29.37	103	2.53	24.30
180 minutes	Z-600	109	2.29	24.80	110	2.59	15.37
	Placebo	102	2.36	24.75	104	2.53	17.62
240 minutes	Z-600	109	2.33	19.17	107	2.58	10.33
	Placebo	102	2.34	18.16	101	2.55	11.23
0 - 4 hour maximum	Z-600	110	2.30	38.81	110	2.59	27.90
	Placebo	104	2.36	37.34	104	2.53	31.94

There were no statistically significant differences in the bronchodilatory effect between either zileuton group (600 mg QID) and placebo. The response to beta-agonist on Double-Blind Day 78 was slightly lower in all three treatment groups than that seen on Single-Blind Day 1, in the same groups. The sponsor ascribes this to an overall chronic improvement in pulmonary function over the course of the trial, leaving less room for improvement.

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SYMPTOM ASSESSMENT [184:104-14]

DAILY SYMPTOM ASSESSMENT [184:104-7]

Patients receiving zileuton 600 mg QID exhibited a statistically significantly larger mean improvement in their daily symptoms than did patients receiving placebo. Symptoms were measured daily, on a four-point scale (0-3, 'none' to 'severe'), and the values were included in the patient diary. Baseline mean daily symptom scores ranged from 1.41 to 1.46 across treatment groups. During the first visit interval (Days 1-15 of Double-Blind treatment) the mean daily symptoms decreased statistically significantly more from baseline in the zileuton 600 mg QID group (-.31, -20.9%) than in the placebo group (-.15, -10.8%). A similar statistically significant difference between the zileuton 600 mg QID group and the placebo group was seen at all visit intervals except Days 16-29. There were no statistically significant differences between the zileuton QID group and the placebo group for mean changes from baseline in Daily symptoms at any time interval.

FIGURE 8 [184:105]

The four-point Daily Symptom Assessment score was averaged over all seven intervals and compared with baseline. This comparison also showed a statistically significant difference between the zileuton 600 mg QID group and placebo, but not the zileuton dose and placebo. The percent change in this table is not the mean percent change of individuals, but is the mean change as a percent of the baseline.

DAILY SYMPTOM ASSESSMENT, MEAN CHANGE AND PERCENT CHANGE FROM BASELINE AVERAGED OVER SEVEN INTERVALS FOR ALL PATIENTS				
Treatment Group	Number	Mean Baseline	Mean Change	Percent Change
Z600	129	1.46	-0.40	-27.6
Placebo	132	1.42	-0.23	-16.1

COMMENT: The average mean change in Daily Symptom Score from baseline, in excess of placebo was -0.17 (Mean Change From Baseline: Z600 = -0.40, Placebo = -0.23). It is uncertain how to translate this into an estimate of clinical improvement in excess of placebo. The average difference between the zileuton 600 mg QID group and the placebo group, -.17, is only 5.6% of the entire three points of the symptom score range of 0-3 scale points.

Subset analysis for patients with severe, moderate and mild flow obstruction at baseline showed that the reduced symptom score of the zileuton 600 mg QID treatment, compared to placebo, was

significant for patients with severe asthma. Qualitative evidence of a dose-proportional effect was present in the severe group. The two other subsets, mild and moderate, showed virtually identical percent changes in daily symptoms for all treatments.

FIGURE 9 [184:107]

NOCTURNAL ASSESSMENT SCORES [184:108-11]

Nocturnal assessment scores were also collected each morning in the daily diary using a four-point scale (0-3, 'slept well' to 'awake all night'). Baseline mean nocturnal symptoms were mild in all groups with scores ranging from 0.90 to 0.93. Differences between the zileuton 600 mg QID group and the placebo group for mean changes from baseline in nocturnal assessment scores were statistically significant for Days 30-43, 58-71 and 72-85 when the zileuton 600 mg QID group decreased by 0.27-0.30 and the placebo group decreased by 0.10-0.13. The zileuton QID group was not significantly different from placebo at any interval, nor for the average of all intervals.

Subgroup analysis of baseline asthma severity echoed previous findings of greater improvement of the zileuton 600 mg QID group compared with placebo, in severe disease. Over a total of 7 intervals, 3 showed a statistically significant difference between zileuton 600 mg QID and placebo groups. The zileuton QID group was not statistically different from the placebo group during any interval.

ASTHMA SYMPTOMS [184:111-13]

The four separate components of the asthma symptom score were cough, nasal congestion, shortness of breath and wheezing which were measured daily on a four-point scale (0-3, 'absent' to 'severe'). Of the seven biweekly intervals, zileuton 600 mg QID was statistically significantly different from placebo for "cough" in 4/7. Differences between the highest zileuton dose and placebo for "shortness of breath" achieved statistical significance in 3/7 intervals. There was one statistically significant difference between the zileuton 600 mg QID group and the placebo group for "nasal congestion" at Days 86-106 (1/7 intervals). There was no statistically significant difference between zileuton 600 mg QID and placebo for "wheeze" in any biweekly interval. There were no statistically significant differences between the zileuton QID group and the placebo group for mean changes from baseline, for any asthma symptom assessment, for any interval.

ACUTE ASTHMA EXACERBATIONS [184:114-16]

Asthma exacerbation information was collected at each visit. An exacerbation was prospectively defined as such by a patient's complaint of worsening of symptoms and by exhibiting one or more of the following parameters: 1) an increase from the previous visit in inhaler use by $\geq 33\%$ of the number of occasions of use; 2) a decrease from the previous visit in the best of three FEV₁₀ measurements by $\geq 20\%$; or, 3) a decrease from the previous AM PEF_R by $\geq 25\%$.

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Percentages of patients with at least one exacerbation that occurred during the Single-Blind period did not differ significantly between treatment groups. During the Double-Blind period, 34.8% of placebo patients experienced at least one acute exacerbation of asthma, versus 36.2% of patients taking Z-600 QID and 26.8% in the zileuton 600 mg QID group. This was not a statistically significant comparison of either zileuton dose with placebo. When broken down by visit at which the exacerbation was diagnosed, in only 1/7 was the zileuton 600 mg QID group statistically different from placebo. The zileuton 600 mg QID dose was not statistically different from placebo during any interval, or over the entire Double-Blind period. A reviewer-synthesized table is presented below to depict these relationships [185:66].

NUMBER (%) OF PATIENTS EXPERIENCING ≥ 1 EXACERBATION OF ASTHMA, IN THE PRE-VISIT INTERVAL, BY TREATMENT GROUP			
Visit	Z-600 N (%)	Z-600 N (%)	Placebo N (%)
During Single-Blind Period	17 (13.0)		21 (15.6)
Day 8	5 (3.9)		10 (7.8)
Day 22	15 (12.1)		14 (11.2)
Day 36	13 (10.6)		8 (6.8)
Day 50	4 (3.4)**		13 (11.2)
Day 64	11 (9.6)		11 (10.6)
Day 78	7 (6.5)		5 (4.8)
Day 92	6 (5.9)		9 (9.1)
During Double-Blind Period	34 (26.8)		46(34.8)
** Significantly different from placebo ($p < 0.05$).			

For acute exacerbation of asthma, subset analyses for patients with severe ($FEV_{1.0} < 50\%$ predicted), moderate (50% to 70%) and mild ($> 70\%$) asthma show qualitative differences that were most marked in the severe baseline group. In this subset, zileuton 600 mg QID was associated with a fewer percentage of patients having ≥ 1 asthma exacerbation than was placebo, but this didn't achieve statistical significance. In fact, no zileuton dose was statistically different from placebo in any baseline severity subset.

CONCURRENT ASTHMA MEDICATIONS [184:116-24]
BETA-AGONIST USE [184:116-20]

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The zileuton 600 mg QID group showed a statistically significantly greater decrease from baseline than did the placebo group in the average daily number of puffs of beta-agonist usage starting at Days 30-43, and the difference remained statistically significant to the end of the study. There were no statistically significant differences between the zileuton QID group and the placebo group for mean changes from baseline in the average daily number of puffs used. A reviewer-synthesized table follows that shows the changes in number of puffs of beta-agonist as an average of all intervals, for zileuton, 600 mg QID QID, and placebo groups. Only the zileuton 600 mg QID group was significantly different from the placebo group for the mean change in number of puffs of rescue medication used, averaged over all intervals.

MEAN CHANGE FROM BASELINE FOR BETA-AGONIST AVERAGE DAILY USAGE (NUMBER OF PUFFS) FOR ALL PATIENTS				
Interval	Treatment	N	Mean Baseline	Mean Change
Average Over All Intervals	Z-600	129	5.88	-1.54
	Placebo	132	6.18	-0.82)

For the endpoint of beta-agonist usage, subset analyses for patients with severe ($FEV_{1.0} < 50\%$ predicted), moderate (50% to 70%) and mild ($> 70\%$) baseline asthma were performed. The graph below indicates that the reduction in beta-agonist use, comparing zileuton to placebo treatment, was greatest for patients with more severe asthma at baseline. A dose-proportional reduction in the use of beta-agonist was seen in both moderate and severe groups. The single statistically significant difference was found in the comparison between the zileuton 600 mg QID treatment and placebo in the severe group.

FIGURE 10 [184:119]

CORTICOSTEROID USE [184:120-4]

Patients who experienced a worsening of their asthma symptoms were permitted a single course of oral or injected corticosteroids. Approximately 11% of the patients who were randomized required oral or injected corticosteroid treatment during the study. Forty-six patients received at least one course of steroids during the trial; 8 (6.1%) in the zileuton 600 mg QID group, 17 (12.7%) in the zileuton QID group and 21 (15.6%) in the placebo group. There was a statistically significant difference between the placebo group and the zileuton 600 mg QID group for the

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percentage of patients requiring corticosteroid rescue medication. No difference between zileuton, QID and placebo groups for this endpoint were found.

Subset analyses, stratified for baseline disease severity, indicates that the percentage of patients requiring corticosteroid rescue was less with zileuton 600 mg QID than placebo and that this effect was most marked in the severe group of baseline asthmatics as is shown in the table below.

PERCENTAGE OF PATIENTS REQUIRING ≥ 1 CORTICOSTEROID TREATMENT(S), STRATIFIED BY BASELINE PERCENT OF PREDICTED FEV_{1,0}			
Severity	Treatment	Total N	n (%)
Severe (FEV _{1,0} < 50% predicted)	Z-600	30	2 (6.7)**
	Placebo	32	11 (34.4)
Moderate (FEV _{1,0} 50%-70% predicted)	Z-600	56	4 (7.1)
	Placebo	66	7 (10.6)
Mild (FEV _{1,0} > 70% predicted)	Z-600	46	2 (4.4)
	Placebo	37	3 (8.1)
** Statistically different from placebo (p < 0.05).			

QUALITY OF LIFE [184:125-130]

The Asthma Quality of Life Questionnaire measures four domains (activity, symptoms, emotion, and environmental exposure) and was administered at the Screening visit and on Double-Blind Days 36 and 92 of the study. Individual domains, and overall scores ranged from 1 = 'worst' to 7 = 'best'. Patients receiving zileuton 600 mg QID had a significant improvement in their overall quality of life with a mean improvement of 0.78 over baseline, as compared to 0.37 in patients receiving placebo. The Minimal Important Difference (MID) correlates with clinical significance and is defined as, "the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management." The MID for the overall quality of life score and for each of the domains is a change in score of 0.5 per item. A change in score of approximately 1.0 is considered a moderate improvement and a change > 1.5 is considered a large improvement. The mean improvement in the quality of life score for the zileuton 600 mg QID group exceeded the MID

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in all four domains and the overall quality of life score. The mean change from baseline for those patients receiving placebo approached the MID of 0.5 only for the symptom domain, and not in any of the other domains, or the overall quality of life score.

Results of the analysis of mean changes from baseline to the final visit in the scores for the four quality of life domains and in the overall score are presented below in tabular form.

MEAN CHANGES FROM BASELINE TO FINAL VISIT (DOUBLE-BLIND DAY 92) IN QUALITY OF LIFE SCORES FOR ALL PATIENTS				
Variable	Treatment	N	Mean Baseline	Mean Change
Symptoms	Z-600	120	4.10	0.89**
	Placebo	119	3.93	0.49
Activities	Z-600	119	4.48	0.65**
	Placebo	118	4.41	0.28
Emotions	Z-600	120	3.99	0.84**
	Placebo	119	3.96	0.32
Environment	Z-600	120	3.98	0.76**
	Placebo	119	4.03	0.30
Overall Score	Z-600	120	4.20	0.78**
	Placebo	119	4.11	0.37
** Significantly different from placebo (p < 0.05).				

There were no statistically significant differences between the zileuton placebo group and the

QID group and the

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COMMENT: Looking at the MID another way, there is only one domain in which the zileuton 600 mg QID group has an average score > 0.5 units higher than the placebo group, and that is "emotions," where the difference from placebo is 0.52. This barely achieves the criterion of clinically important for one domain. The other three domains and the overall score never achieve the MID, as a difference from placebo.

PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) [184:130-6]

The subset of patients who underwent PK/PD testing consisted of 65 patients (20 in the zileuton 600 mg QID group, 24 in the zileuton _____ QID group and 21 in the placebo group). Four patients in the zileuton 600 mg QID group did not complete the study. Six patients in the zileuton _____ QID group prematurely terminated from the study and samples from three other patients were collected on Double-Blind Day 92 but never received at the analytical site. Seven patients in the placebo group did not complete the study and another patient did not participate in the PK/PD testing on Double-Blind Day 92. This left 16 patients in the zileuton 600 mg QID group and 15 patients in the zileuton _____ QID group.

The peak plasma concentrations and the areas under the plasma concentration-time curves during the dosing interval were dose proportional. The mean apparent plasma clearance was similar in the two dosing groups, with a study-wide mean of approximately 550 mL/min. The mean values of some of the zileuton PK/PD parameters are listed in the following table.

PK PARAMETERS FOR THE ZILEUTON GROUPS ON DAYS 1 AND 92 FOR ALL PATIENTS WHO PARTICIPATED IN THE PK/PD TESTING		
Parameters	Z-600	
	Day 1 (N = 20)	Day 92 (N = 16)
T _{Max} (hr)	1.8 ± 1.2	1.8 ± 0.9
C _{Max} (mcg/mL)	5.50 ± 1.51	5.89 ± 1.67
AUC ₀₋₆ (mcg-hr/mL)	15.99 ± 3.90	18.33 ± 4.66
AUC _{0-∞} (mcg-hr/mL)	17.95 ± 3.65	
C _{average} (mcg/mL)	2.66 ± 0.65	3.06 ± 0.78

The percent inhibition of LTB₄ biosynthesis was directly related to zileuton concentration in plasma. This relationship was not affected by differences in a patient's body weight, age, gender, race, smoking history or severity of disease. The zileuton plasma concentration at which 50% of the maximum inhibition of the LTB₄ biosynthesis in whole blood is observed (IC₅₀) was estimated to be equal to 0.47 mcg/ml. Peak zileuton plasma levels were associated with mean LTB₄ inhibition

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of 98% and 92% at steady-state for the 600 mg and zileuton groups, respectively. Trough drug levels at the beginning of the dosing interval were associated with mean inhibition of 40% and 38% at steady-state for the 600 mg and zileuton groups, respectively.

The improvement in FEV_{1,0} in patients who received the 600 QID zileuton dose was immediate, starting within 30 minutes after the first dose and continuing throughout the 6-hour post-dosing interval. The mean time to achieve maximum percent improvement in FEV_{1,0} was 2 to 3 hours later than the time to reach maximum zileuton concentration in plasma after the first dose or maximum LTB₄ inhibition. This suggests a time lag to reach the site of action in the lung. After chronic administration, the improvement in FEV_{1,0} was relatively constant throughout the dosing interval despite large fluctuations between peak and trough zileuton levels or LTB₄ inhibition.

COMMENT: LTB₄ inhibition is virtually the same for both doses of zileuton, as is change in FEV_{1,0}. This seems at odds with the lesser effect of the lower zileuton dose on FEV_{1,0} that is reported throughout this study. A question arises about how representative is this PK/PD sample.

COMPLIANCE MONITORING [184:185-7]

The compliance rate for pill counts was over 75% for all three treatment groups. No zileuton levels were detectable for 4 zileuton patients and 2 placebo patients had isolated instances of detectable zileuton levels. The reason for these results was attributed to plasma tube mislabelling. Overall, the zileuton plasma concentration in each group was proportional to the zileuton dose, both at trough and 2-hours after the first morning dose on double-blind days 1, 8, 64 and 92. Two patients had positive blood theophylline levels. Patient #1172 from the placebo group had a positive test on the first double-blind day. Instructions were given to eliminate theophylline medication and subsequent tests were negative. Patient #1402 from the zileuton 600 mg QID group had a positive test on the first double-blind Days 1, 36 and 92. At least one of these positive tests was attributed to Primatene tablets.

CONCURRENT MEDICATIONS [184:185, 194:172-208]

The distributions of use of various prescription and over-the-counter medications among the three treatment groups was reviewed. These inter-group comparisons were made for individual drugs, as well as for classes of drugs; e.g. H₁-receptor antagonists, anticholinergic drugs, autonomic agents, etc. There was no apparent difference in the distribution of drug use between treatment groups, whether by generic name of the individual drug or by class.

SUMMARY OF EFFICACY RESULTS

The primary efficacy variables, mean change and mean percent change from baseline in trough FEV_{1,0} at each visit, was significantly higher in the zileuton 600 mg QID dose than in placebo at the 1st, 5th and final visits. Significance was not obtained on the 2nd, 3rd or 4th visits with this highest zileuton dose. The mean change and mean percent change for the zileuton 600 mg QID dose

at the final visit were 0.25 L and 13.44%. Comparable values for the placebo group were 0.09 L and 4.96%. The highest zileuton dose, at the final visit, showed a mean change of 0.16 L and a mean percent change of 8.48% difference from placebo. The mean percent change in FEV_{1,0} from baseline, averaged over the all visits, supported the significant difference between placebo and the zileuton 600 mg QID dose. The zileuton QID dose was not significantly different from placebo at any visit, including the final visit.

Analyses of these data in three subsets, derived by the severity of baseline airflow obstruction, was revealing. Virtually all of the improvement in trough FEV_{1,0} from baseline to final visit that distinguished the two doses of zileuton from placebo was evident only in the "severe" baseline asthma subset. Both zileuton doses were significantly different from baseline in this subset of severe flow obstruction.

The change in peak FEV_{1,0} from the pre-double-blind period was measured at 2, 6, 10 and 14 weeks, with serial spiromgrams. The peak FEV_{1,0} was determined by averaging the 2, 3 and 4 hour post-dose FEV_{1,0} values after the morning administration of the treatment drug. No beta-agonist was involved in this. Statistically significant differences from the pre-double-blind period baseline, compared with placebo, were found for this peak FEV_{1,0} at weeks 2, 6 and 14, and at the final determination in the zileuton 600 mg QID group. The zileuton QID dose failed to show significant difference from placebo in peak FEV_{1,0} at any interval. An estimate of the difference between average trough and peak FEV_{1,0} was < 100 mL. This indicates that after chronic dosing with zileuton there was little further improvement in FEV_{1,0} 2-4 hours after taking the first morning dose.

A series of spiromgrams were performed on Double-Blind Day 1, before and for six hours after the first single dose of study medication was given. All treatment groups showed a post-dose rise in FEV_{1,0} that was first measured at 30 minutes. The zileuton 600 mg QID dose was statistically different from placebo in the 2nd, 3rd and 4th hours. The zileuton QID dose was not significantly different from baseline at any time point in this acute effect analysis. Further analyses by subsets of baseline asthma severity showed that a dose proportional effect between the three treatment groups was qualitatively apparent in the moderate and severe baseline asthma groups. This acute effect was modest, relative to a distinct placebo response. The difference between the highest zileuton dose and placebo, in mean change from baseline, was always $\leq$ 0.13 L and was not statistically significant in any subset.

Domiciliary PEFR's, done daily in AM and PM, were each averaged biweekly. The zileuton 600 mg QID dose was significantly better than placebo for 5/7 AM and 7/7 PM biweekly averaged PEFR's and for the average of all 7 intervals for both AM and PM PEFR. Averaged over all intervals, the mean change in the zileuton 600 mg QID dose from placebo was $\leq$ 16.07 L/min for both AM and PM PEFR's. In contrast, the zileuton QID dose was significantly different from placebo only for PM PEFR in the 2 to 15 day (1st) of 7 intervals and did not achieve statistical significance for the average of all intervals in either the AM or PM.

Beta-agonist-induced airflow improvement was determined at baseline and after 78 days of active treatment. There was virtually no difference between groups in mean baseline FEV_{1,0} or mean percent change following beta-agonist. By the 78th double-blind day, with similar baseline FEV_{1,0} values, the placebo group had a greater mean percent change than did the zileuton 600 mg QID group. However, the post-bronchodilator FEV_{1,0} on the 78th day, was higher in the zileuton 600 mg QID group than in the placebo treatment group. This represented a reversal of their relative positions on the 1st (baseline) day when the post-bronchodilator FEV_{1,0} was higher in the placebo group than in the highest zileuton dose group. The sponsor suggests that potential "room for improvement" may explain some of these findings. Daily Symptom Assessment scores, on a four point scale (0-3), were used to show a significant difference between the change from baseline in the three treatment groups at sequentially increasing treatment durations. The zileuton 600 mg QID group was significantly different from placebo at all but one sampling interval, to the 86-106 days interval. As an average over all intervals, the Daily Symptom Assessment mean change of the zileuton 600 mg QID dose was -0.40 and it was -0.23 for the placebo group, a symptom score difference of 0.17 scale points. No significant difference from placebo was found for the zileuton QID dose. Further analysis showed that the only significant difference was between the zileuton 600 mg QID dose and placebo in the severe subset. Nocturnal Assessment scores and Asthma Symptom Assessment scores were corroborative.

The endpoint of number of patients suffering at least one acute asthma exacerbation did not offer a statistically compelling distinction between the three groups.

The reduction in concurrent use of beta-agonist rescue medication statistically distinguished the zileuton 600 mg QID dose from placebo. Subset analyses showed the this reduction in beta-agonist use was most apparent and statistically significant in individuals who had severe asthma at baseline. The zileuton QID group showed no statistically significant difference from placebo. Reduction in courses of oral or injectable corticosteroids was also significantly different in zileuton 600 mg QID and placebo groups. No difference between placebo and the zileuton QID dose group was found.

Quality of Life scores supported a statistically significant difference between placebo and the zileuton 600 mg QID groups, for the overall score and for each of the four domains. However, the MID, expressed as a difference from placebo and the zileuton 600 mg QID groups, was achieved only for the domain of "emotions." No significant difference between placebo and zileuton QID groups was found.

Various PK/PD data were briefly reviewed. The more extensive review of the biopharmacologists will cover this area in greater depth. LTB₄ inhibition was similar in both zileuton dose groups. Neither whole blood LTB₄ inhibition nor serum zileuton levels corresponded closely with the trough or peak FEV_{1,0}.

SAFETY SUMMARY [184:137-81]

Over the course of the study, 488 patients enrolled, 401 entered the double-blind phase and 320 patients completed the study. Treatment-emergent AE's were defined as being reported after the onset of the double-blind treatment had begun and within 14 days after the treatment ended. No deaths were reported during this clinical trial, however, one death was reported for a patient prior to entrance in the study.

Treatment emergent AE's were reported by 99 (75.0%) of patients treated with zileuton 600 mg QID, 99 (73.9%) treated with zileuton QID and 109 (80.7%) of patients who received placebo. The most common over all three groups and in no particular order were: aggravation reaction, flu syndrome, headache and infection.

A total of 45 patients experienced one or more AE's which were classified as severe by the investigator. There were 14 patients (10.6%) in the zileuton 600 mg QID group, 13 (9.7%) in the zileuton QID group and 18 (13.3%) in the placebo group. The frequency distribution of these severe AE's, by COSTART term, was similar to previous reports. Specifically, none of these severe AE's involved abnormal liver function tests or hepatitis and only 6 of the AE's were referable to the GI tract: abdominal pain, abdominal cramp, nausea, nausea/vomiting and dyspepsia (2 cases).

Forty of the patients reporting an AE were prematurely terminated, at least in part, as a result of that AE: 12 (9.1%) patients in the zileuton 600 mg QID group, 12 (9.0%) in the zileuton QID group and 16 (11.9%) in the placebo group. The zileuton 600 mg QID group had two patients with leukopenia (minimum WBC 3.6 and $3.5 \times 10^9/L$) and two with abnormal liver function tests. Two patients with elevated liver function tests were from the zileuton QID group. No early termination because of leukopenia or abnormal liver function tests occurred in the placebo group. Asthma exacerbations were scarce in the zileuton 600 mg QID group (2), more common in the zileuton QID group (5) and most frequent in the placebo group (12).

Five serious AE's resulting in hospitalization, for reasons other than asthma exacerbations, were reported: pyogenic arthritis from trauma while rock-climbing (#1165, Z600), abortion (#1409, surgery for knee injury from a skiing accident (#1189, P), severe depression requiring lithium (#1406, P) and concussion while playing football (#1626, P). Four additional patients were hospitalized due to exacerbations of their asthma.

There were no statistically significant differences between zileuton 600 mg QID group and the placebo group for baseline endocrine parameters. Analyses of mean changes from baseline to various time points in endocrine determinations for male patients revealed no statistically significant differences between either zileuton group and the placebo group. Post-menopausal females with endocrine evaluations numbered only 3, so conclusions drawn from this group are limited. However, there were baseline differences between pre- and post-menopausal women at baseline for LH. Pre-menopausal females showed a significant decline in LH and estradiol from baseline values [184:180].

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STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN TREATMENT GROUPS FOR MEAN CHANGES FROM BASELINE IN ENDOCRINE PARAMETERS IN FEMALES					
Population	Test	Treatment	Number	Mean Baseline	Mean Changes
Pre-Menopausal Females	LH (mu/mL)	Placebo	55	6.21	0.68
		Placebo	55	62.55	10.0
Post-Menopausal Females	FSH (mu/mL)	Z600	3	118.80	-10.27
		Placebo	1	145.20	-91.40
	LH (mu/mL)	Placebo	1	23.80	-12.20

Alterations in male endocrine function were the focus of this investigation because of pre-clinical information. These changes in endocrine parameters of females are of unknown clinical significance.

CONCLUSION

Over virtually all primary and secondary efficacy variables, the zileuton 600 mg QID dose did appear to have a modest effect that was more salutary than the placebo effect. This effect was most apparent in the most severe baseline asthmatics and, depending upon the exact efficacy variable discussed, the effect may have been entirely limited to the severe subset. Virtually no statistically significant findings support the efficacy of the zileuton QID dose in this trial.

Treatment emergent AE's, those that were severe and premature terminations from the clinical trial because of them, were all fairly evenly divided between the three treatment groups. However, the early discontinuation because of leukopenia or abnormal hepatic enzyme elevations were phenomena that were unique to the two groups exposed to zileuton and this is a worrisome safety concern.

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M92-720 "Phase III Study of the Effects of Zileuton QID and 600 mg QID and
Placebo in Moderate Asthma"

OVERVIEW [240:24]

This is a pivotal Phase III study of the effects of zileuton or 600 mg tablets QID or placebo in asthmatics with mild, moderate or severe airway obstruction. It is a randomized, double-blind, parallel-group, placebo-controlled, multi-center, 26-week trial. The study consists of a 10-day, single-blind placebo lead-in period and a 6-month double-blind treatment period. Much of this protocol is very similar, if not identical to the trial design of the other pivotal, Phase III study, M91-685. Differences from, and similarities to, M91-685 are described under the appropriate subject headings.

STUDY POPULATION [240:40]

Patients who met the 18-item study admission criteria for the single-blind period were enrolled in the 10-day lead-in period. After completing the single-blind period, those who met four additional selection criteria for the double-blind period were randomized.

SINGLE-BLIND PERIOD SELECTION CRITERIA [240:41-3]

This was almost completely identical to the criteria outlined in the review of pivotal Phase III trial M91-685. The following are criteria that are unique to the present trial and are different from the criteria referenced in M91-685:

- a. patients with body weights > 20% above the limit of the amended Metropolitan Life tables are proscribed (instead of limiting the acceptable range of -20% to +30%)
- b. FEV₁₀ reversibility, following bronchodilator challenge, is performed "at least 15 minutes after two puffs of inhaled albuterol" (instead of "at least 30 minutes...")
- c. a history of oncologic disease was disqualifying (a new requirement)

ALLOWABLE CONCOMITANT THERAPY [240:53-4, 49]

These were also almost completely identical to the criteria of the other pivotal, Phase III trial, M91-685. The only addition was that antibiotics were considered to be an allowable, short-course treatment for acute exacerbations of asthma.

SINGLE-BLIND, LEAD-IN PERIOD [240:34-5]

Patients who met the single-blind selection criteria began taking their placebo lead-in medication and recording daily, nocturnal, and asthma symptoms, inhaled beta-agonist use, and PEFr's in their diaries at the beginning of Study Day 1, the first day of the single-blind period.

DOUBLE-BLIND PERIOD SELECTION CRITERIA [240:43-4]

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These criteria are identical to those found in M91-685 except that an additional criterion has been added in this study:

“women who were less than two years post-menopausal, those who had tubal ligations, and those who were using IUD, barrier method, Norplant, or oral contraceptives were required to have a negative urine pregnancy test that was confirmed by a serum test”

DOUBLE-BLIND, TREATMENT PERIOD [240:35-6]

Patients who met the double-blind selection criteria were randomized to either zileuton, QID or 600 mg QID, or placebo. They were then administered the initial dose of the double-blind medication at the study site and remained there for at least six hours following the dose. The initial dose was given in the morning. Prior to dosing and at 30, 60, 120, 180, 240, 300 and 360 minutes following the initial dose, the FEV₁₀ and FVC were taken in triplicate.

Study visits were scheduled on Study Day 18 (Double-Blind Day 8) and approximately every 28 days thereafter through Study Day 186 (Double-Blind Day 176). Pulmonary function tests were performed prior to morning dosing and serially over a six-hour period at Weeks 14 and 26 (Double-Blind Days 92 and 176). At Weeks 2, 6, 10 and 18 (Double-Blind Days 3, 36, 64 and 120) pulmonary testing was performed prior to dosing only. A beta-agonist inhalation test was performed at Single-Blind Day 1 and at Week 22 (Double-Blind Day 148) and pulmonary function tests were performed prior to and through 240 minutes post beta-agonist inhalation.

Blood zileuton levels were drawn at Weeks 1, 2, 14 and 26 both before the first morning dose of the treatment drug and just before the 120-minute, post-dosing PFT was performed. Compliance with study medication was monitored by tablet counts and plasma concentrations of zileuton. Patients were given a diary at each visit at which study drug was dispensed. These diaries were used to record a variety of data, as in the other pivotal, Phase III study [240:47-50].

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured for all patients at Double-Blind Days 1, 92 and 176 (or the final visit, if the patient terminated prematurely). In addition, testosterone levels were determined for all males and estradiol levels for all females.

STUDY SCHEMATIC - M92-720										
Study Day	Screen	1	11	18	46	74	102	130	158	186*
Double-Blind Day		-10	1	8	36	64	92	120	148	176
Week			1	2	6	10	14	18	22	26*
Informed Consent	X									
Medical/Asthma History	X									
Physical Examination	X ¹						X ²			X ²

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STUDY SCHEMATIC - M92-720										
Study Day	Screen	1	11	18	46	74	102	130	158	186*
Double-Blind Day		-10	1	8	36	64	92	120	148	176
Week			1	2	6	10	14	18	22	26*
* ...or date of last visit for premature terminations.										
1 height and weight										
2 weight only										
3 if not performed within 12 months										
4 PFT's measured before and at 30, 60, 120, 180, 240, 300 and 360 minutes post-dose										
5 PFT's measured before administration of AM dose (0700) at study center										
6 determination of percent reversibility										
7 PFT's will be done through 240 minutes post beta-agonist inhalation										
8 urine and serum pregnancy tests										
9 serum pregnancy test only										
10 zileuton levels to be drawn prior to dosing and prior to 12-0 minute PFT										

SAMPLE SIZE [240:69]

Calculations were based on a previous one-month study of zileuton and placebo in asthma, M90-460, where a standard deviation of 0.48 L. for the change from baseline in FEV₁₀ was found. It was estimated that approximately 100 patients per treatment group would be required to provide > 90% power at the two-sided 0.05 level for a pairwise comparison between a zileuton group and the placebo group, assuming that the expected difference between these two groups is 0.25 L. for the change from baseline in FEV₁₀. This difference is approximately equal to that observed in M90-460 for the comparison of zileuton 600 mg QID to placebo. It was anticipated that the corresponding difference for this six month study could be expected to be at least this large. An estimate of the standard deviation of the change from baseline in FEV₁₀ based on all data from M92-720 was 0.57 L. A sample size of 120 patients per treatment group was chosen to compensate for loss of power due to unequal enrollment across study sites.

COMMENT: Like the other Phase III study, this pivotal trial was "powered" for a previously discovered mean difference of zileuton from placebo, and for the variance of this difference. This begs the entire question of clinical significance and the degree of difference from placebo that zileuton should show to achieve a clinically meaningful effect.

STATISTICAL ANALYSES [240:46-49]

The same primary and secondary efficacy and safety variables were prospectively declared and defined as in the other Phase III pivotal trial, M91-685. As before changes from baseline were

to be compared among treatment groups using a two-way ANOVA model with effects for investigator, treatment and investigator-treatment interaction.

PATIENT ACCOUNTABILITY [240:70]

Four hundred and fifty-six patients entered the study and 373 of these patients entered the Double-Blind treatment phase of the trial. These 373 were distributed as follows:

125 received 600 mg zileuton QID

126 received

122 received placebo

One hundred and one patients discontinued prematurely leaving 272 patients who completed the Double-Blind phase of the study (72.73%).

BASELINE PARAMETERS [240:72-7]

There were no statistically significant differences for mean baseline airflow variables between either zileuton group, 600 mg QID QID, and the placebo group. No significant difference between groups was found for various symptom scores and measures of beta-agonist use, except for two comparisons. The zileuton QID group was statistically significantly different from placebo for both nocturnal and daily symptom scores. In each case, the placebo group showed lower mean nocturnal and daily symptom scores than did either zileuton QID or 600 mg QID groups. This was felt to be a chance randomization consequence.

CHRONIC "TROUGH" CHANGE IN FEV_{1.0} [240:78-83]

On Double-Blind Days 8, 36, 64, 92, 120, 148 and 176 pulmonary function measurements were made before the morning dose of study medication. This corresponded to the "trough" plasma level of zileuton. The pre-dosing PFT's at each visit were performed after withholding the blinded study medication and any other medications; i.e., albuterol and short-acting antihistamines.

Each zileuton group demonstrated a greater mean improvement in trough FEV_{1.0} from baseline to each visit day than did the placebo group as can be seen in the graph below:

FIGURE 11 [240:80]

Comparing the zileuton 600 mg QID group to placebo, these between-group differences in mean percent change from baseline achieved significance at all visit days, except the 64th. The zileuton QID group was statistically different from placebo only on Double-Blind Day 8.

The mean percent changes from baseline in trough FEV_{1.0}, averaged over the seven visits, were 15.44%, 12.02% and 6.74% for zileuton 600 mg QID, zileuton QID and placebo groups, respectively.

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TROUGH EFFECT BASED ON MEAN CHANGES AND MEAN PERCENT CHANGES FROM BASELINE IN FEV _{1,0} FOR ALL PATIENTS					
Visit	Treatment Group	Number	Mean Baseline (L)	Mean Change (L)	Mean Percent Change (%)
Final Visit	Z600	123	2.28	0.27	15.23
	Placebo	120	2.35	0.14	6.60
Average Of 7 Visits	Z600	123	2.28	n/a	15.44
	Placebo	120	2.35	n/a	6.74

Statistical significance was attained for the zileuton 600 mg QID vs. placebo group for mean percent change from pre-double-blind period baseline for both the final visit and for the average of 7 visits. The QID zileuton dose did not achieve significance for either of these endpoints. The actual mean change from baseline was not statistically significantly different for either of the zileuton doses, relative to placebo, at any visit including the final visit.

COMMENT: The percent change calculated from the mean change and the mean baseline is not the same as the mean percent change. The latter is calculated by taking the mean of each individual percent change.

Subset analyses of trough FEV_{1,0} were performed for the patients with severe (<50% predicted FEV_{1,0} at baseline), moderate (50% - 70% predicted FEV_{1,0} at baseline) and mild (>70% predicted FEV_{1,0} at baseline) airway obstruction. The results suggest that patients with more severe airway obstruction had a greater magnitude of response showing a 39.98% improvement over baseline for the zileuton 600 mg QID group, 19.72% improvement for the zileuton QID group and 13.10% for the placebo group. However, there was not a statistically significant interaction between subsets and treatments.

FIGURE 12 [240:82]

COMMENT: A dose-proportional effect is apparent within the severe subset, the smallest of the three. The only statistically significant difference from placebo occurs in the zileuton QID group, in the moderate subset of baseline disease severity.

CHRONIC "PEAK" CHANGE IN FEV_{1,0} [240:83-5]

The chronic effect of zileuton was also assessed by serial 6-hour pulmonary function measurements at Double-Blind Days 92 and 176. The "peak" improvement in pulmonary function was determined by the two-to-four hour post-dosing average of FEV_{1,0} values.

At Double-Blind Day 92, patients receiving zileuton 600 mg QID demonstrated a 0.48 L (23.16%) mean increase in FEV_{1,0} over pre-Double-Blind period for the 2-4 hour post-dosing average, in comparison with placebo and this was statistically significant. The peak mean increase of this dose on the 176th Double-Blind Day was 0.40 L (20.2%), but this was not significant. At the final visit, the 600 mg QID dose also achieved significance in terms of mean percent change, but not mean change (0.40 L, 8.96%). The zileuton 400 mg QID group did not show a statistically significant difference from placebo group in peak effect at any visit.

FIGURE 13 [240:84]

PEAK EFFECT BASED ON MEAN CHANGES AND MEAN PERCENT CHANGES FROM BASELINE IN FEV _{1,0} FOR ALL PATIENTS AT THE FINAL VISIT					
Visit	Treatment Group	Number	Mean Baseline (L)	Mean Change (L)	Mean Percent Change (%)
Final Visit	Z600	101	2.26	0.40	20.20
	Placebo	97	2.40	0.24	11.24

COMMENT: Both the trough and peak mean FEV_{1,0} change were relative to very similar baselines, the pre-double-blind period, and the two can be roughly compared. The zileuton 600 mg QID group trough FEV_{1,0} showed a mean baseline of 2.28 L and a mean change at the final visit of 0.27 L (sum=2.55 L). This compares with the mean peak baseline of 2.26 L and a mean change of 0.40 L at the final visit (sum=2.66 L). This places the mean change for the zileuton 600 mg QID dose from trough to peak at the final visit of about 0.11 L. Comparable values for the trough placebo mean baseline and mean change at the final visit are 2.35 L and 0.14 L (sum=2.49 L). The peak placebo mean baseline and mean change at final visit are 2.40 L and 0.24 L (sum=2.64 L). Therefore, the mean change for the placebo group from trough to peak at the final visit is about 0.15 L. This implies that chronic dosing with zileuton 600 mg QID had a salutary effect on the trough FEV_{1,0} that did not further improve 2-4 hours after the morning dose, during the final visit, compared with placebo, although the mean peak FEV_{1,0} (baseline + change) at final visit was about 0.22 L higher in the zileuton 600 mg QID group than in the placebo group.

ACUTE BRONCHODILATORY (FIRST-DOSE) EFFECT [240:87-90]

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Over six hours, pulmonary function measurements were obtained after the administration of the first dose of the study medication on Double-Blind Day 1. The first 600 mg dose of zileuton initiated an increase in FEV_{1,0} that was apparent at 30 minutes. The mean change from baseline became statistically significant between 2 and 5 hours after the first dose, comparing either zileuton dose with placebo. The differences in the 2-4 hour mean percent change between active zileuton groups and placebo was always <7% (Z600 = 12.97%, and, in absolute terms, was 0.13 L (Z600=0.27 L, Z400=0.31 L, P=0.18 L). Both zileuton doses were significantly different from placebo for the 2-4 hour average over all visits, whether expressed as mean change or as mean percent change. Interestingly, the lower zileuton dose showed a slightly greater acute effect than did the higher dose. Practically, the two zileuton doses produced very similar effects and the graph suggests that the effect was beginning to wane after 5 hours.

FIGURE 14 [240:89]

Subset analyses of the first dose effect were performed for the patients with severe (<50% predicted FEV_{1,0} at baseline), moderate (50% - 70% predicted FEV_{1,0} at baseline) and mild (>70% predicted FEV_{1,0} at baseline) airway obstruction. The bar graph below presents these data and suggests that the differences between zileuton groups and the placebo group were greatest for the patients with more severe baseline disease, where the effect was dose-proportional. Statistical difference from placebo was achieved by the zileuton 600 mg QID dose in the severe subset and by

FIGURE 15 [240:90]

PEAK EXPIRATORY FLOW RATES [240:91-6]

These were domiciliary determinations that were recorded in the patient diaries twice daily as AM and PM PEFr's and were presented as monthly averages for the three study groups.

The zileuton 600 mg QID group demonstrated statistically significantly greater mean improvements from baseline in AM PEFr's compared with placebo for every visit interval throughout the study. These mean changes from baseline ranged from 23-32 L/min for AM PEFr's. The average mean change from baseline over all seven intervals was 27.40 L/min, 12.84 L/min and 4.85 L/min for zileuton 600 mg QID, zileuton and placebo groups, respectively. The group did not achieve statistically significant difference from placebo for any of the intervals by any measure.

FIGURE 16 [240:93]

The mean change in PM PEFr's were also statistically significantly greater in the zileuton 600 mg QID group compared to placebo at all intervals except during the Day 1-22 and 51-78

intervals. This higher dose was significant for 5/7 intervals. Mean changes from baseline ranged from 14-25 L/min for PM PEFR's, which, expressed as mean percent changes over baseline, ranged from 4-7%. The average mean change from baseline over all seven intervals was 21.40 L/min, 11.74 L/min and 8.08 L/min for zileuton 600 mg QID, zileuton _____ QID and placebo groups, respectively. The zileuton _____ group did not achieve statistically significant difference from placebo for any of the intervals or for the average of all intervals.

FIGURE 17 [240:95]

Values for AM and PM PEFR, mean baseline and mean change averaged over all seven measurement intervals for all three treatment groups are presented in the table below.

AVERAGE OF ALL SEVEN MEASUREMENT INTERVALS OF AM AND PM PEFR's				
PEFR	Treatment Group	Number	Mean Baseline (L/min)	Mean Change (L/min)
AM	Z600	123	362.58	27.40
	Placebo	122	371.80	4.85
PM	Z600	123	409.61	21.40
	Placebo	122	413.41	8.08

RESPONSE TO BETA-AGONIST INHALATION TEST [240:95-6, 241:47-8, 249:175-6]

Improvement in FEV_{1,0} after administration of beta-agonist was assessed on Single-Blind Day 1 and Double-Blind Day 148. On Single-Blind Day 1, 30 minutes after beta-agonist administration, the mean percent change in FEV_{1,0} was 30.05%, 35.54% and 32.82% for the zileuton 600 mg QID, _____ and placebo groups, respectively. On Double-Blind Day 148 the mean response at 30 minutes post-beta-agonist was 19.56%, 20.71% and 25.75% for the zileuton 600 mg QID _____ and placebo groups respectively. The response to beta-agonist on Double-Blind Day 148 was slightly lower in all three treatment groups than that seen on Single-Blind Day 1. This was associated with an overall chronic improvement in lung function measured by trough FEV_{1,0} values. A reviewer synthesized table follows.

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FEV _{1,0} RESPONSE TO INHALED BETA-AGONIST BASED ON PERCENT CHANGE FROM BASELINE TO POST-INHALATION, AT SEQUENTIAL SAMPLING TIMES							
Time Since Beta-Agonist Dose	Group	Single Blind Day 1			Double Blind Day 148		
		N	Baseline Mean	Mean % Change	N	Baseline Mean	Mean % Change
30 minutes	Z-600	90	2.33	30.05	87	2.68	19.56
	Placebo	89	2.36	32.82	87	2.57	25.75
60 minutes	Z-600	89	2.37	29.66	89	2.68	18.60
	Placebo	86	2.33	33.91	89	2.56	26.30
120 minutes	Z-600	89	2.32	26.28	90	2.68	16.16
	Placebo	86	2.33	31.54	90	2.57	22.33
180 minutes	Z-600	89	2.32	20.33	88	2.71	11.81
	Placebo	87	2.32	26.46	87	2.54	17.51
240 minutes	Z-600	89	2.32	16.83	88	2.69	8.62
	Placebo	87	2.32	21.43	86	2.55	12.81
0 - 4 hour maximum	Z-600	90	2.33	34.16	90	2.68	21.83
	Placebo	90	2.35	38.27	90	2.57	29.38

There were no statistical differences between either zileuton group and the placebo group in mean change of FEV_{1,0} from baseline on Single-Blind Day 1, as would be expected because none of the treatment groups had yet received a treatment. On Double-Blind Day 148 the zileuton 600 mg QID group provided a significantly lesser percent FEV_{1,0} response to beta-agonist than placebo at all time intervals. However, the post-bronchodilator FEV_{1,0} on Day 148 was higher for both zileuton doses

than for placebo. This represents a change from Day 1, when the placebo post-bronchodilator FEV_{1.0} was usually the highest of the three groups (table not shown [241:47-8]). The zileuton QID group was not different from placebo for percent change in FEV_{1.0} at any post-beta-agonist time interval.

SYMPTOM ASSESSMENT [240:96-107]

DAILY SYMPTOM ASSESSMENT [240:96-9, 249:181-3]

Patients receiving zileuton 600 mg QID exhibited a statistically significantly larger mean improvement in their daily symptoms than did patients receiving placebo. Symptoms of the day were measured each evening, on a four-point scale (0-3, 'none' to 'severe'), and the values were included in the patient diary. Baseline mean daily symptom scores ranged from 1.30 to 1.44 across treatment groups, without statistically significant differences between groups. During the first visit interval (Days 1-15 of Double-Blind treatment) the mean daily symptoms increased statistically significantly more from baseline in the zileuton 600 mg QID group (-.27, -19.6%) than in the placebo group (-.15, -11.5%). A similar statistically significant difference between the zileuton 600 mg QID group and the placebo group was seen at all visit intervals and for the average of all intervals. The zileuton group showed a reduction in daily symptoms that was statistically different from placebo for 4/7 intervals, Double-Blind Days 51 through 162, and was also significantly different from placebo for the average of all intervals.

FIGURE 18 [240:99]

The four-point Daily Symptom Assessment score was also averaged over all seven intervals and compared with baseline. This comparison also showed a statistically significant difference between both zileuton treatment groups and placebo. The percent change in this table is not the mean percent change of individuals, but is the mean change as a percent of the baseline.

DAILY SYMPTOM ASSESSMENT, MEAN CHANGE AND PERCENT CHANGE FROM BASELINE AVERAGED OVER SEVEN INTERVALS FOR ALL PATIENTS				
Treatment Group	Number	Mean Baseline	Mean Change	Percent Change
Z600	123	1.38	-0.45	-32.3
Placebo	122	1.30	-0.25	-19.4

COMMENT: The average mean change in Daily Symptom Score from baseline, in excess of placebo was -0.20 (Mean Change From Baseline: Z600 = -0.45, Placebo = -0.25). It is uncertain how to translate this into an estimate of clinical improvement in excess of placebo. The average difference

between the zileuton 600 mg QID group and the placebo group, -20, is only 6.7% of the entire three points of the symptom score range of 0-3 scale points.

Subset analyses were performed for patients with severe (<50% predicted FEV_{1,0} at baseline), moderate (50%-70% predicted FEV_{1,0} at baseline) and mild (>70% predicted FEV_{1,0} at baseline) airway obstruction. The results did not show meaningful differences among subsets between zileuton and placebo.

COMMENT: This represents a difference from the other pivotal trial, M91-685, in which subset analysis by baseline disease severity supported a greater effect of zileuton on daily symptom score in patients with more severe disease.

NOCTURNAL ASSESSMENT [240:100-2]

Nocturnal assessment scores, which were an assessment of the patients' previous night's sleep, were reported each morning in the patient diary using a scale of 0-3 ('slept well' to 'awake all night'). Patients receiving zileuton 600 mg QID exhibited statistically significantly greater mean improvements in nocturnal symptoms than did patients receiving placebo at each interval and as an average of all intervals. The zileuton group also showed statistically greater mean improvements in nocturnal symptoms than placebo-treated patients at 6/7 intervals and when analyzed as an average over all intervals.

FIGURE 19 [240:102]

COMMENT: This also represents a difference from the other Phase III trial which showed and small effect of the highest zileuton dose on this secondary outcome variable and no effect of the lower zileuton dose.

Subset analyses were performed for patients with severe (<50% predicted FEV_{1,0} at baseline), moderate (50%-70% predicted FEV_{1,0} at baseline) and mild (>70% predicted FEV_{1,0} at baseline) airway obstruction. No statistically significant differences among subsets between either zileuton dose and placebo were shown.

ASTHMA SYMPTOMS [240:103-6]

The four separate components of the asthma symptom score were cough, nasal congestion, shortness of breath and wheezing which were measured daily on a four-point scale (0-3, 'absent' to 'severe'). Groups were compared by mean changes in asthma symptom scores from baseline at each of seven intervals. Of the seven intervals, zileuton 600 mg QID was statistically significantly different from placebo for "cough" in 1/7. Differences between both zileuton doses and placebo for "shortness of breath" achieved statistical significance in all (7/7) intervals. There was one

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statistically significant difference between the zileuton 600 mg QID group and the placebo group for "nasal congestion" at Days 1-22 (1/7 intervals). "Wheezing" was significantly reduced, compared with placebo, by the zileuton 600 mg QID dose in 6/7 intervals and by the zileuton 400 mg QID dose in 5/7 intervals.

COMMENT: These results are also a bit different than those found in the other Phase III trial. Among many smaller discrepancies, two are worthy of mention. "Wheezing" was reduced by both zileuton doses at most intervals in the current trial and was unrelieved in any interval by any dose of zileuton in M91-685. "Shortness of breath" was relieved by both zileuton doses at all intervals, in the current trial. This was only true of the higher zileuton dose in 3/7 intervals in M91-685. The lower zileuton dose was not different from placebo in any interval in M91-685.

ACUTE ASTHMA EXACERBATIONS [240:107-10, 241:57]

Asthma exacerbation information was collected at each visit and was defined in the Phase III review of trial M91-685. Percentages of patients with exacerbations that occurred during the Single-Blind period did not differ significantly between either of the zileuton groups and the placebo group. During the Double-Blind period, 38.3% of the placebo patients experienced at least one acute exacerbation of asthma, versus 30.8% of patients taking zileuton QID and 26.8% of patients taking zileuton 600 mg QID. This was significant for the zileuton 600 mg QID group compared with patients taking placebo. When viewed from the perspective of visits at which exacerbations were diagnosed, in 2/7 visits the zileuton 600 mg QID group was statistically different from placebo. In 1/7 visits, the zileuton QID group showed significantly fewer acute exacerbations of asthma than placebo. A reviewer-synthesized table is presented below to show these relationships [241:57].

NUMBER (%) OF PATIENTS EXPERIENCING ≥ 1 EXACERBATION OF ASTHMA, IN THE PRE-VISIT INTERVAL, BY TREATMENT GROUP			
Visit	Z-600 N (%)		Placebo N (%)
During Single-Blind Period	8 (6.4)		7 (5.7)
Day 8	4 (3.3)		8 (6.7)
Day 36	14 (12.4)		12 (10.2)
Day 64	12 (11.2)		13 (11.7)
Day 92	9 (8.8)		8 (7.8)
Day 120	6 (6.3)		12 (12.4)
Day 148	3 (3.2)**		14 (15.6)

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NUMBER (%) OF PATIENTS EXPERIENCING ≥ 1 EXACERBATION OF ASTHMA, IN THE PRE-VISIT INTERVAL, BY TREATMENT GROUP			
Visit	Z-600 N (%)		Placebo N (%)
Day 176	4 (4.4)**		13 (15.1)
During Double-Blind Period	33 (26.8)**		46(38.3)
** Significantly different from placebo (p < 0.05).			

COMMENT: This is similar to the findings of M91-685 where the zileuton 600 mg QID group was significantly different from baseline in 1/7 intervals and the zileuton group in none of the 7 intervals.

Additional analyses of the percentage of patients with one or more episodes of acute exacerbation, stratified by baseline percent of predicted FEV₁₀, were performed. These results did not suggest that any difference between zileuton and placebo was more pronounced in patients with more severe baseline airflow.

COMMENT: This subsidiary finding agrees with M91-685, which did not show a clinically significant subset-treatment interaction, although qualitative effects in M91-685 were noted.

CONCURRENT ASTHMA MEDICATIONS [240:111-20] BETA-AGONIST USE [240:111-5]

The zileuton and 600 mg QID groups showed statistically significantly greater decreases from baseline than the placebo group in the mean change in daily number of puffs of beta-agonist usage starting at Days 1-22, and the differences remained statistically significant to the end of the study, at each dose and every interval. A similar, pattern of significance was seen in the mean percent change beta-agonist use. A reviewer-synthesized table follows that shows the mean change in number of puffs of beta-agonist as an average of all intervals, for zileuton, 600 mg QID mg QID, and placebo groups. The mean change (%) is not the same as mean percent change and only represents the mean change from baseline expressed as a percentage.

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MEAN CHANGE FROM BASELINE FOR BETA-AGONIST AVERAGE DAILY USAGE (NUMBER OF PUFFS) FOR ALL PATIENTS				
Interval	Treatment	N	Mean Baseline	Mean Change (%)
Average Over All Intervals	Z-600	122	5.96	-1.65 (27.7)
	Z-400			
	Placebo	122	5.40	-0.42 (7.8)

For the endpoint of beta-agonist usage, subset analysis for patients with severe ($FEV_{1.0} < 50\%$ predicted), moderate (50% to 70%) and mild ($>70\%$) baseline asthma were performed. The graph below indicates that a dose proportional reduction in beta-agonist use with zileuton, compared to placebo, was seen in all three severity subsets. Statistical significance was achieved only in the comparison of the zileuton 600 mg dose with placebo in the moderate flow obstruction subset.

FIGURE 20 [240:114]

COMMENT: These are similar to the findings in M91-685 where only the zileuton 600 mg QID was associated with significantly less beta-agonist use than placebo, by mean change in numbers of puffs used. Subset analysis of the current study shows dose-proportional changes in the mean number of puffs of the rescue medication in each of the three baseline airflow severity subsets.

CORTICOSTEROID USE [240:115-20]

Approximately 13% of the patients who were randomized required corticosteroid treatment during the study. Forty-nine patients received at least one course of steroids during the trial. Ten (8.0%) were in the zileuton 600 mg QID group, 13 (10.3%) in the zileuton group and 26 (21.3%) were in the placebo group. The difference between placebo and each of the zileuton dose groups achieved statistical significance.

Subset analysis, stratified for baseline disease severity, indicates that the percentage of patients requiring corticosteroid rescue was less with zileuton 600 mg QID than placebo and that this effect was marked in the severe and mild groups of baseline asthmatics, as is shown in the table below.

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PERCENTAGE OF PATIENTS REQUIRING ≥ 1 CORTICOSTEROID TREATMENT(S), STRATIFIED BY BASELINE PERCENT OF PREDICTED FEV _{1,0}			
Severity	Treatment	Total N	n (%)
Severe (FEV _{1,0} < 50% predicted)	Z-600	22	1 (4.6)**
	Placebo	25	10 (40.0)
Moderate (FEV _{1,0} 50%-70% predicted)	Z-600	66	7 (10.6)
	Placebo	56	7 (12.5)
Mild (FEV _{1,0} > 70% predicted)	Z-600	37	2 (5.4)
	Placebo	41	9 (22.0)
** Statistically different from placebo (p < 0.05).			

COMMENT: Although statistical significance between zileuton and placebo was only achieved for the 600 mg QID dose in the category of severe disease at baseline, there is a similar trend in the subset of mild baseline flow obstruction.

COMPLIANCE MONITORING [240:189-90]

The compliance rate for pill counts was over 75% for all three treatment groups. No zileuton levels were detectable for isolated instances in 10 zileuton patients., and 1 placebo patient had isolated instance of a detectable zileuton level. The reason for these results was attributed to plasma tube mislabelling. Five patients had positive blood theophylline levels on five occasions. Four of these had levels that were less than 2.8 mcg/mL and the remaining patient had a level of 12.0 mcg/mL on the final double-blind day.

CONCURRENT MEDICATIONS [249:189, 250:91-132]

The distributions of use of various prescription and over-the-counter medications among the three treatment groups was reviewed. These inter-group comparisons were made for individual drugs, as well as for classes of drugs; e.g. H1-receptor antagonists, anticholinergic drugs, autonomic agents, etc. There was no apparent difference in the distribution of drug use between treatment groups, whether by generic name of the individual drug or by class.

SUMMARY OF EFFICACY RESULTS

The primary efficacy variable, the mean change in trough FEV_{1,0} from baseline to final visit, showed the zileuton 600 mg QID dose to be significantly greater than placebo at all visits except Week 10, double-blind Day 64. The mean percent change in trough FEV_{1,0} was significant in the same periods. The zileuton dose was significantly greater than placebo only at Week 2, double-blind Day 8. Only the zileuton 600 mg QID dose showed statistical significance, compared to placebo, at the final visit and as an average of all visits. The mean change in FEV_{1,0} for the 600 mg QID zileuton group at the final visit was 0.27 L, compared to 0.14 L for the placebo group. The mean percent changes were 15.23% for the 600 mg QID zileuton group at the final, compared to 6.60% for placebo. Although a qualitatively dose-proportional effect was present in patients with severe (FEV_{1,0} < 50% predicted) baseline spirometry, a statistically significant difference between the zileuton 600 mg QID group and placebo was achieved only in the moderately severe group.

At double-blind Days 92 and 176, serial spirometry was performed before the morning dose of the treatment medication had been taken, and serially over a 6-hour period. This was compared with a baseline that was determined during the pre-double-blind period. The peak effect was defined as the 2-4 hour post-dosing FEV_{1,0} values. The zileuton 600 mg QID dose produced a mean increase of 0.48 L on the 92nd day and 0.40 L on the 176th day. Only the first of these was statistically different from placebo. The zileuton dose did not achieve significance, compared to placebo, at either the 92nd or 176th Days.

A first-dose effect, measured by FEV_{1,0}, was noted that was quantitatively similar at both zileuton doses and statistically significantly greater than for placebo. This effect reached its peak at 2-4 hours following the first dose. The maximum mean increase over baseline was 0.31 L, 0.33 L and 0.19 L for zileuton 600 mg QID dose, the zileuton QID dose and placebo, respectively, at different times during this 2-hour interval. As an average over the 2-4 hour interval the mean increase over baseline was 0.27 L, 0.31 L and 0.18 L for the zileuton 600 mg QID dose, the zileuton QID dose and for placebo, respectively. The first-dose effect was qualitatively dose-proportional in the subset of patients with severe baseline spirometry. In this subset, the zileuton 600 mg QID dose had a significantly greater acute (first-dose) effect on FEV_{1,0} than did placebo.

The trough spirometry findings were corroborated by domiciliary AM PEFr determinations. AM PEFr's in the zileuton 600 mg QID group were statistically greater than placebo at all seven intervals. The average mean change from baseline over all intervals was 27.40 L/min, 12.84 L/min and 4.85 L/min for the zileuton 600 mg dose, the zileuton dose and for placebo, respectively. PM PEFr's also showed an improvement in all three treatment groups over baseline, with the zileuton 600 mg QID dose significantly greater than placebo in 5/7 intervals. The zileuton QID dose was not statistically different from placebo at any interval for the AM or PM PEFr endpoints.

Improvement in FEV_{1,0} after inhaled beta-agonist administration was assessed on single-blind Day 1 (pretreatment) and double-blind Day 148. The response to beta-agonist on double-blind Day

148 was slightly lower than on single-blind Day 1 for all three treatment groups, but the baseline FEV_{1,0} was also higher on Day 148 than on Day 1 for all three groups.

Patients receiving zileuton 600 mg QID showed a statistically significant reduction in Daily Symptom Assessment scores than did placebo, at all study intervals. The QID zileuton dose caused a lesser reduction in symptoms, but was statistically different from placebo at 4/7 intervals and for the average of all intervals. Similar results were seen with the Nocturnal Assessment symptom score. The zileuton 600 mg QID dose group showed statistically significant mean reduction of nocturnal symptoms compared with placebo at all visits. The QID zileuton dose showed statistically significantly lower mean nightly symptoms, compared with placebo, for 6/7 visits. Some of the components of the Asthma Symptoms score offered some additional insight. Differences in "shortness of breath" between both zileuton doses and placebo achieved statistical significance at all 7 visits. "Wheezing" was significantly reduced, compared with placebo, by the zileuton 600 mg QID dose at 6/7 intervals and by the zileuton dose at 5/7 intervals. "Cough" and "nasal congestion" were only statistically different, comparing the higher zileuton dose with placebo, at 1/7 visits.

The number of asthma exacerbations was statistically less during the entire double-blind period, for the zileuton 600 mg QID group compared with placebo. The D zileuton treatment group did not achieve statistical significance from placebo for this measure. The average daily number of puffs of beta-agonist rescue medication over the entire study was significantly lower for both zileuton doses, compared with placebo. Pre-double-blind baseline spirographic severity, separated into the usual three groups, showed qualitative dose proportionality of beta-agonist use in each subset. The number of rescue oral, or injected, corticosteroid therapy courses was statistically less for both zileuton doses than for the placebo group.

SAFETY [240:121-89]

This topic will be divided between AE's, severe AE's, AE-related premature terminations and abnormal diagnostic tests. Over the course of the study, 458 patients enrolled, 373 entered the double-blind phase and 272 patients completed the study. Treatment-emergent AE's were defined as being reported after the onset of the double-blind treatment had begun and within 14 days after the treatment ended. No deaths were reported in association with this clinical trial.

Treatment emergent AE's were reported by 102 (81.6%) of patients treated with zileuton 600 mg QID, 111 (88.1%) treated with zileuton and 109 (90.2%) of patients who received placebo. The most common over all three groups and in no particular order were: aggravation reaction, back pain, flu syndrome, headache, infection, pain, pharyngitis and sinusitis.

A total of 58 patients experienced one or more AE's which were classified as severe by the investigator. There were 25 patients (20.0%) in the zileuton 600 mg QID group, 23 (18.3%) in the zileuton group and 10 (8.2%) in the placebo group. Six of these 58 patients were prematurely terminated as a result of the AE. The AE's in these patients were: abnormal liver

function test (#1150, Z600), automobile accident (#1470, Z600), abdominal pain (#1511, Z600), acne (#1582, Z600), dyspepsia (#1691) and depression (#1809, Placebo).

Seven patients experienced AE's that required hospitalization: presumed viral pneumonitis (#1322, Z600), automobile accident (#1470, Z600), myocardial infarction (#1264, community acquired bilateral pneumonia (#1273, lifting injury resulting in back surgery (#1263, P), status asthmaticus (#1344, P) and lingual tonsillitis (#1492, P).

Forty-six patients terminated prematurely due, at least in part, to treatment emergent AE's: 15 (12%) were receiving zileuton 600 mg QID, 15 (11.9%) zileuton and 16 (13.95%) were receiving placebo. Elevated liver function tests were reported in 6 of these patients only one of which had a concomitant elevation of bilirubin. Patient #1028 had maximum elevations of ALT = 508 IU/L, AST = 474 IU/L and bilirubin = 1.6 mg/dL (1.4 is ULN) on the 33rd treatment day with zileuton 600 mg QID. She was 55 year old white female who had a history of "infective hepatitis" at age 20 years. She had slight hepatomegaly on physical examination and negative serology for Hepatitis A, B and C. Five days after the study drug was terminated, the liver function tests began to decline and eventually normalized. The remaining 5 patients had ALT, AST or GGT elevations that were all <100 IU/L, in one, and in the range of 100-400 IU/L, in the remaining four. Of the original 6, 4 were in the zileuton 600 mg QID group, 1 in the zileuton ID group and 1 from the placebo group.

Although the sponsor has defined an abnormal liver function test as one that is greater than 3 times the upper limit of normal (>3xULN), 32 patients had liver function test elevations that were >2xULN for one, or more, enzymes in the study. Of these, 12 patients were from the zileuton 600 mg QID group, 8 from the zileuton group and 12 from the placebo group. Two of these patients, both from the placebo group, had some elevation of bilirubin in association to abnormalities of the other enzymes (#1262, #1263).

Patient #1262 entered the study with an elevated GGT of 92 IU/L and a total bilirubin of 1.5 mg/dL. During treatment, this 33 year old white male had a maximum GGT = 117 IU/L and a maximum total bilirubin = 1.5 mg/dL and he was entirely asymptomatic. Patient #1263 had a history of Gilbert's Syndrome and entered the study with a total bilirubin = 3.3 mg/dL. By the end of the study, this patient developed a small elevation of ALT = 103 IU/L and of AST = 57 IU/L with a concomitant total bilirubin = 1.3 mg/dL. The narrative identifies this patient as having developed mononucleosis, but does not describe how that decision was reached or if the patient experienced symptoms.

Analysis of baseline endocrine values showed that there was only one statistically significant difference between either zileuton group and the placebo group. For post-menopausal females, baseline mean values for estradiol between the zileuton group and the placebo group were statistically significantly different). Analyses of mean changes from baseline to various time points for male patients, female patients, pre-menopausal and post-menopausal females revealed no statistically significant differences between either zileuton group and the placebo group [240:184-5].

CONCLUSION

Over a range of primary and secondary endpoints, the zileuton 600 mg QID dose showed modest efficacy, compared with placebo. The effect of this highest zileuton dose was most apparent in subsets with greater baseline airflow obstruction. This association was more often evidenced by dose proportionality than by strict statistical significance, though both were found.

zileuton dose did not achieve statistical separation from placebo for either the primary efficacy variable (FEV_{10}) or the secondary ones (PEFR's), but did appear to favorably influence some of the symptom scores and the endpoint of rescue medication use.

Treatment emergent AE's were most prevalent in the placebo group and, most commonly "aggravation reaction." Severe AE's were over twice as frequent in either zileuton group than in the placebo group. Premature terminations due, in part, to AE's were evenly divided among the three treatment groups. However, there were six terminations due to hepatic enzyme abnormalities, one of which was a frank hepatitis that was most likely drug-related. Five of the six patients terminated because of liver test abnormalities were in zileuton treatment groups. The finding of disproportionate numbers of zileuton-treated patients with hepatic abnormalities corroborates this same finding in the other pivotal trial and underscores our concern.

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M90-460 "Safety and Efficacy of Zileuton (Abbott-64077), 800 mg B.I.D. or 600 mg Q.I.D versus Placebo in the Treatment of Moderate Asthma"

OBJECTIVES

1. determine relationship between zileuton dosage and plasma concentrations and effect on pulmonary function
2. correlate degree of leukotriene inhibition and pulmonary function
3. investigate if zileuton can enhance bronchodilation seen with inhaled beta-agonists

DESIGN

This was a Phase II, randomized, placebo-controlled, parallel-group study of two doses of zileuton capsules.

SUBJECTS

A total of 146 patients completed the single-blind, placebo lead-in period of the study and were randomized to receive one of the three treatments in the double-blind period. The significant entry criteria were: FEV_{1,0} between 40% and 75% of predicted, 15% reversibility after inhaled albuterol and manageable disease with inhaled beta-agonists, alone. The patients ranged in age from 18 to 64 years. There were more males than females (111/31) and Caucasians outnumbered blacks (121/16). Only 5 representatives of other races participated.

DOSAGE

After a one-week, single-blind, placebo lead-in period, the patients were randomized to receive zileuton 600 mg QID, zileuton 800 mg BID or placebo, for a four-week, double-blind period. All patients took four capsules, four times daily that were filled with active drug or placebo from blister packs. Forty-eight received zileuton 600 mg QID, 49 took zileuton 800 mg BID and 48 received placebo QID. The unit of dosing was a 200 mg capsule.

PARAMETERS

Spirometry with beta-agonist reversibility testing was performed at screening. Upon meeting the study criteria the patients were given placebo blister packs, albuterol rescue medication, peak flow meters for BID testing and diaries for daily and nocturnal symptom score recording. The first dose of the double-blind medication was given at the study center with spirometry before dosing and at 30, 60 and 120 minutes following this initial dose. Study visits were scheduled every 7 days through the four weeks, with spirometry at each visit and at one week following the end of the double-blind period. Inhaled beta-agonist reversibility was performed at screening, at weeks 3 and 4 and at the one-week follow-up.

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Pharmacokinetic testing was done on about half of the patients on the mornings of Double-Blind Days -7 (screening), 8 (first double-blind dose day) and 29 (last double-blind dose day). Patients arrived at the study center before first morning dose and submitted to serial blood drawings for plasma zileuton levels and for LTB₄ determinations. Single plasma zileuton levels were performed at each visit as a compliance check. Two four-hour urine samples were collected from each patient for LTE₄ determination, one during the single-blind period and the other on, or just before, the last double-blind day.

RESULTS

Patients receiving zileuton demonstrated improvement in FEV_{1.0} in comparison to the patients receiving placebo. This effect maximized at the third double-blind visit and remained constant through the last double-blind visit. Generally, the dose of 600 mg QID was associated with a slightly greater improvement in the mean FEV_{1.0} than was the dose of 800 mg BID [167:52, 79].

CHANGE FROM BASELINE FEV1.0 AFTER CHRONIC TREATMENT WITH TWO DOSES OF ZILEUTON OR WITH PLACEBO					
Visit Day	Treatment	N	Baseline Mean (L)	Visit Mean (L)	Change Mean (L)
8	Z-600	46	2.39	2.56	0.17
	Z-800	45	2.15	2.32	0.17
	Placebo	47	2.29	2.43	0.14
15	Z-600	46	2.39	2.58	0.19
	Z-800	44	2.19	2.31	0.12
	Placebo	45	2.31	2.44	0.14
22	Z-600	43	2.42	2.72	0.30
	Z-800	44	2.19	2.46	0.27
	Placebo	44	2.34	2.49	0.15
29	Z-600	42	2.39	2.71	0.32
	Z-800	43	2.21	2.45	0.24
	Placebo	42	2.34	2.39	0.05

The trend in mean magnitude of this effect (Z-600 > Z-800 > Placebo) was repeated in the mean morning PEFr values, but not the mean evening PEFr's. It was also repeated in rescue medicine use, as measured by the number of occasions, but not by the number of puffs [167:55-6].

An acute bronchodilatory effect was seen after the first double-blind dose and was greater for the 600 mg dose than for the 800 mg dose, both of which were qualitatively superior to placebo and maximally so at 60 to 120 minutes post-dosing [167:56, 87].

POST-INHALED ALBUTEROL, ACUTE BRONCHODILATORY EFFECTS OF TWO DOSES OF ZILEUTON AND PLACEBO ON FEV _{1,0}					
Time Since Dose (min)	Treatment	N	Baseline Mean (L)	Post-Dose Mean (L)	Change Mean (%)
30	Z-600	46	2.39	2.56	0.17 (7.11)
	Z-800	46	2.21	2.33	0.13 (5.88)
	Placebo	47	2.29	2.34	0.05 (2.18)
60	Z-600	46	2.39	2.74	0.35 (14.64)
	Z-800	46	2.21	2.44	0.23 (10.41)
	Placebo	47	2.29	2.35	0.07 (3.06)
120	Z-600	45	2.41	2.71	0.30 (12.45)
	Z-800	46	2.21	2.45	0.24 (10.86)
	Placebo	47	2.29	2.38	0.09 (3.93)

There were no statistically significant differences in bronchodilatory effect after inhaled beta-agonist between treatment groups at any time point. However, the mean change in FEV_{1,0} after beta-agonist inhalation was smaller for all treatments on double-blind Days 15 and 22, than it was during the placebo, baseline, run-in [167:58, 91].

Three ordinal scale variables, Daily Asthma Symptoms, Nocturnal Symptoms and overall Asthma Assessment, produced similar results. The 600 mg zileuton dose resulted in a significantly larger reduction in symptoms than did placebo. Generally, there was no statistical difference between the 800 mg zileuton dose and placebo [167:58-9].

The analyses of change from baseline and percent change from baseline in urinary leukotriene E₄ revealed that the mean decrease for the zileuton patients was significantly different from the mean increase for the placebo patients as summarized below [167:61]:

CHANGE IN FOUR-HOUR URINARY LTE ₄ FROM BASELINE TO END OF DOUBLE-BLIND PERIOD (AFTER FOUR WEEKS)					
Treatment Group	Baseline Mean (pg/mg Creatinine)	Mean Change from Baseline (pg/mg Creatinine)	Zileuton v. Placebo P-Value	Mean Percent Change From Baseline	Zileuton v. Placebo P-Value
Zileuton 600	101.52	-39.22	0.007	-29.37	0.002
Zileuton 800	101.91	-26.54	0.049	-7.03	0.082
Placebo	91.94	+2.84		+18.51	

Both zileuton doses produced mean changes from baseline that achieved statistical significance, when compared with placebo.

Serum LTB₄ inhibition produced by the 600 mg QID dose, during the double-blind period, was 93.69% - 95.75%. The 800 mg BID dose produced 96.85% - 96.89% inhibition [176:33]. Although pulmonary function tests were performed on the patients who participated in the pharmacokinetic portion of the study, the results of these tests were highly variable. Preliminary investigation of various dynamic models to describe the data were not successful [176:35].

ADVERSE EVENTS

One hundred forty-six patients received the double-blind treatment. One of these inadvertently took the double-blind medication during the single-blind period and was disqualified for efficacy but retained in the analysis of safety. Fifteen patients terminated early, five in each treatment group. Eight discontinued because of an adverse event and 4/8 reported the reason for termination was lack of efficacy, in addition to an adverse event [167:49]. Of the remaining 4/8, two reported an infection, one a sinusitis and one, urticaria [167:63].

Two of the above eight had asthma exacerbations that were considered to be severe. Five additional AE's not associated with premature terminations were rated as severe. Three of these events were recorded in the same patient, who reported three separate episodes of disabling headache. Another patient had a severe headache and one other had a dislocated patella [167:66].

CONCLUSION

The finding of significant flow improvement with this capsule formulation confirms the results of other studies. The three objectives listed above were variously met:

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1. The relationship between zileuton dose and plasma concentration was dose proportional. The relation between zileuton plasma concentrations and pulmonary function measures of airflow was too variable to describe (see #2).
2. There was a rough correlation in the mean degree of leukotriene inhibition (urinary LTE_4) and mean pulmonary function measures. That is the 600 mg QID dose provided greater inhibition and greater improvement in various measures of airflow than did the higher, 800 mg BID dose.
3. No apparent zileuton enhancement of bronchodilation was seen after inhaled beta-agonists.

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M91-597 "Phase II Study of the Effects of Zileuton vs. Placebo on 5-Lipoxygenase Products in Bronchoalveolar Lavage Fluid in Atopic Asthma"

OBJECTIVES

Determine the effect of zileuton, 600 mg QID for 24 hours, or placebo on the allergen-stimulated release of eicosanoids into the bronchoalveolar lavage fluid of mild atopic asthmatics.

DESIGN

This was a randomized, double-blind, placebo-controlled, crossover study of one day of zileuton 600 mg QID.

SUBJECTS

The five study patients ranged in age from 21 to 32 years (mean = 27) with heights from 69 to 72 inches (mean = 70). All patients were male, four were Caucasians and one was black. The study was designed for up to 10 mild atopic asthmatic patients [179:13].

DOSAGE

A dose of 600 mg QID was given before the challenge. The unit dose was a 200 mg capsule.

PARAMETERS

After fulfilling the requirements of methacholine responsiveness by spirometry and wheal produced by allergen skin testing, the subjects took 4 doses of zileuton, or matching placebo, over the course of a day. A pre-allergen bronchoscopic bronchoalveolar lavage (BAL) was carried out 1-2 hours after the last dose of the treatment drug. After this procedure, but before the bronchoscope was withdrawn, an antigen to which the patient had previously shown a reaction, was instilled into a segmental bronchus. A post-allergen lavage was measured 5-20 minutes after the pre-allergen lavage to measure early response to the antigen challenge. Following a three-week washout period, an identical second bronchoscopy was performed on Day 2 after administration of the crossed-over study drug. BAL fluid was to be analyzed for concentrations of LTE_4 , 5-HETE, PGD_2 , TXB_2 , 6-Keto- $\text{PGF}_{1\alpha}$ and PGE_2 . Urine samples were collected just prior to the bronchoscopy and the subsequent two voids post-bronchoscopy (up to 4 hours) for determination of LTE_4 concentration. [179:14].

RESULTS

Two of the 5 patients were unevaluable for all efficacy analyses. Patient #104 was unevaluable because the patient had less than a 20% fall from baseline $\text{FEV}_{1.0}$ in response

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to the methacholine inhalation challenge. Patient #105 was unevaluable for non-compliance caused during Period I [179:22]. Efficacy analyses based on evaluable data were not performed due to insufficient sample size. Results from all 5 patients were evaluated (intent-to-treat), without excisions [179:22, 30]. A blinded interim analysis was performed which indicated no substantial clinical effects for any patient. Subsequently, the study was terminated after 5 patients had completed the protocol [179:25].

Some of the originally planned 5-lipoxygenase metabolites were measured, others were not and still others were added to the assay. Therefore the variables reported will not necessarily conform to those in the "Parameters" section. The baseline (pre-allergen) BAL lavage assay values were not statistically different between treatments [179:26, 34-8].

BASELINE VALUES OF BAL ASSAY RESULTS FOR TWO-PERIOD CROSSOVER ANALYSIS			
BAL Variable	N	Zileuton	Placebo
PGE ₂ (pg/mL)	4	1.16	25.66
PGD ₂ (pg/mL)	4	241.92	272.00
5-HETE (pg/mL)	4	24.27	9.81
15-HETE (pg/mL)	4	21.72	36.47
LTE ₄ (pg/mL)	5	173.88	153.23

There were no significant differences between treatments for any variables comparing the pre-allergen baseline to the post-challenge BAL. There were also no differences between treatments in comparing the post-challenge BAL variables [179:27].

COMMENTS: The above table serves to exemplify the degree of difference between two numbers that is not statistically significant when the "N" is very small.

Urinary LTE₄ showed a significant difference between treatments in mean baseline LTE₄, but not in changes from baseline or in post-challenge values [179:28].

ADVERSE EVENTS

No patients terminated prematurely. Only one of the 5 randomized patients experienced AE's, one while on zileuton (hypotension), and one while taking placebo (influenza-like syndrome). The only vital sign to show a between-group difference was the respiratory rate. The zileuton group had a lower mean respiratory rate (14 B/min) than did

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the placebo group (17 B/min). One clinical laboratory result was significantly different between treatments, the mean platelet count ($Z=270.42 \times 10^9/L$, $P=283.75 \times 10^9/L$) [179:28].

CONCLUSION

The sponsor concludes that the design did not allow for a long enough period of dosing or a long enough period of measurement after allergen challenge [179:30].

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M91-615 "Phase III Study of Zileuton in the Prevention of Exercise-Induced Bronchoconstriction in Asthmatics"

OBJECTIVES

Examine the efficacy and safety of zileuton in preventing exercise-induced bronchoconstriction in patients with exercise-induced asthma.

DESIGN

This was a Phase III, placebo-controlled, randomized, double-blind, two-period crossover trial held in a single center.

SUBJECTS

$FEV_{10} \geq 70\%$ of predicted and exercise-induced fall of 20% from baseline at 80-90% of predicted maximal heart rate, were inclusionary criteria. The patients had to be on no chronic medication other than inhaled beta-agonists. The 24 patients who completed the study ranged in age from 21 to 45 years, with heights ranging from 61 to 78 inches.

DOSAGE

Tablets of 600 mg zileuton, or matching placebo, were taken QID for two days and one additional morning dose was taken after arriving at the test center, on the third day (total = 9 doses), at which time an exercise test was performed. After a 4 to 14 day wash-out period, patients were crossed over to the other arm [180:25, 80].

PARAMETERS

Patients were given baseline and serial spiograms before, and for 60 minutes after, 4-10 minutes of exercise to 80-90% of their age-predicted maximum heart rates, breathing 0% relative humidity compressed air, at room temperature [180:25, 80]. Most parameters were taken at three points: screening, Day 3 of Period 1 and Day 3 of Period 2. Plasma zileuton levels were drawn before exercise and 15 minutes after it on Day 3 of each arm. Laboratory tests were also drawn at the 15 minute, post-exercise sampling interval.

RESULTS

The mean drop in FEV_{10} from its pre-exercise value, as well as the mean percent change from baseline, are presented in the table below. At all sampled time points, the zileuton group was associated with less of a decline in FEV_{10} from baseline than was placebo [180:31].

MEAN FEV_{1,0} CHANGE FROM BASELINE AT VARIOUS TIMES FOLLOWING EXERCISE				
Post-Challenge Time Window	Treatment	N	Within Treatment Change (L)	Within Treatment Change (%)
5 minutes	zileuton	24	-0.55	-14.72
	placebo	24	-0.89	-25.89
10 minutes	zileuton	23	-0.42	-11.20
	placebo	23	-0.80	-23.28
15 minutes	zileuton	22	-0.32	-8.71
	placebo	22	-0.72	-21.43
30 minutes	zileuton	23	-0.19	-5.21
	placebo	23	-0.49	-14.31
45 minutes	zileuton	23	-0.11	-2.99
	placebo	23	-0.29	-8.69
Maximum Decrease	zileuton	24	-0.59	-16.06
	placebo	24	-0.96	-28.16

CONCLUSIONS

Two days of zileuton pre-treatment of patients who have exercise-induced bronchospasm, blunts the exercise-associated drop in FEV_{1,0}, when compared with placebo pre-treatment.

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M91-654 "Effect of Zileuton on Cellular Infiltration and Lung Injury after Local Bronchoscopic Antigen Challenge in Atopic Patients"

OBJECTIVES

Determine the effect of zileuton on inflammatory cell influx and lung injury by local bronchoscopic antigen challenge in atopic patients with a positive reaction to ragweed.

DESIGN

This was a Phase II, randomized, placebo-controlled, double-blind, two-period, crossover study. A one week treatment period of zileuton, 600 mg, or placebo given QID, was followed by a bronchoalveolar lavage (BAL) and an antigen challenge on the eighth day. After another day of treatment, the patient underwent a second BAL on the ninth day. After a two-week washout, this sequence was repeated with the crossover treatment.

SUBJECTS

The ten atopic patients had a range of ages from 22 to 38 years. All ten were Caucasians with $FEV_{10} \geq 75\%$ predicted. There were 9 males and 1 female. All had undergone allergy skin testing with ragweed, at screening, and must have responded with a wheal diameter ≥ 10 mm (= "atopy"). Inhaled steroids, "cromolyn preparations" and theophylline were specifically excluded. Any OTC medication or inhaled beta-agonist use required specific notation on case report forms.

DOSAGE

The dosing unit was a 200 mg capsule. Active drug and identical placebo were administered as 600 mg (3x200 mg) QID for 9 days in each arm.

PARAMETERS

The study consisted of a screening visit and double-blind phase (two one-week treatment periods separated by a two-week washout period). Blood and urine laboratory samples were done at screening and on Day 9 of each period. Spirometry was performed at screening, Day 1, pre-challenge on Day 8 and pre-bronchoscopy on Day 9 of both periods. On Day 8, a BAL was performed and local ragweed antigen was instilled in one lung segment. Twenty-four hours later bronchoscopy with BAL of the antigen-challenged segment was performed. The BAL fluid was evaluated for total cell count, differential cell count, concentrations of urea, albumin, protein, cytokines (IL-4, IL-6), eosinophilic cationic protein (ECP), 5-lipoxygenase products (LTE₄, 5-HETE, 12-HETE and 15-HETE) and eicosanoids (PGD₂, TXB₂, PGF_{2-alpha}, PGF_{2-beta}, PGE₂).

RESULTS

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There was a statistically significant difference between treatments for the change from Day 8 to Day 9 (i.e., from pre-antigen to post-antigen) in total cell count and the eicosanoid, $\text{PGF}_{2-\text{beta}}$, as can be seen in the two reviewer-generated tables below [183:36, 38]. With one exception, lymphocytes in the zileuton group, the cell count on Day 9 was always greater than on Day 8. In every case, this mean change was larger for the placebo group than for the zileuton treatment group.

COMPARISON BETWEEN GROUPS, OF THE PRE- TO POST-ANTIGEN CHALLENGE CHANGE, IN BRONCHO-ALVEOLAR LAVAGE CELL COUNTS						
Variable	Treatment Group	D-8 Mean	D-9 Mean	Mean Change From D-8	Between Groups Mean Diff.	Between Groups P-Value
Basophils ($\times 10^4/\text{ml}$)	Zileuton	0.02	0.40	0.38	-0.121	0.531
	Placebo	0.09	0.59	0.50		
Eosinophils ($\times 10^4/\text{ml}$)	Zileuton	1.06	16.54	15.47	-32.893	0.210
	Placebo	0.61	48.98	48.36		
Lymphocytes ($\times 10^4/\text{ml}$)	Zileuton	0.68	0.32	-0.35	-1.402	0.095
	Placebo	0.69	1.74	1.05		
Macrophages ($\times 10^4/\text{ml}$)	Zileuton	14.27	20.84	6.57	-5.574	0.676
	Placebo	13.10	25.24	12.14		
Neutrophils ($\times 10^4/\text{ml}$)	Zileuton	0.29	3.13	2.84	-22.868	0.531
	Placebo	0.51	26.02	25.71		
Total Cells ($\times 10^6$)	Zileuton	13.18	28.40	15.22	-75.000	0.037
	Placebo	11.06	101.28	90.22		

Analysis of the BAL fluid assay showed some similarities to analysis of cell counts. With one exception, PGE_2 in the zileuton treatment group, the concentration of components was always higher on Day 9 than on Day 8. Moreover, this difference between pre- and post-challenge concentrations was always equal or larger, for the placebo group compared with the zileuton group.

COMPARISON BETWEEN GROUPS, OF THE PRE- TO POST-ANTIGEN CHALLENGE CHANGE, IN BRONCHO-ALVEOLAR LAVAGE FLUID ASSAYS						
Variable	Treatment Group	D-8 Mean	D-9 Mean	Mean Change From D-8	Between Groups Mean Diff.	Between Groups P-Value
Albumin (mcg/ml)	Zileuton	20.2	93.5	73.3	-292.7	0.531
	Placebo	26.2	392.2	366.0		
ECP (mcg/ml)	Zileuton	0.4	12.4	12.1	-10.2	0.144
	Placebo	0.4	22.6	22.2		
E-2 (pg/ml)	Zileuton	10.5	9.7	-0.8	-20.0	0.403
	Placebo	14.9	34.1	19.2		
F-2 Alpha (pg/ml)	Zileuton	11.3	14.4	3.1	-2.1	1.000
	Placebo	15.5	20.8	5.2		
F-2 Beta (pg/ml)	Zileuton	0.6	0.7	0.1	-2.6	0.022
	Placebo	0.6	3.3	2.7		
IL-4 (pg/ml)	Zileuton	0.0	0.1	0.0	0.0	0.676
	Placebo	0.0	0.1	0.0		
IL-8 (pg/ml)	Zileuton	37.1	146.1	109.0	-44.0	0.676
	Placebo	78.0	230.9	152.9		
LTE ₄ (pg/ml)	Zileuton	167.1	301.5	134.3	-752.9	0.060
	Placebo	176.1	1063.3	887.2		
PBD-2 (pg/ml)	Zileuton	165.1	177.5	12.4	-287.9	0.210
	Placebo	147.3	447.5	300.3		
Protein (mcg/ml)	Zileuton	128.5	404.3	275.7	-839.8	0.296
	Placebo	133.4	1248.9	1115.5		
TXB-2 (pg/ml)	Zileuton	13.9	18.7	4.7	-16.4	0.917
	Placebo	17.3	38.4	21.1		

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COMPARISON BETWEEN GROUPS, OF THE PRE- TO POST-ANTIGEN CHALLENGE CHANGE, IN BRONCHO-ALVEOLAR LAVAGE FLUID ASSAYS						
Variable	Treatment Group	D-8 Mean	D-9 Mean	Mean Change From D-8	Between Groups Mean Diff.	Between Groups P-Value
Urea (mcg/ml)	Zileuton	2.3	3.3	1.0	-4.1	0.144
	Placebo	2.3	7.4	5.1		

The only other efficacy finding that achieved statistical significance between groups was the Day 8 urinary LTE₄, both pre- and post-challenge. The urine LTE₄ level was higher in the placebo group than in the zileuton group, both pre- and post-challenge. It rose in both groups from pre- to post-challenge, but rose less in the zileuton arm (170%) than in the placebo arm (773%) [183:44]. The reviewer-generated table below illustrates this point.

DAY 8 URINE LTE ₄ (pg/mg Creatinine)				
Time	Treatment Group	Mean	Mean Difference	Between Groups Diff. P-Value
Pre-Challenge	Zileuton	409.50	-590.20	0.001
	Placebo	999.70		
Post-Challenge	Zileuton	1104.46	-7624.94	0.037
	Placebo	8729.40		

ADVERSE EVENTS

Fourteen patients entered the study and 10 finished it. Four patients terminated prematurely for the following reasons: 2 refused to undergo further bronchoscopy, 1 patient had an upper respiratory infection after the first bronchoscopy and 1 patient had an acute exacerbation of bronchospasm.

AE's occurred in 7 patients during the zileuton treatment period (53.8%) and in 7 patients during the placebo period (58.3%) [183:28]. The most prevalent AE's were chest pain, increased cough and pharyngitis, which occurred as a probable consequence of the bronchoscopy procedure [183:28].

CONCLUSION

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These data suggest that the bronchoscopic antigen challenge was associated with increased cell counts and inflammatory components in both zileuton and placebo treatment groups. This inflammatory response to the challenge was less pronounced in the zileuton group than in the placebo treatment for virtually every item analyzed, though only two reached statistical significance. The Day 8 urinary LTE₄, pre- and post-challenge, showed similar findings.

Virtually every inflammatory index, including cells, 5-lipoxygenase products and eicosanoids, were increased by the antigen challenge, but this increase was blunted by zileuton, compared with placebo.

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These data suggest that the bronchoscopic antigen challenge was associated with increased cell counts and inflammatory components in both zileuton and placebo treatment groups. This inflammatory response to the challenge was less pronounced in the zileuton group than in the placebo treatment for virtually every item analyzed, though only two reached statistical significance. The Day 8 urinary LTE₄, pre- and post-challenge, showed similar findings.

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M92-724 "A Study of the Effects of Zileuton 600 mg QID Compared to Placebo in Nocturnal Asthma"

OBJECTIVES

In a sample of patients with nocturnal asthma, assess the effect of zileuton on:

1. methacholine airway reactivity
2. cellular influx into bronchoalveolar lavage fluid
3. LTB_4 production from alveolar macrophages
4. amount of LTE_4 excreted in urine

DESIGN

This was a randomized, placebo-controlled, double-blind, Phase II, two-period crossover study of zileuton in patients with nocturnal asthma and normal controls. To be included, patients had to have a baseline $FEV_{1.0}$ 40% to 80% of predicted and 15% reversibility after inhaled albuterol. Oral or inhaled steroids, theophylline and inhaled cromolyn were excluded. Stable immunotherapy or nasal steroids were allowed. Short-acting antihistamines were allowed, but were to be discontinued 48 hours before each evaluation visit. The study consisted of a screening visit followed as much as two weeks later by a two-week, single-blind run-in period. The double-blind portion of the protocol was one week for the first period, a one-week washout and one week for the second period.

SUBJECTS

Twelve nocturnal asthmatics and six normal controls entered and completed the double-blind period. These 18 evaluable patients were evenly divided as to gender. They ranged in age from 18 to 46 years. All were Caucasian.

DOSAGE

The zileuton dose was 600 mg QID and the dosing unit was a 600 mg tablet.

PARAMETERS

The major efficacy variables were derived from pulmonary function testing, methacholine challenge and BAL, performed just after the challenge. PFT's were performed at screening and at the beginning and end of each period, one single-blind and two double-blind. Methacholine challenge and BAL were performed during the single-blind period 7 days before the first double-blind period on all normals and on nocturnal asthmatic patients who met selection criteria for Daily Symptom and Nocturnal Assessments. Selection criteria, consisting of beta-agonist use and somewhat greater symptom scores, were required for entry into the double-blind period. PFT's, methacholine challenge and post-challenge BAL were performed at the end (7th day) of each double-blind period. At the end of each

double-blind period PFT's were performed at 2200 hours, repeated after two puffs of albuterol and repeated again, pre-beta-agonist at 0330 hours. Overnight changes in FEV_{1.0} were defined as differences between the post-beta-agonist values at 2200 hours and the pre-beta-agonist value at 0330 hours the next morning. Urine for LTE₄ was collected at the end of the screening period and at the ends of both double-blind periods. Twice daily PEFr's and daily beta-agonist use were recorded. Hematology and chemistry blood work and urinalyses were obtained once each period.

RESULTS

There were no statistically significant differences between treatments in the methacholine log PD₂₀ for the normals or the nocturnal asthmatic patients in either treatment group. Analysis of the BAL cells and mediators between the two treatments of the nocturnal asthmatic patients showed statistical significance only for LTB₄, which was lower while taking zileuton than while taking placebo [299:140, 142, 144].

LEUKOTRIENES, THROMBOXANE & PROTEIN LEVELS FROM BRONCHOALVEOLAR LAVAGE IN NOCTURNAL ASTHMATICS				
Variable	Treatment	N	Mean	ANOVA P-Value
LTB ₄ (pg/ml)	Zileuton	12	8.16	0.015
	Placebo	12	11.17	
LTC ₄ (pg/ml)	Zileuton	12	21.00	0.270
	Placebo	12	30.06	
TXB-2 (pg/ml)	Zileuton	12	27.66	0.234
	Placebo	12	18.65	
Protein (mcg/ml)	Zileuton	9	102.34	0.189
	Placebo	9	69.27	

Protein, thromboxane, LTC₄, total cells and percent eosinophils, lymphocytes, epithelial cells, neutrophils and macrophages were not significantly different between treatments in nocturnal asthmatics. For both normals and nocturnal asthmatics, comparing zileuton to placebo, the former was always associated with lower mean urinary LTE₄ levels than the latter, though this only achieved significance for the 2300-0700 collection in nocturnal asthmatics [299:74].

NDA 020471

FIRM: ABBOTT LABS

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TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

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URINARY LTE ₄ (pg/mg Creatinine)						
Collection Interval	Group	Treatment	N	Mean	Between Treatments	
					Mean	P-Value
1500 - 2300 hours	Nocturnal Asthmatics	Lead-In	8	102.75	-33.08	0.370
		Zileuton	8	48.25		
		Placebo	8	81.33		
	Normal Controls	Lead-In	2	35.00	-18.38	0.113
		Zileuton	5	40.25		
		Placebo	5	58.63		
2300 - 0700 hours	Nocturnal Asthmatics	Zileuton	8	33.60	-88.67	0.046
		Placebo	8	122.27		
	Normal Controls	Zileuton	6	40.10	-32.50	0.109
		Placebo	6	72.60		

Among a host of secondary efficacy endpoints, the only one to achieve statistical significance was beta-agonist use, which was lower in zileuton-treated than in placebo-treated nocturnal asthmatics. Specifically, the overnight fall in FEV₁₀ in nocturnal asthmatics was not statistically different between placebo and treatment arms [299:59].

CHANGE IN FEV ₁₀ OVERNIGHT FROM POST-BETA-AGONIST AT 2200 HOURS TO PRE-BETA-AGONIST AT 0330 HOURS					
	Treatment	N	2200 Mean (L)	0330 Mean (L)	Mean % Change Within Patients
Nocturnal Asthmatics	Zileuton	10	3.10	2.44	-28.6
	Placebo	10	3.02	2.12	-45.6
Normal Controls	Zileuton	5	3.92	3.78	-3.58
	Placebo	5	3.89	3.80	-2.53

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ADVERSE EVENTS

Among the twelve nocturnal asthma patients, 12 AE's were reported during zileuton treatment and 8, during placebo. Six headaches were reported under each of the two types of treatment. Placebo treatment was associated with 2 reports of pain. Zileuton-treated asthmatics made the following reports: 2 myalgias, 1 flu-syndrome, 1 flatulence, 1 eczema and 1 voice alteration.

Among the six normal controls, 2 AE's were reported during zileuton treatment and 3 during placebo. One episode of headache and one of cystitis were reported during the zileuton arm. Three headaches were reported during the placebo arm.

CONCLUSION

A trend is present showing a salutary effect of zileuton on indices of inflammation in patients with nocturnal asthma. This trend is not as apparent in pulmonary function and symptom score endpoints.

Some of the primary endpoints achieved statistical significance, like BAL LTB₄ and urinary LTE₄, others did not; e.g. methacholine challenge. In the zileuton group, the overnight mean FEV_{1,0} declined less and the mean nocturnal symptom score was less than in the placebo group, but neither of these comparisons approached statistical significance.

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M92-763 "Phase III Study of the Effects of Zileuton 400 mg QID and 600 mg QID Compared to Theophylline in the Treatment of Moderate Asthma

OBJECTIVES

Compare the safety and efficacy of zileuton 400 mg and 600 mg QID to theophylline in the treatment of moderate asthma.

DESIGN

This was a Phase III, randomized, double-blind, parallel-group, multi-center, 13-week study. Patients must have had a history of asthma, an FEV_{1,0} of 40 to 80% of predicted when tested off all asthma medication and showed 15% FEV_{1,0} reversibility after inhaled albuterol challenge. After up to three weeks of theophylline titration to a blood level of 8-15 mcg/ml. (Period I), the patients were exposed to a one-week, single-blind, placebo lead-in (Period II). Period III was the double-blind, three-armed treatment with either zileuton 400 mg QID, zileuton 600 mg QID or theophylline (SLO-BID®) BID titrated to give a therapeutic blood level. More precise timing of treatment drug dosing than BID or QID was not provided. Patients were dropped from the study if titration to a therapeutic serum level required $\geq$ 400 mg of SLO-BID® twice daily or required more than 20 days. Titration was also carried out during the double-blind period by an unblinded third party. To maintain the blind, mock titration was performed on the theophylline placebo patients [4/17/95 1:31]. A single course of prednisone with a maximum dose of 60 mg QD for $\leq$ 7 days could be used for an exacerbation, but a second such course was disqualifying [4/17/95 1:46].

SUBJECTS

A total of 471 patients entered the study and 377 patients were randomized into the double-blind period. Of these, 129 received zileuton 400 mg QID, 124 received zileuton 600 mg QID and 124 received theophylline twice daily. Sixty-four patients discontinued the double-blind period prematurely leaving 313 patients who completed the entire study. All three treatment groups had a small preponderance of females (56-61%) and Caucasians comprised the majority of each treatment (85-91%). Ages ranged from 18 to 60 years with a mean in the mid-30's [4/17/95 1:73].

DOSAGE

Zileuton was supplied as 400 and 600 mg tablets, or identical placebo's, all individually encased in single-dose blister packs. Theophylline was supplied by Rhone-Poulenc-Rorer Pharmaceuticals as SLO-BID® 100 mg capsules. An identical placebo was supplied by Abbott.

PARAMETERS

Spirograms were performed at the Screening Visit, on Study Days 1 and 71 (prior to dosing and serially through 240 minutes post-beta-agonist), on Study Days 8, 43 and 99 (prior to dosing and serially through 360 minutes post-dosing) and on Study Days 15, 29 and 57 (immediately prior to

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dosing) [4/17/95 1:30]. The primary efficacy variable was the FEV₁₀ at scheduled Study Visits and the secondary efficacy variables were the twice daily domiciliary PEF_R determinations. Other efficacy variables included beta-agonist rescue medication use, frequency of asthma exacerbations, response to beta-agonist challenge and several scales (asthma, nocturnal symptoms, asthma symptoms, quality of life). Blood was drawn for zileuton and theophylline levels, as well as for various safety labs. ECG's, vital signs and physical examinations were also part of the safety follow-up [4/17/95 1:43, 76].

RESULTS

An acute, first-dose bronchodilatory effect on FEV₁₀, compared with baseline, was seen in all three treatment arms. The zileuton QID dose group showed the largest effect and this was statistically significantly greater than the increase seen with theophylline at 3/7 serial post-first-dose time points.

The primary efficacy variable, the AM trough FEV₁₀, was measured at all six Study Visits (Days 8, 22, 36, 50, 64 and 92) and compared with the pre-double-blind baseline as is shown in the reviewer synthesized table below [4/17/95 1:86].

AM TROUGH FEV ₁₀ BASED ON MEAN CHANGE AND MEAN PERCENT CHANGE FROM PRE-DOUBLE-BLIND BASELINE					
Visit Day	Treatment	N	Mean Baseline (L)	Mean Change (L)	Mean Change (%)
8	Z-600	119	2.25	0.24	11.29
	Theo	121	2.14	0.33	16.60
22	Z-600	119	2.25	0.16	7.92
	Theo	121	2.14	0.27	13.53
36	Z-600	119	2.25	0.19**	9.92**
	Theo	121	2.14	0.36	18.62
50	Z-600	119	2.25	0.21**	9.80**

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AM TROUGH FEV ₁₀ BASED ON MEAN CHANGE AND MEAN PERCENT CHANGE FROM PRE-DOUBLE-BLIND BASELINE					
Visit Day	Treatment	N	Mean Baseline (L)	Mean Change (L)	Mean Change (%)
	Theo	121	2.14	0.37	20.31
64	Z-600	119	2.25	0.25	11.21
	Theo	121	2.14	0.30	16.55
92	Z-600	119	2.25	0.27	12.13**
	Theo	121	2.14	0.36	20.24
Final	Z-600	119	2.25	0.27	12.19**
	Theo	121	2.14	0.36	20.24

** statistically significant, zileuton dose vs. theophylline

The originally-defined primary efficacy variable, the AM trough FEV₁₀, showed that theophylline produced about half again greater change from the pre-double-blind baseline as did either of the two zileuton doses. The superiority of theophylline over both zileuton doses is significant, when expressed as the mean percent change from baseline to the final visit.

The sponsor suggests disregarding this prospectively defined primary efficacy variable, the chronic AM trough improvement in FEV₁₀ comparing the 600 mg QID zileuton dose with theophylline, because of the long half-life of the theophylline formulation (6-12 hours) and the relatively shorter half-life of zileuton (2 hours).

COMMENT: This is, by definition, a post-hoc explanation and isn't convincing for several reasons. First, in the case of zileuton the PD variable, FEV₁₀ has a poorly typed, but delayed relation to zileuton blood levels, making the half-life of zileuton in the blood relatively unimportant. Second, the PK/PD characteristics of both drugs were known when the trial was designed. The current argument doesn't explain why these same facts failed to be appreciated when the primary efficacy variable was selected. Third, this same efficacy variable that the sponsor now wishes to abandon has shown statistically significant increases in both pivotal trials over placebo.

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Substitute primary efficacy variables favored by the sponsor are chronic improvement in FEV_{1,0} measured on double-blind days 36 and 92 serially through 6 hours post-morning dosing, of each treatment drug. The baseline for this comparison is the pre-double-blind dosing period. The improvement variables are the 2-4 hour FEV_{1,0} averages, when acute zileuton doses have the highest blood levels, and the 0-6 hour FEV_{1,0} maximum. These are presented in a reviewer synthesized table below and are termed the "Chronic Average Effect" and the "Chronic Maximum Effect" by the sponsor [4/17/95 2:15-7].

CHANGE AND PERCENT CHANGE OF FEV _{1,0} FROM PRE-DOUBLE-BLIND BASELINE ON DOUBLE-BLIND DAYS #36 AND #92					
VISIT/Time Since Dose	Treatment Group	N	Baseline Mean (L)	Post-Dose Mean (L)	Mean Percent Change (%)
DAY #36					
2-4 Hour Avg	Z-600	107	2.23	2.67	21.21
	Theo	105	2.11	2.54	23.17
0-6 Hour Max	Z-600	108	2.23	2.85	30.24
	Theo	105	2.11	2.75	33.70
DAY #92 (The Final Visit)					
2-4 Hour Avg	Z-600	108	2.23	2.70	22.26
	Theo	105	2.11	2.54	23.84
0-6 Hour Max	Z-600	108	2.23	2.86	30.16
	Theo	105	2.11	2.73	33.71

All represented changes of FEV_{1,0} in each treatment group from baseline were statistically significant. No significance was shown for the change from baseline of the theophylline group compared with the change from baseline of either zileuton dose groups.

The AM domiciliary PEF values averaged over two-week intervals and compared with the pre-double-blind baseline period mirror the findings of the AM trough FEV_{1,0}. That is, each

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treatment was superior to the baseline, and theophylline treatment generally resulted in larger increases from baseline than did either dose of zileuton. This achieved statistical significance in about half of the comparisons. For unexplained reasons, the zileuton _____ dose provided larger mean, and mean percent, changes from baseline than did the zileuton 600 mg QID dose [4/17/95 1:90].

Daily symptoms (0-3 scale) were averaged over two-week intervals, like the PEF's, and the mean for each treatment was compared with the mean of that treatment group during the pre-double-blind baseline. All treatment groups showed mean symptom reductions that were statistically significant compared with baseline at every one of the six averaged intervals. At every interval, theophylline showed a larger mean reduction in symptoms than did either of the zileuton treatments. This difference between theophylline and zileuton was statistically significant at 2/6 intervals [4/17/95 1:93]. An identical result was found for nocturnal symptom assessment (0-3 scale, also). That is, mean scores of every treatment at every interval were statistically significantly reduced from the pre-double-blind baseline. Theophylline showed a greater mean reduction at every interval than did either zileuton group and this change was statistically significant favoring theophylline over one, or both zileuton doses in 2/6 intervals [4/17/95 1:95].

Asthma symptoms numbered four (cough, wheezing, shortness of breath, nasal congestion) and each was rated on a 0-3 scale, daily. Differences between groups in symptom reduction favored the superiority of theophylline for reducing wheezing and shortness of breath, in some of the intervals, and favored zileuton _____ over theophylline for nasal congestion in half the intervals [4/17/95 1:96].

The percentage of patients with acute worsening of asthma symptoms occurring during the single-blind period did differ significantly between the zileuton 600 mg QID group and the theophylline group (16.8% and 27.4% for the zileuton 600 mg QID and theophylline groups, respectively). During the double-blind period, 72.1% of theophylline patients experienced an acute worsening of asthma, versus 77.3% of patients taking zileuton 600 mg QID and 74.2% of patients in the zileuton _____ group [4/17/95 1:97].

All three treatment groups demonstrated statistically significant decreases from baseline in the number of puffs of beta-agonist used for all visit intervals. The theophylline group showed a statistically significantly greater decrease in beta-agonist use than the zileuton _____ group in 4/6 visit intervals and a greater decrease than the zileuton 600 mg QID group in 3/6 visit interval. There were no statistically significant differences between groups in numbers of patients who underwent a course of oral or injected steroids [4/17/95 1:98-100].

ADVERSE EVENTS

A total of 37 patients prematurely terminated from the study due at least in part to AE's. Five of these 37 terminated early due to associated non-treatment emergent AE's (Z600 = 3, _____ = 1, Theo = 1). Of the remaining 32, whose premature termination was due to an AE during treatment, the division among arms was Z600 = 7, _____ and Theo = 14.

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The number in each treatment arm with AE's judged to be "severe" by the investigator were as follows: Z600 = 11 patients and Theo = 11 (8.9%). There were 7 patients with serious AE's that resulted in hospitalization. Of these 7, one was in the zileuton 600 mg QID group, one was in the zileuton group, three were in the theophylline group and two were withdrawn from the study prior to receiving any drug.

Clinical laboratory test results were remarkable only for liver function abnormalities. There were 25 patients with liver function elevations noted during the trial that were at least 2xULN (Z600 = 9, Theophylline = 8). Six of these prematurely terminated because of abnormal liver tests (Z600 = 2, Theophylline = 2). In the remaining 19 patients, the liver function test elevations either resolved while on the study drug or decreased to normal levels when the study drug was discontinued [4/17/95 1:102-4]. There were five patients with abnormally low WBC's (Z600 = 2, Theophylline = 1), but the lowest recorded value for this parameter was 2.1×10^9 cells/L. [4/17/95 1:132-3].

CONCLUSIONS

The originally defined primary efficacy variable, the AM trough FEV_{10} , showed that theophylline produced about half again greater change from the pre-double-blind than did either of the two zileuton doses. Retrospectively-defined, FEV_{10} -derived efficacy variables showed equivalence of both zileuton doses and theophylline.

The AM domiciliary PEF values averaged over two-week intervals and compared with the pre-double-blind baseline period mirrored the findings of the AM trough FEV_{10} . That is, each treatment was superior to the baseline and theophylline treatment generally resulted in larger increases from baseline than did either dose of zileuton.

Daily symptoms (0-3 scale) were averaged over two-week intervals, like the PEF's, and all treatment groups showed mean symptom reductions that were statistically significant compared with baseline at every one of the six averaged intervals. At every interval, theophylline showed a larger mean reduction in symptoms than did either of the zileuton treatments. An identical result was found for nocturnal symptom assessment (0-3 scale). That is, mean scores of every treatment at every interval were statistically significantly reduced from the pre-double-blind baseline. Theophylline showed a greater mean reduction at every interval than did either zileuton group.

The percentage of patients with acute worsening of asthma symptoms occurring during the single-blind period did differ significantly between the zileuton 600 mg QID group and the theophylline group (16.8% and 27.4% for the zileuton 600 mg QID and theophylline groups, respectively).

All three treatment groups demonstrated statistically significant decreases from baseline in the number of puffs of beta-agonist used for all visit intervals. The theophylline group showed a statistically significantly greater decrease in beta-agonist use than the zileuton group in 4/6 visit intervals and a greater decrease than the zileuton 600 mg QID group in 3/6 visit interval.

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Over all efficacy endpoints, theophylline seems to have out-performed both zileuton doses. A curious phenomenon was the lack of a dose-proportional response for zileuton. The 360 mg QID dose frequently produced a greater change in the efficacy outcomes than did the higher, 600 mg QID dose. Safety outcomes showed little difference among the three treatment arms.

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M92-785 "Phase III Study of the Effects of Zileuton vs. Placebo on Bronchial Hyperresponsiveness in Asthma Patients"

OBJECTIVES

Determine the effect of zileuton or placebo on non-specific airway hyperresponsiveness of very mild asthma patients as assessed by two broncho-provocation modes, the methacholine and ultrasonically nebulized distilled water challenge tests [302:18].

DESIGN

This was a randomized, double-blind, parallel-group, placebo-controlled, multicenter, 3-month study of zileuton 600 mg QID against placebo in patients with mild asthma, assessed by airflow measures, symptom scores and methacholine challenge. Patients were screened and, 14 days later, those meeting admission criteria were randomized to zileuton or placebo arms. On all study days, the treatment drug dose was to have been withheld and administered at the site after trough procedures were completed. Visits were spaced at about every two weeks.

SUBJECTS

A total of 118 patients were randomized into the study; 58 received zileuton 600 mg QID and 60 received placebo QID. Entrance criteria included an FEV₁₀ $\geq$ 80% of predicted off theophylline and beta agonists, methacholine-induced reduction in FEV₁₀ of $\geq$ 20% and the requirement to have used a beta-agonist within the preceding 30 days. Both nasal steroids and immunotherapy could be continued during the study, if the treatment was chronic and stable. All 118 patients who were randomized received the double-blind drug. Twenty-seven patients discontinued prematurely from the study. Patient ages ranged from 18 to 65 years with a mean value of about 30 years of age. Females were in the majority and accounted for about 60% of both treatment groups. Caucasians accounted for 85-90% of the population [302:32-4, 58].

DOSAGE

Zileuton was supplied as white 600 mg final formulation tablets. Placebo tablets were identical in appearance to the active drug. Both were distributed in bottles of 68 tablets each [302:24].

PARAMETERS

Visits were spaced at about every two weeks and a spirogram was done at each. About every other visit, a methacholine or distilled water challenge was administered and blood for zileuton or theophylline levels was drawn. Only a subset of centers performed distilled water challenge and then only to patients who had shown a positive response to this challenge in the pre-double-blind period. Patient diaries were used to record twice-daily PEFr's, Daily Symptom Assessment (0-3 scale), Nocturnal Assessment (0-3 scale) and number of puffs of beta-agonist rescue-medication use.

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Acute exacerbations of asthma were prospectively defined by symptoms, inhaler use, FEV₁₀ or PEFER changes and was tracked by the investigator along with exacerbations requiring steroids.

The primary efficacy variable was the response to the methacholine challenges. The secondary efficacy variable was the response to distilled water challenges. [302:18, 26-9, 36].

RESULTS

Methacholine challenges were done at baseline, three times within the double-blind period and once during the post-study period. There were no statistically significant differences between the zileuton and placebo groups for mean changes from baseline in log PD₂₀ for any of the methacholine study visits. Ultrasonically nebulized distilled water challenges (UNDW) to determine PI₂₀ (length of exposure to UNDW required to induce a 20% decrease from the best control effect) was only positive in 24 of 118 patients and only 22 underwent one or more additional UNDW challenges. There were no statistically significant differences between the zileuton and placebo groups for mean changes in log PI₂₀ for any of the UNDW study visits.

There were no statistically significant differences between the zileuton and placebo groups in percent change from baseline in trough FEV₁₀ value at any of the visit days. Neither did AM or PM PEFER, Daily Symptom Assessment, Nocturnal Assessment or beta-agonist use measurements show any difference between placebo and zileuton treated groups, expressed as final monthly averages [302:62-4].

ADVERSE EVENTS

The percentage of patients who had at least one treatment-emergent AE was 86.2% for zileuton and 75.0% for placebo. The most common AE's were headaches, aggravation reactions, infections and flu syndrome. The incidences of these AE's were similar for both treatment groups. Seven patients (12.1%) in the zileuton group and five patients (8.3%) in the placebo group experienced one or more severe treatment-emergent AE's. One of these events resulted in hospitalization and was therefore classified as serious. Twelve patients were prematurely terminated from the study due to treatment-emergent AE's. Two patients (3.3%) were in the placebo group and ten patients (17.2%) were in the zileuton group. There were no deaths. There were no statistically significant differences between the zileuton and placebo group for mean changes from baseline for any of the liver function enzyme results. The only statistical difference of clinical relevance in any laboratory parameter is that the zileuton group demonstrated a reduction in percent eosinophils whereas the placebo group demonstrated an increase.

CONCLUSION

In this study of asthmatics with very mild disease, no difference between zileuton 600 mg QID and placebo was found for any efficacy outcome. Safety variables showed no clinically significant differences between the two treatment groups.

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M92-797 "Phase III Study of the Effects of Zileuton and 600 mg QID Compared to Beclomethasone in Moderate Asthma"

OBJECTIVES

Compare efficacy and safety of two doses of zileuton against an inhaled steroid positive control in moderate asthmatics for three months.

DESIGN

This was a Phase III, randomized, double-blind, parallel-group, active-control, multi-center 14-week trial. Patients were to have a baseline FEV₁₀ between 40% and 80% of predicted, with 15% FEV₁₀ reversibility following inhaled albuterol. Allowable respiratory medication included inhaled beta-agonist and chronic, stable treatment with nasal steroids or immunotherapy. After screening and a one-week single-blind placebo run-in period, patients were randomized to one of three arms: 1) zileuton 600 mg QID or, 2) zileuton 300 mg BID or, 3) beclomethasone inhaler 2 puffs (42 mcg/puff) QID. An otherwise unmanageable exacerbation could be handled with oral or intravenous steroids at a maximum dose of 60 mg of Prednisone for one week, with tapering over the ensuing week. Only one such steroid burst was permitted during the trial. A second use of steroids would necessitate withdrawal from the study.

SUBJECTS

Three hundred seventy patients met the inclusion criteria and were randomized in the double-blind period and 293 patients completed it [320:58-9]. These evaluable patients ranged in age from 18 to 61 years and in height from 58 to 82 inches. Fifty-five percent were female and 89.7% were Caucasian [320:63].

DOSAGE

Zileuton 600 mg tablets, and U.S.-approved beclomethasone, 42 mcg/puff were used. Two different placebo pills were necessary because the two active doses of zileuton were dissimilar in appearance.

PARAMETERS

Spirograms were performed at the screening visit, on Study Days 1 and 85 (prior to dosing and at 30, 60, 120, 180 and 240 minutes post-beta-agonist), on Study Days 8, 43 and 99 (immediately prior to dosing and at 30, 60, 120, 180, 240, 300 and 360 minutes post-dosing) and on Study Days 15, 29, 57 and 71 (immediately before dosing). Twice daily domiciliary PEFr's were recorded in the patient diary as were daily, nocturnal and asthma symptom scores and each instance of beta-agonist rescue medication use. Plasma zileuton levels were drawn on Study Days 8, 43 and 99 to insure compliance. Clinical laboratory safety

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information was drawn on Study Days 8, 29, 57 and 99. Blood for zileuton and theophylline was drawn on three study days within the double-blind period to monitor compliance with the protocol.

RESULTS

Chronic trough improvement in pulmonary function was determined by obtaining pulmonary function measurement in the morning, before the first daily dose of the double-blind medication. The results are shown in the reviewer-constructed table below [320:67].

TROUGH EFFECT ON FEV ₁₀ , MEAN CHANGES AND PERCENT CHANGES FROM BASELINE					
Visit Day	Treatment	N	Baseline Mean (L)	Mean Change (L)	Mean % Change
8	Z-600	121	2.25	0.20	11.87
	Beclomethasone	121	2.24	0.48	25.58
36	Z-600	121	2.25	0.23	12.28
	Beclomethasone	122	2.24	0.59	30.97
64	Z-600	121	2.25	0.28	14.65
	Beclomethasone	122	2.24	0.63	32.60
92	Z-600	121	2.25	0.26	14.74
	Beclomethasone	122	2.24	0.60	31.24

All three treatment groups demonstrated statistically significant mean improvements in trough FEV₁₀ compared to pre-Double-Blind baseline at every visit. This was seen as early as one week and persisted throughout the three month period. At every visit, beclomethasone showed a significantly greater mean change from baseline, compared to either dose of zileuton [320:67].

There appears to be a Day 1, first-dose, acute bronchodilatory effect of zileuton that is greater for the larger dose than the smaller and is quantitatively similar between the mg dose and beclomethasone. This effect peaks at the second to the fourth hours following ingestion, and accounts for a change from baseline FEV₁₀ of up to 20% [320:79].

Mean morning and evening PEF's, over two-week intervals, confirmed the spiographic findings. Each treatment drug showed improvement over baseline for each time interval and beclomethasone achieved statistical superiority over each dose of zileuton [320:80-4].

Beta-agonist responsiveness was determined immediately on the first single-blind Day, before the first treatment, and again on Study Day 85, double-blind Day 78. On the latter occasion, the baseline spirogram was determined before the patient had taken the first daily dose of the double-blind treatment. Two hours later, two puffs of albuterol were administered and serial PFT's were performed. On each of the days, each of the treatments at all of the serial sampling times, showed a significant increase in FEV₁₀ over the baseline measurement for that day. The baseline means for Study Day 1 were lower than for Study Day 85, a measure of baseline improvement with treatment. The beta-agonist responsiveness on Study Day 85 was less than on Study Day 1, but the post-challenge mean FEV₁₀ was higher. With a few exceptions at individual time points, the mean changes from baseline did not serve to separate any treatment from any other [321:64-5].

Daily symptom scores, nocturnal symptom scores and beta-agonist use all followed a similar pattern. At most sampling intervals, each treatment showed significantly lower symptom scores than at baseline. In terms of mean change from baseline, the two zileuton doses were not statistically different. Beclomethasone achieved statistically greater reductions in symptom scores than either dose of zileuton [320:85-94].

ADVERSE EVENTS

Thirty-six patients prematurely terminated from the study due to treatment-emergent AE's; 15 (12.1%) from the zileuton 600 mg group, 14 (11.5%) from the zileuton 400 mg group and 7 (5.7%) from the beclomethasone group. The most common AE's associated with these withdrawals were aggravation reaction/asthma exacerbation, headache and infection [320:95, 97].

There were 34 severe AE's; 16 (12.9%) in the zileuton 600 mg group, 6 (4.9%) in the zileuton 400 mg group and 12 (9.7%) in the beclomethasone group. The distribution of AE types was similar to the 36 patients who suffered treatment-emergent AE's [320:95, 100-1].

Three serious AE's resulting in hospitalization were reported, one in each of the treatment groups, one pneumonia, an appendicitis and a miscarriage. In addition, one patient from the zileuton 600 mg group and one from the beclomethasone group were hospitalized due to an acute exacerbation of their asthma [320:95, 104].

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CONCLUSIONS

Both doses of zileuton and inhaled beclomethasone raised trough $FEV_{1.0}$ above baseline, when given chronically. These three treatments also reduced daytime and nighttime symptom scores and rescue medication use. For each of these endpoints, both doses of zileuton and beclomethasone achieved a statistically significant change from baseline. For most endpoints, the mean change from baseline was greater with beclomethasone than with either dose of zileuton.

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M92-800 "The Effect of Chronic Treatment With Zileuton on the Male Reproductive System in Normal Volunteers"

OBJECTIVES

Assess the effect of zileuton on prostate and testicular size; sperm count, motility and morphology; and serum concentrations of testosterone, luteinizing hormone (LH), dihydrotestosterone (DHT) and follicle-stimulating hormone (FSH).

DESIGN

This was a double-blind, randomized, placebo-controlled, parallel-group, 18-week, multicenter trial

SUBJECTS

A total of 43 healthy, sexually mature, adult male volunteers were enrolled in this study at two different sites. Thirty subjects were randomized to receive zileuton and 13 to placebo. One of the subjects was enrolled to replace a subject who experienced an adverse event on Day 2 of the study and who was withdrawn. Forty subjects completed the entire study. Their ages ranged from 18 to 52 years. The 42 subjects included 34 Caucasians and 8 "others" [162:28, 51].

DOSAGE

Subjects took zileuton 600 mg, or its placebo, QID at about 0700, 1300, 1800 and 2300 hours for a period of 18 weeks. The unit dose was a 600 mg tablet [162:28, 35].

PARAMETERS

Screening was followed, within 28 days, by a single baseline visit (Day -1). Subjects visited the study center every two weeks following the initial dose of the treatment regimen. Prostate and testicular ultrasound and Prostate Specific Antigen (PSA) were determined at baseline, Week 8 and Week 18. Only patients free of varicocele and prostatic enlargement were eligible for the study. Following 24 hours of sexual abstinence, semen analysis was performed at baseline, Week 4, Week 12 and Week 18. An endocrine profile was determined at baseline, Week 4, Week 8 and Week 18. The profile was comprised of three collections of blood at each indicated visit which were pooled and analyzed for testosterone, DHT, LH and FSH. Plasma zileuton was determined about 2 hours following the first morning dose (0700) every two week from Week 2 through Week 18. Semen analysis for zileuton was determined at Weeks 4, 12, 18 and 1 month post-dosing. Safety was assessed through periodic measurements of vital signs, physical examinations, laboratory tests, ECG's and interviews [162:33-4].

RESULTS

An extensive analysis of these results was performed under consultation with the Division of Endocrinology by Dr. Jean Fourcroy. The complete consultation is provided as an attachment to this review and a short summary follows. No statistical or clinical changes in prostatic or testicular size were noted. Specifically, there was no evidence of decreasing testicular size with drug therapy. No statistical or clinical changes in PSA level were noted.

A few small, but statistically significant changes in spermatozoan structure and function were noted. The changes were increased motility (Weeks 12 and 18) and sperm concentration (Week 4) from baseline in the placebo group. There were increased cytoplasmic droplets in the zileuton subjects at all time points compared with the placebo group. These were well within the normal range and less than 1%.

No difference between groups was noted for testosterone, FSH and LH. DHT showed a significant difference but remained within clinical parameters ($Z=41.8$ ng/dL, $P=52.8$ ng/dL). The semen zileuton concentrations were approximately 1 mcg/mL which is at the limit of assay. There did not appear to be an accumulation of zileuton in the seminal fluid.

ADVERSE EVENTS

The number and percentage of subjects reporting any AE were 19 (63.3%) for the zileuton group and 7 (53.8%) of the placebo group. The most frequently reported AE's were headache, dizziness, pharyngitis and rhinitis. The number of subjects reporting mild to moderate headache were 6 for the zileuton group (20.0%) and 1 for the placebo group (7.7%). Subject #301 experienced a severe headache while taking zileuton. Dizziness was reported by 2 zileuton subjects (6.7%) and 3 placebo subjects (23.1%). Seven zileuton subjects (23.3%) and 4 placebo subjects (30.8%) complained of pharyngitis. Five zileuton (16.7%) subjects reported rhinitis. One placebo subject (7.7%) experienced a single episode of a transient reduction in libido. This symptom resolved spontaneously.

Two zileuton subjects reported urticaria. Subject #203 experienced a rash after 6 doses. The study drug was withdrawn, he received Benadryl and the rash resolved. Subject #109 developed urticaria after ingesting Robitussin. A single dose of self-administered chlorpheniramine resulted in complete resolution and the subject continued to take zileuton.

Three subjects withdrew. Subject #203 was addressed in the preceding paragraph. One subject withdrew because of an incompatible work schedule. A third subject quit because of personal reasons [162:66-7].

CONCLUSIONS

These are examined in detail in the attached consultation. The consultant concludes that there is no evidence the drug is toxic to developing or post-maturation sperm in this 18-week study and does not appear to be secreted or concentrated in the seminal fluid. The

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consultant notes that 18 weeks is little more than one cycle in spermatogenesis. She recommends post-approval surveillance of possible reproductive changes with chronic use of zileuton including semen evaluation after 3 or 4 cycles of treatment.

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M88-265 "Assessment of the Pharmacokinetic Interaction Between Abbott-64077 and Theophylline"

OBJECTIVES

Define the potential effect of concomitant administration of Abbott-64077 on theophylline pharmacokinetics (PK) under steady-state conditions, and evaluate the safety of such concomitant administration.

DESIGN

This was a randomized, double-blind, two-period, multiple-dose, crossover, Phase I study which included an initial single oral dose of theophylline during screening and serial plasma samples for PK.

SUBJECTS

Sixteen healthy, non-smoking, adult male volunteers were split into two groups of 8 each, with each group receiving a different sequence of regimens.

DOSAGE

The subjects were randomly assigned to receive either 800 mg doses of zileuton or placebo BID for a total of nine consecutive doses. The unit dosage form of the active drug was a 200 mg capsule. Zileuton, or placebo, was administered about 2 hours before breakfast and 1.5 hours following dinner.

PARAMETERS

Within two weeks of starting the study, all subjects were given a single 200 mg dose of theophylline. Serial blood specimens were drawn over the ensuing 10 hours. In order to qualify for the study, the peak theophylline plasma concentration had to be < 7.5 mcg/mL and the theophylline half-life had to be < 10 hours. The half-life estimate for one subject was slightly greater than 10 hours (11.76) but the subject was retained [136:15, 215]. During each study period, all subjects were administered 200 mg doses of theophylline QID for a total of 17 doses over a 5-day period beginning on the morning of Study Day 1. The subjects were randomly assigned to additionally receive either 800 mg doses of zileuton, or placebo, BID for a total of nine consecutive doses, also beginning on the morning of Study Day 1. A 15-day washout separated the two periods. In the second study period, the subjects received the alternate treatment [136:17].

The major thrust of this study was the PK information derived from serial blood samples taken on the 5th day of the double-blind period. Routine ECG's, hematology, and chemistry labs were performed at baseline and again at the completion of a study period. Most of the remainder of the monitoring consisted of vital signs and physical examinations.

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These individuals were institutionalized for the duration of the study periods, and, so, were under some degree of constant scrutiny.

RESULTS

The peak and trough concentration of theophylline after concurrent treatment with zileuton were significantly higher than those after theophylline alone, and the peak levels appeared significantly later after zileuton co-administration [136:436].

FIGURE 21 [136:436]

The statistically significant increase of the systemic exposure from theophylline, when co-administered with zileuton, was caused by a significant decrease of the apparent theophylline clearance. This gave rise to a longer elimination half-life. Furthermore, the theophylline $t_{1/2}$ during the interaction might have been underestimated since the $t_{1/2}$ was estimated at the terminal part of the theophylline profile, where the effect of the zileuton was small, and no systemic zileuton levels could be measured. Even with a possible underestimation of the theophylline $t_{1/2}$ after zileuton co-administration, the increase of the $t_{1/2}$ due to the interaction was highly significant [136:22-3].

THEOPHYLLINE PHARMACOKINETICS (Mean $\pm$ S.D.) IN HEALTHY SUBJECTS WHO WERE CO-ADMINISTERED ZILEUTON OR PLACEBO		
Pharmacokinetic Parameters	Without Zileuton	With Zileuton
C_{Max} (mcg/mL)	12.14 ($\pm$ 2.74)	20.99 ($\pm$ 3.46)
T_{Max} (hr)	0.9 ($\pm$ 0.5)	1.4 ($\pm$ 0.5)
C_{Min} (mcg/mL)	7.06 ($\pm$ 2.09)	15.92 ($\pm$ 3.69)
AUC (mcg-hr/mL)	56.59 ($\pm$ 14.44)	108.93 ($\pm$ 22.37)
$t_{1/2}$ (hr)	6.74 ($\pm$ 1.30)	8.36 ($\pm$ 1.74)

Period effects were not statistically significant.

WITHDRAWALS & ADVERSE EVENTS

Fourteen of the 16 subjects reported a total of 44 AE's, all of which were judged to be "possibly" or "probably" drug-related. During theophylline-placebo treatment, 8 of 15 subjects reported a total of 8 AE's, so the majority of AE's were reported during theophylline-zileuton treatment. In fact, side effects that are generally recognized as being

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theophylline-related were apparent during theophylline-zileuton treatment. These side effects included headache, tachycardia, nausea and vomiting [136:27].

Three subjects terminated prematurely from the double-blind portion of the protocol. One vomited several times on Study Day 3, had an evanescent and low grade temperature and was found to have a spot blood theophylline level of 21.8 mcg/mL. A second subject developed sinus tachycardia and elevated blood pressure (160/110) on Study Day 2. He was discontinued from the study and a blood theophylline level drawn 11.5 hours later was found to be 13.5 mcg/mL. The last early withdrawal, developed headache and nausea on Study Day 3. The blood theophylline level was 20.5 mcg/mL [136:25].

CONCLUSION

The concomitant administration of zileuton and theophylline causes a decrease in theophylline clearance. As a result, the mean theophylline peak plasma concentration increased 73% over that attained when theophylline was administered alone. The increase of the elimination half-life of theophylline from a mean of 6.74 hours to 8.36 hours was significant. Co-administration of theophylline and zileuton will require careful attention to alterations in clearance of the former, caused by the latter. Extra caution will be required if zileuton is to be given at the Phase III dose of 600 mg QID, which is a 1/3 greater daily dose than was used in this study.

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M90-541 "Assessment of the Pharmacokinetic and Pharmacodynamic Interaction Between Zileuton (Abbott-64077) and Warfarin"

OBJECTIVES

Assess the potential effects of concomitant administration of zileuton and warfarin on the anticoagulant effect and on the steady-state pharmacokinetics of warfarin.

DESIGN

This was a randomized, double-blind, multiple-dose, parallel-group Phase I study. Healthy subjects took warfarin for 9 days with the dose titrated to give a target prothrombin time (PT) of 14 to 18 seconds. For 4 more days the patients were maintained on a stable dose of warfarin and their PT checked at the end of this period to insure that they maintained the target PT. The double-blind portion of the protocol began on the 15th day when the group was randomized to receive either placebo or zileuton, in addition to their stable warfarin dose. This double-blind period lasted for 7 days.

SUBJECTS

Twenty-four normal subjects entered the double-blind period. The range of their ages was 19 to 39 years. Their heights ranged from 63 to 74 inches. Four were Black and twenty were Caucasian.

DOSAGE

The double-blind dose of zileuton was 600 mg QID and the dosing unit was a 200 mg capsule. Warfarin was made by Dupont and supplied as 1, 2, 5 and 10 mg crystalline warfarin sodium per tablet.

PARAMETERS

The thrust of this study was to establish the pharmacokinetics of warfarin, as affected by zileuton. Multiple serial blood drawings for warfarin enantiomers were done on the 13th and 14th days (baseline), and on the 19th and 20th days. This latter time was chosen because it represented a steady-state level after chronic administration of 5 days on zileuton or placebo treatment. The patients were institutionalized for the duration of the study. Some blood, ECG's and urinalyses were performed.

RESULTS

The PK data are presented as individual means ($\pm$ SD) for R- and S-enantiomers when warfarin was administered alone (Day 14) and following concomitant multiple dose administration with either zileuton or placebo (Day 20) [141:8, 23].

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MEAN ($\pm$ SD) STEADY-STATE PHARMACOKINETIC PARAMETERS FOR R- AND S-WARFARIN ADMINISTERED ALONE, WITH ZILEUTON OR WITH PLACEBO				
Parameters	Zileuton/Warfarin (n=9)		Placebo/Warfarin (n=11)	
	Warfarin Alone (Day 13-14)	Warfarin + Zileuton (Day 19-20)	Warfarin Alone (Day 13-14)	Warfarin + Placebo (Day 19-20)
R-WARFARIN				
T_{Max} (hr)	4.2 ($\pm$ 3.1)	5.2 ($\pm$ 3.9)	2.0 ($\pm$ 0.8)	2.3 ($\pm$ 2.3)
C_{Max} (ng/mL)	1059 ($\pm$ 598)	1184 ($\pm$ 691)	1374 ($\pm$ 529)	1394 ($\pm$ 455)
C_{Min} (ng/mL)	650 ($\pm$ 357)	807 ($\pm$ 483)	722 ($\pm$ 214)	813 ($\pm$ 356)
AUC_{0-24} (ng-hr/mL)	18994 ($\pm$ 10359)	23109 ($\pm$ 13812)	22244 ($\pm$ 6773)	24207 ($\pm$ 8724)
$t_{1/2}$ (hr)	n/a	71 ($\pm$ 17)	n/a	58 ($\pm$ 14)
S-WARFARIN				
T_{Max} (hr)	3.3 ($\pm$ 2.6)	2.3 ($\pm$ 1.3)	2.1 ($\pm$ 0.8)	2.2 ($\pm$ 2.3)
C_{Max} (ng/mL)	704 ($\pm$ 394)	642 ($\pm$ 362)	965 ($\pm$ 372)	857 ($\pm$ 248)
C_{Min} (ng/mL)	348 ($\pm$ 159)	340 ($\pm$ 167)	402 ($\pm$ 116)	365 ($\pm$ 117)
AUC_{0-24} (ng-hr/mL)	11215 ($\pm$ 5420)	11008 ($\pm$ 5516)	13604 ($\pm$ 3915)	12555 ($\pm$ 3514)
$t_{1/2}$ (hr)	n/a	47 ($\pm$ 17)	n/a	35 ($\pm$ 8)

There is a statistically significant increase in the AUC of the R-enantiomer when zileuton is co-administered with warfarin, compared to warfarin alone. This increase appears qualitatively greater than the slight increase seen with warfarin and placebo, compared to warfarin alone.

Pharmacodynamic data are listed below as the mean ($\pm$ S.D.) morning and evening prothrombin times for the average of Days 13 and 14 (warfarin alone) and those for the average of Days 19 and 20 (warfarin with zileuton or placebo) [141:8].

MEAN ($\pm$ SD) MORNING AND EVENING PROTHROMBIN TIMES WHEN WARFARIN IS ADMINISTERED ALONE, WITH ZILEUTON OR WITH PLACEBO				
	Zileuton/Warfarin (n=13)		Placebo/Warfarin (n=11)	
	Warfarin Alone	Warfarin + Zileuton	Warfarin Alone	Warfarin + Placebo
Pre-dose (morning)	17.5 ($\pm$ 1.7)	19.8 ($\pm$ 3.5)	18.1 ($\pm$ 1.4)	18.8 ($\pm$ 1.9)
12 hr. post-dose (evening)	17.1 ($\pm$ 1.9)	19.1 ($\pm$ 3.8)	17.3 ($\pm$ 1.5)	17.5 ($\pm$ 1.8)

There appears to be a distinct increase in the mean PT with warfarin and zileuton compared with warfarin alone. This is of greater magnitude than the very slight increase in PT with warfarin and placebo, compared with the preceding baseline warfarin-alone period.

WITHDRAWALS & ADVERSE EVENTS

Twenty-four subjects entered the double-blind period. Four of 13 who entered the warfarin/zileuton treatment group were dropped because of PT exceeding protocol limitations ($\geq$ 23 seconds). Dropouts occurred after 5-11 doses of zileuton on double-blind days 2-3 [141:22]. No patients receiving placebo withdrew early.

Ten AE's were reported by 6 subjects during the double-blind portion of the study. Of these 6, 5 received zileuton and 1 received placebo. The 5 zileuton patients reported 9/10 of the AE's: anxiety, insomnia, alert upon awakening, lightheadedness, drowsiness, tiredness (some reported more than once). All AE's were followed until they remitted.

CONCLUSION

Although the analysis of covariance results did not detect significant differences in the pharmacokinetics of R- and S-warfarin between the zileuton/warfarin and placebo/warfarin groups, within-group analysis detected a significant effect of chronic Q6H dosing of zileuton on the pharmacokinetics of R-warfarin. This resulted in an increased warfarin AUC which was accompanied by an increase in the morning and evening prothrombin times. This PK interaction may have been underestimated because four subjects with PT's >23 seconds were prematurely dropped from the study, thus eliminating them from representation in the pharmacokinetic characterization. Zileuton doses of 600 mg Q6H given with warfarin QD should be monitored frequently with PT's and dose titration of warfarin should be anticipated.

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M90-543 "Efficacy of Oral Contraceptives in Volunteers Receiving Concomitant Zileuton"

OBJECTIVES

Examine the effect of oral zileuton, 600 mg QID, and concomitantly administered oral contraceptives in women on plasma:

1. ethinylestradiol
2. progestogen
3. progesterone

Secondary objectives were to study the effect of zileuton on plasma follicle stimulating hormone (FSH), luteinizing hormone (LH) and sex globulin binding hormone (SGBH) in women using oral contraceptives. (N.B. The SGBH was never evaluated because of inadequate amounts of plasma collected to assay it [143:54].)

DESIGN

This was a placebo-controlled, multiple-dose, double-blind, parallel-group study in healthy female volunteers who had regularly used fixed-combination oral contraceptives for a minimum of 6 months with no AE's and with good compliance. All subjects were monitored for 3 complete menstrual cycles. The beginning of a cycle was defined by the first day of oral contraceptive pill administration. A monitored cycle consisted of 21 days of contraceptive pill-taking and the following 7 days, in which no contraceptive pill was taken. On the first day of the first cycle, subjects entered a single-blind lead-in period and received three capsules of placebo QID in addition to their oral contraceptive medicine. The beginning of the second cycle was the beginning of the double-blind period with 2/3 of the subjects receiving zileuton and the remaining third, the placebo in addition to their usual oral contraceptive, the latter taken between 2000 and 2200 hours. Zileuton, or its placebo, was taken continuously through cycles 2 and 3 with the last dose administered on the 21st day of the 3rd cycle [143:12, 18].

SUBJECTS

Thirteen subjects entered the double-blind segment of the study and received zileuton and 6 subjects received placebo. They ranged in age from 19 to 32 years and all were Caucasian except for one whose race was listed as English/Dominican Republic. The inclusion criteria of compliance was that subjects, "...will have a history of compliance with oral contraceptives and have a regular 28-30 day menstrual cycle defined as no breakthrough bleeding during oral contraception and normal withdrawal bleed during the pill-free period."

DOSAGE

Zileuton was given in a dose of 600 mg QID and the individual dosing unit was the 200 mg capsule. This was an English study and some of the fixed-combination oral

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contraceptives are not approved in this country. These fixed-combination oral contraceptives included: Eugynon 30®, Femodene®, Marvelon®, Microgynon 30®, Minulet®, Ovan 30® and Ovranelle®.

PARAMETERS

Blood samples were collected for determination of ethinylestradiol, progestogen, progesterone, FSH, LH and SHBG on Days 5, 6, 7, 19, 20 and 21 at 0800 during cycles 1 and 3. Plasma zileuton levels were measured on Days 5 and 21 during the 2nd and 3rd cycles for compliance. In addition, on Day 21 of cycle 3, serial plasma samples for the determination of zileuton pharmacokinetics were obtained from 1/5 to 24 hours post-dosing [143:19]. Laboratory safety studies were done twice each cycle; pregnancy tests, once per cycle and physical exams 2-3 times each cycle.

RESULTS

Mean plasma hormones levels in the follicular phase of the menstrual cycle (Days 5, 6 and 7) are presented for each hormone in cycle 1, oral contraceptive only, and cycle 3 where the oral contraceptive was given either with zileuton or with placebo in this reviewer-synthesized table [143:42-6].

MEAN ($\pm$ SD) PLASMA HORMONE LEVELS DURING FOLLICULAR PHASE (DAYS 5-7) OF MENSTRUAL CYCLES 1 & 3				
Hormone	N	Cycle 1 Mean ($\pm$ SD) (OC only)	Cycle 3 Mean ($\pm$ SD) (OC + Zileuton or Placebo)	Double- Blind Treatment
ethinylestradiol (pg/mL)	13	29.80 ($\pm$ 17.99)	36.67 ($\pm$ 16.41)	Zileuton
ethinylestradiol (pg/mL)	5	35.89 ($\pm$ 18.92)	33.63 ($\pm$ 13.62)	Placebo
progestogen (ng/mL)	13	2.87 ($\pm$ 2.06)	3.05 ($\pm$ 2.21)	Zileuton
progestogen (ng/mL)	5	2.64 ($\pm$ 1.24)	2.98 ($\pm$ 1.23)	Placebo
FSH (U/L)	13	2.19 ($\pm$ 0.74)	1.60 ($\pm$ 0.76)	Zileuton
FSH (U/L)	5	2.17 ($\pm$ 1.33)	1.36 ($\pm$ 1.20)	Placebo
LH (U/L)	13	2.97 ($\pm$ 1.52)	2.75 ($\pm$ 1.74)	Zileuton
LH (U/L)	5	3.47 ($\pm$ 2.17)	2.71 ($\pm$ 2.65)	Placebo
progesterone (nmol/L)	12	0.98 ($\pm$ 0.93)	1.63 ($\pm$ 1.14)	Zileuton
progesterone (nmol/L)	5	0.68 ($\pm$ 0.58)	0.53 ($\pm$ 0.52)	Placebo

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The differences in ethinylestradiol between baseline and zileuton or placebo treatments, co-administered with oral contraceptives, did not achieve statistical significance. By inspection, there was no effect of zileuton on the plasma progestogen. FSH declines in the 3rd cycle, consistent with the suppressing effect of oral contraceptives, but the explanation for the greater decline in the placebo treatment group is not apparent. Plasma LH appears little effected by different cycles and treatment groups in the follicular phase. The mean plasma concentrations of progesterone in the follicular phase increased significantly from cycle 1 to 3 in the zileuton group but not in the placebo group [143:53].

A table describing the average hormonal results of the luteal phase (Days 19 - 21) has been synthesized by the reviewer [143:47-51].

MEAN ($\pm$ SD) PLASMA HORMONE LEVELS DURING LUTEAL PHASE (DAYS 19-21) OF MENSTRUAL CYCLES 1 & 3				
Hormone	N	Cycle 1 Mean ($\pm$ SD) (OC only)	Cycle 3 Mean ($\pm$ SD) (OC + Zileuton or Placebo)	Double- Blind Treatment
ethinylestradiol (pg/mL)	13	37.09 ($\pm$ 19.26)	47.81 ($\pm$ 23.73)	Zileuton
ethinylestradiol (pg/mL)	5	50.69 ($\pm$ 23.56)	49.53 ($\pm$ 17.00)	Placebo
progestogen (ng/mL)	13	4.02 ($\pm$ 2.46)	4.16 ($\pm$ 2.65)	Zileuton
progestogen (ng/mL)	5	4.03 ($\pm$ 1.95)	4.35 ($\pm$ 2.08)	Placebo
FSH (U/L)	13	0.45 ($\pm$ 0.22)	0.35 ($\pm$ 0.12)	Zileuton
FSH (U/L)	5	0.73 ($\pm$ 0.60)	0.79 ($\pm$ 0.78)	Placebo
LH (U/L)	13	1.48 ($\pm$ 0.61)	1.22 ($\pm$ 0.64)	Zileuton
LH (U/L)	5	1.52 ($\pm$ 0.79)	1.59 ($\pm$ 1.36)	Placebo
progesterone (nmol/L)	12	1.13 ($\pm$ 0.80)	1.61 ($\pm$ 1.00)	Zileuton
progesterone (nmol)	5	0.52 ($\pm$ 0.47)	0.63 ($\pm$ 0.68)	Placebo

The luteal phase ethinylestradiol appears to increase after zileuton was added to the oral contraceptive, although the double-blind plasma level is not different from the double-blind placebo-group level. Progestogen rose slightly from cycle 1 baseline in both zileuton and placebo double-blind period treatment groups. Neither within group, nor between group changes in the FSH or LH are significant. Progesterone increased when zileuton was added

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to oral contraceptive in the 3rd cycle of the luteal phase, which also occurred in the follicular phase.

Trough morning zileuton concentrations measured on Days 5 and 21 of cycle 2 and on Days 5 and 21 of cycle 3 indicate that the subjects were compliant with their dosing schedule. For the last dose interval of the study (Day 21 of cycle 3), the mean C_{Max} was 3.25 mcg/mL and the mean AUC was 12.69 mcg-hr/mL. [143:54].

WITHDRAWALS & ADVERSE EVENTS

Of the 19 patients who entered the double-blind period, only 1 was withdrawn for an episode of cystitis. All, but one subject who received placebo, had at least one AE. The most frequently reported mild AE's were headache, asthenia, infection and metrorrhagia. Moderately severe AE's included flu-like syndrome, abdominal pain, pinworm infection, cough, nausea, vomiting and break-through bleeding. Half of the moderately severe AE's were reported in the placebo group.

CONCLUSION

Subjects receiving oral contraceptives and concomitant zileuton showed no clinically significant changes in plasma concentrations of ethinylestradiol, progesterone, FSH and LH. A small but statistically significant rise in plasma progesterone concentration during the luteal phase of the menstrual cycle was noted in the zileuton group when comparing cycle 1 and cycle 3 values. However, the mean for each subject was well below values observed in ovulating females (16-80 nmol/L) [143:56].

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M91-617 "Assessment of the Pharmacokinetic Interaction Between Zileuton (Abbott-64077) and Prednisone"

OBJECTIVES

Evaluate the effect of concomitant steady-state zileuton administration and a single 40 mg dose of prednisone on the pharmacokinetics of prednisolone, the active metabolite of prednisone. Also evaluate the effect of the single prednisone dose on the steady state pharmacokinetics of zileuton.

DESIGN

This was a randomized, placebo-controlled, double-blind, 2-period crossover, Phase I study. Zileuton 600 mg QID, or placebo, was given for 7 days. On the 6th day a single 40 mg prednisone dose was administered and serial blood samples were drawn. An 8 day washout separated the blinded treatments, zileuton or placebo.

SUBJECTS

Sixteen healthy, non-smoking adult male volunteers between 19 and 40 years of age and with heights ranging from 64 to 75 inches participated. They were institutionalized for each of the 7-day periods and received the zileuton/placebo at controlled times, beginning at 0600 hours.

DOSAGE

The zileuton dose was 600 mg QID and the unit dose was a 200 mg capsule. The four dosing times were 0600, 1200, 1800 and 2400 hours. Prednisone was given as a single dose of 40 mg, two Deltasone 20 mg tablets given at 0600 hours on the 6th Day.

PARAMETERS

Blood samples, for zileuton and for prednisolone, were collected prior to dosing on Study Day 1. Samples for zileuton were taken on Days 5 and 6, before the morning dose, and serially through 6 hours thereafter. Samples for prednisolone were obtained on Day 6, before dosing, and serially through 30 hours thereafter.

RESULTS

The pharmacokinetic parameters of prednisolone and zileuton, presented as mean ($\pm$ standard deviation), obtained in this study are presented below [144:17].

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PHARMACOKINETICS OF ZILEUTON, WITH OR WITHOUT PREDNISONE, AND OF PREDNISOLONE, WITH AND WITHOUT ZILEUTON				
Parameters	Prednisolone PK		Zileuton PK	
	Placebo	Zileuton	Day 5 (pre-prednisone)	Day 6 (with prednisone)
T_{Max} (hr)	1.4 ($\pm$ 0.8)	1.2 ($\pm$ 0.5)	2.3 ($\pm$ 0.8)	1.7 ($\pm$ 0.7)
C_{Max} (ng/mL)	401 ($\pm$ 46)	422 ($\pm$ 58)		
C_{Max} (mcg/mL)			6.28 ($\pm$ 1.83)	5.89 ($\pm$ 1.40)
AUC_{0-6} (mcg-hr/mL)			22.92 ($\pm$ 7.05)	19.86 ($\pm$ 4.97)
AUC_{0-} (ng-hr/mL)	2312 ($\pm$ 487)	2372 ($\pm$ 391)		
$t_{1/2}$ (hr)	2.8 ($\pm$ 0.3)	2.9 ($\pm$ 0.4)		

The peak plasma prednisolone concentration was reached on average at 1.3 hours post-dosing with placebo and zileuton. After 3 hours, the plasma concentration declined, becoming undetectable at 24 hours, except in one patient. Patient #6 had received concomitant zileuton and had detectable prednisolone levels 30 hours post-dosing. The only statistically significant finding in prednisolone PK between placebo- and zileuton-treated patients, was the $t_{1/2}$. Although significant, this mean increase in the $t_{1/2}$ of 3.6% was felt to be clinically of little import and it was concluded that multiple doses of zileuton have little effect on the PK of plasma prednisolone, following oral prednisone.

Small, but statistically significant decreases in mean AUC_{0-6} and T_{Max} of zileuton were seen on Day 6 compared with Day 5. These decreases, observed with the co-administration of prednisone, were possibly due to randomness in morning zileuton pre-dose mean concentrations, which were significantly lower on the morning of the prednisone dose (2.06 mcg/mL) than on the prior morning (3.15 mcg/mL) [144:18].

WITHDRAWALS & ADVERSE EVENTS

Fourteen of the 16 subjects in the study reported a total of 45 AE's. Of these 45, 18 events (40%) were reported by a single subject who experienced 11 events during or after placebo and 7 events during zileuton dosing. Generally, AE's were more frequent when subjects were taking zileuton alone, though the number of subjects reporting these AE's was no greater for the zileuton-alone portion of the protocol than it was for the placebo-alone period [144:20].

NUMBER AND PATIENTS REPORTING ADVERSE EVENTS			
Treatment	Study Days	Number of AE's	Number Of Subjects Reporting AE's (%)
Zileuton	Days 1-5 (zileuton alone)	19	7 (44%)
	Days 6-7 (zileuton/prednisone)	6	5 (31%)
Placebo	Days 1-5 (placebo alone)	13	7 (44%)
	Days 6-7 (placebo/prednisone)	6	2 (13%)

The most AE's reported were CNS-related; headache, disorientation, dizziness, a "fuzzy" feeling and abnormal dreams (4 zileuton- and 6 placebo-subjects). The next most frequent AE's were lower digestive system-related events, flatulence, indigestion, nausea and decreased appetite (3 zileuton- and 3 placebo-treated patients). A total of 10 subjects (62.5%) in the zileuton group and 8 placebo-subjects (50%) reported at least one AE [144:26].

CONCLUSION

The pharmacokinetics of prednisolone after oral administration of 40 mg of prednisone in healthy volunteers were not changed by seven days of 600 mg zileuton QID as compared with placebo. The steady-state plasma concentrations of zileuton were slightly lower on the day of co-administration with prednisone (Day 6) than on the previous, pre-prednisone day (Day 5). A small but statistically significant decrease in zileuton AUC was noted on Day 6, when co-administered with prednisone. This may be attributable to the lower baseline zileuton concentration on that day, or to the concomitant administration of prednisone. The former interpretation is favored by the sponsor.

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M92-789 "Assessment of the Pharmacokinetic Interaction Between Zileuton (Abbott-64077) and Propranolol"

OBJECTIVES

Assess the effects of concomitant administration of multiple doses of zileuton on propranolol pharmacokinetics and to evaluate the safety of co-administering these two agents.

DESIGN

This was an open-label, multiple-dose, 8-day, Phase I study in which each subject served as his own control. A single oral dose of propranolol was given at 0800 on Days 1 and 7. Zileuton Q6H was begun at 0800 on Day 3 for a total of 20 doses, the last of which was given at 0200 on Day 8. The subjects were confined for the duration of the trial.

SUBJECTS

Sixteen healthy adult male subjects were enrolled in, and completed, the study. The ages of the subjects ranged from 18 to 33 years, with heights ranging from 66 to 74 inches. Fourteen of the subjects were Caucasian and two were black.

DOSAGE

Zileuton was given at the dose of 600 mg QID for 5 days (Study Days 3-8) at 0800, 1400, 2000 and 0200. The unit dose was a 600 mg tablet. Propranolol was given as a single 80 mg tablet at 0800 on Days 1 and 7. The dosing unit was a single 80 mg tablet manufactured by Wyeth-Ayerst Laboratories.

PARAMETERS

Serial, 24-hour blood samples were taken for propranolol PK after a single 80 mg dose given on Day 1 (baseline) and on Day 7 (with zileuton). To help distinguish among any reduction in propranolol clearance due to an effect of zileuton on cytochrome P₄₅₀IID6, cytochrome P₄₅₀IICmp or glucuronyl transferase, 4-hydroxy-propranolol plasma concentrations were also measured. Additional labs, vital signs and ECG's were periodically determined as safety measures.

RESULTS

Serum levels of propranolol and of 4-hydroxy propranolol are presented as a function of time since dose for zileuton- and placebo-treated subjects [145:26].

FIGURE 22 [145:26]

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The mean ($\pm$ standard deviation) pharmacokinetic parameters of propranolol and 4-hydroxy-propranolol after dosing with propranolol alone and with 600 mg of zileuton Q6H are shown in the table below [145:27].

PK PARAMETERS OF PROPRANOLOL AND 4-HYDROXY PROPRANOLOL WITH AND WITHOUT CO-ADMINISTRATION OF ZILEUTON				
Parameter	Propranolol		4-OH-Propranolol	
	Propranolol Alone	Propranolol With Zileuton	Propranolol Alone	Propranolol With Zileuton
T _{Max} (hr)	1.4 ($\pm$ 0.7)	1.9 ($\pm$ 1.0)	0.9 ($\pm$ 0.2)	1.3 ($\pm$ 0.6)
C _{Max} (ng/mL)	93.15 ($\pm$ 41.31)	142.24 ($\pm$ 64.65)	40.24 ($\pm$ 27.19)	44.31 ($\pm$ 33.44)
AUC _{0-∞} (ng*hr/mL)	517.2 ($\pm$ 312.1)	1056.4 ($\pm$ 755.7)	104.1 ($\pm$ 65.9)	139.09 ($\pm$ 89.06)
t _{1/2} (hr)	3.56 ($\pm$ 0.56)	4.54 ($\pm$ 1.26)	2.41 ($\pm$ 0.67)	3.40 ($\pm$ 0.77)
AUC Ratio (4-OH-P/P)			0.29 ($\pm$ 0.21)	0.24 ($\pm$ 0.19)

Co-administration of propranolol and zileuton increased the C_{Max} of propranolol from 93 to 142 ng/mL, doubled the AUC_{0-∞} and increased the elimination half-life (t_{1/2}) by 28%. The AUC_{0-∞} and t_{1/2} of 4-OH-propranolol were also significantly increased with propranolol and zileuton co-administration. The mean AUC ratio decreased from 0.29 to 0.24 in the presence of zileuton. If the cytochrome P₄₅₀IID6 oxidation of 4-OH-propranolol was unaffected by zileuton and if the metabolite's elimination clearance was also unaffected, the AUC of the metabolite should have increased proportional to the increase in the AUC of propranolol. The less than expected increase in AUC for 4-OH-propranolol and the statistically significant change in the AUC ratio implies that either the P₄₅₀IID6 pathway was inhibited or the elimination of the metabolite was increased in the presence of zileuton [145:39-40].

The standing heart rate decreased significantly from its same-day pre-dose value comparing Day 1 to Day 7 at 2, 4 and 6 hours after the propranolol dose. Lesser but similar changes were noted in the supine heart rate. The same-day, pre- and post-propranolol standing heart rates differed by a mean of 1.5 beats/min. on Day 1 (before zileuton) and by a mean of 11 beats/min. on Day 7 (after zileuton) [145:28]. This pharmacodynamic (PD)

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effect parallels the PK effect. For another possible PD effect see the development of sinus bradycardia under "ADVERSE EVENTS," below.

WITHDRAWALS & ADVERSE EVENTS

Four subjects (25%) reported an AE while taking propranolol alone, 8 subjects (50%) in the zileuton monotherapy segment and 6 subjects (38%) during the co-administration period. Headache was the most common AE. The only other AE experienced by more than one subject during any segment was sore throat (pharyngitis), which was reported by 1 subject (6%) during the propranolol segment, 3 subjects (19%) during the zileuton segment and no subjects during the combination-drug segment [145:33].

On Day 7, 2 hours after the first propranolol dose and multiple zileuton doses, ECG's showed changes from the pre-study period in 8/16. In 5/16 similar changes were seen on Day 8, 24 hours after the last propranolol dose. The pre-study ECG's were normal or showed early repolarization. In all cases on Days 7 and 8 the ECG change was interpreted as the development of sinus bradycardia [145:34, 204-7].

One subject developed mildly elevated ALT (69 U/L) and GGT (110 U/L) on Day 8, having had normal LFT's at pre-study [145:37].

CONCLUSION

The metabolism of a single, 80 mg dose of propranolol was significantly inhibited by 600 mg doses of zileuton Q6H for 4 to 5 days. Inhibition of P₄₅₀IID6 mediated oxidation was at least partially responsible for this decreased clearance of propranolol. Increased beta-blocking activity of propranolol occurred in the presence of zileuton, probably as a consequence of this change in propranolol PK.

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M92-821 "Assessment of the Pharmacokinetic Interaction Between Zileuton (A-64077) and Digoxin"

OBJECTIVES

Assess the potential effects of concomitant administration of multiple doses of zileuton on digoxin pharmacokinetics.

DESIGN

This was a double-blind, multiple-dose, two-period crossover study. Each subject received zileuton or placebo Q6H beginning at 0800 on Day 1 through 1400 on Day 14. Additionally, subjects received digoxin 0.25 mg during both study periods on Days 1 through 11 with the 0800 dose of zileuton or placebo. After an 8-day washout interval, subjects received the alternate regimen during the second period.

SUBJECTS

Twelve healthy, adult, non-smoking males between 21 and 44 years of age participated in the study. Ten were Caucasian, one was black and the twelfth was oriental [149:3].

DOSAGE

Zileuton 600 mg Q6H was provided as a 600 mg tablet as the unit dose. Digoxin was given QAM as a single tablet of 0.25 mg, and was manufactured by Burroughs-Wellcome.

PARAMETERS

Digoxin serum levels were sampled each morning before the 0800 dose of digoxin or zileuton/placebo. In addition, on the 11th day, after the last dose of digoxin, serial blood samples were drawn for 72 hours to map the decline of the chronically dosed but now discontinued digoxin, while zileuton/placebo Q6H continued throughout the collection period. Routine chemistries, hematology, urinalyses and ECG's were periodically obtained.

RESULTS

Digoxin PK taken from the trough level, before the last AM dose on the 11th day, through 72 hours later shows almost identical plasma concentration, time-decay curves, whether digoxin was taken concomitantly with zileuton or with placebo [148:9, 31].

FIGURE 24 [148:9, 31]

The mean PK parameters were also very similar, as might be supposed from the concentration-time curves [148:8].

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MEAN PHARMACOKINETIC PARAMETERS OF DIGOXIN AFTER THE LAST DOSE FOLLOWING DAILY DOSING FOR ELEVEN DAYS				
Parameter	Treatment Means (n=12)		Treatment Means (n=10)	
	Digoxin & Placebo	Digoxin & Zileuton	Digoxin & Placebo	Digoxin & Zileuton
C_{Max} (ng/mL)	1.314 ($\pm$ 0.266)	1.345 ($\pm$ 0.261)	1.356 ($\pm$ 0.240)	1.348 ($\pm$ 0.213)
T_{Max} (hr)	1.43 ($\pm$ 0.49)	0.95 ($\pm$ 0.30)	1.47 ($\pm$ 0.52)	0.89 ($\pm$ 0.27)
AUC_{0-24} (ng*hr/mL)	14.27 ($\pm$ 3.21)	14.06 ($\pm$ 2.95)	14.69 ($\pm$ 3.18)	14.09 ($\pm$ 2.93)
$t_{1/2}$ (hr)	34.9 ($\pm$ 5.0)	37.1 ($\pm$ 6.1)	34.7 ($\pm$ 5.1)	37.8 ($\pm$ 6.2)

The number of patients in each group were not equal. At 0800 on Day 11 Period 2, two subjects were dosed incorrectly, hence removed from the final calculation of PK parameters. Only the mean T_{Max} shows a difference in digoxin PK between zileuton- and placebo-treated patients. Those who received zileuton and digoxin had a statistically significantly shorter T_{Max} than did those taking digoxin and placebo. As a single PK difference, this was considered to be clinically unimportant because of the long duration of action of digoxin [148:9-10]. No other PK parameters were significant.

WITHDRAWALS & ADVERSE EVENTS

Two patients were withdrawn from the efficacy analysis because they had been given the wrong treatment drug, but all were retained in the AE analysis. The number and percent of subjects reporting any AE was 5 (42%) for the zileuton/digoxin regimen and 8 (67%) for the placebo/digoxin regimen. Headache was the most frequent AE and was seen in 2 (17%) of those taking the zileuton/digoxin regimen and 3 (25%) of patients on the placebo/digoxin regimen. All other AE's were reported by one subject. Only one subject experienced a severe AE and that was a syncopal episode in a placebo/digoxin patient. Two patients showed elevated liver enzymes and both were in the placebo/digoxin group.

CONCLUSION

The concomitant administration of zileuton and digoxin had no effect on the PK of digoxin.

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M92-824 "Assessment of the Pharmacokinetic Interaction Between Zileuton and Phenytoin"

OBJECTIVES

Assess the effect of zileuton on phenytoin pharmacokinetics and evaluate the safety of concomitant administration of these two agents.

DESIGN

This was a double-blind, multiple-dose, randomized, 2-period crossover study. During each study period, the subjects were to receive 600 mg of zileuton or placebo Q6H beginning at 0800 on Day 1 through 1400 on Day 8. On Day 5 of both periods, each subject received a single 300 mg oral dose of phenytoin, along with the 0800 zileuton or placebo dose. A 9-day interval separated the last dose in Period 1 and the first dose in Period 2. Period 2 was identical to Period 1 except that the alternate treatment was given, zileuton or placebo.

SUBJECTS

Twenty-one healthy, adult, non-smoking male volunteers between 19 and 38 years of age, were enrolled and 18 successfully completed the study. Fifteen subjects were evaluable for PK analysis.

DOSAGE

The dose of zileuton was 600 mg QID and the unit dose was a 600 mg tablet. Phenytoin was given as a single dose of 300 mg and the unit dose was a 100 mg capsule manufactured by Parke-Davis.

PARAMETERS

Phenytoin PK data was gathered before and serially for 72 hours after a single oral dose was administered coincident with, and after 5 days of chronic dosing with, either zileuton or placebo. The usual blood samples, urine tests, vital signs and ECG's were also obtained before, during and after the double-blind period.

RESULTS

The mean ($\pm$ standard deviation) pharmacokinetic parameters of phenytoin for all 15 evaluable subjects, after administration with or without zileuton are tabulated below [146:9]

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SINGLE-DOSE PHENYTOIN PHARMACOKINETICS WHEN CO-ADMINISTERED AFTER, AND IN CONJUNCTION WITH, CHRONIC ZILEUTON TREATMENT		
Parameter	With Placebo	With Zileuton
T _{Max} (hr)	7.1 (± 2.8)	6.1 (± 5.6)
C _{Max} (mcg/mL)	4.3 (± 1.3)	4.3 (± 1.2)
AUC _{0-t} (mcg*hr/mL)	141 (± 72)	151 (± 87)
AUC _{0-∞} (mcg*hr/mL)	164 (± 137)	183 (± 179)

The curves of plasma concentration as a function of time since administration, were almost superimposable [146:31].

FIGURE 23 [146:31]

Analysis of variance indicated that none of the PK parameters of phenytoin was statistically different between the two dosing regimens. Ninety-five percent confidence intervals for the ratio of regimen medians for both C_{Max} and AUC_{0-∞}, overlap unity and extend over a narrow range, suggesting the absence of interaction. Analysis based on a Michaelis-Menten model and using the data of all 20 subjects who received the phenytoin dose of one or both of the regimens did not give any evidence of a difference in phenytoin PK for the two regimens [146:32-3].

WITHDRAWALS & ADVERSE EVENTS

One subject (#9) was replaced with subject (#21) because of the protocol violation of smoking. Subject #3 did not enter Period 2 and subject #7 was withdrawn from Period 2 (placebo), both because of rash development. An additional three subjects (#6, #18 and #20) were not included in the PK analysis due to a large number of missing values that resulted from insufficient blood sample volumes for reanalysis.

The number and percent of subjects reporting any adverse event were 10 (47.6%) for the zileuton/phenytoin regimen and 12 (63.2%) for the placebo/phenytoin regimen. Adverse events reported by >2 subjects are presented in the table below [146:33-4].

ADVERSE EVENTS THAT OCCURRED IN > 2 SUBJECTS		
Adverse Event	Zileuton/Phenytoin	Placebo/Phenytoin
Headache	4 (19%)	3 (15.8%)
Rash	2 (9.5%)	3 (15.8%)

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ADVERSE EVENTS THAT OCCURRED IN > 2 SUBJECTS		
Adverse Event	Zileuton/Phenytoin	Placebo/Phenytoin
Dizziness	2 (9.5%)	2 (10.5%)
Pharyngitis	2 (9.5%)	3 (15.8%)

One subject on the zileuton/phenytoin regimen complained of diarrhea and that was the only AE referable to the Digestive System reported in this study [146:36]. Two patients were found to have above normal eosinophil differential counts. One had 11% eosinophils at screening, which declined during the study, and was attributed to his seasonal allergies. Another subject had 8% eosinophils at screening which also decreased slightly during the course of the study [146:41].

CONCLUSION

Zileuton given at a dose of 600 mg QID for 5 to 8 days did not have any appreciable effect on concomitantly administered single-dose phenytoin.

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M93-054 "Assessment of the Pharmacokinetic Interaction between Zileuton (ABT-077) and Terfenadine"

OBJECTIVES

Assess the potential interaction effect of zileuton on terfenadine pharmacokinetics, the morphology of the TU complex and the duration of the QTc interval in determining the safety of co-administration of these two compounds.

DESIGN

This was a single-period, multiple-dose, open-label study. Subjects relieved Q12H terfenadine beginning at 0800 on Day 1 and ending with the 0800 dose on Day 10. Zileuton was given Q6H beginning at 0800 on Day 6 and continuing through 0200 on Day 11.

SUBJECTS

Twelve, healthy, non-smoking, adult male subjects between 23 and 40 years of age (mean, 31 years) participated in this study. These were comprised of 5 Caucasians, 4 blacks, 2 Hispanics and 1 black-Hispanics [150:28, 162].

DOSAGE

Zileuton was administered in a dose of 600 mg Q6H and the unit dose was a 600 mg tablet. The terfenadine dose was 60 mg Q12H and the unit dose was the 60 mg tablet.

PARAMETERS

Twelve-hour serial blood samples were taken for baseline terfenadine and carboxyterfenadine PK on Day 5, before zileuton was begun. On Day 10, 24-hour serial blood samples for terfenadine and carboxyterfenadine PK were determined. ECG's were obtained at the end of the terfenadine dosing period, before the next scheduled BID dose.

RESULTS

The terfenadine plasma concentration, as a function of time since last terfenadine dose, co-administered with zileuton or placebo is shown on the following graph [150:31].

FIGURE 25 [150:31]

A similar graph of concentration against time was also plotted for the major terfenadine metabolite, carboxyterfenadine [150:32].

FIGURE 26 [150:32]

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The PK parameters that derived from these concentration-time graphs indicate a greater effect of zileuton on terfenadine than on carboxyterfenadine parameters as is shown in the table below [150:9, 29].

PHARMACOKINETICS OF TERFENADINE AND ITS MAJOR METABOLITE WHEN CO-ADMINISTERED WITH ZILEUTON OR PLACEBO				
Parameters	Terfenadine		Carboxyterfenadine	
	Alone	with Zileuton	Alone	with Zileuton
TROUGH (ng/mL)	1.827 ($\pm$ 1.131)	2.431 ($\pm$ 1.271)	82.4 ($\pm$ 28.7)	71.7 ($\pm$ 22.0)
C _{Max} (ng/mL)	5.092 ($\pm$ 3.311)	6.599 ($\pm$ 3.230)	294.5 ($\pm$ 85.3)	244.2 ($\pm$ 72.2)
T _{Max} (hr)	2.3 ($\pm$ 1.1)	2.5 ($\pm$ 0.5)	1.7 ($\pm$ 0.5)	2.5 ($\pm$ 0.7)
AUC ₀₋₁₂ (ng*hr/mL)	41.17 ($\pm$ 27.72)	51.58 ($\pm$ 29.25)	1682.4 ($\pm$ 461.5)	1616.0 ($\pm$ 441.5)
t _{1/2} (hr)		21.2 ($\pm$ 12.0)		9.5 ($\pm$ 1.1)

Co-administration of terfenadine with zileuton resulted in approximately a 25% increase in the mean AUC₀₋₁₂, a 30% elevation in the mean C_{Max} and a 33% elevation in the mean TROUGH of terfenadine. The T_{Max} of terfenadine was not significantly different after the addition of zileuton. The only carboxyterfenadine PK parameter to achieve statistical significance was the mean T_{Max}, which was higher with concomitant zileuton administration.

C_{Max} terfenadine levels for 11 of 12 baseline subjects were below 10.0 ng/mL. The single exception was Patient #9 who had a level of 14.02 ng/mL at baseline, on terfenadine alone. Eleven of these subjects had paired-terfenadine PK samples, the first taken at Day 5, before the addition of zileuton, and the second at Day 10, during terfenadine coadministration with zileuton. In 8 of the 11 paired PK samples, C_{Max} increased after zileuton had been added to terfenadine. Two of these values exceeded 10 ng/mL (Patient #9 = 13.25, Patient #11 = 10.56) [150:271].

QTc intervals calculated from 12-lead ECG's obtained prior to the first dose of terfenadine and zileuton (morning of Day 6) were not statistically significantly different from the QTc intervals after dosing with zileuton on Days 7 to 11 of the study. In fact, the median changes in the QTc intervals on Day 7 to Day 11 compared to Day 6 were all negative and ranged from -1 to 12 milliseconds.

WITHDRAWALS & ADVERSE EVENTS

The number and percent of subjects reporting any adverse event were 3 (25%) for terfenadine alone and 5 (42%) for terfenadine with zileuton. Rhinitis was the most frequently reported adverse event and was reported by two subjects (17%) receiving

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terfenadine with zileuton. All other AE's were reported by only one subject. One subject (#12) showed a three-beat run of ventricular tachycardia detected by cardiac monitor on Day 2 of zileuton/terfenadine dosing. The episode was experienced as a palpitation and resolved spontaneously. The QT interval, at the time, was unchanged from baseline [150:223]. No Day-10 PK data are available for this patient because of early termination, but we know that his C_{Max} of Day-5 (baseline) was 2.61 ng/mL [150:271]. This patient was the single premature termination from the study. One other subject (#2) developed an accelerated junctional arrhythmia on Day 8 of the study which spontaneously resolved. This subject had QT intervals that were consistently slightly shorter than at baseline throughout the study period [150:39, 218]. His terfenadine C_{Max} was 3.77 ng/mL on Day-5 (baseline) and was 5.98 ng/mL at Day-10 [150:271]. Six of the subjects had liver enzyme elevations (ALT) that were observed after the last dose of either study medication. The highest of these elevations was 174 (normal is 10-55 IU/L) [150:44].

CONCLUSION

PK effects of zileuton on terfenadine achieved statistical significance for the mean AUC and the mean C_{Max} . Specifically, the individual subject's terfenadine C_{Max} rose in 8/11 (72.7%) of subjects when zileuton was coadministered. These PK changes seemed to have little effect on the ECG, and specifically the QT or QTc, that was taken at trough serum terfenadine levels.

COMMENT: Because ECG parameters were assessed at trough terfenadine levels which was not felt to be a good assessment of safety, a second interaction study was requested and follows.

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M94-166 "Assessment of the Pharmacokinetic Interaction between Zileuton (ABT-077) and Terfenadine"

This was submitted 21 October 1994, apart from the NDA, and references in the square brackets are understood to mean the volume and page number of this submission rather than the NDA or the Safety Update.

OBJECTIVES

1. assess potential interaction effect of zileuton on terfenadine pharmacokinetics
2. compare the effects of terfenadine alone and the combination of zileuton and terfenadine on the electrocardiogram morphology of the TU complex and the duration of the QTc interval
3. assess safety of co-administration of these two compounds

DESIGN

This was a double-blind, randomized, placebo-controlled, two-period, crossover study.

SUBJECTS

Sixteen healthy, non-smoking, adult male subjects were enrolled in this study with 15 subjects completing the entire study. They ranged in age from 18 to 48 years, with a mean age of 28. Fourteen subjects were Caucasians and two were black [1:11, 39].

DOSAGE

During each of two study periods, all 16 subjects received terfenadine, 60 mg Q12H, in an open-label fashion beginning at 0800 on Study Day 1 and continuing through 0800 on Study Day 7. Additionally, all 16 subjects received zileuton, 600 mg Q6H, or matching placebo beginning with the 0800 dose on Study Day 1 and concluding with the 1400 hour dose on Study Day 10. A two-week interval separated the crossover periods [1:12].

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ASSESSMENT OF PHARMACOKINETIC INTERACTION BETWEEN ZILEUTON AND TERFENADINE (M94-166)				
Sequence Group	Number Of Subjects	Regimen		
		Period 1	Washout	Period 2
A	8	T (Days 1-7) 13 doses Z (Days 1-10) 36 doses	2 Weeks	T (Days 1-7) 13 doses ZP (Days 1-10) 36 doses
B	8	T (Days 1-7) 13 doses ZP (Days 1-10) 36 doses	2 Weeks	T (Days 1-7) 13 doses Z (Days 1-10) 36 doses
T = Terfenadine Z = Zileuton ZP = Zileuton Placebo				

PARAMETERS

Vital signs were determined at the following intervals throughout the study: screening, Day -1, prior to morning dosing on Study Days 1-9 in both periods and during the exit examination.

A daily blood sample was obtained immediately before the 0800 dosing on Study Days 1 through 6, for determination of terfenadine and its acid metabolite. On Study Day 7 of both periods samples were collected sequentially at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours for terfenadine and the acid metabolite. In Period 1 only on Day 1, and in both periods on Day 7, prior to the 0800 dosing a blood sample was collected for terfenadine protein binding determination. On Days 5, 6 and 7 an additional sample was obtained for zileuton determinations.

All subjects had a laboratory profile performed during screening, upon confinement (Day -2) and prior to the conclusion of confinement on Study Day 10 in each period. Included in this fasting laboratory screen were:

CBC, WBC differential, platelet count, glucose, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, LDH, total protein, albumin, total cholesterol, calcium, sodium, potassium, chloride, bicarbonate, magnesium, uric acid and hepatitis B surface antigen

In addition, all subjects had a urine drug and alcohol test obtained at screening and Day -2 during each period.

During the study all subjects had a 12-lead ECG's with rhythm strip performed at screening, upon confinement (Day -2), daily prior to morning dosing and according to the

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following schedule, which is the same for both periods. The times are relative to terfenadine dosing:

Day -1 0, 1, 2, 4, 6, 8 and 12 hours after
Day 7 0, 1, 2, 4, 6, 8, 12 and 24 hours after

Blinded tracings were analyzed for QT interval and the QTc was calculated using Bazett's formula. All subjects were continuously monitored via telemetry from Study Day 1 through Study Day 10 in each period.

RESULTS - PHARMACOKINETIC

These were calculated excluding results of the one subject who prematurely discontinued the study after completing only the first period. These data are presented in the table below as mean $\pm$ standard deviation for Day 7 [1:41].

DAY 7 PHARMACOKINETICS OF TERFENADINE AND ITS ACID METABOLITE, WITH AND WITHOUT CONCOMITANT ZILEUTON				
Parameter	Terfenadine PK		Carboxyterfenadine PK	
	Terfenadine with Placebo	Terfenadine with Zileuton	Terfenadine with Placebo	Terfenadine with Zileuton
C_{TROUGH} (ng/mL)	1.518 $\pm$ 1.062	1.934 $\pm$ 1.468	84.6 $\pm$ 32.1	90.1 $\pm$ 28.8
C_{Max} (ng/mL)	3.398 $\pm$ 1.674	4.589 $\pm$ 2.455	236.2 $\pm$ 85.9	274.7 $\pm$ 82.5
T_{Max} (hours)	2.6 $\pm$ 1.9	2.2 $\pm$ 1.4	2.3 $\pm$ 0.7	2.5 $\pm$ 0.6
AUC_{0-12} (ng*hr/mL)	27.27 $\pm$ 16.03	36.97 $\pm$ 23.53	1537 $\pm$ 421	1737 $\pm$ 416
$T_{1/2}$ (hours)	21.4 $\pm$ 5.5	22.1 $\pm$ 6.1	13.1 $\pm$ 4.0	16.6 $\pm$ 8.9

Co-administration of terfenadine with zileuton resulted in a 36% increase in the mean AUC_{0-12} of terfenadine and an elevation in mean C_{Max} of 35%, both statistically significant. Zileuton co-administered with terfenadine resulted in approximately a 13% increase in mean AUC_{0-12} and 16% increase in mean C_{Max} of carboxyterfenadine compared to co-administration of terfenadine and placebo. These findings also reached statistical significance [1:42].

The maximum terfenadine serum level that was found in this study was the C_{Max} of subject #9 (9.6 ng/mL) which was obtained during coadministration of zileuton and terfenadine [3:40-47].

Zileuton coadministration with terfenadine resulted in a small decrease in the percent protein-binding of terfenadine, compared with placebo (baseline=99.71%, zileuton Day-

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7=99.68%, placebo Day-7=99.72%). This difference did not quite achieve statistical significance. The mean bound percent of terfenadine at baseline was similar for the placebo and zileuton groups [1:42].

RESULTS - ELECTROCARDIOGRAPHIC

The two primary variables for the QTc interval analysis were the maximum observed QTc measurement and the average QTc interval for the Day-7 terfenadine dose interval. The average QTc interval is the area under the QTc interval versus time curve (AUC) divided by the length of the dose interval (12 hours).

MEAN AND MEAN MAXIMUM QTc FOR DAY 7 WITH 95% CONFIDENCE INTERVALS				
Variable	Adjusted Means		Difference of Means	
	Terfenadine with Zileuton	Terfenadine with Placebo	Estimate	95% Confidence Interval
Average (msec)	402.4	400.2	2.27	-2.11 to 6.64
Maximum (msec)	417.1	417.9	-0.79	-9.62 to 8.05

Adjustments to the mean were made for the possibility of period effects. The difference between the adjusted means of the regimens was small and not statistically significant.

Individual QTc results in Appendix C.12.2 were examined by the reviewer and QTc values ≥ 440 were counted. Of the 16 patients enrolled, 5 had one, or more QTc ≥ 440 . The number of discrete ECG's with QTc ≥ 440 was 8. The longest of these QTc intervals was .454 and six of the eight ECG's had intervals that were $\leq .445$. Three of the five patients were in zileuton-dosing days 1-7, and they also accounted for five of the eight prolonged QTc intervals.

Three of the abnormal ECG's probably were unrelated to zileuton. One was an isolated occurrence on treatment day -2, before any active therapy had begun. Another occurred on the 25th treatment day, during the washout period between crossing arms and after the patient had been exposed to zileuton placebo. The last happened on the 25th treatment day, during the washout period and 18 days after the last dose of zileuton had been taken [2:129-90].

WITHDRAWALS & ADVERSE EVENTS

Sixteen patients were enrolled and 15 completed the study. Subject #4 completed Period I during which he received terfenadine plus placebo but did not return for Period II. Attempts were made to reach him but were unsuccessful [1:39; 2:222].

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AE's were reported by 9/15 (60%) of patients taking zileuton-terfenadine and in 10/16 (62.5%) of patients taking placebo-terfenadine [1:46-7]. During the zileuton-terfenadine regimen, AE's that were reported by more than one subject were: headache (3), somnolence (2) and epistaxis (2). The only AE reported by more than one subject during the placebo-terfenadine treatment was anxiety (2) [1:46-7].

CONCLUSION

Co-administration of terfenadine and zileuton had a pronounced effect on the pharmacokinetics of terfenadine and its acid metabolite, increasing the AUC of each 35% and 16%, respectively. This was associated with a less impressive pharmacodynamic effect on the QTc interval.

INTEGRATED SUMMARY OF EFFICACY

Two pivotal trials, M91-685 and M92-720, demonstrated that zileuton 600 mg QID was statistically superior to placebo in the change of the trough FEV_{1,0} from baseline. The change from baseline of the zileuton 600 mg QID group in excess of that seen in the placebo group was modest, 130-160 mL and 8.48-8.63%. This was corroborated by the morning domiciliary PEFr determinations which showed statistically significant improvement for the zileuton 600 mg QID group over baseline and in excess of the change seen in the placebo group. Peak, post-morning-dose FEV_{1,0} and evening PEFr's generally showed improvement over baseline too, and the zileuton 600 mg QID group achieved statistical significance over placebo in most of these endpoints.

Somewhat unexpectedly, a first-dose effect on the FEV_{1,0} was demonstrable in the zileuton 600 mg QID group over the placebo group at 2-4 hours post-dosing and it achieved statistical significance. Responsiveness to beta-agonist challenge was preserved in zileuton-treated patients. By the end of the study, beta-agonist-induced improvement in FEV_{1,0} was greater in the placebo group than in the zileuton 600 mg QID group, but this improvement occurred from a lower baseline in the placebo group. The likely explanation is that the baseline for zileuton-treated patients had improved, as a consequence of zileuton treatment, and there was less room for improvement following the beta-agonist. Another measure of efficacy, the number of puffs of beta-agonist rescue medication reduced more in the zileuton 600 mg QID group than in the placebo group in both pivotal trials. Generally, the various symptom scores, including Quality Of Life, supported an effect of the zileuton 600 mg QID group over the placebo group.

Retrospective analyses of various of these endpoints, with patients stratified by baseline FEV_{1,0}, showed that much of the differential effect of zileuton compared with placebo was within the subgroup with severe baseline flow obstruction (FEV_{1,0} < 50 % predicted). This finding was not constant for every endpoint and it was a post hoc comparison that could not be meaningfully tested for statistical significance. Protocol M92-785 provided information that gave some support to the concept of greater efficacy in patients with more severe baseline disease. This was a provocation challenge study in very mild asthmatics who had 15% FEV_{1,0} responses to beta-agonist but had baseline FEV_{1,0} values of $\geq$ 80% predicted. This three-month trial of over 100 patients failed to show any effect of zileuton on trough FEV_{1,0}, not the primary efficacy endpoint but one that is in common with the two pivotal trials. If greater effect in a sample with severe baseline flow obstruction is postulated, then small-to-no effect in patients with minimal disease should also be expected and this was the finding in M92-785.

PK/PD analysis was done on a subset of patient in M91-685 and showed a direct relation between blood zileuton level and plasma LTB₄ inhibition. Acutely, there was a 2-3 hour time lag between the maximum blood zileuton level and the maximum improvement in FEV_{1,0}. After chronic administration, improvement in FEV_{1,0} was relatively constant throughout the dosing interval despite large fluctuations between peak and trough blood zileuton levels and LTB₄ inhibition. Protocol M90-460, a four-week study of over 100 patients, was done to study the

relationship between blood zileuton concentrations, blood leukotriene inhibition and pulmonary function. This PK/PD trial showed a rough correlation between the mean leukotriene inhibition (urinary LTE₄) and mean pulmonary function measures and a dose proportional relation between zileuton dose and zileuton plasma concentration. However, the relation between zileuton blood concentrations and pulmonary function measures of airflow was too variable to describe. Inter-patient variability of trough blood zileuton levels in the two pivotal trials was large and produced coefficients of variation (ratio of standard deviation to mean) between 1.25 and 2.4, considering both zileuton doses in both pivotal trials [IND 3/24/95 1:41-2].

Efficacy in the particular subgroup of exercise-induced bronchospasm (EIB) was studied under protocol M91-615, where 24 patients underwent a crossover design consisting of two days of treatment followed by exercise tests. After, minimally, a four-day washout, the patient was crossed over to the other treatment, zileuton or placebo, and again given an exercise test. Zileuton clearly resulted in some blunting of the bronchoconstrictor effect of exercise, as manifested by the FEV_{1.0}. A placebo-controlled crossover study (M92-724) of 12 mild-to-moderate asthmatics with nocturnal manifestations was carried out to measure the effect of zileuton on methacholine challenge, cellular and mediator indices of inflammation in BAL fluid and blood and urinary leukotriene excretion. Zileuton produced a trend toward blunting indices of inflammation in patients with nocturnal asthma and this occasionally achieved statistical significance. This trend was not as apparent in pulmonary function and symptom score endpoints.

Bronchoscopic segmental antigen challenge and subsequent BAL were performed in protocol M91-654 to measure cellular injury in ten atopic patients in a placebo-controlled, randomized, double-blind, two-period crossover design. The bronchoscopic antigen challenge was associated with increased cell counts and inflammatory components in both zileuton and placebo treatment groups. This inflammatory response to the challenge was less pronounced in the zileuton group than in the placebo treatment for virtually every item analyzed, though only two reached statistical significance. A similar study (M91-597) was stopped early because of lack of effect that may have been caused by too short of a dosing period and/or too short of a period of measurement after the antigen challenge.

Two protocols allowed comparison of zileuton with other asthma treatments. M92-797 was a parallel-group design of two zileuton doses, 600 mg QID, and one positive comparator, beclomethasone, 42 mcg/puff 2 puffs QID by inhalation, in patients with moderate asthma. This was a Phase III trial of close to 300 patients over 14 weeks. Both doses of zileuton and inhaled beclomethasone raised trough FEV_{1.0} above the baseline, when given chronically. These three treatments also reduced daytime and nighttime symptom scores and rescue medication use. For each of these endpoints, both doses of zileuton and beclomethasone achieved a statistically significant change from baseline. For most endpoints, the mean change from baseline was greater with beclomethasone than with either dose of zileuton and this difference between treatments was statistically significant.

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A 13-week study of the same two doses of zileuton against oral theophylline was performed in over 300 mild-to-moderate asthmatics in protocol M92-763. SLO-BID® was the positive comparator and was titrated to a therapeutic serum level throughout all periods of the study. The morning trough $FEV_{1.0}$ showed that theophylline produced about half again greater change from the pre-double-blind baseline than did either of the two zileuton doses. The superiority of theophylline over both zileuton doses was statistically significant when expressed as the mean percent change from baseline to the final visit for the trough $FEV_{1.0}$.

Taken together, these studies paint the picture of zileuton as an asthma medication with modest but definite efficacy, when given at a dose of 600 mg QID. The magnitude of this efficacy seems to be at least equalled, and perhaps exceeded by theophylline. A small dose of inhaled beclomethasone was obviously superior to either of the two zileuton doses. The zileuton dose of _____ occasionally showed statistical significance in some subdivisions of endpoints, but for the most part it must be regarded as not having demonstrated efficacy.

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INTEGRATED SAFETY SUMMARY

INTRODUCTION

The safety data presented here are derived from 93 clinical studies, 4 of which are ongoing. Forty-two are Phase I trials, 4 of which were single-dose, and the remainder were multiple-dose studies. There were 51 Phase II/III trials, 20 of which involved asthmatic patients. The remaining 31 were in patients with allergic rhinitis, rheumatoid arthritis, ulcerative colitis, psoriasis and "others." [Safety Update (SU) 1:120].

The number of subjects exposed to at least one dose of zileuton in Phase I trials was 712. Information from 2573 zileuton patients participating in Phase II/III or uncontrolled studies are included in this report. Of these, 1457 patients received zileuton in asthma trials, 677 in ulcerative colitis studies, and 439 in non-asthma and non-ulcerative colitis trials. Of the total number of patients exposed to zileuton, 2063 received the drug in domestic studies and 510, in foreign trials [SU 1:120].

DEMOGRAPHICS

These demographic data are based on the variables of age, gender, race, height and weight. The break down of these variables for ASTHMA-ONLY and for ALL patients exposed to zileuton in parallel or uncontrolled Phase II/III trials is given in the table below [SU 1:125].

DEMOGRAPHICS OF ZILEUTON-TREATED PATIENTS IN PARALLEL-GROUP, OR UNCONTROLLED, PHASE II/III TRIALS		
VARIABLE	ALL (n = 2573)	ASTHMA-ONLY (n = 1457)
Female (%)	52.1	54.8
Male (%)	47.9	45.2
Black (%)	4.8	5.6
Caucasian (%)	84.8	84.8
Other (%)	10.4	9.6
Age, mean (range), years	37.7 (16.0-78.0)	34.5 (16.0-70.0)
Height, mean (range), inches	67.9 (55.5-97.0)	66.8 (56.3-97.0)
Weight, mean (range), lbs.	161.4 (87.0-360.0)	161.0 (89.0-278.0)

COMMENT: The patients were evenly split between the two sexes with only a small excess of females. Preponderantly, these data were derived from Caucasians, with about 7/8 of the population

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comprised of this ethnic group. The asthmatics were slightly younger than the ALL patient population, otherwise demographic characteristics were very similar.

The demographic distribution of placebo-controlled, parallel, Phase II/III ASTHMA-ONLY studies was similar with but a few minor changes. These 1338 patients (doesn't include uncontrolled or active controlled trials) were divided into 491, who took the placebo, and 847, who received zileuton. The slight gender predominance is shifted to the females in both placebo and zileuton groups. There are slightly more blacks who were given placebo (8.2%) than were given zileuton (5.2%) [SU 1:126].

When ALL Phase II/III placebo-controlled, parallel-group studies were examined, they had a distribution of demographic variables that was very similar to the previous table, except for a slight male gender excess in the placebo patients (53% males, 47% females). These trials involved 2858 patients divided into 1101 who took placebo, and 1757, who received zileuton [SU 1:127].

Active-control, parallel group, Phase II/III studies accounted for 1150 patients, divided into 744, who took zileuton, and 406, who received the active control. This active-control subgroup has about equal gender participation. However, the zileuton group is slightly weighted toward females (57.4% female, 42.6% male) [SU 1:128].

Only 106 patients participated in crossover study designs. These were weighted more heavily toward males patients (69.81%) and were younger (mean = 29.6 years) than other subgroups [388:129, 389:127, SU 1:129].

EXPOSURE

Considering ALL parallel-group, Phase II/III, controlled or uncontrolled trials, a total of 1457 patients received ≥ 1 dose of zileuton, in ASTHMA trials, and 2573 patients took the drug, in ALL studies taken together. For these zileuton-treated, ASTHMA-ONLY and ALL patients the treatment duration is summarized in the table below [SU 1:130, 132].

DURATION OF ZILEUTON EXPOSURE IN PARALLEL-GROUP, CONTROLLED, OR UNCONTROLLED, PHASE II/III TRIALS		
INTERVAL, days	ASTHMA, number (%)	ALL, number (%)
1 - 14	77 (5.3)	192 (7.5)
15 - 30	148 (10.2)	516 (20.1)
31 - 60	216 (14.8)	473 (18.4)
61 - 90	200 (13.7)	265 (10.3)
91 - 150	469 (32.2)	539 (20.9)
151 - 210	174 (11.9)	287 (11.2)

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DURATION OF ZILEUTON EXPOSURE IN PARALLEL-GROUP, CONTROLLED, OR UNCONTROLLED, PHASE II/III TRIALS		
INTERVAL, days	ASTHMA, number (%)	ALL, number (%)
> 210	173 (11.9)	301 (11.7)
TOTAL	1457	2573

Counting ALL trials, 588 patients have received zileuton for > 150 days and 228 patients have taken zileuton for > 300 days (this latter figure can not be derived from the table) [SU 1:132].

Zileuton exposure can also be classified by dosage received, rather than duration, and this is presented for ALL parallel Phase II/III controlled or uncontrolled studies in the table below. Note that the number sums to > 2573 because some of the patients received more than one treatment regimen. For this reason percentages are not provided [SU 1:132].

ZILEUTON EXPOSURE, BY DOSE REGIMEN, FOR ALL PARALLEL-GROUP, CONTROLLED OR UNCONTROLLED, PHASE II/III TRIALS	
ZILEUTON REGIMEN	NUMBER OF PATIENTS
600 mg QID (2400 mg/day)	1602
600 mg TID (1800 mg/day)	52
800 mg BID (1600 mg/day)	394
300 mg TID (900 mg/day)	29
800 mg QD (800 mg/day)	36
200 mg QID (800 mg/day)	70

The most frequent regimen was 600 mg QID, followed next in frequency by 800 mg BID.

The distribution of patients exposed to zileuton and to placebo in ALL parallel, placebo-controlled, Phase II/III studies by indication and by number of studies are summarized in the following table [SU 1:135].

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ZILEUTON EXPOSURE, BY STUDY INDICATION, IN ALL PLACEBO-CONTROLLED, PHASE II/III TRIALS			
INDICATION	NUMBER OF STUDIES	NUMBER OF PATIENTS (%)	
		ZILEUTON	PLACEBO
Asthma	8	847 (48.2)	491 (44.6)

Zileuton exposure also occurred in actively-controlled trials. In fact 744 patients were exposed when ALL active-control, parallel, Phase II/III studies are taken together. Of these, 420 were exposed for > 90 days to doses of 200 mg BID (7) and 600 mg QID (243) [SU 1:137].

A total of 614 patients were exposed to zileuton during uncontrolled Phase II/III clinical trials. Most of these (495) received the highest dose, 600 mg QID [SU 1:138].

SUMMARY OF DEMOGRAPHICS

Taken together, the 2573 ALL Phase II/III controlled and uncontrolled zileuton study patients were fairly evenly divided between genders. About 85% of the enrolled patients were Caucasians. The mean patient age was in the mid-30's and ranged from 16 to 78 years of age. About 56% (1446) of ALL patients were studied for > 3 months and over 300 patients were studied for >210 days. Over 1600 of those patients who took zileuton were exposed to 600 mg QID, the highest dose.

ADVERSE EVENTS

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"Only treatment-emergent adverse events, those which had an onset during the study drug dosing period, are included in the analyses of safety data...Adverse events (AE's) that occurred during the course of the study were reported in detail on appropriate case report forms...The description of the adverse event included time of onset, duration, severity, alternative etiology, the investigator's assessment of the relationship of the adverse event to the study drug, whether the event was intermittent, and any required treatment. Severity of an adverse event was assessed using the following definitions: mild (an event that was transient and easily tolerated), moderate (an event that caused discomfort and interrupted usual activities), and severe (an event that caused considerable interference with the patient's usual activities and was potentially incapacitating or life-threatening)...

"Generally, laboratory abnormalities were not recorded as AE's unless values reached clinically significant levels as judged by the investigator or patients were discontinued prematurely from the study due to the laboratory abnormality. An adverse event was not reported in a patient if the patient had a previous history of the adverse event in question unless the event was more frequent or more severe than previously reported. Clusters of adverse events related to a single condition or syndrome were combined into a single adverse event best describing the condition or syndrome...Adverse events, coded by COSTART term and body system, were evaluated from all Phase I/II/III studies for this summary" [388:139-40, SU 1:139].

Of ALL zileuton-treated patients (2573) in parallel or uncontrolled Phase II/III studies, 1955 patients (76.0%) experienced at least one treatment-emergent AE during zileuton treatment. The percentage (80.6%) in ASTHMA Phase II/III studies was slightly greater [SU 1:143]. Any adverse events with a frequency $\geq 2\%$ in any treatment group in parallel or uncontrolled Phase II/III studies are presented in the table below [SU 1:144].

MOST FREQUENT AE'S IN ZILEUTON-TREATED, PARALLEL-GROUP, CONTROLLED OR UNCONTROLLED, PHASE II/III TRIALS		
Adverse Event (COSTART Term)	Asthma Studies, n (%) (N = 1457)	All Studies, n (%) (N = 2573)
Abdominal Pain	66 (4.5)	129 (5.0)
Accidental Injury	58 (4.0)	88 (3.4)
Aggravation Reaction	624 (42.8)	624 (24.3)
Asthenia	65 (4.5)	132 (5.1)
Back Pain	82 (5.6)	130 (5.1)
Chest Pain	31 (2.1)	53 (2.1)
Fever	42 (2.9)	70 (2.7)
Flu Syndrome	159 (10.9)	258 (10.0)

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MOST FREQUENT AE'S IN ZILEUTON-TREATED, PARALLEL-GROUP, CONTROLLED OR UNCONTROLLED, PHASE II/III TRIALS		
Adverse Event (COSTART Term)	Asthma Studies, n (%) (N = 1457)	All Studies, n (%) (N = 2573)
Headache	395 (27.1)	666 (25.9)
Infection	364 (25.0)	519 (20.2)
Pain	125 (8.6)	176 (6.8)
Diarrhea	54 (3.7)	106 (4.1)
Dyspepsia	108 (7.4)	183 (7.1)
Nausea	87 (6.0)	196 (7.6)
Vomiting	22 (1.5)	53 (2.1)
Arthralgia	35 (2.4)	65 (2.5)
Myalgia	74 (5.1)	99 (3.8)
Dizziness	57 (3.9)	128 (5.0)
Insomnia	51 (3.5)	84 (3.3)
Somnolence	32 (2.2)	48 (1.9)
Bronchitis	55 (3.8)	75 (2.9)
Cough Increased	30 (2.1)	58 (2.3)
Pharyngitis	143 (9.8)	189 (7.3)
Rhinitis	89 (6.1)	125 (4.9)
Sinusitis	108 (7.4)	135 (5.2)
Rash	57 (3.9)	109 (4.2)
Urinary Tract Infection	37 (2.5)	59 (2.3)
Dysmenorrhea	31 (3.9)	37 (2.8)
Any Sign/Symptom	1175 (80.6)	1955 (76.0)

In asthmatics, "aggravation reaction" had the highest incidence, consistent with the worsening of their labile and underlying condition, while "headache" and "infection" were numbers two and three in relative incidence. Taking ALL studies together, "headache" was number one, "aggravation

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reaction" second and "infection" was in third place. There were slightly more frequent AE's in the ASTHMA studies overall and this appeared to be largely due to the excess in "aggravation reactions."

COMMENT: No comparable listing of AE's for placebo patients was found in the statistical tables that were said to reference this summary table [391:168-86]. No doubt this is deliberate, since these data are derived from controlled and uncontrolled zileuton patients, together. Any placebo data would have to have been derived from, and compared within, the controlled trials, and these will be covered later.

When the overall incidence of AE's in Phase II/III studies was tabulated by study category, AE reporting rates were similar in all study categories except for crossover trials, where the patient numbers were too small to provide reliable estimates [SU 1:145].

OVERALL INCIDENCE OF AE'S IN PHASE II/III TRIALS, BY STUDY CATEGORY				
STUDY CATEGORY	ASTHMA STUDIES		ALL STUDIES	
	Zileuton n (%)	Control n (%)	Zileuton n (%)	Control n (%)
Placebo-controlled	631 (74.5)	369 (75.2)	1249 (71.1)	751 (68.2)
Active-controlled	436 (87.4)	204 (82.3)	605 (81.3)	307 (75.6)
Single-dose, crossover	n/a	n/a	12 (18.8)	8 (12.9)
Multiple-dose, crossover	n/a	n/a	26 (66.7)	21 (55.3)
Uncontrolled	274 (80.6)	n/a	469 (76.4)	n/a

There was a trend for the zileuton-treated patients to have slightly more AE's than the control groups, within each study category, but this is neither invariant nor marked. Sample sizes for study categories do not sum to the total because patients may have been enrolled in more than one study and category.

Considering only placebo-controlled, parallel-group, Phase II/III studies, the same three AE's lead in frequency of occurrence, in both ASTHMA-ONLY and ALL trials, "aggravation reaction," "headache" and "infection." A statistically significant difference, between zileuton and placebo, was found for dyspepsia in both ASTHMA-ONLY (Z=7.6%, P=2.9%) and in ALL trials (Z=6.8, P=3.8%). Insomnia was significantly more common in zileuton-treated patients than in placebo patients in ALL trials (Z=3.1%, P=0.8%) and in the ASTHMA-ONLY studies (Z=3.1%, P=1.0%). In the ASTHMA-ONLY studies, zileuton was associated with significantly more abdominal pain

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and dizziness than placebo. These same statistically significant findings did not extend to ALL studies where zileuton was associated with significantly more rash than was placebo [SU 1:149]. The following table lists all AE's occurring in $\geq 2\%$ of patients in any treatment group [SU 1:147-8].

AE INCIDENCE IN PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III TRIALS				
Adverse Events (COSTART term)	ASTHMA STUDIES		ALL STUDIES	
	Zileuton n (%)	Placebo n (%)	Zileuton n (%)	Placebo n (%)
Abdominal pain	41 (4.8)	12 (2.4)	83 (4.7)	43 (3.9)
Accidental Injury	27 (3.2)	10 (2.0)	42 (2.4)	23 (2.1)
Aggravation reaction	232 (27.4)	147 (29.9)	232 (13.2)	147 (13.4)
Asthenia	34 (4.0)	12 (2.4)	87 (5.0)	46 (4.2)
Back pain	49 (5.8)	26 (5.3)	77 (4.4)	41 (3.7)
Chest Pain	19 (2.2)	6 (1.2)	n/a	n/a
Fever	24 (2.8)	12 (2.4)	47 (2.7)	23 (2.1)
Flu syndrome	71 (8.4)	42 (8.6)	142 (8.1)	78 (7.1)
Headache	217 (25.6)	118 (24.0)	440 (25.0)	271 (24.6)
Infection	189 (22.3)	106 (21.6)	294 (16.7)	178 (16.2)
Pain	59 (7.0)	26 (5.3)	97 (5.5)	48 (4.4)
Diarrhea	24 (2.8)	14 (2.9)	59 (3.4)	45 (4.1)
Dyspepsia	64 (7.6)	14 (2.9)	119 (6.8)	42 (3.8)
Flatulence	n/a	n/a	28 (1.6)	29 (2.6)
Nausea	39 (4.6)	18 (3.7)	122 (6.9)	60 (5.4)
Arthralgia	17 (2.0)	4 (0.8)	40 (2.3)	16 (1.5)
Myalgia	30 (3.5)	14 (2.9)	48 (2.7)	19 (1.7)
Dizziness	28 (3.3)	7 (1.4)	85 (4.8)	47 (4.3)
Insomnia	26 (3.1)	5 (1.0)	54 (3.1)	9 (0.8)
Bronchitis	25 (3.0)	11 (2.2)	35 (2.0)	15 (1.4)

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AE INCIDENCE IN PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III TRIALS				
Adverse Events (COSTART term)	ASTHMA STUDIES		ALL STUDIES	
	Zileuton n (%)	Placebo n (%)	Zileuton n (%)	Placebo n (%)
Pharyngitis	63 (7.4)	37 (7.5)	90 (5.1)	61 (5.5)
Rhinitis	45 (5.3)	27 (5.5)	68 (3.9)	37 (3.4)
Sinusitis	56 (6.6)	33 (6.7)	72 (4.1)	44 (4.0)
Rash	28 (3.3)	13 (2.6)	64 (3.6)	25 (2.3)
Dysmenorrhea	15 (3.4)	15 (5.9)	19 (2.1)	18 (3.5)
Urinary Tract Infection	19 (2.2)	6 (1.2)	n/a	n/a
ANY SIGN/SYMP TOM	631 (74.5)	369 (75.2)	1249 (71.1)	751 (68.2)
TOTAL NUMBER ('N')	847	491	1757	1101

Three additional AE's occurred with statistically significantly greater incidences in the zileuton group for ALL studies although the overall incidence was < 2%. These were "taste perversion" (Z=13 patients, P=1), "peripheral edema" (Z=17 ulcerative colitis patients, P=0) and "pruritis" (Z=31 patients, P=9) [SU 1:150].

Most adverse events were considered mild or moderate in nature. A total of 97 (11.5%) zileuton-treated ASTHMA patients experienced AE's considered "severe" compared to 37 (7.5%) placebo-treated asthmatics. Corresponding numbers for ALL studies were 156 (8.9%) and 70 (6.4%). The following table accounts for the most common "severe" AE's and is extracted from a larger table supplied by the sponsor [SU 1:156-7].

MOST FREQUENT "SEVERE" AEs, BY COSTART TERM, IN PHASE II/III, PLACEBO-CONTROLLED STUDIES				
Adverse Event (COSTART term)	ASTHMA STUDIES		ALL STUDIES	
	Zileuton n (%)	Placebo n (%)	Zileuton n (%)	Placebo n (%)
Abdominal pain	5 (0.6)	0 (0)	11 (0.6)	6 (0.5)
Accidental injury	4 (0.5)	3 (0.6)	6 (0.3)	4 (0.4)
Headache	21 (2.5)	17 (3.5)	36 (2.0)	24 (2.2)

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MOST FREQUENT "SEVERE" AE'S, BY COSTART TERM, IN PHASE II/III, PLACEBO-CONTROLLED STUDIES				
Adverse Event (COSTART term)	ASTHMA STUDIES		ALL STUDIES	
	Zileuton n (%)	Placebo n (%)	Zileuton n (%)	Placebo n (%)
Infection	11 (1.3)	5 (1.0)	11 (0.6)	6 (0.5)
Pain	6 (0.7)	3 (0.6)	9 (0.5)	4 (0.4)
Dyspepsia	2 (0.2)	2 (0.4)	2 (0.1)	6 (0.5)
Insomnia	2 (0.2)	0 (0)	3 (0.2)	0 (0)
Bronchitis	1 (0.1)	2 (0.4)	2 (0.1)	2 (0.2)
Pharyngitis	5 (0.6)	0 (0)	5 (0.3)	0 (0)
Dysmenorrhea	1 (0.2)	3 (1.2)	2 (0.2)	3 (0.6)
ANY SIGN/SYMPTOM	97 (11.5)	37 (7.5)	156 (8.9)	70 (6.4)
TOTAL NUMBER ('N')	847	491	1757	1101

The most frequently reported "severe" adverse event was headache in both ASTHMA and ALL studies, and in both study populations, the incidence in zileuton and placebo patients was similar. Dyspepsia, which occurred significantly more frequently among ALL and ASTHMA-ONLY zileuton patients than placebo patients, was characterized as "severe" slightly more commonly in placebo patients. Insomnia, also more frequent among zileuton patients, was more commonly "severe" in that group [SU 1:157].

Examining AE's in placebo-controlled, parallel-group, Phase II/III studies plotted against dosage regimen fails to demonstrate any relation between dose and AE incidence. In fact the 1200 mg QID regimen has more recorded AE's (80.4%) than does the 600 mg QID regimen (73.5%) [SU 1:159].

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TOTAL AE'S IN PLACEBO-CONTROLLED, PHASE II/III TRIALS				
ADVERSE EVENT	Zileuton Patients n (%)	Zileuton Dosage Regimens		Placebo Patients n (%)
		800 mg BID (1600 mg/d) n (%)	600 mg QID (2400 mg/d) n (%)	
ANY SIGN/SYMPTOM	631(74.5)	22 (44.9)	349 (73.5)	369 (75.2)
TOTAL NUMBER ('N')	847	49	475	491

COMMENT: The very narrow dose range makes finding a dose related effect on categorical data; i.e., the number of AE's, difficult. Hence, the lack of this association does not imply proof of its absence.

In actively-controlled trials, the incidence of AE's was similar to those seen in the parallel-group, placebo-controlled Phase II/III studies. That is headache and infection were the top two. Not surprisingly, pharyngitis was seen more frequently in the active-control, beclomethasone group than the zileuton group [SU 1:200-1].

AE's were tabulated against time intervals during which they were first experienced. Percentages by time interval indicate the risk of initially experiencing an AE within that time interval, given that the AE had not been experienced within a previous time interval. The following table was synthesized by the reviewer and presents these exposure data [SU 1:173-5].

AE INCIDENCE BY TIME INTERVAL (Days) FOR LONGEST CONTINUOUS ZILEUTON EXPOSURE IN PARALLEL-GROUP OR UNCONTROLLED STUDIES								
Study Type	1-14 n (%)	15-30 n (%)	31-60 n (%)	61-90 n (%)	91-150 n (%)	151-210 n (%)	> 210 n (%)	Overall
Asthma-Only	672 (46.1)	232 (29.6)	154 (30.4)	70 (26.2)	27 (16.8)	15 (27.3)	5 (31.3)	1175 (80.6)
All	1146 (44.5)	402 (28.2)	233 (25.7)	91 (21.3)	46 (18.5)	19 (19.0)	14 (35.0)	1950 (75.8)

In general, most AE's had a greater rate of onset during the first two weeks of zileuton experience than during any other time interval. Thereafter, the incidence of AE's decreased. The apparent jump in incidence at the longest exposure time is likely to be a factitious result of a very small sample size [388:169-74, 391:187, 198, SU 1:172-7]. The incidence percentages were based on the population "at risk" during each interval.

Prevalence is defined as having a particular AE present during a given interval. All AE's were captured during the specified time period without regard to their onset. The prevalence rate of AE's among zileuton patients in placebo-controlled asthma studies was 47.3% during the first two weeks and remained stable through the > 150-day time interval. The overall prevalence of AE's was similar between treatment groups ($Z=76.4\%$, $P=78\%$) for the asthma studies. A similar pattern was observed for all placebo-controlled studies [SU 1:177-8, SU 5:95-152]. Like incidence, prevalence was based on the "at risk" population during each interval.

Previously found significant AE's, dyspepsia and insomnia, were re-examined for overall incidence or incidence in any time interval during zileuton and placebo exposure in both Phase II/III or uncontrolled ASTHMA and ALL trials. Both dyspepsia and insomnia had a greater incidence of onset during the first two weeks of dosing than during any other time interval. After two weeks, the incidence of dyspepsia and insomnia onset in zileuton patients decreased, but generally continued to be greater than in placebo patients [SU 1:177].

A number of statistically significant interactions between demographic subgroups and AE incidence were uncovered, but none seem clinically meaningful. For example younger aged patients showed statistical differences between zileuton and placebo for a number of AE's, that was not apparent in patients of age > 60 years. However, 99% of ALL patients were less than 60 years of age, so this apparent demographic finding, is probably attributable to the widely differing numbers of patients in each group [388:178, 182, 185, SU 1:180-3, 186-9]. Other subgroup analyses were performed on AE incidence in: 1) patients with baseline inhaled beta-agonist use, dichotomized at 6 puffs/day, 2) concomitant terfenadine use, 3) concomitant acetaminophen use, and 4) baseline liver function tests [388:185-93, SU 1:189-98]. In none of these cases was there any great difference between placebo and zileuton groups within the strata. A stratification of ulcerative colitis, ASTHMA and ALL patients did show some minor differences, but the bearing that they have to the present NDA is not apparent [SU 1:198-200].

The relationship between AE's and zileuton blood levels was investigated in the two pivotal trials, M91-685 and M92-720. The peak levels were obtained 1-3 hours post-dosing which is supposed to be an interval in which peak zileuton levels should occur in most patients. Zileuton patients were grouped as having a high zileuton blood level or as having a not high zileuton blood level. High was defined as ≥ 7.5 mcg/ml which would be approximately 1.5 times the expected estimated mean C_{Max} in patients receiving zileuton 600 mg QID. Overall, the percentage of patients experiencing one or more AE's was similar between groups. Two AE's, vomiting and leukopenia, had a statistically significantly higher incidence in the ≥ 7.5 mcg/ml group than in the < 7.5 mcg/ml group. For both of these events the actual number of patients experiencing the event was small. These results suggest that zileuton blood levels, in general, were not a factor in determining AE's [388:200-1, 390:335, SU 1:202-3].

AE'S STRATIFIED BY HIGH & LOW BLOOD ZILEUTON LEVELS FROM THE TWO PIVOTAL TRIALS, M91-685 AND M92-720		
Adverse Event (COSTART) term	< 7.5 mcg/ml n (%)	≥ 7.5 mcg/ml n (%)
Vomiting	2 (0.5)	3 (4.9)
Leukopenia	0 (0)	2 (3.3)
Total Number of AE's	327 (79.0)	49 (80.3)
Total Number of Patients	414 (100)	69 (100)

SUMMARY OF ADVERSE EVENTS

About 76% of ALL zileuton-treated patients in Phase II/III studies experienced at least one treatment-emergent AE. The number is slightly higher, about 81%, for the ASTHMA-ONLY trials. The three most frequent AE's, for all treatment regimens including placebo, were "aggravation reaction", "headache" and "infection." By study category (e.g., active control, single-dose crossover, etc.) for similar sized trials, AE reporting rates were very similar and there was a trend for the zileuton-treated patients to have a slightly higher AE incidence than the control patients. In parallel-group, placebo-controlled, Phase II/III ALL and ASTHMA-ONLY trials, AE's significantly different in zileuton and placebo arms, were "dyspepsia" and "insomnia." Other AE's with less statistically firm difference between treatment groups were "abdominal pain," "dizziness" and "rash." Three AE's with an incidence of < 2% were nonetheless statistically different when treatment groups were compared. These were "taste perversion," "peripheral edema" (limited to ulcerative colitis patients) and "pruritis," all of which were more frequent in patients treated with zileuton than in control patients.

A total of 97 (11.5%) zileuton ASTHMA patients experienced AE's classified as severe compared with 37 (7.5%) placebo patients. Corresponding numbers for ALL studies were 156 (8.9%) and 70 (6.4%). The most frequently reported, severe AE was headache in both ASTHMA-ONLY and ALL studies. No relation between magnitude of dose and incidence of AE's was found.

AE's in zileuton-treated cases had the greatest rate of onset, incidence in at-risk patients, during the first two weeks of zileuton treatment. The number of patients with AE's present during any given interval, the prevalence, was slightly less than 50% for the first two weeks and remained at about the same level through all time intervals including the longest (> 150 days).

Demographic subgroup stratification showed some statistically significant, but clinically dubious associations. Finally, the higher zileuton blood levels, dichotomized at

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7.5 mcg/ml, were associated with more leukopenia and vomiting, but absolute numbers were very small.

SPECIAL SAFETY CATEGORIES

DEATHS

Seven deaths occurred worldwide in over 4000 patients enrolled in Phase II/III trials; four of these patients had received zileuton. A 43-year-old female with ulcerative colitis developed gall bladder cancer 18 months after receiving zileuton. She had received 800 mg BID for 18 days and then placebo for 2 months. After 11 months exposure to zileuton 600 mg QID, a 50-year old man in an ulcerative colitis trial, developed rectal carcinoma and died of post-operative complications. A 59-year old man with a history of myocardial infarction and coronary artery bypass surgery died in cardiogenic shock after 14 months of zileuton 600 mg QID for the treatment of ulcerative colitis. His death is presumed due to another myocardial infarction. A 30-year old received 5 days of zileuton 600 mg QID at least 9 months before his death from a complication of AIDS.

Three of the deaths were not associated with zileuton. An 80-year old female in a ulcerative colitis trial who received placebo, died a sudden death that is postulated to be secondary to either a myocardial infarction or a pulmonary embolism. A 26-year old female, who was enrolled but who had never received any study drug, died of anoxic encephalopathy secondary to an exacerbation of asthma. Finally, an 18-year old male on theophylline, died in cardio-respiratory arrest secondary to status asthmaticus [SU 1:206-9].

PREMATURE TERMINATION DUE TO ADVERSE EVENTS

Of a total of 2573 zileuton-treated patients from ALL studies, 263 (10.2%) were terminated from the trials because of an adverse event. A similar, total statistic from the ASTHMA studies is 168/1457 (11.5%) which is broken down into the most common reasons for termination [SU 1:211]. Many of these AE's represent joint events and are not necessarily the additive sum of individual components, because one patient may have reported more than one AE. The following table contains only the most frequent (≥ 2 patients) AE's causing early termination in either ASTHMA or ALL studies.

SOME OF THE MOST FREQUENT AE's, LEADING TO TERMINATION, FROM ALL/ASTHMA PARALLEL-GROUP OR UNCONTROLLED, PHASE II/III STUDIES		
Adverse Event	Asthma, n (%) (N=1457)	All, n (%) (N=2573)
Abdominal Pain	6 (0.4)	12 (0.5)
Accidental Injury	1 (0.1)	2 (0.1)

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SOME OF THE MOST FREQUENT AE's, LEADING TO TERMINATION, FROM ALL/ASTHMA PARALLEL-GROUP OR UNCONTROLLED, PHASE II/III STUDIES		
Adverse Event	Asthma, n (%) (N=1457)	All, n (%) (N=2573)
Aggravation Reaction	77 (5.3)	77 (3.0)
Allergic Reaction	2 (0.1)	2 (0.1)
Asthenia	3 (0.2)	6 (0.2)
Back Pain	0 (0.0)	2 (0.1)
Chest Pain	2 (0.1)	6 (0.2)
Face Edema	0 (0.0)	2 (0.1)
Flu Syndrome	1 (0.1)	3 (0.1)
Headache	5 (0.3)	16 (0.6)
Infection	9 (0.6)	10 (0.4)
Malaise	1 (0.1)	3 (0.1)
Migraine	2 (0.1)	2 (0.1)
Myocardial Infarction	1 (0.1)	3 (0.1)
Palpitation	2 (0.1)	3 (0.1)
Tachycardia	1 (0.1)	4 (0.2)
Anorexia	1 (0.1)	2 (0.1)
Diarrhea	4 (0.3)	8 (0.3)
Dyspepsia	6 (0.4)	8 (0.3)
GGT Increase	1 (0.1)	2 (0.1)
Gastroenteritis	1 (0.1)	2 (0.1)
Hepatitis	4 (0.3)	4 (0.2)
LFT's Abnormal	10 (0.7)	17 (0.7)
Nausea	5 (0.3)	15 (0.6)
Nausea & Vomiting	0 (0.0)	2 (0.1)
Vomiting	2 (0.1)	5 (0.2)

SOME OF THE MOST FREQUENT AE's, LEADING TO TERMINATION, FROM ALL/ASTHMA/ PARALLEL-GROUP OR UNCONTROLLED, PHASE II/III STUDIES		
Adverse Event	Asthma, n (%) (N=1457)	All, n (%) (N=2573)
Leukopenia	2 (0.1)	4 (0.2)
AST Increased	5 (0.3)	5 (0.2)
ALT increased	5 (0.3)	7 (0.3)
Arthralgia	0 (0.0)	4 (0.2)
Agitation	1 (0.1)	2 (0.1)
Anxiety	0 (0.0)	2 (0.1)
Confusion	0 (0.0)	2 (0.1)
Depression	4 (0.3)	8 (0.3)
Dizziness	4 (0.3)	9 (0.3)
Insomnia	6 (0.4)	7 (0.3)
Nervousness	3 (0.2)	4 (0.2)
Somnolence	2 (0.1)	3 (0.1)
Thinking Abnormal	1 (0.1)	2 (0.1)
Bronchitis	5 (0.3)	5 (0.2)
Dyspnea	0 (0.0)	4 (0.2)
Pneumonia	2 (0.1)	3 (0.1)
Rhinitis	5 (0.3)	5 (0.2)
Sinusitis	3 (0.2)	4 (0.2)
Maculopapular Rash	2 (0.1)	3 (0.1)
Pruritis	1 (0.1)	7 (0.3)
Rash	5 (0.3)	16 (0.6)
Sweating	1 (0.1)	2 (0.1)
Urticaria	5 (0.3)	6 (0.2)
Conjunctivitis	0 (0.0)	2 (0.1)

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SOME OF THE MOST FREQUENT AE's, LEADING TO TERMINATION, FROM ALL/ASTHMA PARALLEL-GROUP OR UNCONTROLLED, PHASE II/III STUDIES		
Adverse Event	Asthma, n (%) (N=1457)	All, n (%) (N=2573)
Vaginitis	0 (0.0)	2 (0.1)
ANY SIGN/SYMPTOM	168 (11.5)	263 (10.2)

In the zileuton arms of the ASTHMA studies, the most common reasons for early termination because of an AE were: aggravation reaction (n=77, 5.3%), LFT's Abnormal, AST increased or ALT increased (n=15, 1.0%) and infection (n=9, 0.6%) [SU 1:211]. The zileuton treated patients in ALL studies had similar AE's that were the most frequent reason for early termination: aggravation reaction (n=77, 3.0%), LFT's abnormal, AST increase or ALT increase (n=24, 0.9%), abdominal pain (n=12, 0.5%), nausea (n=15, 0.6%), headache (n=16, 0.6%) and rash (n=16, 0.6%) [SU 1:212].

COMMENT: The categories of combined hepatic dysfunction; "LFT's Abnormal," "AST Increased" and "ALT Increased" appear to exclude frank "Hepatitis."

A table comparing the AE's that led to early terminations is presented below and shows some disparities between zileuton and placebo [SU 1:214-16].

AE's LEADING TO PREMATURE TERMINATION FOR ≥ 2 PATIENTS IN EITHER TREATMENT GROUP IN PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES				
Adverse Events	Asthma Studies		All Studies	
	Zileuton N = 847 n (%)	Placebo N = 491 n (%)	Zileuton N = 1757 n (%)	Placebo N = 1101 n (%)
Abdominal Pain	2 (0.2)	1 (0.2)	8 (0.5)	4 (0.4)
Aggravation Reaction	24 (2.8)	26 (5.3)	24 (1.4)	26 (2.4)
Allergic Reaction	2 (0.2)	0 (0.0)	2 (0.1)	0 (0.0)
Asthenia	2 (0.2)	0 (0.0)	5 (0.3)	0 (0.0)
Back Pain			2 (0.1)	1 (0.1)
Chest Pain	2 (0.2)	0 (0.0)	3 (0.2)	1 (0.1)
Fever			1 (0.1)	2 (0.2)

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AE's LEADING TO PREMATURE TERMINATION FOR ≥ 2 PATIENTS IN EITHER TREATMENT GROUP IN PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES				
Adverse Events	Asthma Studies		All Studies	
	Zileuton N = 847 n (%)	Placebo N = 491 n (%)	Zileuton N = 1757 n (%)	Placebo N = 1101 n (%)
Flu Syndrome			3 (0.2)	1 (0.1)
Headache			11 (0.6)	2 (0.2)
Infection	4 (0.5)	6 (1.2)	4 (0.2)	7 (0.6)
Malaise			3 (0.2)	0 (0.0)
Myocardial Infarction			2 (0.1)	0 (0.0)
Tachycardia			3 (0.2)	1 (0.1)
Diarrhea	3 (0.4)	0 (0.0)	7 (0.4)	3 (0.3)
Dyspepsia	3 (0.4)	1 (0.2)	4 (0.2)	2 (0.2)
Hepatitis	3 (0.4)	0 (0.0)	3 (0.2)	0 (0.0)
LFT's Abnormal	4 (0.5)	1 (0.2)	7 (0.4)	1 (0.1)
Nausea	3 (0.4)	1 (0.2)	13 (0.7)	4 (0.4)
Vomiting			4 (0.2)	1 (0.1)
Leukopenia	2 (0.2)	0 (0.0)	4 (0.2)	0 (0.0)
ALT Increase			2 (0.1)	0 (0.0)
Arthralgia			2 (0.1)	1 (0.1)
Anxiety			2 (0.1)	0 (0.0)
Confusion			2 (0.1)	0 (0.0)
Depression	2 (0.2)	1 (0.2)	4 (0.2)	1 (0.1)
Diplopia			0 (0.0)	2 (0.2)
Dizziness	2 (0.2)	0 (0.0)	7 (0.4)	4 (0.4)
Insomnia	3 (0.4)	0 (0.0)	4 (0.2)	0 (0.0)
Nervousness	2 (0.2)	0 (0.0)	3 (0.2)	0 (0.0)

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AE's LEADING TO PREMATURE TERMINATION FOR ≥ 2 PATIENTS IN EITHER TREATMENT GROUP IN PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES				
Adverse Events	Asthma Studies		All Studies	
	Zileuton N = 847 n (%)	Placebo N = 491 n (%)	Zileuton N = 1757 n (%)	Placebo N = 1101 n (%)
Bronchitis	2 (0.2)	0 (0.0)	2 (0.1)	0 (0.0)
Dyspnea			2 (0.1)	0 (0.0)
Pharyngitis	0 (0.0)	2 (0.4)	0 (0.0)	2 (0.2)
Rhinitis	3 (0.4)	0 (0.0)	3 (0.2)	0 (0.0)
Sinusitis	2 (0.2)	1 (0.2)	3 (0.2)	1 (0.1)
Maculopapular Rash			2 (0.1)	0 (0.0)
Pruritis			6 (0.3)	1 (0.1)
Rash	2 (0.2)	1 (0.2)	9 (0.5)	2 (0.2)
Urticaria	3 (0.4)	0 (0.0)	4 (0.2)	0 (0.0)
Conjunctivitis			2 (0.1)	0 (0.0)
ANY SIGN/SYMP TOM	78 (9.2)	41 (8.4)	137 (7.8)	61 (5.5)

The combined early termination rate was almost 1% more for zileuton than for placebo in ASTHMA studies (Z=9.2%, P=8.4%). In ALL trials, zileuton had about 2% excess of early terminations because of AE's (Z=7.8%, P=5.5%). For zileuton patients in ALL studies, aggravation reaction (n=24, 1.4%), nausea (n=13, 0.7%), headache (n=11, 0.6%) and abdominal pain (n=8, 0.5%) were the most commonly cited reasons for study termination [SU 1:215-6].

COMMENT: Hepatic abnormalities are classified under several terms, "hepatitis," "LFT's abnormal" and "ALT Increase." They are similar in that each of these AE's is more frequent in zileuton-treated patients than those receiving placebo. The true incidence cannot be determined from these data because more than one AE may be used to describe a single event.

Several AE's are notable for having occurred in $\leq 2\%$ of the general study population, yet having been cited as the reason for termination by ≥ 2 patients [388:209-10]. These include: abnormal LFT's, AST or ALT (24); hepatitis (4); GGT increase (2); pruritis (7); urticaria (6);

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maculopapular rash (3); sweating (2); myocardial infarction, tachycardia or palpitations (10); depression (8); nervousness (4); and, migraine, agitation, anxiety, confusion and thinking abnormally were reasons for 2 each to drop out. Other early withdrawals were for reasons of: dyspnea (4); pneumonia (3); malaise (3); leukopenia (4); and, two each for gastroenteritis, nausea and vomiting, accidental injury, allergic reaction, anorexia, conjunctivitis, facial edema and vaginitis [SU 1:212].

For ALL placebo-controlled studies in which AE's led to premature discontinuation for ≥ 2 patients in either group, more patients discontinued due to an adverse event having an onset during the first two weeks of the study than during any other time interval, followed by the second two-week period. This pattern was the same for ASTHMA-ONLY placebo-controlled, parallel-group, Phase II/III trials [SU 1:218]. The percentages refer to the "at risk" population.

ONSET OF AE CAUSING EARLY TERMINATION FROM ALL PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III TRIALS		
	ZILEUTON (n=1757) total dropouts = 137 (7.8%)	PLACEBO (n=1101) total dropouts = 61 (5.5%)
Weeks 1 & 2	60 (3.4%)	27 (2.5%)
Weeks 3 & 4	35 (2.1%)	10 (0.9%)

SERIOUS AE's RESULTING IN 10-DAY SAFETY REPORTS

These totaled 6, 5 of which were in the group receiving zileuton. Among these were 2 miscarriages, 1 cholecystitis/cholecystectomy, 1 depression with suicidal ideation and 1 case of urticaria with significantly elevated liver enzymes ($>20X$ upper limit of the normal range). These abnormal liver function tests returned to within normal limits within one month after zileuton was discontinued. One patient, who had received placebo, developed pyelonephritis and renal insufficiency [388:219-20, SU 1:224-5].

HOSPITALIZATIONS

A total of 87 patients were hospitalized with admission occurring during the active treatment periods of the studies. In addition, 7 patients had a hospital admission during the single-blind, or lead-in, periods of the NDA studies. An additional 22 patients were hospitalized sometime after the final dose of the study medication [SU 1:226]. Thirty-two of the 116 hospitalizations were associated with the control treatment, active or placebo, and the remaining 84 were from the zileuton treatment group [SU 23:510].

MALIGNANCIES

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Twenty-two (22) cases of dysplasia or malignancy were reported while patients were enrolled in, or after they had finished, zileuton studies. Fourteen (14) of these occurred in patients who participated in ulcerative colitis studies. This latter group had a high preponderance of the expected types of dysplastic syndromes, 9/14 had bowel or rectal carcinoma or dysplasia. The remaining cases in the ulcerative colitis patients were:

1. small cell lung cancer in a smoker
2. CLL & non-Hodgkin's lymphoma
3. gall bladder cancer
4. subcutaneous lipoma
5. benign breast neoplasm

The 8 non-ulcerative colitis patients had a host of non-related neoplasms:

1. abnormal Pap smear (repeat smear was negative)
2. carcinoma of the hand (history of sun exposure)
3. bilateral nasal polyps
4. lipoma of the forehead
5. hyperplasia (Pt #307; Study M92-785)
6. nasal polyps (two cases)
7. malignant breast neoplasm

COMMENT: The types of dysplasia/neoplasia are relatively clearly defined and do not seem to similar to one another [SU 1:226-30]. On the other hand, of the 22 cases of dysplasia or malignancy, 19 were associated with zileuton, 1 with placebo, 1 with theophylline and 1 was still blinded as to treatment [SU 1:228-30].

SUMMARY OF SPECIAL SAFETY CATEGORIES

Deaths were about equal in the zileuton group and the control arms. Premature termination due to AE's in both ASTHMA and ALL subjects who took zileuton, were most frequently caused by an aggravation reaction and second most frequently caused by abnormal indices of hepatic function. Comparison of zileuton and placebo groups shows about a 2% excess AE termination rate in the zileuton group compared with placebo. Terminations due to AE's were more likely to happen in the first 4 weeks of treatment in both control and zileuton groups, based on the "at risk" population in each interval. Five of six of the 10-day Safety Reports involved zileuton patients, but whether this was a chance finding is unknown. Neoplasms appeared in greater number in ulcerative colitis patients and were usually found in the bowel, as would be predicted from the natural history of the disease. Of the 22 neoplasms, some exposure to zileuton was present in 19, but in 9 this exposure was $\leq$ 3 months.

CLINICAL LABORATORY DETERMINATIONS

HEMATOLOGY CHANGES

This included such traditional members of the CBC as hemoglobin, hematocrit, red blood cells (RBC), platelets and white blood cells (WBC). The differential cell count was also represented: neutrophils (%), lymphocytes (%), monocytes (%), basophils (%), eosinophils (%), total eosinophils, granulocytes (%), bands (%) and atypical lymphocytes (%). This section also included some coagulation parameters, the prothrombin time (PT) and the activated partial thromboplastin time (PTT) [SU 1:234-5].

Six zileuton patients discontinued prematurely due to changes in hematology parameters; 5 were discontinued prematurely due to decreased WBC, which was associated with thrombocytopenia in one [SU 1:282].

ZILEUTON PATIENTS WHO PREMATURELY TERMINATED DUE TO LOW WHITE BLOOD CELL COUNTS (WBC x10 ⁹ /L)							
Trial/Patient	Age	Sex	Baseline		Minimum		Comments
			Day	WBC	Day	WBC	
							is"
							"

Three of these five were reported to have had some evidence of decreased WBC at other times [388:266, SU 2:1]. One of the six withdrew prematurely because of hypochromic anemia [SU 1:236].

Mean change, from baseline to final visit for both the ASTHMA-ONLY group and ALL trials showed similar results. The percent eosinophils, total eosinophils, white blood cell count, RBC count and hemoglobin all decreased more in the zileuton group than in the placebo arm [SU 1:238]. A table of these representative values in the ASTHMA-ONLY studies is presented below [SU 1:239].

MEAN CHANGE FROM BASELINE TO FINAL VISIT IN HEMATOLOGY PARAMETERS FOR PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III, ASTHMA STUDIES						
Parameter	Zileuton			Placebo		
	N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
Hemoglobin	814	14.45	-0.11	473	14.44	0.04
Hematocrit	812	44.4	-0.46	473	44.23	-0.10
RBC	789	4.86	-0.06	459	4.87	-0.02
Platelets	809	256.06	-3.60	467	261.93	-0.39
WBC	814	6.29	-0.01	473	6.45	0.20
% Neutrophils	811	55.38	0.93	471	56.01	0.16
% Lymphocytes	811	30.95	-0.52	471	30.76	-0.17
% Monocytes	811	5.83	0.26	471	5.82	0.11
% Basophils	811	0.87	0.04	471	0.87	0.02
% Eosinophils	811	5.36	-0.75	471	4.99	-0.12
Total Eosinophils	670	364.19	-54.82	370	340.35	-3.55
% Bands	758	0.19	-0.04	411	0.25	-0.11
% Atypical Lymphs	548	2.09	0.09	306	1.97	0.16

A table of the hematology variables in ALL placebo-controlled trials showed basic similarity to the last table. The only statistically significant differences between placebo and zileuton that was common to both tables were percent eosinophils, total eosinophils and WBC count [SU 1:239].

Percent eosinophils and total eosinophil count in the female population showed statistically significant differences between treatment groups due to decreases in the zileuton group and smaller decreases, or increases, in the placebo group for both ASTHMA and ALL studies [SU 1:241].

Age-stratified subgroup analysis, dichotomized at age 60, for ALL trials indicated that the greater decrease in percent eosinophils with zileuton over placebo was found in the younger ($\leq$ 60 years) age group. The over-60 patients in the zileuton group had a baseline percent eosinophils that was less than the younger group and was increased at the final visit [SU 1:243]. Stratification by race for ALL trials, showed similar directional changes in percent eosinophils which only showed statistical significance in Caucasians [SU 1:245].

COMMENT: Patients over the age of 60 years constituted less than 1% of the study population for both ASTHMA-ONLY and ALL trials and Caucasians comprised 85-90% of the patients in each. Therefore, this statistically significant subgroup analyses by age and ethnicity reflects the large numbers of Caucasians and patients under the age of 60.

Shift table analyses categorized patients in two groups: 1) those with low or normal baseline values who shifted to high, at the final visit, and 2) those with high or normal baseline values who shifted to low, at the final visit. Zileuton and placebo groups were compared by means of this shift table. Results for ASTHMA-ONLY and ALL trials were similar. The studies showed a greater number of patients in the zileuton group shifting from low or normal to high percent eosinophils, than was seen in the placebo group [SU 1:251].

A frequency count of patients with abnormally low hematology values was carried out by primary and secondary criteria. The primary method included all patients with a laboratory value at any time during the study (up to 3 days post-dosing) that was both 1) below the lower limit of normal, and 2) below the baseline value of the patient. The secondary method further reduced the number of primary method patients by excluding patients with hematology values, at the final visit, that had either 1) fallen within the normal reference range, or 2) were greater than the baseline value for that individual [1:234, 256].

COMPARISON OF PRIMARY AND SECONDARY METHODS OF ANALYSIS FOR THE NUMBERS AND PERCENTAGES OF PATIENTS WITH ABNORMALLY LOW VALUES IN PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA STUDIES								
Variable	PRIMARY				SECONDARY			
	Zileuton		Placebo		Zileuton		Placebo	
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
WBC	815	13 (1.6)	473	0 (0.0)	815	8 (1.0)	473	0 (0.0)
RBC	797	19 (2.4)	463	5 (1.1)	797	10 (1.3)	463	2 (0.4)
Hct	815	28 (3.4)	473	12 (2.5)	815	12 (1.5)	473	4 (0.8)
Platelets	813	1 (0.1)	471	0 (0.0)	813	0 (0.0)	471	0 (0.0)

Abnormally low hematology variables were more often associated with zileuton, at any dose, than with placebo. The WBC achieved statistical significance for both primary and secondary methods, in a comparison between zileuton and placebo.

In actively-controlled trials, mean changes from baseline to final visit in total and percent eosinophils showed a much greater decline with the active-control arm, compared with the

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zileuton group. This was presumed to be due to the eosinopenic effect of beclomethasone on eosinophils. [SU 1:262].

SUMMARY OF HEMATOLOGY CHANGES

Eosinophil parameters, percent eosinophils and total eosinophils, were the only hematology parameters that showed an obvious decreasing trend in the zileuton group that was statistically significant in more than one analysis favoring an effect of zileuton versus placebo. In actively-controlled trials, decreased eosinophil parameters were more prevalent in the active-control group possibly because the active control was the eosinopenic drug, beclomethasone.

Trends toward lower WBC with zileuton were not universal over all methods of analysis but occasionally reached statistical significance. However, 5 patients did discontinue the protocol because of abnormally low WBC. Three of these had histories consistent with previously low WBC.

HEPATIC CHEMISTRY CHANGES

BASELINE-TO-FINAL-VISIT CHANGES:

Increases in mean GGT and AST were about 5%, and ALT about 9%, in excess of placebo for ALL placebo-controlled, parallel, Phase II/III trials, baseline to final visit. However, none of these reached statistical significance between zileuton and placebo groups [SU 2:4-5].

BASELINE AND FINAL VISIT MEAN VALUES FOR LIVER TESTS FROM ALL PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III TRIALS						
Laboratory Values	Zileuton			Placebo		
	N	Baseline	Mean Change	N	Baseline	Mean Change
GGT (IU/L)	1618	22.65	2.51	996	22.93	1.21
AST (IU/L)	1638	21.24	2.19	1024	20.61	0.75
ALT (IU/L)	1621	21.68	3.17	1012	20.69	1.09
Alk. Phos (IU/L)	1671	87.96	1.57	1051	88.52	0.62
LDH (IU/L)	1660	175.25	1.88	1039	186.17	-1.45
Bilirubin (mg/dL)	1663	0.57	-0.01	1049	0.58	-0.01

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When a subset of these, the ASTHMA studies, was considered, similar trends were found with a change in magnitude of the zileuton-group mean ALT to about 22% in excess of the placebo group. In contrast to this elevation in the zileuton group, the mean ALT declined slightly in the placebo group. Differences between zileuton and placebo achieved statistical significance for GGT, AST and ALT [SU 2:3].

BASELINE AND FINAL VISIT MEAN VALUES FOR LIVER TESTS FROM PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS						
Laboratory Values	Zileuton			Placebo		
	N	Baseline	Mean Change	N	Baseline	Mean Change
GGT (IU/L)	764	21.62	2.39	418	22.29	-0.08
AST (IU/L)	814	20.30	3.01	474	20.02	0.03
ALT (IU/L)	814	20.93	4.37	473	21.05	-0.36
Alk. Phos (IU/L)	813	79.54	-1.23	473	78.30	-2.31
LDH (IU/L)	813	169.31	1.18	471	176.03	-2.89
Bilirubin (mg/dL)	814	0.62	0.00	475	0.63	0.00

No relation between dose and degree of mean enzyme elevation was found in ALL parallel-group, placebo-controlled, Phase II/III trials [SU 2:5, 17, 20].

Liver enzyme values were classified into five categories and the number of patients in each category, at baseline and at the final visit, was presented as a cross-tabulation for ASTHMA studies by zileuton dose regimen. From these, the numbers of patients who had increased, or decreased, by one, or more, categories for each liver test could be determined and the dose regimens compared. No clear relation between daily dose and percent worsening or ratio of number worsening to number improving was found in these cross-tabulations [SU 14:264-271, 276-79].

COMMENTS: The dose range was narrow (1600-2400 mg/day) and the liver enzyme categories were fairly wide: I, <normal; II, within normal range; III, 1-3 times the Upper Limit of Normal (1-3xULN); IV, 3-8xULN; and V, ≥ 8xULN. Nevertheless, dose-relatedness to mean changes in liver enzymes, and categorical frequency changes of enzyme values, from baseline to final visit, was not shown.

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Shift table analysis of placebo-controlled, parallel-group, Phase II/III ASTHMA trials, showed that the number of patients with normal, or low, enzyme levels at baseline, and high (>ULN) levels at the final visit was a trend similar to the mean changes. That is, zileuton was associated with increases in the mean values of liver tests, when compared to placebo. ALT and GGT show 1.7 and 2.3% shifts in excess of placebo, respectively. Changes in the same direction were seen in alkaline phosphatase (1.6% in excess of placebo) and AST (1.4% in excess of placebo [SU 2:17]).

NUMBER (%) OF PATIENTS WITH LIVER TESTS $\leq$ NORMAL, AT BASELINE, WHO HAD LIVER TESTS $>$ NORMAL, AT FINAL VISIT, FOR PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS		
Laboratory Value	Placebo	Zileuton (all doses)
GGT	8 (2.1)	31 (4.4)
AST	20 (4.4)	45 (5.8)
ALT	22 (5.1)	51 (6.8)
Alkaline Phosphatase	7 (1.5)	24 (3.1)
LDH	13 (2.9)	19 (2.5)
Total Bilirubin	13 (2.9)	11 (1.4)

Analysis of ALL parallel-group, placebo-controlled, Phase II/III showed similar findings [SU 2:20].

COMMENT: Apparently, the percent of individuals shifting from low, or normal, enzyme levels at baseline to high levels at the final visit is small relative to the percent increase in the mean enzyme levels between these two time points. One possible interpretation is that those patients taking zileuton who do develop enzyme elevations, show large increases, relative to placebo.

WORSENING OF ABNORMAL, BASELINE LIVER FUNCTION TESTS

Each liver function test that was $>$ ULN, at baseline, was followed and the final visit value was recorded. This final visit value was then categorized into groups: $<$ normal, within normal range, 1-3xULN, 3-8xULN and $>$ 8xULN. Based on movements between these broad categories, judgement was made if the abnormal, baseline value remained in the same category, worsened or improved. These machinations were carried out for ALL placebo-controlled, parallel-group, Phase II/III trials and the subset dealing with ASTHMA patients. The table below shows those liver function tests that were abnormal at baseline and worsened (shifted by $\geq$ 1 category) at the

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final visit [SU 2:18, 21]. The table below was synthesized by the reviewer from the two referenced tables.

NUMBER (%) OF PATIENTS WITH LIVER TESTS > ULN, AT BASELINE, WHO BECAME WORSE (SHIFTED BY ≥ 1 CATEGORY), AT THE FINAL VISIT FOR PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III TRIALS				
LIVER FUNCTION TESTS	ALL		ASTHMA	
	Zileuton	Placebo	Zileuton	Placebo
GGT	8/122 (6.6)	1/73 (1.4)	5/55 (9.1)	0/29 (0.0)
AST	2/56 (3.6)	0/30 (0.0)	1/33 (3.0)	0/20 (0.0)
ALT	4/118 (3.4)	0/61 (0.0)	2/68 (2.9)	0/40 (0.0)
Alk. Phos.	0/114 (0.0)	0/63 (0.0)	0/41 (0.0)	0/16 (0.0)
LDH	1/62 (1.6)	0/51 (0.0)	1/36 (2.8)	0/19 (0.0)
Bilirubin	0/37 (0.0)	0/42 (0.0)	0/21 (0.0)	0/20 (0.0)

The sponsor concludes a small number of patients, the majority in the zileuton group, with elevated liver tests at baseline had a further increase to a worse category during treatment.

COMMENT: Worsening of already abnormal liver functions is more prevalent in zileuton-treated patients than in the placebo group. The ALT, AST and GGT are the liver tests that are most affected.

BASELINE-TO-MAXIMUM CHANGE:

Mean changes from baseline to post-baseline maximum value for liver tests in ASTHMA trials was submitted 22 November 1994 as a response to an information request. A reviewer-synthesized table of these data is presented below [11/22/94 0:56-61].

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BASELINE AND POST-BASELINE MAXIMUM MEAN VALUES FOR LIVER TESTS FROM PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS						
Laboratory Values	Zileuton			Placebo		
	N	Baseline	Mean Change	N	Baseline	Mean Change
GGT (IU/L)	777	21.67	6.52	424	22.34	3.35
AST (IU/L)	834	20.32	7.81	483	19.99	4.63
ALT (IU/L)	835	20.96	11.00	482	21.02	5.53
Alk. Phos (IU/L)	833	79.43	6.76	482	78.05	4.70
LDH (IU/L)	833	169.35	20.42	480	176.10	16.32
Bilirubin (mg/dL)	834	0.62	0.15	483	0.63	0.13

In comparison with baseline-to-final-visit mean changes in liver enzymes, the changes shown above are greater in magnitude for both zileuton and placebo groups. No inferential statistical test results were supplied, but the relative changes in zileuton and placebo groups in mean liver enzymes is similar to that seen in baseline-to-final-visit tables.

In placebo-controlled, parallel-group, Phase II/III ASTHMA trials, the number of patients with normal, or low, enzyme levels at baseline, and maximal high (> ULN) levels at sometime during the study, shows a trend similar to the baseline-to-final-visit changes. ALT and GGT show 2.1 and 4.0% incidence shifts, respectively, in excess of placebo [SU 2:8]. (N.B. The phrase "at maximum percent of the upper limit of normal" is an attempt to report post-baseline maximum values for patient-samples evaluated at different laboratories with different normal reference ranges.)

NUMBER (%) OF PATIENTS WITH LIVER TESTS $\leq$ NORMAL, AT BASELINE, WHO HAVE LIVER TESTS $>$ NORMAL, AT MAXIMUM PERCENT OF THE UPPER LIMIT OF NORMAL FOR PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS		
Laboratory Value	Placebo	Zileuton (all doses)
GGT	19 (4.8)	63 (8.8)
AST	47 (10.2)	93 (11.6)
ALT	51 (11.5)	104 (13.6)
Alkaline Phosphatase	13 (2.8)	41 (5.2)

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NUMBER (%) OF PATIENTS WITH LIVER TESTS $\leq$ NORMAL, AT BASELINE, WHO HAVE LIVER TESTS $>$ NORMAL, AT MAXIMUM PERCENT OF THE UPPER LIMIT OF NORMAL FOR PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS		
Laboratory Value	Placebo	Zileuton (all doses)
LDH	29 (6.3)	49 (6.2)
Bilirubin _r	20 (4.3)	34 (4.2)

The percent of patients with elevated GGT, ALT, AST and Alkaline Phosphatase at the time of maximum percent of the upper limit of the normal range was greater for the zileuton group compared to the placebo group.

COMMENT: Baseline-to-maximal-elevation plots showed the same general findings as did baseline-to-final-visit. Elevated liver tests during treatment were more frequent and had higher mean values with zileuton-, than with placebo-treated patients.

Liver enzyme values were separated into five classes; below normal, within normal range, from 1-3xULN, from 3-8xULN and $\geq$ 8xULN, at baseline and again at final visit, or at maximum post-baseline value. Cross-tabulations of these categories at baseline and at the maximum post-baseline value, were carried out for ASTHMA studies and the number of patients who had changed by $\geq$ 1 category was noted, both those who had improved and those who had worsened. A reviewer-synthesized table of baseline to maximum post-baseline value is provided [SU 14:318-25, 330-33].

SELECTED LIVER ENZYME VALUES FROM PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III ASTHMA STUDIES: A CROSS-TABULATION OF BASELINE VALUES AND MAXIMUM POST-BASELINE VALUES, BY DOSAGE REGIMEN					
Liver Enzyme	Zileuton Dose	Total Number	# Worse (%)	# Better (%)	Ratio #W/#B
AST	600 QID	475	55 (11.6)	11 (2.3)	5.00
AST	800 BID	49	5 (10.2)	0 (0.0)	undefined
ALT	600 QID	475	58 (12.2)	11 (2.3)	5.27
ALT	800 BID	49	5 (10.2)	1 (2.0)	5.00

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SELECTED LIVER ENZYME VALUES FROM PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III ASTHMA STUDIES: A CROSS-TABULATION OF BASELINE VALUES AND MAXIMUM POST-BASELINE VALUES, BY DOSAGE REGIMEN					
Liver Enzyme	Zileuton Dose	Total Number	# Worse (%)	# Better (%)	Ratio #W/#B
GGT	600 QID	475	41 (8.6)	6 (1.3)	6.83
GGT	800 BID	49	2 (4.1)	0 (0.0)	undefined

No clear relation between daily dose and percent worsening or ratio of number worsening to number improving was found in these cross-tabulations.

WORSENING OF ABNORMAL, BASELINE LIVER FUNCTION TESTS

Each liver function test that was >ULN, at baseline, was followed and the maximum, post-baseline value was recorded. This was then categorized into groups: 1-3xULN, 3-8xULN and >8xULN, and patients whose liver enzyme values changed categories, were recorded. The table below shows the number of patients with liver function tests >ULN at baseline that worsened (shifted by ≥ 1 category) at the maximum post-baseline measurement [SU 2:11].

NUMBER (%) OF PATIENTS WITH LIVER TESTS >ULN, AT BASELINE, WHO BECAME WORSE (SHIFTED BY ≥ 1 CATEGORY), AT MAXIMAL ELEVATION FOR PARALLEL-GROUP, PLACEBO-CONTROLLED, PHASE II/III ASTHMA TRIALS		
LIVER TESTS	Zileuton	Placebo
GGT	7/57 (12.3)	0/30 (0.0)
AST	1/34 (2.9)	0/20 (0.0)
ALT	6/69 (8.7)	0/40 (0.0)
Alk. Phos.	0/43 (0.0)	0/16 (0.0)
LDH	1/38 (2.6)	0/19 (0.0)
Bilirubin	1/21 (4.8)	0/20 (0.0)

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The sponsor interprets these data to show that in a few cases, zileuton-treated patients who had liver test elevations at baseline, had a more severe elevation at the time the maximal value was obtained.

COMMENT: This table mirrors the trend seen in the baseline-to-final-visit table. Baseline elevated liver tests more frequently became more elevated at follow-up in the zileuton group than in the placebo group. When worsening of already abnormal liver functions is defined as it was here, the phenomenon is virtually unique to zileuton-treated patients, and is almost unknown in the placebo group. This number of patients worsening is greater for GGT and ALT in the baseline-to-maximum-post-baseline change than it was in the baseline-to-final-visit comparison.

ACTIVE CONTROL TRIALS

Baseline-to-final-visit liver functions were compared between zileuton and the beclomethasone active control in asthmatics. ALT, AST and GGT elevations were seen in the zileuton group, but not with the active control [SU 2:22]. The magnitude of the mean rise of the various enzymes in zileuton-treated patients was similar to findings reported elsewhere in this document.

DURATION OF EXPOSURE

Baseline-to-final-visit liver functions in combined parallel or uncontrolled Phase II/III ASTHMA trials were recorded by time interval, according to longest continuous exposure to zileuton as seen in this reviewer synthesized table [SU 18:173-4, 176]. (N.B. The phrase "longest continuous exposure" means with no break in using the active drug that was > 30 days.)

BASELINE AND FINAL VISIT MEAN LIVER TESTS IN PARALLEL OR UNCONTROLLED ASTHMA STUDIES BY TIME INTERVAL, ACCORDING TO LONGEST CONTINUOUS EXPOSURE									
Day	AST (IU/L)			ALT (IU/L)			GGT (IU/L)		
	N	Baseline	Mean Change	N	Baseline	Mean Change	N	Baseline	Mean Change
30	1372	20.04	1.08	1369	20.64	2.66	1320	22.34	0.88
60	1178	19.91	2.68	1178	20.35	3.66	1129	21.85	1.44
90	713	19.79	0.66	713	19.98	1.06	675	21.57	1.26
120	724	19.81	1.03	724	20.72	1.05	717	22.18	0.66
150	335	19.93	-0.02	335	21.04	-0.56	333	21.64	0.40
180	324	19.78	1.67	324	20.78	2.64	324	21.86	1.77

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BASELINE AND FINAL VISIT MEAN LIVER TESTS IN PARALLEL OR UNCONTROLLED ASTHMA STUDIES BY TIME INTERVAL, ACCORDING TO LONGEST CONTINUOUS EXPOSURE									
Day	AST (IU/L)			ALT (IU/L)			GGT (IU/L)		
	N	Baseline	Mean Change	N	Baseline	Mean Change	N	Baseline	Mean Change
210	180	19.48	-0.18	180	19.68	0.19	180	20.18	1.04
240	157	19.36	0.29	157	19.46	-0.10	157	20.29	0.76
270	143	19.36	0.15	143	19.87	0.17	143	20.12	1.90
300	133	19.3	0.44	133	20.02	0.81	133	20.38	1.81
330	121	19.4	-0.67	121	19.93	-0.61	121	20.17	1.59
360	122	19.42	-0.23	122	20.29	-0.78	122	20.15	2.22

The patient numbers drop precipitously from about 1300 patients at 30 days to just over 100 at 360 days. For ALT and AST, the mean change at succeeding visits was greater than baseline through the 120th day. The mean change at the final visit became less than the baseline at the 150th day visit. The mean change of the GGT was positive (greater than baseline) for every time period through one year. For each of the three enzymes, one of the largest differences from baseline seemed to be at the 60th day. The GGT showed another period of large increase from baseline from the 270th day visit onward.

COMMENT: The numbers of patients exposed for each longer duration is statistically large through the 360th day. The decline in patient numbers with increasing duration of exposure is most obviously due to more numerous short trials with cumulatively more people. A lesser contribution to the decline is undoubtedly a result of early termination of patients, many of whom dropped out because of AE's, some because of abnormal liver tests.

INCIDENCE OF PATIENTS WITH CLINICALLY SIGNIFICANT ABNORMALITIES

A total of 4.6% (119/2573) of patients treated with zileuton had one, or more, elevated (3xULN, or 2xULN for Bilirubin) liver functions. Considering only single liver function abnormalities, ALT was the most commonly elevated in 66 patients, followed by GGT (but not ALT >3xULN) in 28, Bilirubin (>2xULN, but not ALT >3xULN) in 16 and AST (but not ALT >3xULN) in 9 [SU 2:11]. The sponsor proposes eliminating 53 of these cases from consideration for a variety of reasons: AST lacks specificity, GGT is too sensitive and 7/16 patients with elevated Bilirubin levels had Gilbert's Syndrome [SU 2:27; SU 24:11]. The sponsor does not further elaborate on these 53 patients who are to be eliminated from further analysis.

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COMMENT: The sponsor argues that, only those patients with elevated ALT's $\geq 3xULN$ represent hepatic dysfunction. This is at odds with the recommendation of an international consensus committee that concluded conjugated bilirubin or ALT $> 2xULN$ were appropriate single-test criteria for drug-induced hepatic injury (Journal of Hepatology, 1990; 11:272-6) [412:38-9,42; SU 24:10]. This reference was supplied by the sponsor as an authority for the definitions of "hepatocellular," "cholestatic," and "mixed" patterns of drug-induced liver dysfunction. The remaining criterion of drug-induced liver injury, advanced by this consensus committee, was a combined increase in AST, alkaline phosphatase and total bilirubin provided one of them was $> 2xULN$. The sponsor was equally silent about using this second criterion. Please refer to a later section of this area of the review, "ADDITIONAL ASSESSMENT" for another analysis of data submitted by the sponsor. We reject the sponsor's narrow interpretation of drug-induced hepatic injury as an evaluation criterion and regard it as particularly misleading as a surveillance tool. We note that even using the sponsor's narrow definition, there is a high number of liver function abnormalities associated with zileuton.

The sponsor further contends that 6 of these remaining 66 patients should also be excluded from consideration as having zileuton-associated hepatotoxicity because they achieved the criterion ALT $\geq 3xULN$ only at baseline before any active drug was administered [SU 2:29]. Six more patients were eliminated for a variety of reasons; viral-induced hepatitis (2), baseline ALT $> 2xULN$ and history of ALT elevations (1), baseline ALT $> 2xULN$ and history of primary sclerosing cholangitis (1), elevated ALT at baseline which nearly normalized on study drug (1) and small ALT elevation that was emphasized as a percent of the ULN when the normal reference range changed [SU 2:29].

The remaining 54 patients with ALT $\geq 3xULN$ represent 2.1% of all patients exposed to zileuton in ALL Phase II/III studies. There was no obvious trend to suggest that the incidence of liver injury was greater for one indication than others. Among the categories for race, the non-Caucasian-non-black group had a slightly higher incidence (3.4%) than the Caucasians (2.0%) or the blacks (0.8%) [SU 2:29].

The onset of ALT $\geq 3xULN$ ranged from 6 to 202 days from the start of zileuton administration and had a median time of 35 days. Thirty-nine (72%) had the onset by the 60th day. Forty-one (76%) had the onset by the 91st day and 90% had the onset by the 166th day [SU 2:30; SU 24:14].

COMMENT: Proposed labelling suggests monitoring liver functions monthly, for three months, and then periodically [1:86]. About one quarter of the ALT elevations $\geq 3xULN$ occurred more than 3 months after the zileuton was first administered, but this is not an entirely accurate picture. The percent developing abnormal liver functions should be taken as a percent of those at risk to develop them. There were many patients enrolled in short trials and many fewer enrolled in

lengthy ones, thus giving the possibly mistaken impression that most abnormal liver functions developed early in the course of treatment.

An enzyme pattern consistent with "hepatocellular disease" was found in 49 (91%) of the 54 cases [SU 2:30]. The definition of "hepatocellular" disease was taken as [412:38-9,42; SU 24:10].

- ALT $\geq$ 2xULN, only, OR...
- ALT $\geq$ 5xAlkaline Phosphatase

On follow-up, 51 (94%) of the 54 patients had their ALT decrease to $<$ 2xULN. The median time to this index of resolution was 20 days, with a range of 4-241 days. Two of the remaining 3 had $>$ 50% decreases in ALT levels on follow-up, but no further determinations were made. One other patient who did not normalize had a history of liver disease prior to entering the study [SU 24:16].

Spontaneous zileuton-associated ALT elevations to $>$ 2xULN were seen in 18/54 (33%) continuing to take the study drug. Twenty-three patients were discontinued from zileuton at the time of the elevation, 6 had their elevation on the last day of the study drug and 2 showed the elevation after the study drug had been stopped [SU 2:32].

Among the 54 patients, 15 (28%) showed symptoms at the time of the ALT abnormality, that may have been attributable to liver injury. These included nausea, vomiting, abdominal pain, flu-like symptoms, hepatitis (2) and jaundice (1). These last two conditions, which occurred in two patients, are worthy of more detailed treatment.

Patient #302 in M90-460 was discontinued due to moderate urticaria after 24 days on zileuton 800 mg BID. The urticaria was accompanied by an increase in ALT to $>$ 20xULN. Bilirubin never exceeded the normal reference range. The ALT for this patient normalized by 35 days post-treatment. The second case of hepatitis was Patient #1271/313 in ongoing study M92-738, who received zileuton for 11 weeks, laid off for 2 and resumed taking the drug for 2 additional doses. He was asymptomatic, but had an elevated ALT $>$ 40xULN and an elevated bilirubin of 2xULN. A liver biopsy showed chronic granulomatous hepatitis and the liver tests were near normal within 3 weeks. His case is complicated by ingestion of wild mushrooms, administration of homeopathic treatments for joint pain and the use of naproxen. The jaundiced patient, #1682 in M92-797 discontinued zileuton 400 mg QID after 42 days. She became symptomatic on the 37th day with nausea, vomiting and flu-like symptoms. The maximum ALT elevation ($>$ 30xULN) was on the 42nd day of treatment. The jaundice and elevated bilirubin were maximal on the 7th post-treatment day ($>$ 12xULN) and almost all liver tests had normalized by 71 days post-treatment [SU 2:31]. Two of these patients, #1271/313 and #1682, will be described in greater detail later.

COMMENT: "Hepatitis" is an accepted COSTART term and is used to describe AE's without any attempt at specificity. That is, this term may equally be applied to any patient with any degree of signs or symptoms referable to the liver, at the discretion of the investigator.

RISK FACTORS FOR DEVELOPING ALT $\geq$ 3xULN:

Zileuton blood levels were seldom higher than expected on the maximum dose of 600 mg QID ($\geq$ 7.5 mcg/ml). In only 5/54 were serum level elevations above this limit seen and, in no case, did these levels coincide with ALT $\geq$ 3xULN [SU 2:32].

Patients on zileuton with baseline elevations of ALT were at 3.2 times greater risk of developing ALT $\geq$ 3xULN than were zileuton patients with a normal baseline ALT [SU 2:34]. Elevated serum chloride or a decreased serum carbon dioxide, at baseline, had statistical association with patients who would eventually develop ALT $\geq$ 3xULN in ALL but not in ASTHMA patients. The physiological reason for this is not obvious and the finding may be a chance occurrence [SU 2:34].

ADDITIONAL ASSESSMENT

The line listings for 101 patients in Appendix 8 were analyzed for any abnormalities of total bilirubin, in relation to patterns of other liver enzyme elevations, to assess possibly clinically consequential hepatic dysfunction [SU 23:276-309].

Twenty total bilirubin elevations greater than the upper limit of normal were identified. Four bilirubin elevations occurred in the absence of increases in any other liver tests. These cases were assumed to represent a benign hyperbilirubinemia, such as Gilbert's Syndrome. Six additional patients were found in which the elevated bilirubin either preceded or did not further increase when other liver tests elevated above normal. These bilirubin elevations were assumed to be unrepresentative of zileuton associated hepatic injury.

The 10 remaining cases represent elevations of total bilirubin that were associated with other liver enzyme elevations. In four of these cases the total bilirubin elevation was $>2xULN$. In the remaining 6 cases the total bilirubin was greater than normal and $\leq 2xULN$. Seven of these 10 were eliminated as showing liver test abnormalities that were not clearly associated with zileuton, for a variety of reasons. The remaining three patients were identified as having had hepatic injury that was probably associated with zileuton. An additional patient has recently been reported from an Italian trial, and this case is included to make a total of four cases.

PROBABLE ZILEUTON-ASSOCIATED HEPATIC INJURY

1. Patient #685/1682 (briefly described earlier)

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Trial M92-797 Patient #1682 [SU 23:303] (EACH VALUE IN THE TABLE IS A FACTOR xULN)						
Rx Day	A.P.	T. Bili	AST	ALT	LDH	GGT
-14	0.56	0.36	1.05	0.32	0.73	0.27
1	0.47	0.50	0.71	0.32	0.70	0.33
22	0.51	0.71	0.95	0.61	0.72	0.30
42	1.31	6.57	28.62	33.13	1.32	1.18
49	1.57	12.79	8.71	11.13	0.99	0.94
76	0.65	3.57	1.14	0.94	0.69	0.58
112	0.39	1.07	0.76	0.42	0.73	0.30

This case had normal enzymes until the Treatment Day 42 when abrupt elevations in all liver tests occurred with hyperbilirubinemia. The enzymes declined as the bilirubin continued to a maximum of 12.79xULN.

On Treatment Day 42, this 28 year old female who was taking zileuton complained of fatigue, nausea, itching, headache, nasal congestion and body aches that had begun 6 days before. Scleral icterus was noted and the study drug was discontinued. Clinical jaundice was found during an examination one week later. Subsequently, a gall bladder sonogram, blood work for autoimmune disease and Wilson's Disease and serologies for Hepatitis A, B and C were all normal.

2. Patient #1028

Trial M92-720 Patient #1028 [SU 23:290] (EACH VALUE IN THE TABLE IS A FACTOR xULN)						
Rx Day	A.P.	T. Bili	AST	ALT	LDH	GGT
-18	0.84	0.50	0.62	0.38	0.54	0.75
1	0.84	0.57	0.76	0.40	0.51	0.67
7	0.92	0.64	0.86	0.45	0.56	0.84
33	1.81	1.14	12.81	12.70	1.61	3.00
38	2.09	0.50	7.41	9.25	0.90	3.20
263	0.70	0.50	0.62	0.35	0.60	0.71

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Normal enzymes preceded the maximum elevations which were accompanied by a modest increase in bilirubin to above the normal range.

On Treatment Day 27 this 56 year old male patient complained of increasing tiredness over the past month. On Treatment Day 38 the patient was found to have right upper quadrant tenderness and slight liver enlargement and he was prematurely discontinued from the study, stopping his treatment with zileuton 600 mg QID. Two determinations of a viral etiology for his condition failed to implicate Hepatitis A, B or C.

3. Patient #3502

Trial M91-581 Patient #3502 [SU 23:287] (EACH VALUE IN THE TABLE IS A FACTOR xULN)						
Rx Day	A.P.	T. Billi	AST	ALT	LDH	GGT
-1	4.01	0.94	1.63	1.25	0.50	6.97
1	3.65	0.98	1.59	1.17	0.50	7.90
14	0.83	0.84	1.22	0.97	0.52	8.73
56	3.48	0.35	1.63	2.10	0.78	4.93
112	3.81	1.82	6.30	5.07	0.85	10.77
182	3.33	0.29	1.52	1.97	0.68	6.60
209	2.88	0.36	1.70	2.17	0.52	6.30
224	2.39	0.34	0.26	0.23	0.22	5.47
262	2.69	0.37	1.15	1.07	0.59	5.10
317	2.89	0.31	2.15	2.60	0.54	4.37

This case had baseline liver test abnormalities, with cholestatic features, and these continued throughout the trial. On the 112th treatment day acute liver enzyme elevations with hyperbilirubinemia to 1.82xULN occurred, consistent with a superimposed hepatocellular insult and consistent with a zileuton effect on hyperbilirubinemia.

This patient was a 19 year old female who took zileuton 600 mg QID who completed the study normally. On the 3rd and 11th Treatment Days she complained of epigastric pain which was considered to be menstrual in origin. On the 15th Treatment Day, she complained of menstrual cramps. Her only other AE was tonsillitis on the 167th Treatment Day. None of these symptoms are concordant in time with the acute hepatocellular enzyme elevations.

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4. Patient #1271/313 (briefly described earlier)

Trial M92-738 Patient #1271/313 [FAX 3/3/95] (NORMAL RANGE OF VALUES IS GIVEN BELOW EACH TEST)						
Date	AST (0-37)	ALT (0-40)	T. Bili. (<1.0)	A.P. (50-250)	LDH (230-460)	GGT (11-49)
04/27/94	20	17	0.73	167	361	10
05/04/94	20	16	0.64	158	353	9
07/04/94	34	46	0.74	164	352	12
08/18/94	1237	1650	1.99	533	1038	292
08/23/94	980	1430	2.52	538	n/a	324
08/26/94	355	705	1.49	386	n/a	241
08/29/94	176	388	1.37	352	n/a	227
09/02/94	80	170	1.26	284	n/a	176
09/08/94	37	61	1.09	235	n/a	112
09/27/94	27	28	1.48	189	339	66
11/02/94	22	26	1.15	161	n/a	27

The tests are normal until 07/04/94 when a trivial increase in the ALT was first noted. On 08/18/94, all liver tests were elevated with some hyperbilirubinemia. The next set of tests occurred over a month later and showed a slight reduction in ALT and AST, fairly stable alkaline phosphatase and GGT and a total bilirubin that peaked at 2.52 mg/dL. Thereafter, a continuous decline in all liver tests was seen with eventual normalization for all except the total bilirubin.

This 53 year old female received zileuton 600 mg QID for asthma and showed significant liver test abnormalities on 08/18/94. During the prior approximately one month, she had taken oral homeopathic medications. Seven injections of homeopathic medications were taken at the end of July and the beginning of August. About 3 weeks before the elevations, she experienced nausea and vomiting after eating mushrooms with dinner guests, none of whom suffered any ill effects. On 07/28/94 she discontinued the study medication at the suggestion of her private physician. On 08/17 and 08/18 she took two doses of zileuton before the study terminated normally. She reported suffering daily, mild fatigue and nausea, during the treatment interruption, and brownish discoloration of her urine from 08/07 to 08/16/94. She had taken naproxen, 1-2 500 mg tablets/day for 4 of the days during her treatment interruption.

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On 08/23/94 she was hospitalized where an abdominal sonogram was negative as were blood tests for Hepatitis A, B and C. A liver biopsy was performed on 09/05/94. This was interpreted as showing chronic granulomatous hepatitis. A panel of hepatologists concluded that:

1. She suffered hepatocellular injury consistent with a drug or toxin.
2. Toxic mushroom ingestion was an unlikely etiology because other diners had suffered no ill effects, she had not experienced diarrhea and there was no evidence of renal toxicity.
3. The 4 doses of naproxen could have caused the liver injury, though the treatment duration was short and this drug more commonly causes a cholestatic picture.
4. Homeopathic medicines were not reported to cause liver injury, but the uncontrolled ingredients make liver-injury a possibility.
5. Naproxen or zileuton were the most likely etiologies.

These cases indicate that enzyme elevations with concomitant hyperbilirubinemia can be seen with zileuton and that this may be symptomatic. The magnitude of serious liver injury may be estimated by examining the number of patients in the zileuton and placebo treatment groups who had normal ALT's at baseline that became varying degrees of abnormal sometime during the course of the trial. Some of this information has been provided in shift tables earlier in this review, but this next table is a slightly different representation of it [11/22/94 0:29, 35, 41, 47].

CROSS-TABULATION OF NUMBER (%) ALT VALUES THAT WERE $\leq$ 1xULN AT BASELINE VS. MAXIMUM POST-BASELINE VALUES FOR PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES						
Study/Treatment	N	($\leq$ 1)xULN	(1-2) N	(2-3)xULN	(3-4)xULN	(>4)xULN
ASTHMA/Zileuton	847	662 (78.0)	81 (9.6)	7 (0.8)	3 (0.3)	13 (1.5)
ASTHMA/Placebo	491	391 (79.6)	43 (8.8)	7 (1.4)	1 (0.2)	0 (0.0)
ALL/Zileuton	1757	1359 (77.3)	145 (8.3)	14 (0.8)	7 (0.4)	18 (1.0)
ALL/Placebo	1101	880 (79.9)	78 (7.1)	13 (1.2)	1 (0.1)	1 (0.1)

The table shows that elevations of ALT >4xULN in ALL placebo-controlled, parallel-group, Phase II/III studies occur 1 in 1000 times among patients taking placebo and 1 in 100 times for patient who were taking zileuton. The relative risk for this level of ALT elevation is 10/1 for zileuton/placebo patients.

SUMMARY OF HEPATIC CHEMISTRY CHANGES:

Elevations in ALT, AST and GGT were consistently reported in association with zileuton more than placebo, whether expressed as changes in mean enzyme levels or in

categorical shift-tables. Zileuton-associated hepatic enzyme elevations were not dose-related, over a small range of doses, but were more prevalent in patients who had pre-existing liver enzyme elevations. The sponsor focuses on $ALT \geq 3xULN$ as defining zileuton-associated hepatotoxicity. Data analysis suggests that elevations of $GGT \geq 3xULN$, with or without elevations of other enzymes, occur about as commonly. Reference to recommendations made by an international consensus committee on drug-induced liver injury suggests that the sponsor has defined this entity too narrowly.

Sixty-six patients were identified who met the sponsor's $ALT \geq 3xULN$ criterion. The sponsor recommended eliminating 6 of this group because they only met the ALT criterion at baseline. A additional 6 patients were eliminated based on vagaries of their individual cases, leaving 54 patients with sponsor-defined hepatotoxicity.

The modal period for hepatic enzyme elevations, on zileuton, was the second month after treatment was initiated. The median for $ALT \geq 3xULN$ was day 35, with a range of 6 to 202 days. Fully 24% of these peak elevations occurred after the 91st day, the third month. The timing of these liver test abnormalities reveals little because the "at-risk" population was not taken into consideration. Symptoms compatible with hepatic inflammation were found in 28% of patients with peak $ALT \geq 3xULN$. Spontaneous decline of the ALT to the criterion of $< 2xULN$ occurred in 28% of patients while still taking the study drug. On follow-up, 94% of patients with peak $ALT \geq 3xULN$ declined to $< 2xULN$. The median time for this endpoint was 20 days, with a range of 4 to 241 days.

Four cases of hepatic enzyme elevations with hyperbilirubinemia were reported to focus on the clinical aspects of these enzyme elevations. The ALT elevations occurred with ten-fold the frequency in zileuton-treated patients, compared with those patients who received placebo.

Even when narrowly defined, zileuton-associated hepatotoxicity is frequent ($> 2\%$), can occur late, is quite often symptomatic and can be slow to resolve.

RENAL AND ELECTROLYTE CHEMISTRY CHANGES

Analysis of mean changes from baseline comparing zileuton to placebo in both ASTHMA-ONLY studies, as well as ALL studies, showed small changes. The variables studied in this section were sodium, potassium, chloride, calcium, phosphorous, BUN, creatinine, uric acid, urine pH, urine specific gravity and creatinine clearance.

In the ASTHMA-ONLY trials, PO_4 and uric acid showed the only statistically significant differences between zileuton and placebo groups [SU 2:40].

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SUMMARY OF MEAN CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III ASTHMA STUDIES OF ZILEUTON						
Parameter	Zileuton			Placebo		
	N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
Sodium	815	139.86	0.10	475	139.66	0.35
Potassium	815	4.30	-0.08	475	4.30	-0.08
Chloride	812	104.63	-0.19	474	104.40	-0.05
CO ₂	695	23.63	-0.35	411	23.96	-0.42
Calcium	814	9.30	-0.08	473	9.31	-0.06
Inorganic Phosphorous	809	3.56	0.00	472	3.55	0.07
BUN	812	15.18	0.04	471	15.25	0.12
Creatinine	815	1.07	0.00	473	1.08	0.00
Uric Acid	812	5.09	-0.09	471	5.19	0.00
Urine pH	809	5.88	-0.15	467	5.85	0.00
Specific Gravity	795	1.02	0.00	451	1.02	0.00

When ALL studies were taken together, the uric acid and urine pH fell minimally (<2.5%), in the zileuton group, and rose slightly (<1.2%), in the placebo group and these were the only comparisons between zileuton and placebo that reached statistical significance [SU 2:42].

SUMMARY OF MEAN CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR ALL PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES OF ZILEUTON						
Parameter	Zileuton			Placebo		
	N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
Sodium	1671	140.13	0.00	1052	140.09	-0.06
Potassium	1671	4.30	-0.06	1052	4.27	-0.03
Chloride	1127	104.66	0.09	707	104.31	-0.33

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Parameter	Zileuton			Placebo		
	N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
CO ₂	1506	25.24	-0.51	950	25.40	-0.30
Calcium	1670	9.26	-0.05	1049	9.25	-0.03
Inorganic Phosphorous	1663	3.54	0.00	1047	3.53	0.04
BUN	1670	14.90	-0.33	1048	15.01	-0.21
Creatinine	1673	1.02	0.01	1052	1.04	-0.01
Uric Acid	1666	5.05	-0.03	1045	5.10	0.05
Urine pH	1646	5.78	-0.13	1019	5.77	0.02
Specific Gravity	1593	1.02	0.00	972	1.02	0.00

When sub-group analysis by gender was done, the small effects that were noted, in both the ASTHMA-ONLY and ALL studies, were mostly seen in males. Statistically significant differences between zileuton and placebo were observed for inorganic phosphorous in males, uric acid in males and urine pH in both sexes. These differences were primarily due to increases in the placebo group and decreases or essentially no change in the zileuton group [SU 2:44].

SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN TREATMENT GROUPS FOR MEAN CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE III/III STUDIES, STRATIFIED BY GENDER								
Studies	Parameter	Sex	Zileuton			Placebo		
			N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
Asthma Only	Inorganic PO ₄	M	388	3.55	0.02	228	3.52	0.14
		F	421	3.58	-0.02	244	3.57	0.02
All Studies	Uric Acid	M	830	5.78	-0.03	554	5.80	0.09
		F	836	4.32	-0.03	491	4.31	0.01

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SUMMARY OF STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN TREATMENT GROUPS FOR MEAN CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES, STRATIFIED BY GENDER								
Studies	Parameter	Sex	Zileuton			Placebo		
			N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
	Urine pH	M	822	5.77	-0.12	544	5.78	0.04
		F	824	5.79	-0.15	475	5.77	0.00

Other such sub-group analyses showed that these effects achieved statistical significance in patients < 60 years-of-age and in Caucasians [SU 2:44-7].

COMMENT: The vast majority of the study population was < 60 years-of-age and Caucasian, so these sub-group findings are understandable.

Analyses of treatment duration, dichotomized at 8 weeks, showed inconsistent results in chemistry change from baseline to the final visit for ALL placebo-controlled, Phase II/III trials [397:303, 309:327, 335, 309:306, 314, 324, 330, SU 2:48].

Shift table analyses of < above-normal to above-normal and of > below-normal to below-normal, from baseline to final visit for both ASTHMA-ONLY and ALL studies did not reveal any marked differences between treatments. In ALL studies, the percentage of patients shifting was never more than 2.0% greater in the zileuton group than in the placebo arm [SU 2:52-4].

SUMMARY OF SHIFT CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR ALL PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III TRIALS								
Parameter	Low or Normal Baseline Shifted to High Final				High or Normal Baseline Shifted to Low Final			
	Zileuton		Placebo		Zileuton		Placebo	
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
Sodium	1652	18 (1.1)	1039	3 (0.3)	1628	24 (1.5)	1023	24 (2.3)
Potassium	1627	22 (1.4)	1029	11 (1.1)	1645	16 (1.0)	1038	24 (2.3)
Chloride	1069	24 (2.2)	666	16 (2.4)	1109	15 (1.4)	696	8 (1.1)

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SUMMARY OF SHIFT CHANGES FROM BASELINE TO FINAL VISIT IN RENAL AND ELECTROLYTE CHEMISTRY PARAMETERS FOR ALL PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III TRIALS								
Parameter	Low or Normal Baseline Shifted to High Final				High or Normal Baseline Shifted to Low Final			
	Zileuton		Placebo		Zileuton		Placebo	
	N	n (%)	N	n (%)	N	n (%)	N	n (%)
CO ₂	1480	12 (0.8)	918	19 (2.1)	1282	174 (13.6)	822	95 (11.6)
Calcium	1658	13 (0.8)	1044	4 (0.4)	1609	38 (2.4)	1006	28 (2.8)
Inorganic PO ₄	1608	47 (2.9)	1006	28 (2.8)	1625	35 (2.2)	1018	25 (2.5)
BUN	1635	21 (1.3)	1030	8 (0.8)	1615	40 (2.5)	1005	28 (2.8)
Creatinine	1648	7 (0.4)	1031	7 (0.7)	1635	14 (0.9)	1030	10 (1.0)
Uric Acid	1614	32 (2.0)	999	22 (2.2)	1628	30 (1.8)	1015	9 (0.9)
Urine pH	1572	24 (1.5)	967	38 (3.9)	1608	13 (0.8)	991	4 (0.4)
Spec. Gravity	1546	40 (2.6)	939	22 (2.3)	1494	49 (3.3)	904	30 (3.3)

The summary of shift changes from baseline to final visit for renal parameters for ASTHMA studies is not substantially different from the table presented above.

Mean changes from baseline to final visit in active-controlled, Phase II/III trials showed small changes in serum sodium. There was a greater mean decrease in the control group than was seen in the zileuton group, but no mean change was greater than 0.6 mEq/L [SU 2:62].

Creatinine clearance was analyzed in 176 patients in 8 Phase I, placebo-controlled, studies. Mean changes, from baseline to final visit, failed to show any statistically significant difference between zileuton and placebo groups. Most of these studies lasted < 8 days [388:324-5, SU 2:67]. Creatinine clearance was also measured in 50 patients in the open-label, asthma protocol M92-744, an extension of the placebo-controlled, double-blind study, M91-685. A 10.6% drop was noted, from the first (112.61 ml/min), but not necessarily pre-zileuton, to last (100.72 ml/min) clearance determination in patients taking zileuton. This did not achieve statistical significance [SU 2:68].

M92-801, an open-label ASTHMA extension of M92-720, accumulated 113 patients and demonstrated the reverse trend. Creatinine clearance showed a 1.22 % increase from the first (91.89 ml/min), but not necessarily pre-zileuton, to last (93.01 ml/min) clearance in patients taking zileuton. The first and last creatinine clearance were not statistically significantly different from one another [SU 2:68].

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Another study also showed a decrease in creatinine clearance. An eight-week, double-blind, parallel-group, placebo-controlled study in patients with Systemic Lupus Erythematosus (M91-594) incorporated 32 patients, half of whom received zileuton, 600 mg QID. The mean clearance decreased from 104.3 ml/min, at baseline, to 99.4 ml/min, at the final visit, a 4.7% decrease in the zileuton group. The placebo group showed a mean increase in clearance, from baseline to final visit of 90.8 to 95.6. There was no statistically significant difference between the two treatment groups for mean change from baseline [SU 2:69]. The sponsor performed a sub-group analysis, comparing patients in each arm at three different durations of exposure to either zileuton or to placebo. There were only small groups in each category, from 11 to 16 patients, but clearance declined the most at the 15th day, relative to placebo, less of a decline was noted at the 57th day, and a decline in clearance similar to that seen on the 15th day was noted at the final visit. At each of the intervals, the equally small placebo groups showed increases in clearance from baseline. None of these findings were statistically significant [407:336].

RESULTS OF ANALYSIS OF CHANGES FROM BASELINE IN CREATININE CLEARANCE (ml/min) EVALUATIONS M91-594					
Visit	Treatment	N	Baseline Mean	Visit Mean	Mean Change (SEM)
Day 15	Zileuton	13	106.2	96.7	-9.76 (9.8)
	Placebo	16	90.8	96.8	6.0 (8.8)
Day 57	Zileuton	13	105.3	104.2	-1.2 (7.5)
	Placebo	11	93.2	94.3	1.1 (8.2)
Final	Zileuton	16	104.3	99.4	-4.9 (6.3)
	Placebo	16	90.8	95.6	4.9 (6.3)

Gross and microscopic urinalysis results were only compared for three of the parallel, placebo-controlled, ASTHMA trials, M91-685, M92-720 and M92-785, which all used the same reference laboratory. No apparent difference between zileuton and placebo groups could be demonstrated by analysis of abnormal results for urine casts, glucose, RBC or WBC [388:326, 405:341-5]. The only one of these endpoints to achieve statistical significance was the dipstick urine protein, due to a greater number of placebo patients with abnormally high values [SU 2:71].

SUMMARY OF THE NUMBERS AND PERCENTAGES OF PATIENTS IN STUDIES M91-685, M92-720 OR M92-785 WITH ≥ 1 ABNORMALLY HIGH URINALYSIS FINDINGS					
Parameter	Abnormally High Criteria	N		n (%)	
		Zileuton	Placebo	Zileuton	Placebo
Casts	$\geq$ positive	9	4	8 (89)	4 (100)
Glucose	> negative	549	308	7 (1.3)	3 (1.0)
Protein	> 1+	549	308	2 (0.4)	6 (1.9)
RBC	> 5	549	308	41 (7.5)	24 (7.8)
WBC	> 5	549	308	37 (6.7)	15 (4.9)

COMMENT: It should be kept in mind that a reportedly abnormal dipstick for urine proteins would have to achieve the criterion of $\geq 2+$ protein to be considered abnormal and to have been reported [412:6-7].

No large or consistent changes in 24-hour urine protein determinations was found in M92-744 and M92-801 [2/16/95 0.1-4].

Summaries of the mean changes from baseline using the duration of the longest continuous zileuton exposure for ASTHMA-ONLY and ALL groups, did not reveal any large differences in renal and electrolyte variables at any duration. There were slightly greater differences from baseline in the ASTHMA-ONLY patients, but the mean changes in ALL patients were in the same direction and in the same variables. No mean change from baseline exceeded 1 mEq/L or 1 mg/dL [SU 2:72-4].

Shift table categorical changes, from baseline to final visit, in a parameter from $\leq$ normal to $>$ normal and for $\geq$ normal to $<$ normal were analyzed for parallel-group or uncontrolled Phase II/III studies where the end of the longest continuous duration was the final visit. The greatest percentage of patients shifting occurred for CO₂, with 193 of the asthma patients (20.5%) and 271 of the total population (14.8%) shifting from either a high or normal baseline level to a low final level. This same directional shift in CO₂ was also noted in the placebo group of placebo-controlled trials [SU 2:75-6].

SUMMARY OF RENAL & ELECTROLYTE CHEMISTRY CHANGES

There were no consistent significant changes in renal and electrolyte chemistry parameters found across all study designs. Moreover, the changes observed were small and probably not clinically meaningful. None of the zileuton patients prematurely discontinued a study due to abnormal renal or electrolyte chemistry results.

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METABOLIC/NUTRITIONAL CHEMISTRY CHANGES

This section presents results for the chemistry variables glucose, total protein, albumin, cholesterol and triglycerides. ASTHMA studies revealed only a small decrease in cholesterol from baseline in the zileuton group (-2.12 mg/dL) and a small increase in the placebo group (0.93 mg/dL). This was a statistically significant effect [SU 2:80]. When ALL studies were analyzed, small increases in both glucose and albumin were seen in patients receiving zileuton and these also achieved statistical significance. In the zileuton group, the mean glucose increased 2.0% while decreasing 0.06% in the placebo arm. The mean serum albumin did not change in the zileuton arm and declined by 0.7% in the placebo group [SU 2:82].

SUMMARY OF MEAN CHANGES FROM BASELINE TO FINAL VISIT FOR METABOLIC/NUTRITIONAL CHEMISTRY PARAMETERS FOR PLACEBO-CONTROLLED PARALLEL GROUP PHASE III/III STUDIES						
Parameter	Zileuton			Placebo		
	N	Baseline Mean	Mean Change	N	Baseline Mean	Mean Change
Glucose**	1666	91.08	1.80	1044	91.56	-0.06
Total Protein	1632	7.14	0.03	1016	7.16	-0.03
Albumin**	1671	4.31	0.00	1041	4.34	-0.03
Cholesterol	1669	188.50	-2.79	1052	191.26	-1.32
Triglycerides	1563	129.57	-0.83	966	130.15	0.10

** = statistically significant (p < 0.05)

There were no statistically significant differences between treatments for any parameter, when analyzed between genders [SU 2:83]. Differences in both albumin and glucose were significant only in patients $\leq$ 60 years of age and in Caucasians [SU 2:84-5].

COMMENT: As was discussed before, the vast majority of the study population was < 60 years-of-age and Caucasian, so the findings of these sub-group analyses are probably inconsequential.

Actively controlled trials showed statistically significant differences between zileuton and the active control for albumin, in the ASTHMA trials. In ALL studies, total protein, albumin and triglycerides were significantly different between treatment groups [SU 2:96-7]. The small significant findings in ASTHMA and ALL studies did not appear clinically meaningful.

Mean exposure duration revealed some differences between placebo and zileuton groups but these changes were small and not felt to be of clinical relevance [SU 2:103].

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FIRM: ABBOTT LABS

3 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

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Shift tables of the five variables from < above-normal to above-normal, and from > below-normal to below-normal, failed to distinguish placebo group from zileuton in either ASTHMA-ONLY or ALL trials [SU 2:90]. The results for ALL placebo-controlled, parallel-group, Phase II/III patients are presented below as representative of these unrevealing data.

SUMMARY SHIFT CHANGES FROM BASELINE TO FINAL VISIT IN METABOLIC/NUTRITIONAL CHEMISTRY PARAMETERS FOR ALL PLACEBO-CONTROLLED, PARALLEL-GROUP, PHASE II/III STUDIES								
Parameter	Low or Normal Baseline Shifted to High at Final Visit				High or Normal Baseline Shifted to Low at Final Visit			
	N	Zileuton n (%)	N	Placebo n (%)	N	Zileuton n (%)	N	Placebo n (%)
Glucose	1569	85 (5.4)	965	48 (5.0)	1608	43 (3.0)	1015	41 (3.9)
Total Protein	1581	31 (2.0)	981	30 (3.1)	1604	25 (1.6)	994	17 (1.7)
Albumin	1634	24 (1.5)	1011	15 (1.5)	1631	20 (1.2)	1009	22 (2.2)
Cholesterol	1286	90 (7.0)	820	72 (8.8)	1630	26 (1.6)	1016	20 (2.0)
Triglycerides	1319	89 (6.7)	814	65 (8.0)	1530	24 (1.6)	934	20 (2.1)

SUMMARY OF METABOLIC/NUTRITIONAL CHEMISTRY RESULTS

No consistently significant changes in these variables was observed, although certain differences did appear in analyses with large numbers of patients. There were no premature terminations due to metabolic/nutritional chemistry results.

ENDOCRINE CHANGES

This section of the Safety Update is unchanged since the submission of the original Integrated Safety Summary with the NDA [SU 2:106-21].

Follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estradiol were analyzed for placebo-controlled, active-controlled, longest continuous exposure and all zileuton exposure for Phase II/III studies. The types of analyses included mean changes from baseline, shift changes from baseline to final visit and the incidence of abnormal values. The last were defined as values outside of the normal range. All analyses were stratified by gender [388:353-4].

Analyzed for ASTHMA-ONLY trials, or for ALL clinical trials, the mean change from baseline to final visit did not reveal any consistent or statistically significant results [388:354-5,

406:149-56]. A separate analysis of the subgroup with ulcerative colitis also failed to distinguish any differences between zileuton and placebo [388:355, 406:157-60].

Shift tables for endocrine variables of ALL trials failed to show any statistically significant differences between zileuton and placebo for changes from < high to high, or from > low to low. For male patients, a smaller percentage of zileuton patients (3.9%) than placebo patients (5.6%) shifted from high or normal testosterone, at baseline, to low, at final visit [388:356].

Abnormally high or low, as it applies to these tests of endocrine function are defined by the sponsor to be < 1xULN and > 1xLLN, respectively [412:7]. Patients with at least one value in either category were enumerated to compare zileuton and placebo [388:357, 359]. Considering only ASTHMA studies, there was no difference between placebo and zileuton for any of the measured variables. Looking at these same variables over ALL Phase II/III trials, more placebo patients had at least one high LH measurement than did zileuton patients ($P = 3.1\%$, $Z = 0.9\%$). The incidence of abnormally low testosterone levels was greater in the placebo group (10.6%) than in the zileuton group (6.7%) [388:359, 406:173, 176].

Actively-controlled studies showed no differences in means, from baseline to final visit [388:360, 406:193-6]. Incidence analysis of abnormally high and low values from these actively-controlled trials was similarly unrevealing [388:361, 406:197-204].

When arrayed as baseline endocrine function measurements against the value at visits in 30-day zileuton exposure increments, up to one year, the only significant results were not dramatic nor sustained. At the 90-, 120- and 150-day visits FSH values in female subjects were significantly lower than at baseline in both ASTHMA and ALL trials. At the 150-day visit, estradiol and LH in females were significantly lower than at baseline for both ASTHMA-ONLY and for ALL studies [388:362-3, 406:221-3]. Uncontrolled studies were analyzed by incidence of abnormally high or low values, but the small number of patients in the analysis adds nothing to the analyses that have gone before [388:364].

SUMMARY OF ENDOCRINE CHANGES

Pre-clinical toxicology studies in male beagle dogs indicated that zileuton could cause prostatic and testicular atrophy in immature and mature dogs. However, an effect of zileuton on the human reproductive system was not evident in subsequent clinical trials: M88-151, where 29 normal males received 800 mg BID or placebo for 21 days, and M92-800, where 28 subjects received zileuton 600 mg QID, and 14 received placebo, for 18 weeks. In M88-151, no obvious clinically significant trends were observed for semen analysis, prostatic and testicular size, sexual function and complete endocrine profiles. M92-800 did show an effect on sperm motility, but not on prostatic or testicular size or endocrine chemistry variables [388:364-5]. The endocrine safety variables, reported here, confirm these negative studies.

VITAL SIGNS

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These consisted of blood pressure, pulse rate, respiration rate, temperature and weight. In most presentations blood pressure is described as either standing, sitting or supine. In a few cases, the position of obtaining blood pressure was not defined. In those cases, it was assumed that blood pressure was measured in the sitting position. In order to simplify presentation of blood pressure data, sitting blood pressures were combined with standing blood pressures. This means that the standing blood pressure category will include data with three different origins, true standing blood pressure, true sitting blood pressure and assumed sitting blood pressure. Abnormally high or low values for these vital sign parameters are defined in the following table [SU 2:122-3].

PARAMETER	ABNORMALLY LOW	ABNORMALLY HIGH
Systolic Blood Pressure	< 80 mm Hg	$\geq$ 40 mm Hg increase from baseline
Diastolic Blood Pressure	< 50 mm Hg	$\geq$ 20 mm Hg increase from baseline
Pulse Rate	< 50 beats/minute	> 120 beats/minute
Weight	$\geq$ 7% decrease from baseline	$\geq$ 7% increase from baseline

Mean changes from baseline to final visit reached statistical significance in both ASTHMA-ONLY and ALL trials in only one category, standing pulse rate. A very small decrease in the zileuton group was accompanied by an even smaller decrease, or a very small increase in the placebo group. The magnitude of these mean changes was < 1 beat/minute and they are of doubtful clinical significance [SU 2:125-6].

Tabulation of the incidence of abnormally low and high vital sign parameters in zileuton and placebo groups in ASTHMA ONLY and ALL trials showed only one variable in which there was a statistically significant difference between treatments, standing bradycardia [SU 2:126]. This difference was primarily a function of the influence of ASTHMA patients. Closer scrutiny of the 20 patients, taken from ALL trials, who were bradycardic revealed that 14/20 had only one standing pulse rate in the bradycardic range and 5 of the remaining 6 had been bradycardic before zileuton treatment had begun. These 20 were largely male (17/20) and young (male mean age = 26.5 years; 10/17 males $\leq$ 30 years old) [SU 2:128].

Mean change from baseline to final visit in the ASTHMA-ONLY, active-control group showed no statistically significant changes in any safety parameter. Beclomethasone and theophylline were the only comparators that were used in ASTHMA, active-controlled trials [SU 2:128-9].

Analyses of uncontrolled trials were not revealing. The summary of longest continuous exposure to zileuton, defined as it has been for chemistry parameters, failed to show any large or consistent change, though occasional statistically significant differences between zileuton and placebo were seen [SU 2:136-7].

SUMMARY OF VITAL SIGNS

In the placebo-controlled parallel Phase II/III studies the only consistent findings were a change in the standing pulse rate and incidence of standing bradycardia, which were found in both ASTHMA and ALL studies.

Overall, there was no consistent and meaningful changes in vital signs across all study designs. The changes that did occur were small and largely unassociated with symptoms.

PHYSICAL EXAMINATIONS

These showed no consistent or meaningful-differences between zileuton and placebo or, within the zileuton group, between body systems, when the analysis was confined to those examinations that were normal at baseline and became abnormal at some other visit summing "clinically significant" and "not clinically significant" categories [SU 2:138-46]. ASTHMA-ONLY, placebo-controlled, ALL-placebo-controlled and uncontrolled trials were separately analyzed and were similarly unrevealing.

ELECTROCARDIOGRAMS

In most studies standard 12-lead ECG's were obtained prior to study initiation and again at end of treatment. In many studies additional ECG's were done at intervals during treatment. Information obtained at screening included: heart rate, PR-, QRS- and QT-intervals. In addition, interpretation was requested: normal, abnormal and not clinically significant or abnormal and clinically significant; a yes/no answer to whether ST-segment or T-wave abnormalities were present; and, a description of abnormalities. The incidence of patients with a normal baseline who changed to abnormal in both ASTHMA-ONLY and ALL studies failed to show any difference between placebo and zileuton groups. Similarly, the prevalence of patients with abnormal baseline ECG's that changed during treatment were not different between placebo and zileuton groups [SU 2:147-8].

Cardiovascular system adverse event codes were reported in 5 patients who received zileuton in placebo-controlled parallel Phase II/III studies, who had normal baseline ECG's and who experienced "clinically significant" ECG changes during the study. One asthmatic had sinus bradycardia and Sick Sinus Syndrome was eventually diagnosed. Another asthmatic had RBBB on ECG that may not have been changed from baseline. An ulcerative colitis patient had a wandering atrial pacemaker. A patient with rheumatoid arthritis developed QT interval prolongation for heart rate. That is, her QT interval remained at 400 msec while his heart rate increased from 68 to 84 be. s/min. An SLE patient showed asymptomatic premature supraventricular contractions. All of these patients were taking 600 mg QID of zileuton [SU 2:149-51].

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Also, six patients showed "clinically significant" changes from baseline abnormal ECG's. A hypertensive asthmatic developed more pronounced T-wave inversions in the anterolateral leads, which was a nonspecific finding possibly linked to her hypertension. A second asthmatic developed RBBB and failed to contribute a follow-up ECG (Z = 600 mg QID). A third asthmatic developed an ECG pattern consistent with right ventricular hypertrophy and no follow-up ECG was done (Z = 600 mg QID). An ulcerative colitis patient had a single finding of sinus bradycardia that was attributed to atenolol (Z = 800 mg BID). Another ulcerative colitis patient, a 34-year old female, developed sinus tachycardia, ischemia and diffuse non-diagnostic T waves. Follow-up showed resolution with non-diagnostic T wave changes (Z = 600 mg QID). A rheumatoid arthritis patient had baseline LAD and developed possible Wolff-Parkinson-White Syndrome. Her ECG normalized at the final visit (Z = 600 mg QID) [SU 2:152].

Of the patients who received zileuton in either active-controlled or uncontrolled trials, 2 patients experienced a "clinically significant" change from an abnormal baseline and 2 had a change from a normal baseline. A 18 year old male with RBBB on his baseline ECG was found to have bradycardia (rate = 32) with wandering pacemaker and junctional escape (Z = 800 mg one dose). A 64-year old rheumatoid arthritis patient had a junctional rhythm with ST segment and T wave abnormalities that remitted when Diltiazem was discontinued (Z = 600 mg QID). A 75-year old female rheumatoid arthritis patient with a baseline heart rate of 75 beats/minute developed sinus bradycardia and a rate of 49 beats/min, which resolved spontaneously (Z = 800 mg BID). A 21-year old asthmatic female had episodic dizziness during the study and two syncopal episodes which were attributed to a vasovagal mechanism. She was found to have marked sinus bradycardia at several times during the trial with 40 beats/min as the lowest heart rate [SU 2:153-4].

For ASTHMA-ONLY and ALL Phase II/III placebo-controlled trials there was no difference between placebo and zileuton groups in mean ST-segment/T-wave changes, or QTc, QRS- and PR-intervals. In fact the mean QTc-interval decreased non-significantly in each zileuton group [SU 2:161-2]. Of the 34 patients who were identified as having "potentially clinically significant" QTc changes, 16 increased and 18 shortened [SU 2:167-71].

Concurrent terfenadine and zileuton use, in patients who had both a baseline ECG and one taken within 48 hours after terfenadine dosing, occurred in only 18 patients in the entire data base. None of these had QTc prolongation, based on age and gender norms [SU 2:173].

SUMMARY OF ECG CHANGES [388:407-8]

No systematic effects on ECG parameters were found by any of the analytic techniques. Investigation of zileuton-terfenadine interactions was troubled by small numbers of patients and a ECG sampling interval that was somewhat diverse.

CONCLUSION

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Taken together, the 2573 ALL Phase II/III controlled and uncontrolled zileuton study patients were fairly evenly divided between genders. About 85% of the enrolled patients were Caucasians. The mean patient age was in the mid-30's and ranged from 16 to 78 years of age. About 56% (1446) of ALL patients were studied for > 3 months and over 300 patients were studied for > 210 days. Over 1600 of those patients who took zileuton were exposed to 600 mg QID, the highest dose.

About 76% of ALL zileuton-treated patients in Phase II/III studies experienced at least one treatment-emergent AE. There was a trend for the zileuton-treated patients to have a slightly higher AE prevalence than the control patients. In parallel-group, placebo-controlled, Phase II/III ALL and ASTHMA-ONLY trials, AE's that were statistically significantly different in zileuton and placebo arms, were "dyspepsia" and "insomnia."

A total of 97 (11.5%) zileuton-treated ASTHMA patients experienced AE's classified as severe compared with 37 (7.5%) placebo patients. Corresponding numbers for ALL studies were 156 (8.9%) and 70 (6.4%). The most frequently reported, severe AE was headache in both ASTHMA-ONLY and ALL studies. No relation between magnitude of a very narrow dose range and prevalence of AE's was found.

AE's in zileuton-treated cases had the greatest rate of onset (incidence) during the first two weeks of zileuton treatment. The number of patients with AE's present during any given interval, the prevalence, was slightly less than 50% for the first two weeks and remained at about the same level through all time intervals including the longest (> 150 days). Both incidence and prevalence percentages were based on the "at risk" population.

Higher zileuton blood levels, dichotomized at 7.5 mcg/ml, were associated with more leukopenia and vomiting, but absolute numbers were very small. These two were the only associations with dichotomized dose that achieved statistical significance.

Deaths were about equal in the zileuton group and the control arms. Premature termination due to AE's in both ASTHMA and ALL subjects who took zileuton, were most frequently caused by an aggravation reaction and second most frequently caused by abnormal indices of hepatic function. Comparison of zileuton and placebo groups showed about a 2% excess AE termination rate in the zileuton groups compared with placebo. Terminations due to AE's were more likely to happen in the first 4 weeks of treatment in both control and zileuton groups, based on the "at risk" population in each interval.

Neoplasms appeared in greater number in ulcerative colitis patients and were usually found in the bowel, as would be predicted from the natural history of the disease. Of the 22 neoplasms, some exposure to zileuton was present in 19, but in 9 this exposure was $\leq$ 3 months.

Eosinophil parameters, percent eosinophils and total eosinophils, were the only hematology parameters that showed an obvious decreasing trend in the zileuton group that was statistically significant in more than one analysis favoring an effect of zileuton versus placebo. In actively-controlled trials, decreased eosinophil parameters were more prevalent in the active-control group possibly because the active control was the eosinopenic drug, beclomethasone.

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Trends toward lower WBC with zileuton were not universal over all methods of analysis but occasionally reached statistical significance. However, 5 patients did discontinue the protocol because of abnormally low WBC. Three of these had histories consistent with previously low WBC.

Elevations in ALT, AST and GGT were consistently reported in association with zileuton more than placebo, whether expressed as changes in mean enzyme levels or in categorical shift-tables. Zileuton-associated hepatic enzyme elevations were not dose-related, over a small range of doses, but were more prevalent in patients who had pre-existing liver enzyme elevations. The sponsor focused on $ALT \geq 3xULN$ as defining zileuton-associated hepatotoxicity. Data analysis suggested that elevations of $GGT \geq 3xULN$, with or without elevations of other enzymes, occurred about as commonly. Reference to recommendations made by an international consensus committee on drug-induced liver injury suggests that the sponsor has defined this entity too narrowly.

Sixty-six patients were identified who met the sponsor's $ALT \geq 3xULN$ criterion. The sponsor recommended eliminating 6 of this group because they only met the ALT criterion at baseline. An additional 6 patients were eliminated based on vagaries of their individual cases, leaving 54 patients with sponsor-defined hepatotoxicity. Fully 24% of these peak elevations occurred after the 91st day, the third month. The timing of these liver test abnormalities reveals little because the "at-risk" population was not taken into consideration. Symptoms compatible with hepatic inflammation were found in 28% of patients with peak $ALT \geq 3xULN$. Spontaneous decline of the ALT to the criterion of $< 2xULN$ occurred in 28% of patients while still taking the study drug. On follow-up, 94% of patients with peak $ALT \geq 3xULN$ declined to $< 2xULN$. The median time for this endpoint was 20 days, with a range of 4 to 241 days. Four cases of hepatic enzyme elevations with hyperbilirubinemia were reported to focus on the clinical aspects of these enzyme elevations. Even when narrowly defined, zileuton-associated hepatotoxicity is frequent ($> 2\%$), can occur late, is quite often symptomatic and can be slow to resolve.

There were no consistent significant changes in renal and electrolyte or metabolic and nutritional chemistry parameters found across all study designs. Moreover, the changes observed were small and probably not clinically meaningful. None of the zileuton patients prematurely discontinued a study due to abnormal renal, electrolyte, metabolic or nutritional chemistry results.

Pre-clinical toxicology studies in male beagle dogs indicated that zileuton could cause prostatic and testicular atrophy in immature and adult dogs. However, an effect of zileuton on the human reproductive system was not evident in subsequent clinical trials: M88-151, where 29 normal males received 800 mg BID or placebo for 21 days, and M92-800, where 28 subjects received zileuton 600 mg QID, and 14 received placebo, for 18 weeks. In M88-151, no obvious clinically significant trends were observed for semen analysis, prostatic and testicular size, sexual function and complete endocrine profiles. M92-800 did show an effect on sperm motility, but not

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on prostatic or testicular size or endocrine chemistry variables. The endocrine safety variables, reported here, confirm these negative studies.

In the placebo-controlled parallel Phase II/III studies the only consistent findings were a change in the standing pulse rate and incidence of standing bradycardia, which were found in both ASTHMA and ALL studies. Overall, there was no consistent and meaningful changes in vital signs across all study designs. The changes that did occur were small and largely unassociated with symptoms.

No systematic effects on ECG parameters were found by any of the analytic techniques. Investigation of coincidental zileuton-terfenadine interactions was troubled by small numbers of patients and a ECG sampling interval that was somewhat diverse.

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Safety Update II (SU2) 7/28/95

INTRODUCTION

This includes safety data from all studies completed and unblinded since 15 August 1994, the closing date of the first integrated Safety Update. SU2 includes an additional 364 patients (355 with complete baseline data) exposed to zileuton who derive from two asthma studies, M92-798 and M92-858, and one rheumatoid arthritis (RA) trial, M93-016. A brief description of each of the studies follows.

M92-798 was a 14-week, multi-center European study with three arms that compared low-dose inhaled beclomethasone (200 mcg BID) plus either of two oral zileuton doses: 600 mg QID) with high-dose inhaled beclomethasone alone (). About 100 patients per study arm completed the trial.

M92-858 was a Phase-II European crossover study of zileuton 600 mg QID and placebo in aspirin-sensitive asthmatics. Each of the two double-blind period lasted 6 weeks and 40 patients completed the study.

M93-016 was a Phase-II, placebo-controlled, double-blind, 24-week, randomized withdrawal study of RA patients conducted at multiple U.S. centers. Patients received open-label zileuton 600 mg QID for 12 weeks. Responders then entered the blinded portion of the protocol and received either zileuton, at the same dose, or placebo. About 100 patients entered Part I and 30 Part II.

SPECIAL SAFETY CATEGORIES

Safety information has been combined by this reviewer for presentation. No deaths occurred in these three studies and in this time frame. Eight patients were hospitalized; three for asthma exacerbations, one for abdominal pain, one for hypertension and heart failure, one for pericarditis, one urogenital abnormality and one patient for bladder carcinoma. This last patient was the only case of a new malignancy found in SU2 [7/28/95 1:117]. Six of the 8 hospitalized patients were receiving zileuton [7/28/95 1:121]. However, numbers of patients exposed to zileuton and control over time were not equal, as can be seen from the study summaries above, and favored greater zileuton exposure.

In study M92-798, 50 patients discontinued prematurely. These were fairly evenly distributed among the three equal-sized arms. Sixteen of these terminated prematurely because of AE's and these latter were also evenly distributed among the three arms [7/28/95 1:21]. All 40 patients who entered the double-blind period of study M92-858 completed the study; i.e. no premature terminations [7/28/95 1:53]. The two-part RA withdrawal study provided 47/106 drop outs from the first Part, 8 because of AE's. In Part II, 19/59 withdrew early, 2 because of AE's [7/28/95 1:69].

A total of 22 patients who had been exposed to zileuton terminated prematurely from the three studies covered in SU2. Four of these patients quit with abnormal LFT's, one of whom also

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had abdominal pain. Nine discontinued because of aggravation reactions and 5 quit with GI complaints (dyspepsia, nausea or diarrhea) [7/28/95 1:102-3].

COMBINED LOW WBC RESULTS

The incidence of WBC values $\leq 2.8 \times 10^9/L$ in zileuton-treated patients was 3/355 (0.8%) and the lowest recorded value from these 3 patients was $2.6 \times 10^9/L$ in a patient with RA and Felty's Syndrome. The number of patients with WBC showing a $\geq 50\%$ decrease from baseline for patients treated with zileuton was an additional 6 (1.7%) and the lowest value recorded from these 6 patients was $3.4 \times 10^9/L$. Four of these 6 had baseline elevated WBC and one normalized while on the study drug [7/28/95 1:106-7].

COMBINED ELEVATED LFT RESULTS

Drug-induced liver disease has been defined by the sponsor to mean ALT $\geq 3 \times \text{ULN}$, with or without elevations of other LFT's. Four (1.1%) of the 355 zileuton-treated patients met this criterion. Two of these 4 cases had maximum ALT's $\geq 8 \times \text{ULN}$ (individual maximums: 401 and 557 IU/L) but none achieved a maximum Total Bilirubin $\geq 2.0 \text{ mg/dl}$. The onset of ALT $\geq 3 \times \text{ULN}$ was 8 to 43 days and the mean time to resolution of 3 of the patients who stopped the medication was 21 days. The fourth patient continued to take zileuton and resolved to ALT $\leq 2 \times \text{ULN}$ while on the study drug. One of these 4 had symptoms of abdominal pain associated with nausea and vomiting 1-2 weeks prior to the discovery of the abnormal ALT's. The other 3 patients were asymptomatic.

One other patient, not included among the 4, had LFT elevations found one month after completion of zileuton treatment, during placebo treatment, that appeared to be compatible with the diagnosis of pre-existing chronic cholelithiasis, not solely related to zileuton. In support of this contention, she was 68 years of age, obese, had a history of cholelithiasis and chronic dyspepsia. Her baseline LFT's were normal and she had no history of alcohol use. After 84 days on zileuton 600 mg QID and 30 double-blind treatment days she had maximum elevations in AST (14.2xULN), ALT (13.1xULN), GGT (23.5xULN), alkaline phosphatase (2.1xULN) and a normal total bilirubin. The double-blind treatment drug was discontinued. A gall bladder ultrasound confirmed the history of cholelithiasis, her enzymes normalized and she underwent a laparoscopic cholecystectomy [7/28/95 1:107-8, 12:1-135].

LFT's other than, or in addition to, ALT have also shown elevations $\geq 3 \times \text{ULN}$. In fact, the most commonly elevated LFT is the GGT, a previously finding [7/28/95 1:113].

COMBINED RESULTS: ZILEUTON PATIENTS WITH LIVER TEST ELEVATIONS				
Degree of Elevation	ALT	AST	GGT	Alkaline Phosphatase
$3 \times \text{ULN} \leq \text{LFT} < 8 \times \text{ULN}$	2	3	11	2
$8 \times \text{ULN} \leq \text{LFT}$	2	1	1	0

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COMBINED RESULTS: ZILEUTON PATIENTS WITH LIVER TEST ELEVATIONS				
Degree of Elevation	ALT	AST	GGT	Alkaline Phosphatase
TOTAL (USULN $\pm$ LFT)	4	4	12	2

CONCLUSION

SU2, with information on an additional 364 patients exposed to zileuton, has provided slightly more information that confirms findings of the integrated safety summary and first update. The most frequent AE's causing premature terminations in patients taking zileuton are aggravation reactions, abnormal LFT's and GI complaints. Although low WBC's are seen in patients exposed to zileuton, the low counts are neither very frequent, nor very low and are often plausibly explainable by other mechanisms. Abnormal LFT's continue to be associated with zileuton. High transaminase elevations are found, some of which are accompanied by symptoms, but total bilirubin is usually little effected. Thus far, we have encountered only one case of hepatitis with frank jaundice in over 5000 exposed persons, including the 2500 patients from the ongoing surveillance study (M94-199).

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M94-199 "Long-Term Surveillance Study of Zileuton Plus Usual Care Versus Usual Care in Patients with Asthma" (INTERIM ANALYSIS SUBMITTED 8/17/95)

OBJECTIVES

Evaluate the long-term safety, and in particular effects on liver function, of zileuton, in a large population of patients with asthma.

DESIGN

This is a 12-month, open-label, randomized, multi-center study of chronic asthmatics exposed to usual care (UC) alone or zileuton plus usual care (Z+UC), 600 mg QID. After a screening visit the patients were randomized to one of the two groups on Day 1. They were restudied after two and four weeks (first month), then monthly through the fifth month, and at the end of the seventh, tenth and twelfth months.

SUBJECTS

Three thousand patients were to be enrolled and randomized in a ratio of 5:1, 2500 patients exposed to Z+UC and 500 patients exposed to UC. To be eligible for the study an asthmatic patient must have demonstrated an $FEV_{10} \geq 35\%$ of predicted and had at least 15% reversibility of this measure after inhaled beta-agonist. All patients were to have been ≥ 16 years of age without clinically significant laboratory abnormalities or medical history that might affect their participation in the study. Patients were allowed to remain on their usual asthma medications with the exception of chronic oral corticosteroid use. Patients were allowed limited bursts of systemic corticosteroids as rescue medication during the study to treat acute exacerbations of their asthma [8/17/95 1:9]. Patients taking theophylline could be randomized to either group. If placed in the Z+UC arm, the theophylline dose was reduced by half and titrated, subsequent to the addition of zileuton, to achieve peak serum levels between 8 and 15 mg/L [8/17/95 1:11].

At screening, the ALT level must have been $< 1.5xULN$ and the total bilirubin level < 2.0 mg/dL. The latter condition could be waived if the patient had a documented history of Gilbert's Syndrome. During the study liver function tests $> ULN$ were assessed by the investigator and Abbott Laboratories. In cases of $ALT \geq 3xULN$, the patient was contacted to determine if possible risk factors and associated signs and symptoms of liver injury were present. Elevations of all liver functions $> 3xULN$, except bilirubin, were recorded as AE's. This included AST, ALT, GGT, LDH and alkaline phosphatase. Total bilirubin levels > 2.0 mg/dL were recorded as AE's [8/17/95 1:9].

DOSAGE

Patients in the Z+UC treatment group received oral doses of 600 mg QID of the study drug throughout the study.

PARAMETERS

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Chemistry laboratory studies and hematology blood work were taken at each study visit and an algorithm for managing abnormalities of ALT was undertaken. ALT was considered to be the deciding indicator of hepatic dysfunction. The algorithm began with the recording of an ALT $\geq$ 3xULN as an AE and proceeded as follows, contingent upon the presence or absence of symptoms and the actual magnitude of the ALT elevation [8/17/95 1:10].

1. 3xULN $\leq$ ALT < 5xULN and asymptomatic - retest in one week
 - a. ALT < 3xULN - continue in study with original timing of visits
 - b. 3xULN $\leq$ ALT < 5xULN and asymptomatic - see #1
 - c. ALT $\geq$ 5xULN or symptomatic - D/C
 - (1) Asymptomatic - F/U in one week
 - (2) Symptomatic - F/U in 48 hours
2. 5xULN $\leq$ ALT < 8xULN and asymptomatic - D/C and retest in one week
3. ALT $\geq$ 8xULN or symptomatic - D/C and retest in 48 hours

RESULTS

The demographic parameters of this trial show that 61% of the participants were female, the mean age of all patients enrolled was 43 years with a range of 15 to 86 years, and 8.7% were over the age of 65 years. The population was predominantly Caucasian, approximately 91%. These findings were similar in both Z+UC and UC arms of the study. In comparison with data from the Integrated Safety Summary I, this study had a slightly higher proportion of females and Caucasians and the mean age was about 6 years older [8/17/95 1:16].

The cumulative number of patients having any exposure for $\geq$ sequential time periods is shown in the table below [8/17/95 1:17]:

SUMMARY OF TREATMENT EXPOSURE IN M94-199 FOR ZILEUTON-PLUS-USUAL-CARE (Z+UC) AND USUAL-CARE-ALONE (UC) ARMS		
Exposure	Z+UC	UC
Any	2458	489
$\geq$ 25 days ($\geq$ 1 month)	2137	458
$\geq$ 55 days ($\geq$ 2 months)	1929	442
$\geq$ 85 days ($\geq$ 3 months)	1797	431
$\geq$ 115 days ($\geq$ 4 months)	1687	420
$\geq$ 145 days ($\geq$ 5 months)	1258	333
$\geq$ 175 days ($\geq$ 6 months)	1018	248
$\geq$ 205 days ($\geq$ 7 months)	142	52

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There have been three deaths in this trial so far and all in the Z+UC arm. Two have been from status asthmaticus and one from a myocardial infarction. The latter occurred in a 71 year-old with pre-existing heart disease. Full narrative summaries and case report forms are submitted under with the Safety Update II [8/17/95 1:22-3].

As of the 23 June 1995 cut-off, 1003 patients had prematurely terminated from study M94-199. Ninehundred-seven of these were randomized to the Z+UC arm and 96 to the UC arm. Fourhundred-thirtyfour Z+UC patients dropped out because of AE's (17.7%) as compared with 9 dropouts in the UC arm for AE's (1.8%). Dropouts in both arms for reasons other than AE's were comparable (Z+UC = 19.2%, UC = 17.8%). The most frequently reported AE's leading to premature termination for zileuton patients were: aggravation reaction (3.3%), nausea (2.4%) and abnormal LFT's (2.0%). This generally agrees with the most frequent AE's causing premature termination in the asthma studies of the Integrated Safety Summary Update I (ISS-1) which were: aggravation reaction (5.3%) and abnormal LFT's (1.0%). The number of premature terminations due to AE's is almost ten times greater in the Z+UC arm, compared to the UC arm [8/17/95 1:18].

COMMENT: This represents quite an increase in the ratio of zileuton-to-placebo AE's over what was reported in the Integrated Safety Summary and first Safety Update. ASTHMA trials showed 9.2% premature terminations due to AE's in the zileuton group and 8.4% in the placebo arm [SU 1:214-16].

A total of 136 hospitalizations have been reported which have been fairly evenly divided between the two arms (Z+UC = 4.7%, UC = 4.3%). Of the total number of hospitalizations, 71 were attributable to asthma exacerbations. Hospitalizations because of asthma affected 56 (2.3%) of the Z+UC arm patients and 15 (3.1%) of the patients enrolled in the UC arm [8/17/95 1:24].

So far, seven malignancies have been reported, six in the Z+UC group and one in the UC arm. There have been four cases of breast cancer, one of prostate cancer, one of rectal cancer and one with a malignancy of the thyroid gland [8/17/95 1:25-7]. The relative frequency of malignancies in the two groups closely reflects the 5:1 assignment frequency of patients to the two study arms.

Despite protocol requirements for contraceptive use in women of child-bearing potential, there were a total of 13 pregnancies, 11 in the Z+UC arm. All patients were prematurely terminated from the study at an estimated 2-6 weeks of gestation. In the Z+UC group, there have been 5 induced abortions, 2 spontaneous abortions and one delivery of a normal baby girl. Three of the pregnancies in this arm are ongoing [8/17/95 1:27-8].

Counts of patients with any liver function test > ULN at the maximum value of that test were enumerated for both study arms in the following reviewer synthesized table [8/17/95 2:223-4].

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SINGLE LFT's > 1xULN (FROM TABLES 18.1 & 18.2)		
Elevated Parameters	Z+UC (n = 2458) Count (%)	UC (n = 489) Count (%)
ALT Alone	132 (5.37)	21 (4.29)
AST Alone	40 (1.63)	5 (1.02)
GGT Alone	191 (7.77)	35 (7.16)
LDH Alone	73 (2.97)	17 (3.48)
Alk. Phos. Alone	76 (3.09)	19 (3.89)
NONE	1446 (58.83)	313 (64.01)

The last line of the table shows that there were over 5% fewer patients in the Z+UC arm without some kind of LFT elevation than were in the UC arm. When larger departures from normal ranges are defined, certain LFT elevations show separation between the two arms. In this case, the definition of abnormal ALT, AST, GGT and Alkaline Phosphatase was $\geq 3 \times \text{ULN}$ and $>$ baseline value. One hundred fifty-two patients were identified to have ≥ 1 abnormal LFT by these criteria. An additional 21 patients had an elevated total bilirubin alone ≥ 2.0 mg/dL providing a total of 173 patients with one or more abnormal LFT value. The following table shows the only two LFT's to achieve statistical significance in comparison of the two treatment arms. The total numbers ('n') given for each arm are the number of patients eligible, meaning that they had baseline, and at least one post-baseline value for analysis [8/17/95 1:33].

SUMMARY OF PATIENT NUMBERS WITH LFT $\geq 3 \times \text{ULN}$ AND $>$ BASELINE		
Elevated Parameters	Z+UC (n = 2358) Count (%)	UC (n = 448) Count (%)
ALT	92 (3.9)	3 (0.7)
AST	47 (2.0)	1 (0.2)

Subgroup analysis of these data by gender and age dichotomized at 65 years seems to indicate that females over the age of 65 years had a disproportionate incidence of ALT elevations [8/17/95 1:36].

These analyses of abnormal LFT's do not exactly conform to any of the analyses previously performed in the ISS-1 report, however the following do and allow for comparisons to be made. The first table shows the number of patients with liver tests $\leq$ normal, at baseline, who had liver tests $>$ normal at the final visit for both Z+UC and UC alone groups [8/17/95 2:133-8].

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NUMBER (%) OF PATIENTS WITH LIVER TESTS $\leq$ NORMAL, AT BASELINE, WHO HAD LIVER TESTS $>$ NORMAL, AT FINAL VISIT		
Laboratory Value	UC	Z+UC
GGT	17 (4.2%)	118 (5.9%)
AST	12 (2.7%)	117 (5.4%)
ALT	27 (6.1%)	167 (8.1%)
Alkaline Phosphatase	7 (1.6%)	59 (2.8%)
LDH	16 (3.7%)	101 (4.7%)
Total Bilirubin	4 (0.9%)	22 (1.0%)

These findings compare favorably with ISS-1 asthma trials. In the current surveillance study, the percent of zileuton-treated patients with baseline normal ALT's which became elevated at the final visit is higher than in ISS-1 (Surveillance = 8.1% vs. ISS-1 = 6.8%), but the difference between zileuton-treated and control groups for this same measure is comparable (Surveillance = 2.0%, ISS-1 = 1.7%).

A similar comparison is shown below for liver tests that were normal at baseline and became elevated ($>$ ULN) at some time during treatment [8/17/95 2:145-56]. Though the terminology is different, this reviewer synthesized table is directly comparable to the ISS-1 shift table of "normal to maximum percent of the upper limit of normal."

NUMBER (%) OF PATIENTS WITH LIVER TESTS $\leq$ NORMAL, AT BASELINE, WHO HAD LIVER TESTS $>$ NORMAL, AT MAXIMUM POST-BASELINE VALUES		
Laboratory Value	UC	Z+UC
GGT	46 (10.5%)	280 (12.8)
AST	42 (8.8%)	339 (14.2%)
ALT	62 (13.6%)	450 (19.5%)
Alkaline Phosphatase	24 (5.1%)	169 (7.1%)
LDH	25 (5.2%)	182 (7.8%)
Total Bilirubin	11 (2.3%)	60 (2.4%)

Like the baseline-to-final-visit shift table, the percentage of zileuton-treated patients with baseline normal ALT's which became elevated sometime post-baseline is higher in this surveillance

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trial than in ISS-1 (Surveillance = 19.5% vs. ISS-1 = 13.6%). In addition, the difference between zileuton-treated and control groups for this measure is larger in the surveillance study (Surveillance = 5.9%, ISS-1 = 2.1%). A higher incidence of AST abnormalities in zileuton-treated patients was also seen in comparison of the surveillance study with the ISS-1 report (Surveillance = 14.2% vs. ISS-1 = 11.6%) and the difference between zileuton treated and control groups is also larger (Surveillance = 5.4%, ISS-1 = 1.4%).

Each liver function test that was $>$ ULN, at baseline, was followed and the maximum, post-baseline value was recorded and categorized into groups: 1-3xULN, 3-8xULN and $>$ 8xULN. The table below shows the number of patients with liver function tests $>$ ULN at baseline that worsened (shifted by ≥ 1 category) at maximum post-baseline measurements [8/17/95 2:160-5].

NUMBER (%) OF PATIENTS WITH LIVER TESTS $>$ ULN, AT BASELINE, WHO BECAME WORSE (SHIFTED BY ≥ 1 CATEGORY), AT MAXIMAL POST-BASELINE ELEVATION		
Liver Test	Z+UC	UC
GGT	0 (0%)	0 (0%)
AST	3 (13.0%)	0 (0%)
ALT	5 (10.4%)	1 (9.1%)
Alkaline Phosphatase	0 (0%)	0 (0%)
LDH	1 (3.7%)	0 (0%)
Total Bilirubin	0 (0%)	0 (0%)

Numbers of patients with abnormal LFT's at baseline were small and further worsening is only reflected by the enzymes AST and ALT. This finding corroborates the ISS-1 report that small numbers of patients, the majority in the zileuton-treated group, with elevated liver tests at baseline had further increases to a worse category during treatment.

Analysis of mean changes of various LFT's from baseline to each visit, comparing the two study arms, show the greatest differences between arms for the enzymes ALT and AST. Statistically significant differences between arms, in changes from baseline to each visit, were seen through the end of the third month for ALT and through the end of the fourth month for AST [8/17/95 1:31]. Perhaps a better approximation of the attack rate is the conditional probability of the first incidence of an ALT elevation at the monthly intervals (Monthly Hazard Rate). This is based on the number of patients at risk, defined as the number who had not previously developed an elevation of ALT ≥ 3 xULN and for whom blood draws were still being performed as of that interval [8/17/95 1:36-7].

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MONTHLY HAZARD RATE ALT $\geq$ 3xULN BY LIFE TABLE METHOD			
Visit Interval (Days)	ALT $\geq$ 3xULN "N"	P(1st occurrence of ALT $\geq$ 3xULN)	Probability Standard Error
2-35	43	2.1%	0.31%
36-65	24	1.3	0.26
66-95	6	0.3	0.14
96-125	8	0.5	0.14
126-155	6	0.5	0.18
156-185	1	0.1	0.10
186-216	4	0.8	0.40

This shows that the hazard rate drops after the second month remaining approximately constant through the 6th month of exposure. The apparent increase in the hazard rate during the 7th month is associated with a small number of patients exposed in this interval and is accompanied by a larger standard error. In addition to these 92 cases of ALT $\geq$ 3xULN, there have been 7 new cases in the Z+UC group since the June 23, 1995 data cut-off date. All of these cases occurred after five months of exposure. These might influence the monthly hazard rate function shown in the table above but, without knowing the total number of at-risk patients from which the 7 derived, exact calculations cannot be made [8/17/95 1:39].

Of the original 92 Z+UC patients who developed ALT $\geq$ 3xULN, 86 had their first occurrence during treatment and 6 patients had their first occurrence of ALT elevations 2-15 days after discontinuing the study drug [8/17/95 1:37]. Of the 92 cases of ALT $\geq$ 3xULN, 59 patients (64%) including the 6 patients with a first ALT elevation off-drug have prematurely terminated from the study, either before or after 23 June 1995. Fifty seven of these 59 have since resolved to ALT $<$ 2xULN with a mean time to resolution of 22.5 days and a range of 1-101 days [8/17/95 2:47].

Resolution of abnormal ALT's was followed in 53 patients whose initial ALT was between 3 and 5xULN and occurred while the patient was taking the study drug:

1. Resolved on drug (ALT $<$ 2xULN) N = 28
 - a. Continued in study N = 25
 - b. Prematurely terminated N = 3
2. Not resolved on study drug (ALT $\geq$ 2xULN) N = 25
 - a. Continued in study N = 5
 - b. Prematurely terminated N = 20
 - (1) Resolve off drug (ALT $<$ 2xULN) N = 16
 - (2) Not resolved by 6/23/95 (ALT $\geq$ 2xULN) N = 4

About half of these patients failed to resolve on the study drug and 20 (80%) of those terminated prematurely. Thirteen of the 20 had further elevations of ALT $> 5xULN$ and 7 withdrew with ALT levels $3-5xULN$ [8/17/95 1:44]. Possibly these 7 became symptomatic. Retrospective analysis points out that there appears to be a longer time to resolution for those patients who had their first incidence of ALT $\geq 3xULN$ on drug for greater than 3 months (mean resolution time 52.7 days), regardless of the magnitude of the elevation, and a shorter time to resolution for those who had their initial elevation within 3 months of starting the study drug (mean resolution time 17.5 days) [8/17/95 1:46]. Although the greatest magnitude of ALT elevation ($\geq 15xULN$) had a longer mean time to resolution (33 days), numbers were small (2 people) and other degrees of maximum ALT elevation all showed similar mean resolution times of about 21 days with a range of 1-90 days [8/17/95 1:45].

There were 6 patients who had ALT $\geq 3xULN$ in combination with an elevated bilirubin $> 1xULN$ at the same visit and all of these came from the Z+UC arm of the study. The maximum bilirubin elevations for any of these patients was 2.5 mg/dL (Patient #3212). Two others of these six (total = 3/6) had a maximum bilirubin value > 2.0 mg/dL, patient #2305 and patient #2770 both with total bilirubin = 2.10 mg/dL [8/17/95 1:42-3, 49]. Frank jaundice was not appreciated in any of these cases. Two of these had likely alternate etiologies for their LFT elevations. Patient #1808 had cholelithiasis on ultrasound and the AST/ALT elevations were accompanied by elevations of similar magnitude in GGT and Alkaline Phosphatase. Patient #2770 showed the maximum total bilirubin elevation (2.1 mg/dL) in the pre-treatment period and had only lesser elevations (maximum = 1.80 mg/dL) during treatment, a possible case of Gilbert's Syndrome.

One unusual aspect of elevated LFT's was seen in three patients and was referred to by the sponsor as "rebound." Patients #1898, #2812 and 3612 all showed initial reduction of elevated LFT levels, coincident with termination of the study drug. Between 15 and 31 days after premature termination from the study, discontinuation of zileuton and declining LFT's, the LFT's again began to rise. The magnitude of this rebound was greater than the initial elevations in 2/3 of these cases [8/17/95 1:54, 2:309, 338, 363].

Extensive follow-up of hematology parameters failed to show any definitive effect of zileuton on any of these parameters. A total of 21 patients, 18 in the Z+UC and 3 in the UC arms, had one or more abnormally low WBC counts ($\leq 2.8 \times 10^9/L$). This is similar to the 5:1 Z+UC to UC randomization ratio at admission. Three zileuton patients (#1362, #3492 and #3530) prematurely discontinued treatment due to leukopenia, two more (#2126 and #2458) were prematurely terminated due to thrombocytopenia and one patient (#1496) was discontinued due to anemia. One of the three patients with leukopenia had a preponderance of atypical lymphocytes on differential WBC and the WBC returned to normal within one week, suggesting the possibility of a viral etiology. Another of the leukopenic patients failed to resolve after treatment cessation, was seen by a Hematologist and diagnosed as having African-American Neutropenia. One of the two patients with thrombocytopenia had a history of Idiopathic Thrombocytopenia (ITP) during pregnancy and ITP is proposed as a possible alternate causation. The other thrombocytopenic patient entered the study with low platelets (110K) and counts declined further during 1.5 months of treatment (54K, 53K, 18K). Although the

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platelet counts recovered somewhat after cessation of zileuton, counts remained generally low and fluctuating through the three months of follow-up. The single anemic patient, a female, entered the study with low hemoglobin and hematocrit values and microcytosis. She terminated on the 11th treatment day and normalized her hematologic parameters on iron supplementation [8/17/95 1:57-67].

CONCLUSION

Premature terminations due to AE's were proportionally more common in the zileuton arm than had previously been shown by the ISS-1 report. The latter indicated approximately equal proportions of zileuton and control patients from ASTHMA studies terminated early because of AE's. This surveillance study reports almost a ten-times greater number of early discontinuations due to AE's in the Z+UC group, compared with the UC arm. Specific AE's causing early termination in the surveillance trial were similar in frequency to those reported in the ISS-1 report. No explanation for this phenomenon is readily apparent from examination of the data, nor is one suggested by the sponsor.

This surveillance study has confirmed abnormal tests of hepatic function occur in conjunction with zileuton administration. Abnormal ALT's have been the focus of the sponsor's attention, though abnormal GGT elevations are at least as common. ALT elevation which are $\geq 3xULN$ and $>$ baseline ALT occurred in 3.9% of patients taking Z+UC, as compared with 0.7% in the UC arm. This indicates an excess of 3.2% ALT elevations in Z+UC over UC alone patients. This is greater than the 1.7% patients with ALT elevations seen in zileuton group in excess of the number of patients with elevations in the placebo group from Safety Update I. Some of the ALT elevations have accompanying symptoms: e.g., abdominal pain, and some also show small elevations of total bilirubin (≤ 2.5 mg/dL in this study).

The attack rate of newly developing ALT elevations $\geq 3xULN$ was addressed in terms of the monthly hazard rate. This was 2.1% in the first month after beginning zileuton, 1.3% in the second month and then varied between 0.1% and 0.5% per month through the sixth month. Possibly an increase in this monthly hazard rate occurred in the seventh month (0.8%), but numbers of patients were small and the standard error of the probability estimate was relatively large. Ignoring the vagaries of the seventh month, these data imply a relatively constant attack rate of new onset ALT elevations to $\geq 3xULN$, following the initial two months of greater hazard, which may continue for as long as patients take the drug, but certainly lasts through seven months of continuous exposure to zileuton.

Underscoring this concern about the incidence of late ALT elevations is the report of 7 new cases of ALT $\geq 3xULN$ in the Z+UC group after the reporting cut-off date of 23 June 1995, all of which occurred after five months of zileuton exposure. The sponsor hasn't provided a denominator of patients at risk, but one doubts that these new cases will reduce the monthly hazard rate after five months and may well serve to increase it.

A further complication is the report 6/92 patients with ALT $\geq$ 3xULN, had their first abnormal value between 2 and 15 days after zileuton had been discontinued. This would imply that hepatic enzyme surveillance should be extended for some time after the drug is stopped, that is $\geq$ 2 weeks.

Sixty-four percent of patients with ALT elevations $\geq$ 3xULN eventually terminated prematurely from the study. A subset of these comprises 53 asymptomatic patients with ALT's between 3-5xULN who were allowed to continue to take the treatment drug and who were followed. About half of this smaller group failed to resolve while the patient continued to receive zileuton. Eighty percent of this non-resolving half terminated prematurely either because of further ALT elevations or, possibly, development of symptoms. Taken together, 23/53 asymptomatic patients with ALT elevations of 3-5xULN terminated prematurely, most usually from further ALT elevations.

Complicating the issue of resolution of the abnormal ALT's, is the finding of "rebound" phenomena in 3/92. That is ALT elevations occurred, zileuton was stopped and those elevations began to resolve. But, before returning to the normal range, the ALT began increasing again, while the patient was off of zileuton, achieving elevations that were higher than the originally abnormal ALT elevation in 2/3 patients who showed this "rebound" phenomenon.

In summary, continuous zileuton treatment implies the continuing monitoring of hepatic enzymes, at least through the seventh month of use. At present, there is no indication that this surveillance can be safely discontinued at any time after the patient begins to take the drug. In fact, there is some evidence of an increasing hepatic dysfunction attack rate in the last month, the seventh, for which data is available. Hepatic enzyme surveillance maybe required until some time after the patient has discontinued taking zileuton because of the 6/92 (6.5%) ALT elevations which have been reported to have begun as late as 15 days after stopping the drug. A smaller percentage (3/92 = 3.3%) have shown a "rebound" phenomenon that makes monitoring possibly required, even after abnormal ALT elevations have begun to return to normal.

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NDA #20471

REVIEW OF PROPOSED LABELLING

Comments on this topic will not be offered until the New Drug Application is adequate for approval, in other respects.

OVERALL CONCLUSIONS

The sponsor has presented adequate data from clinical studies to demonstrate the efficacy of zileuton 600 mg QID in improving various indices of air flow and symptoms. There is inadequate data to support efficacy of the lower zileuton dose. Efficacy of the larger zileuton dose is modest. Both oral theophylline and inhaled beclomethasone show significantly greater improvement in trough morning air flow than zileuton 600 mg QID.

Pharmacokinetic interactions of zileuton with common drugs have been seen. Serum levels of theophylline, warfarin, propranolol, terfenadine and the major active terfenadine metabolite all increase when co-administered with zileuton. These pharmacokinetic interactions are of sufficient magnitude to predict clinical relevance.

Clinical safety concerns center around liver test elevations. Only one case of hepatitis with frank jaundice has been reported but transaminase elevations, sometimes > 1000 IU/L, have been seen. Concomitant troubling findings have been reports of abdominal pain, sometimes in the right upper quadrant, and minor elevations of total bilirubin, seldom > 2.5 mg/dL. Though the elevated liver test attack rate is highest in the first two months after treatment is initiated, it becomes constant for the next four months and even shows a possible increase in the last month for which we have data, the seventh month of the ongoing surveillance study, M94-199.

Some pre-clinical findings have been disappointing. Pharmacologic toxicity studies have been interpreted as showing that zileuton is a genotoxic carcinogen in animals. This subject is more completely addressed in the pharmacologist's review.

RECOMMENDATIONS

Zileuton is not approvable for safe use, based upon the pre-clinical findings of genotoxicity and carcinogenicity. Unless these findings are shown not to apply to man, any future demonstration of safety will be difficult.

If zileuton proves to be safe for human use in the future, elevated liver test attack rate information from M94-199, the safety surveillance study, will be crucial in determining laboratory follow-up intervals and duration. Therefore, we require that the safety surveillance study, M94-199, be completed.


Raymond F. Anthracite, M.D., Medical Review Officer

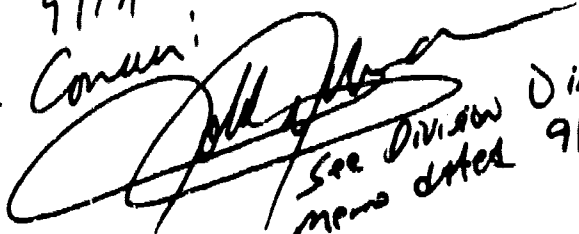
9/19/95
Concur:

See Division Director's memo dated 9/20/95

FIGURE 1

Figure 3. Trough Effect

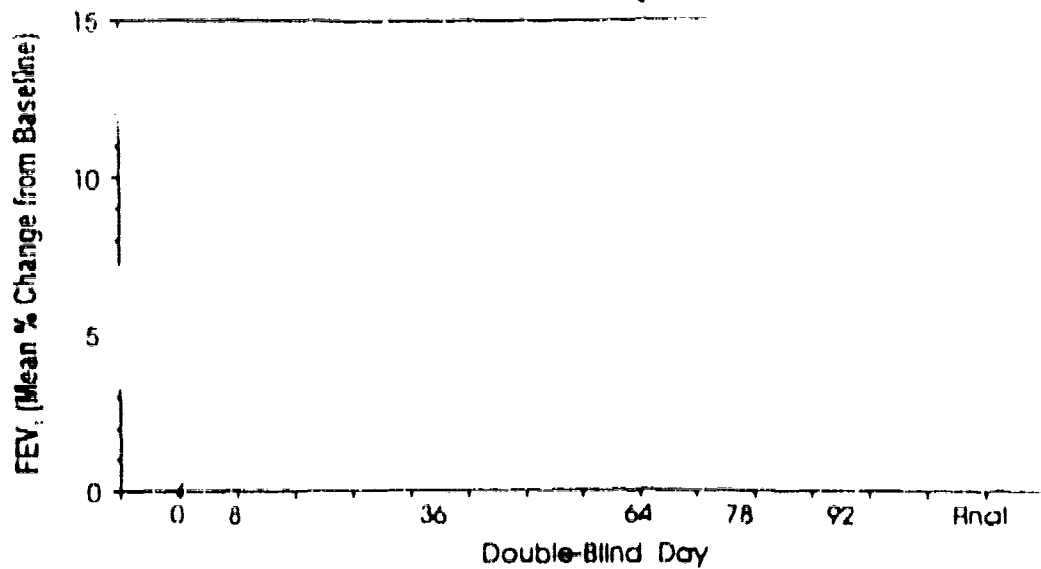


FIGURE 2

Figure 4. Trough Effect For Subsets of Patients with Mild, Moderate and Severe Asthma

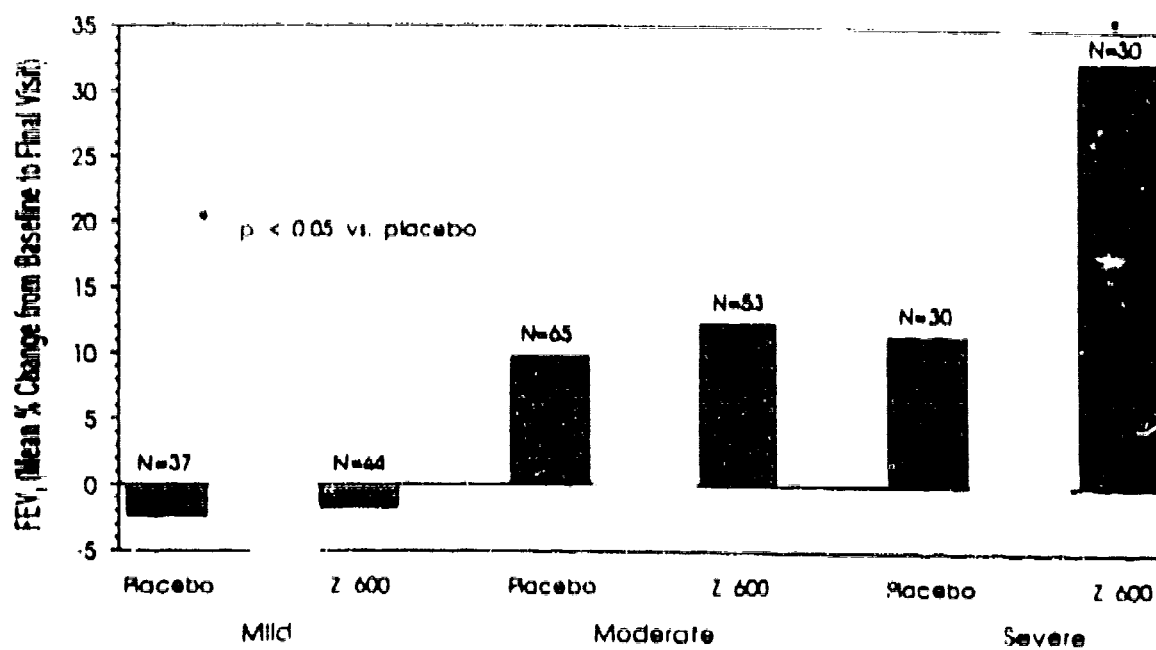


FIGURE 3

Figure 5. Peak Effect

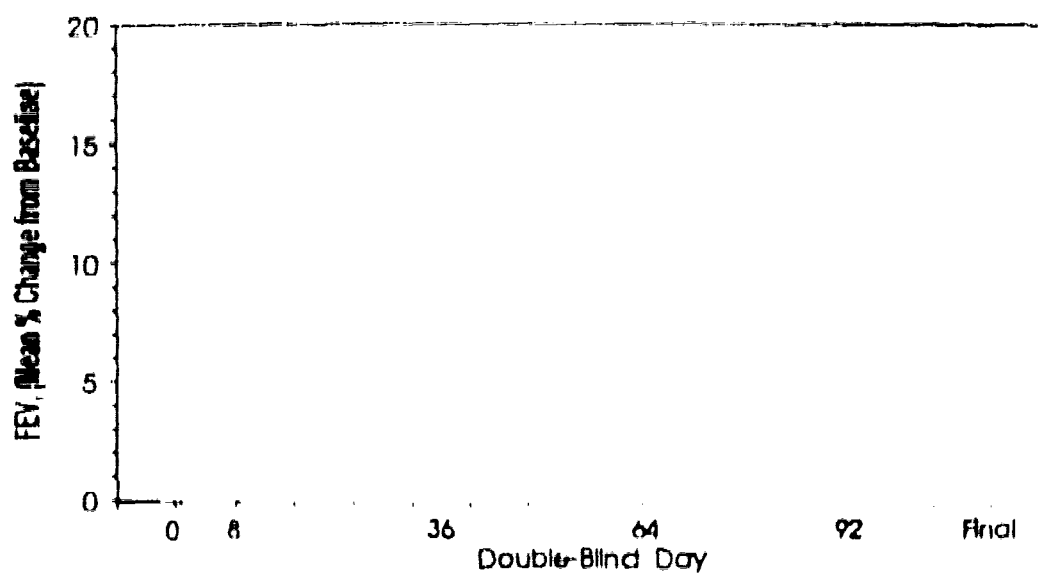


FIGURE 4

Figure 8. First Dose Effect

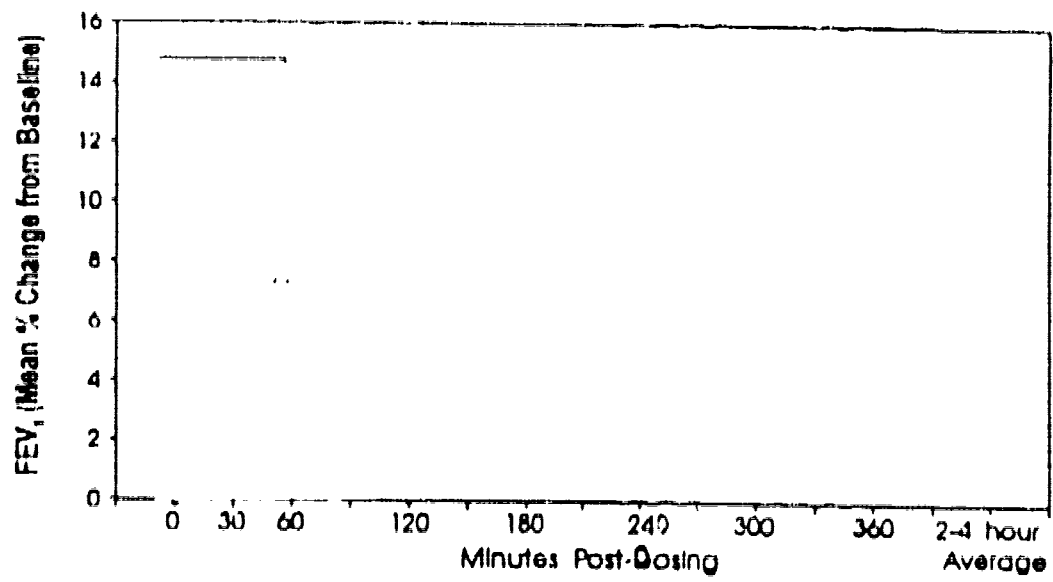


FIGURE 5

Figure 9. Acute Effect For Subsets of Patients with Mild, Moderate and Severe Asthma

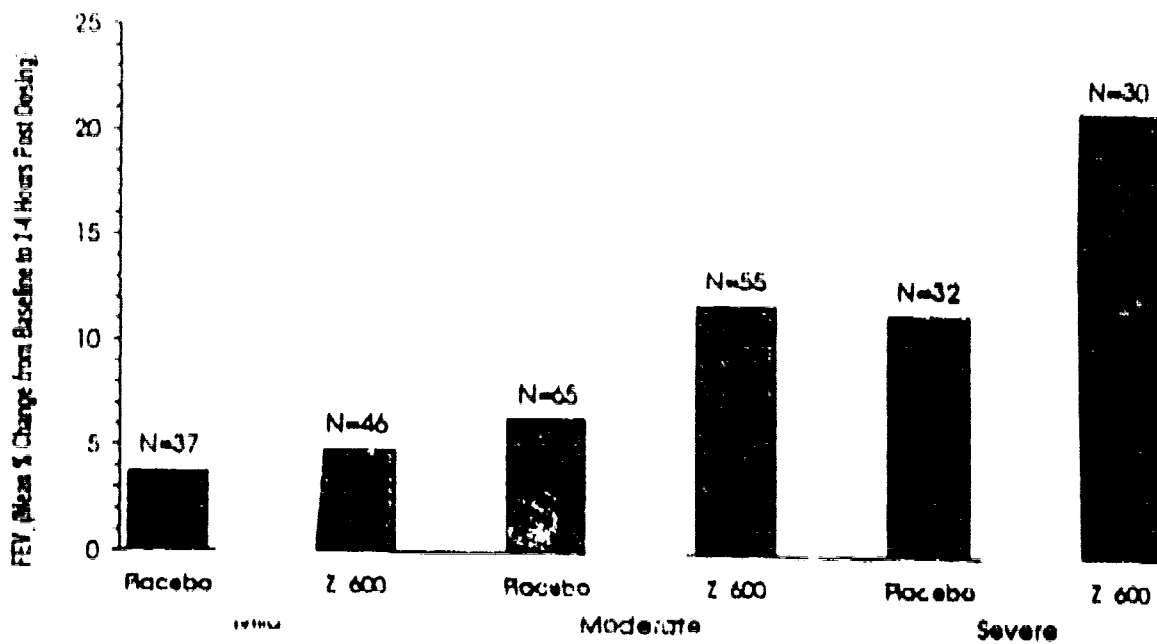


FIGURE 6

Figure 10. AM Peak Expiratory Flow Rates

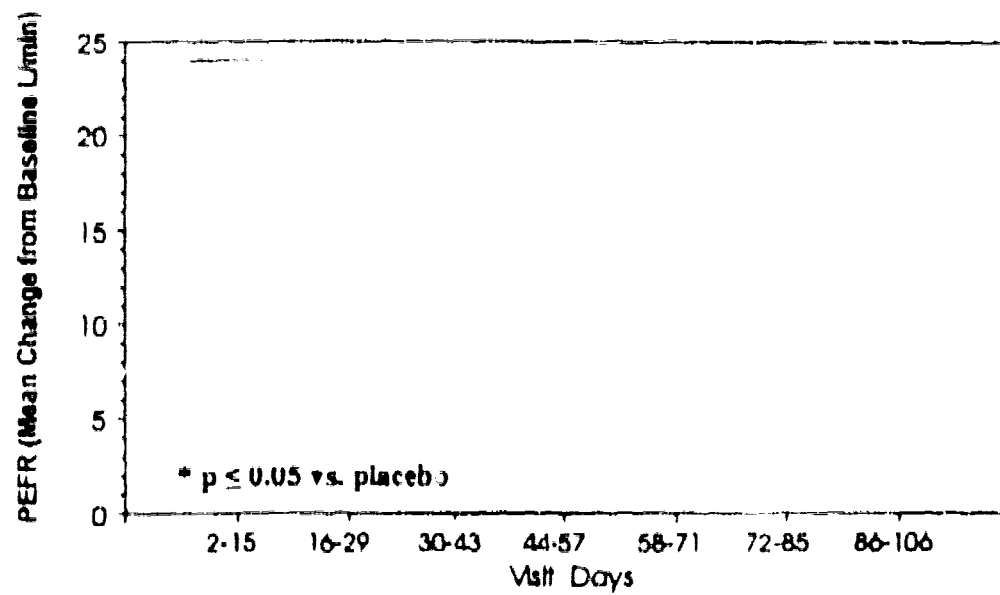


FIGURE 7

Figure 11. PM Peak Expiratory Flow Rates

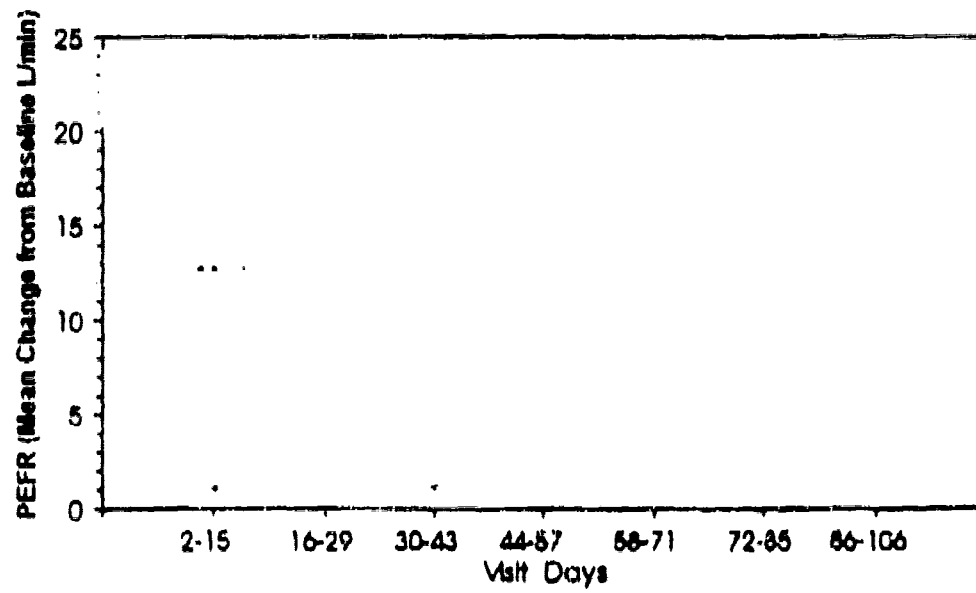


FIGURE 8

Figure 12. Daily Symptoms

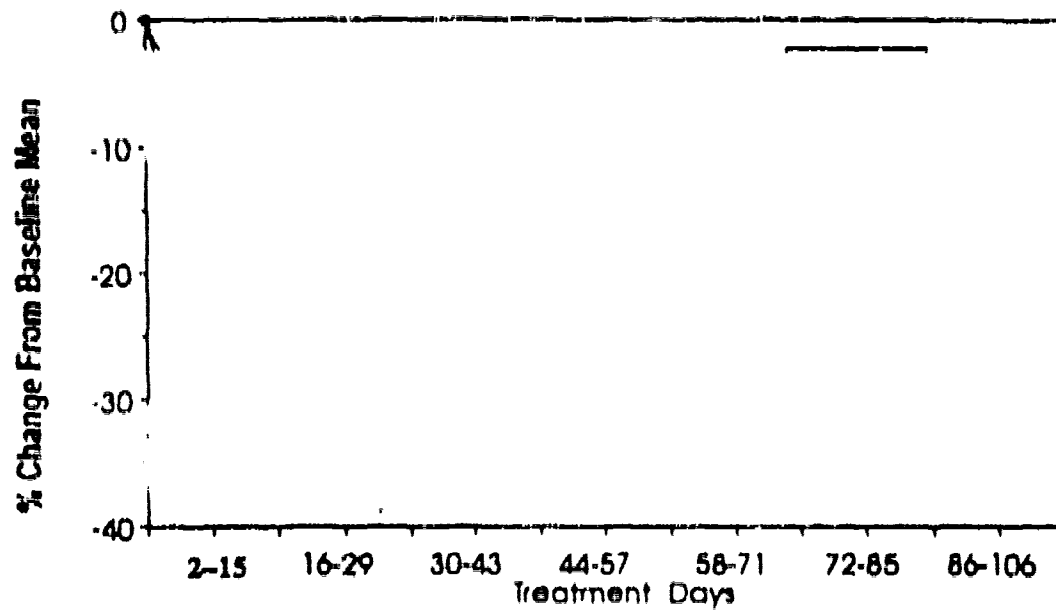


FIGURE 9

Figure 13. Daily Symptoms for Subsets of Patients with Mild, Moderate, and Severe Asthma

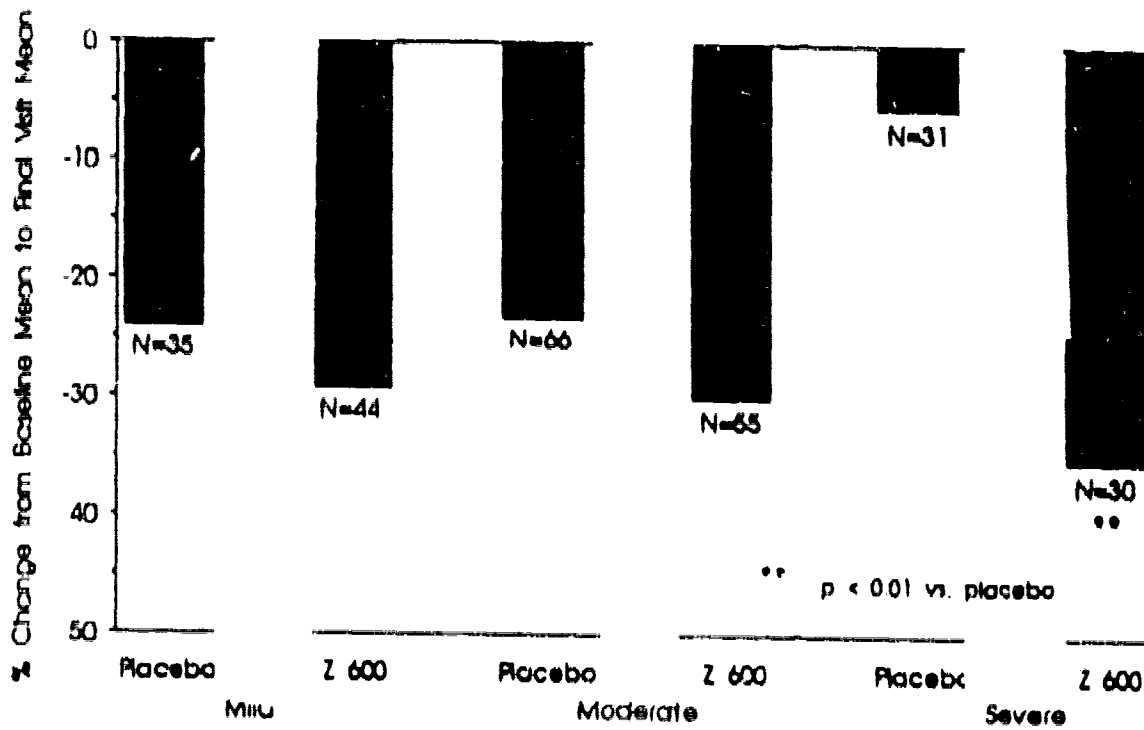


FIGURE 10

Figure 18. Daily Beta-Agonist Use for Subsets of Patients with Mild, Moderate, and Severe Asthma

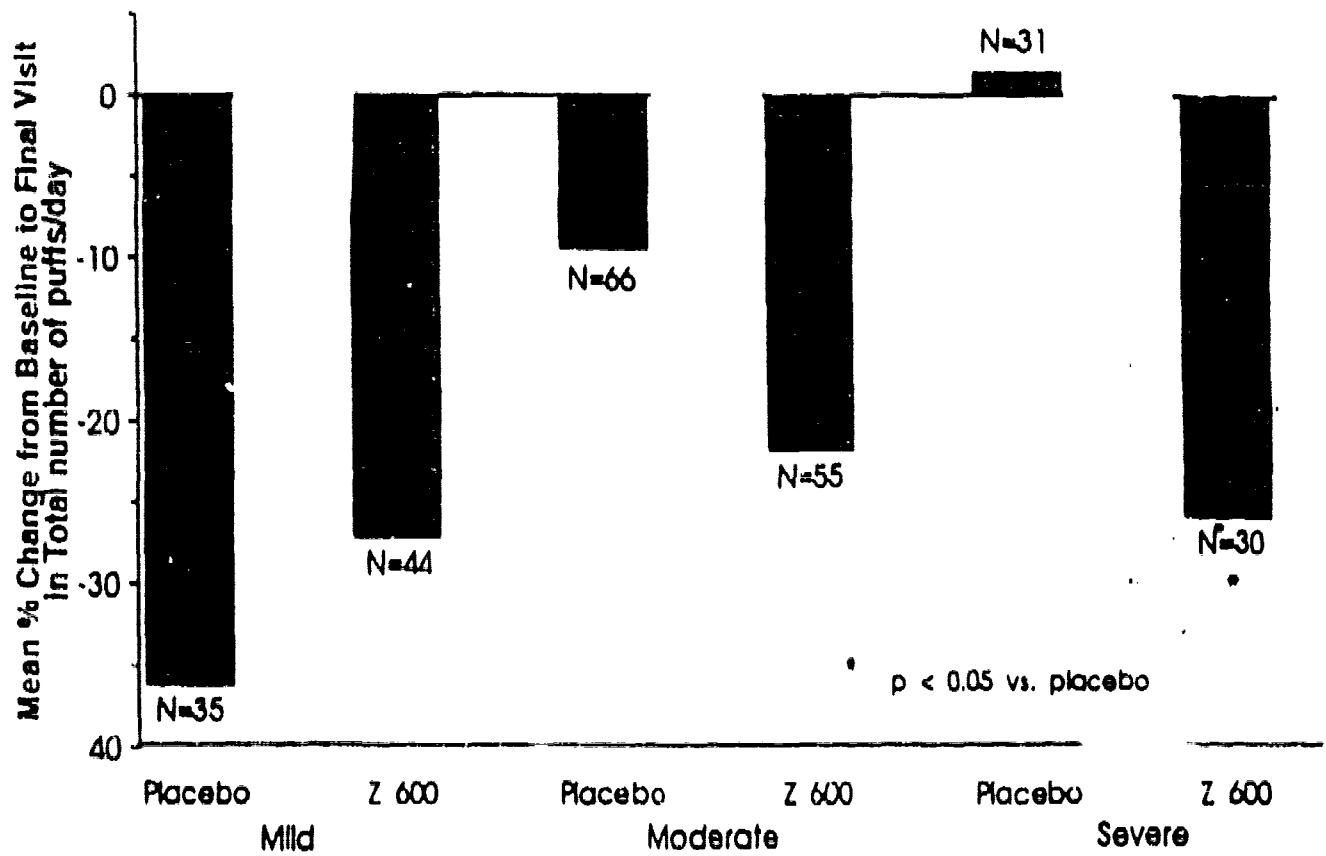


FIGURE 11

Figure 3. Trough Effect

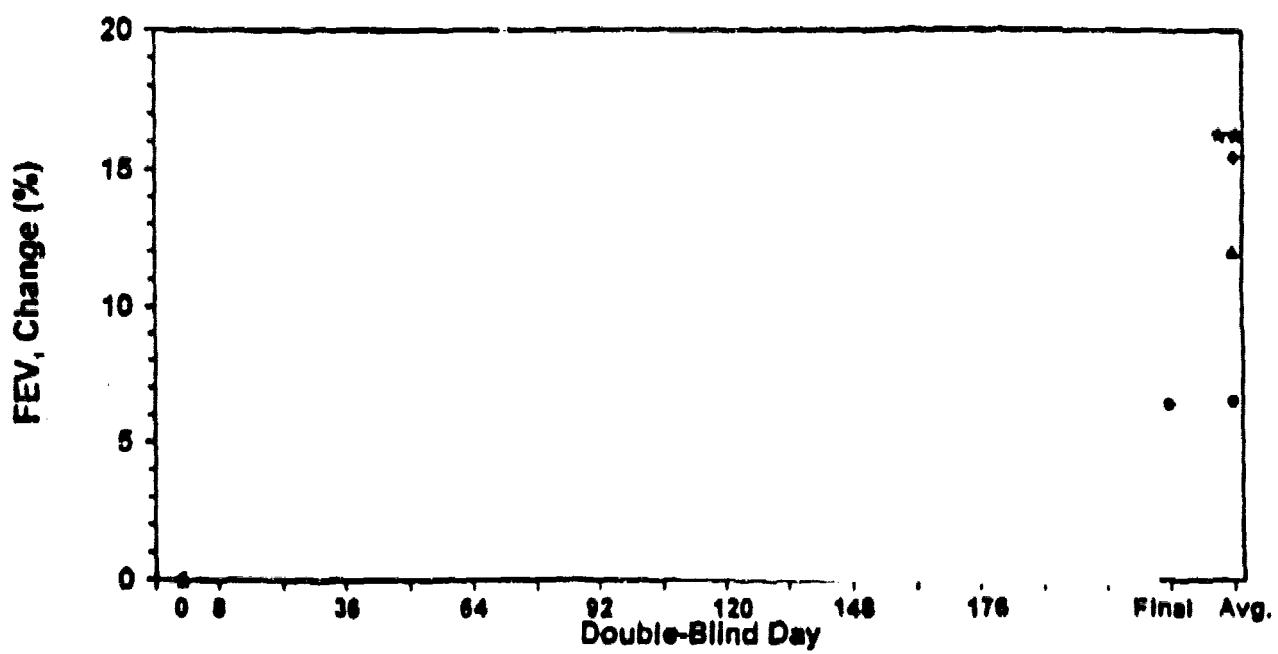


FIGURE 12

Figure 4. Trough Effect for Subsets of Patients with Mild, Moderate and Severe Airway Obstruction

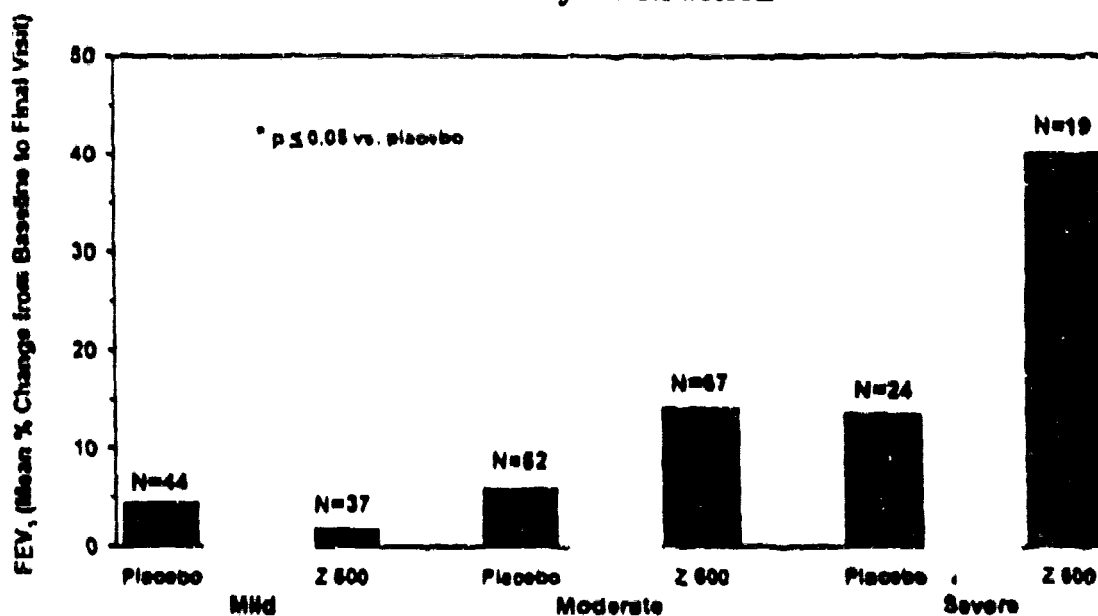


FIGURE 13

Figure 5. Peak Effect

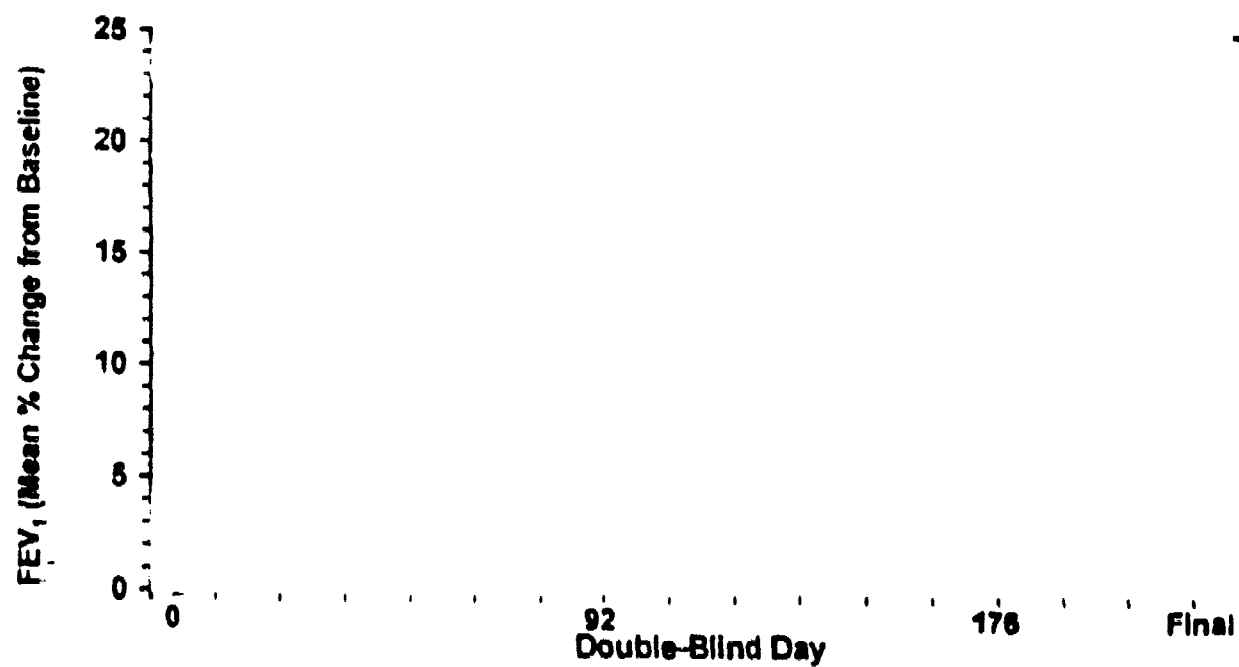


FIGURE 14

Figure 8. First Dose Effect

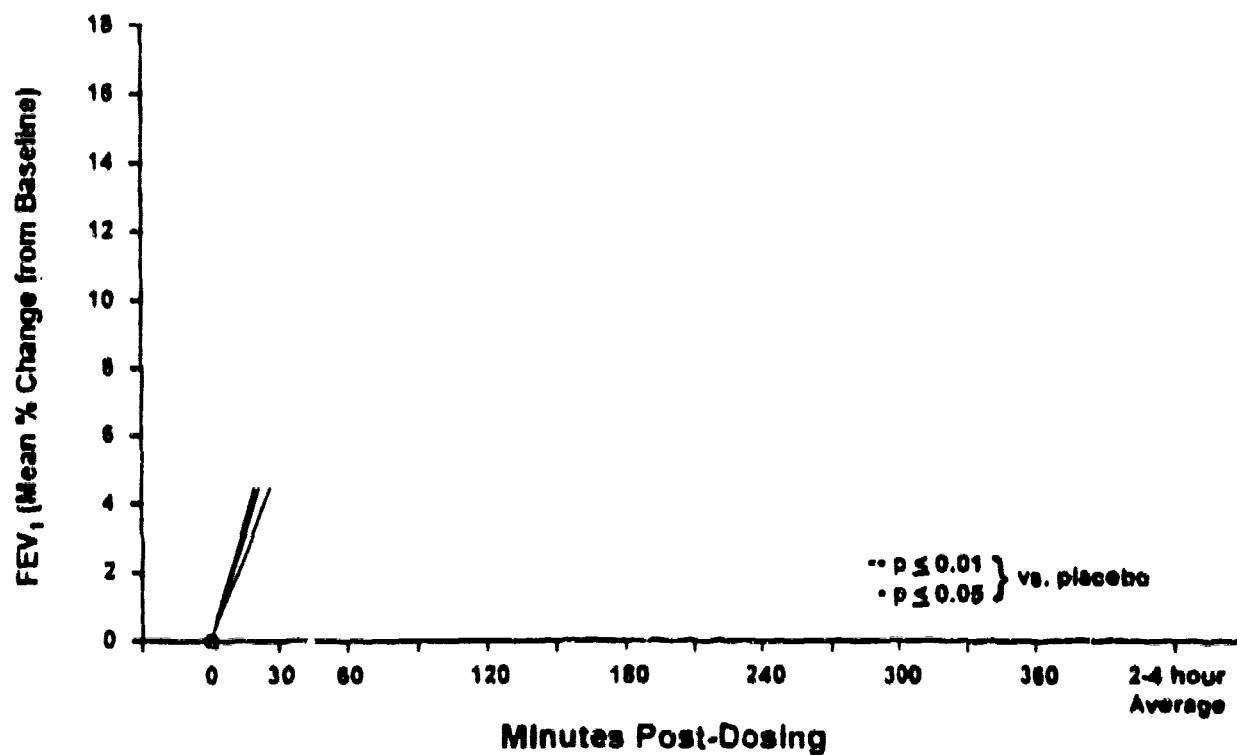


FIGURE 15

Figure 9. First Dose Effect for Subsets of Patients with Mild, Moderate and Severe Airway Obstruction (2-4 Hour Average)

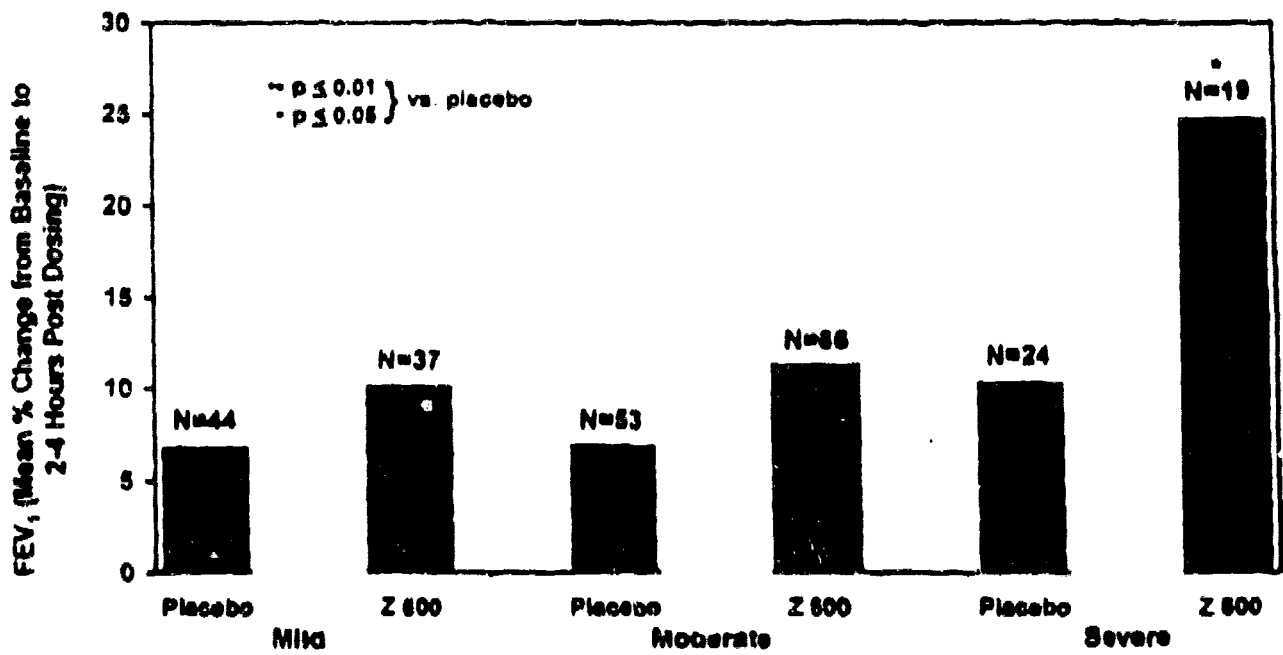


FIGURE 16

Figure 10. AM Peak Expiratory Flow Rates

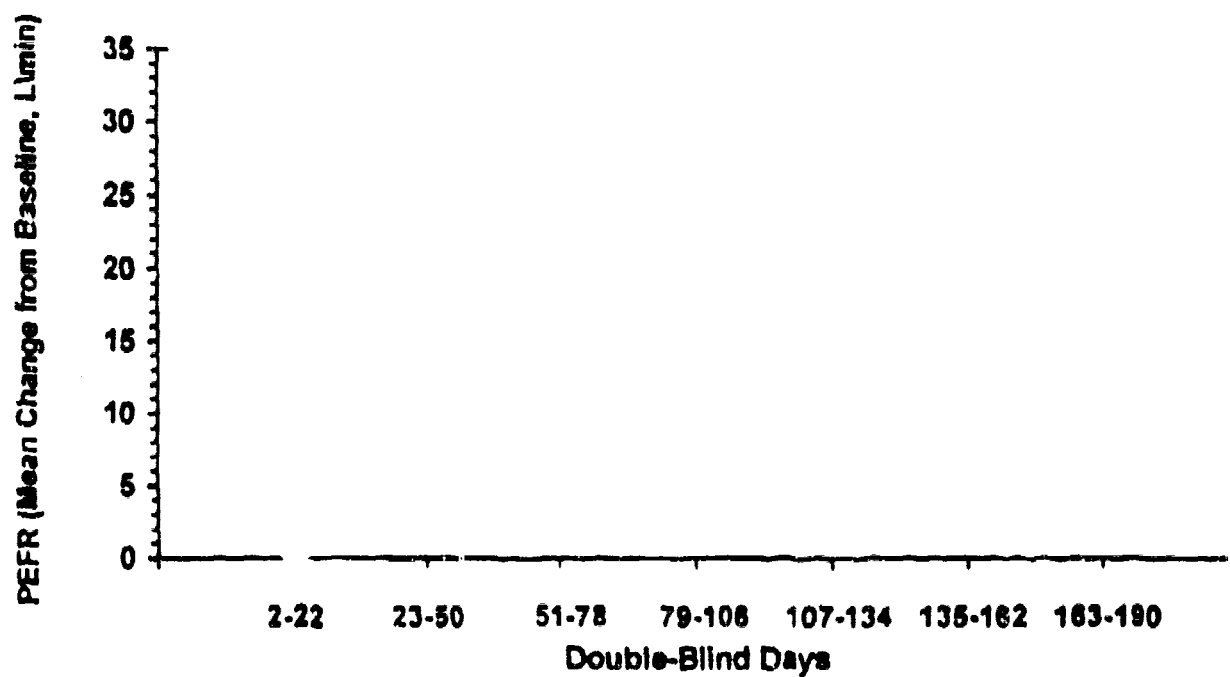


FIGURE 17

Figure 11. PM Peak Expiratory Flow Rates

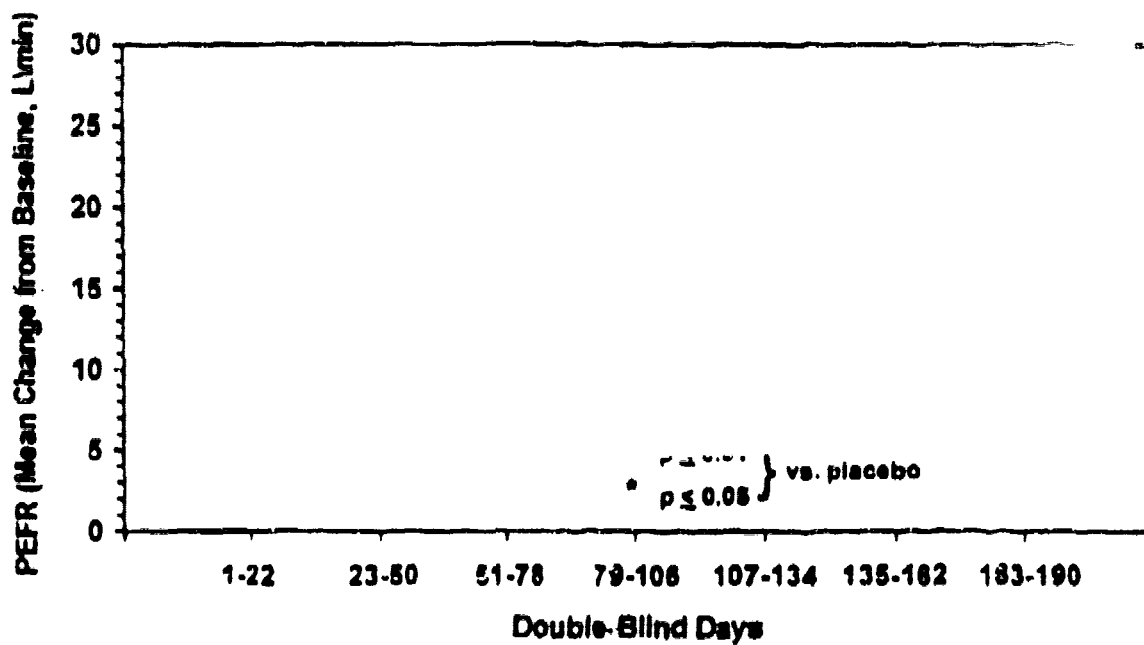


FIGURE 18

Figure 12. Overall Daily Symptoms

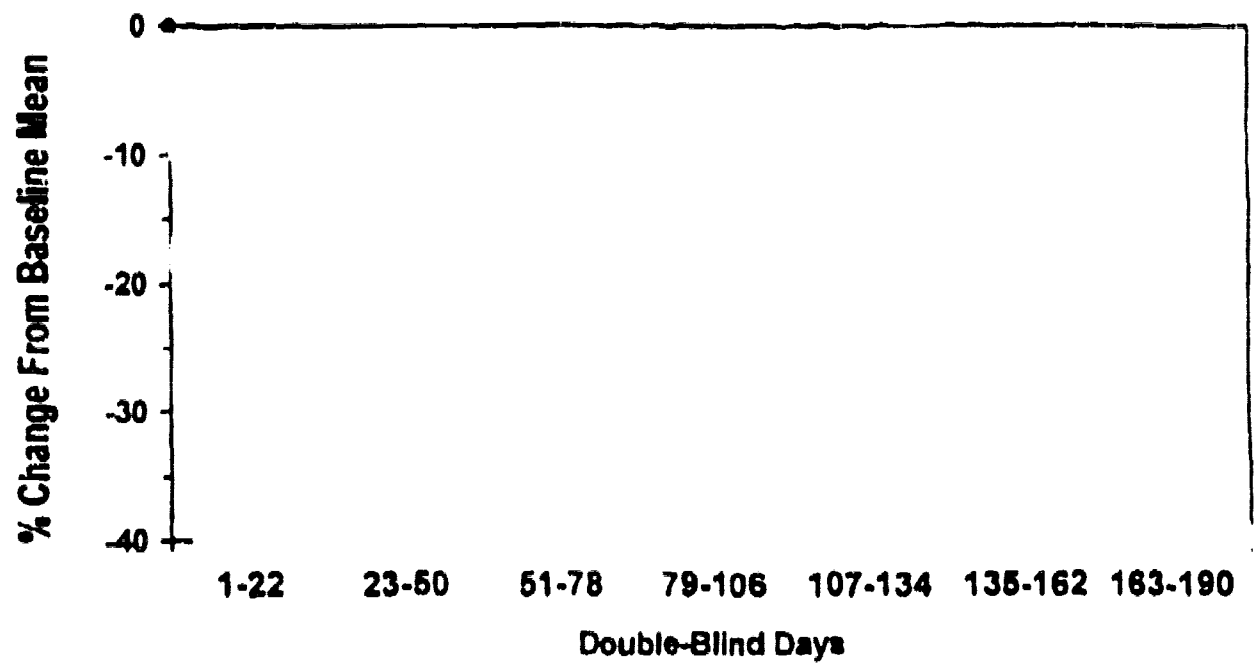


FIGURE 19

Figure 13. Overall Nocturnal Symptoms

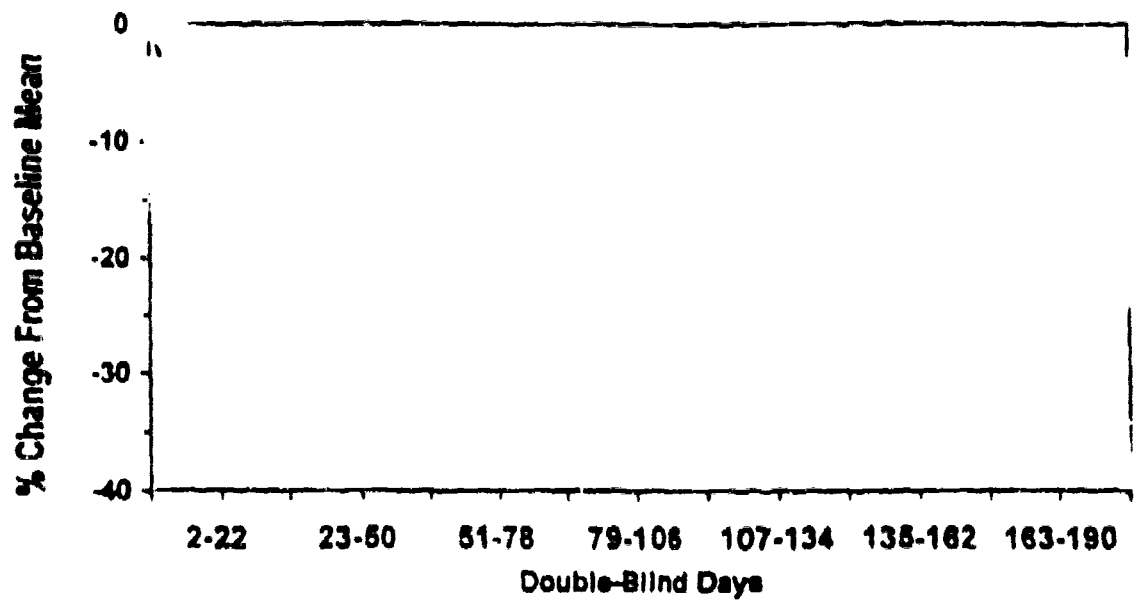


FIGURE 20

Figure 17. Daily Beta-Agonist Use for Subsets of Patients with Mild, Moderate and Severe Airway Obstruction

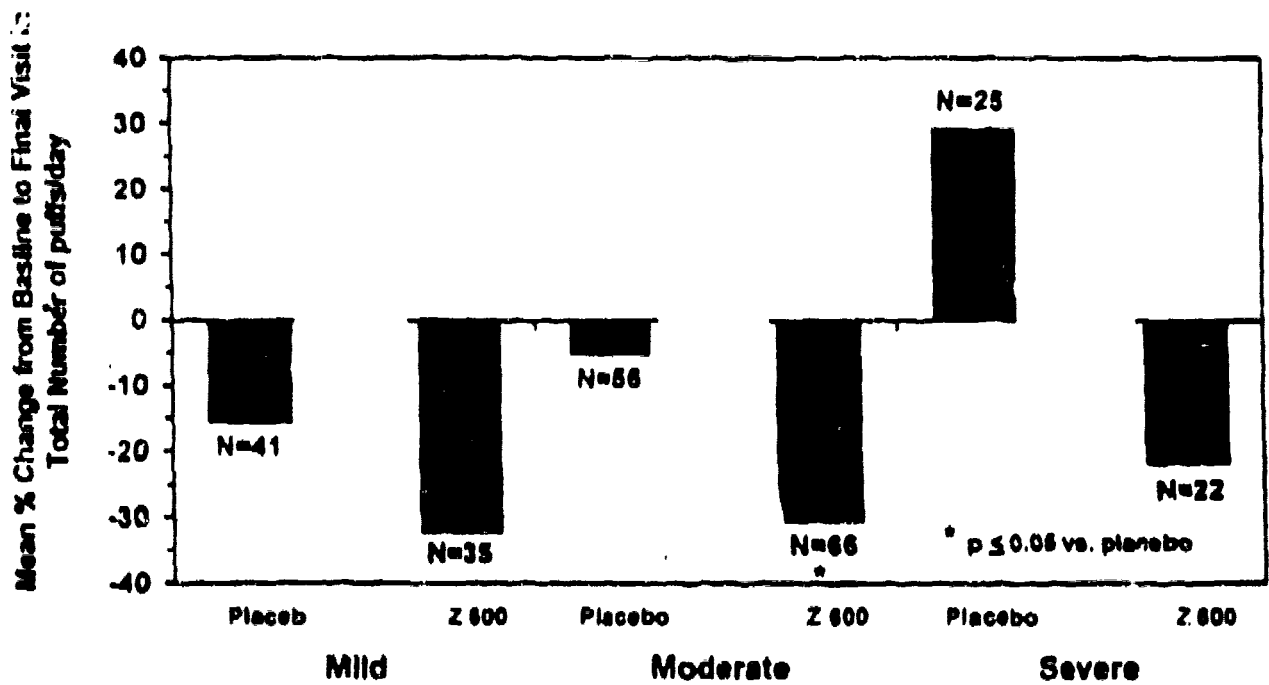


FIGURE 21

Mean Plasma Concentrations of ABBOTT-64077 and Theophylline in Man on Day 5 after Oral Administration of 200 Mg Theophylline Q.I.D. with and without 800 Mg ABBOTT-64077 B.I.D.

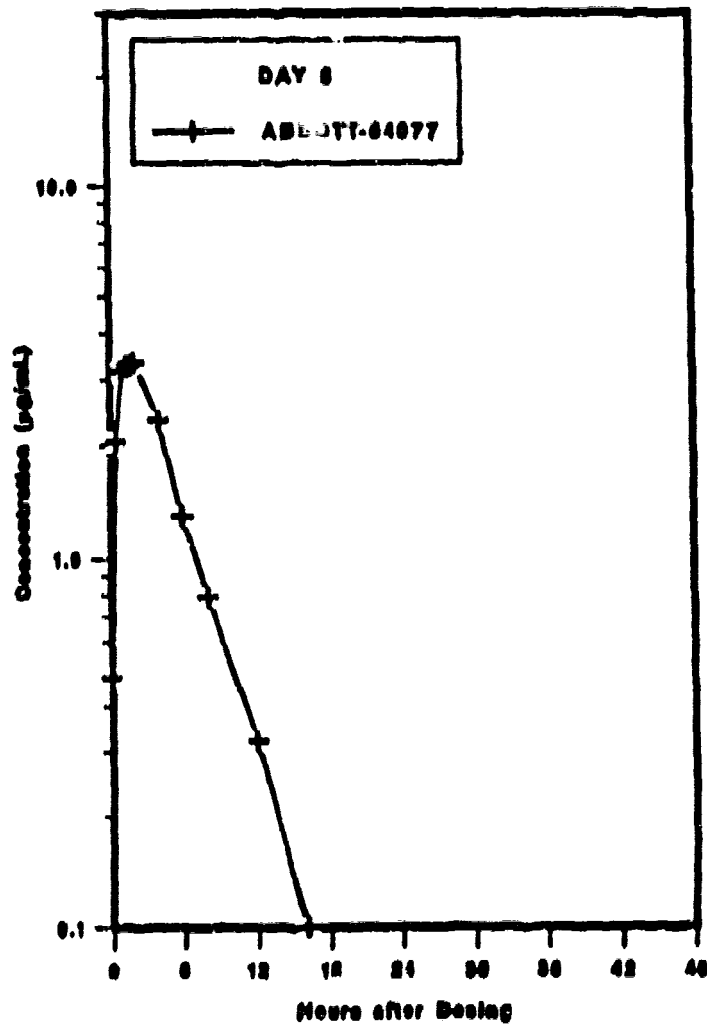
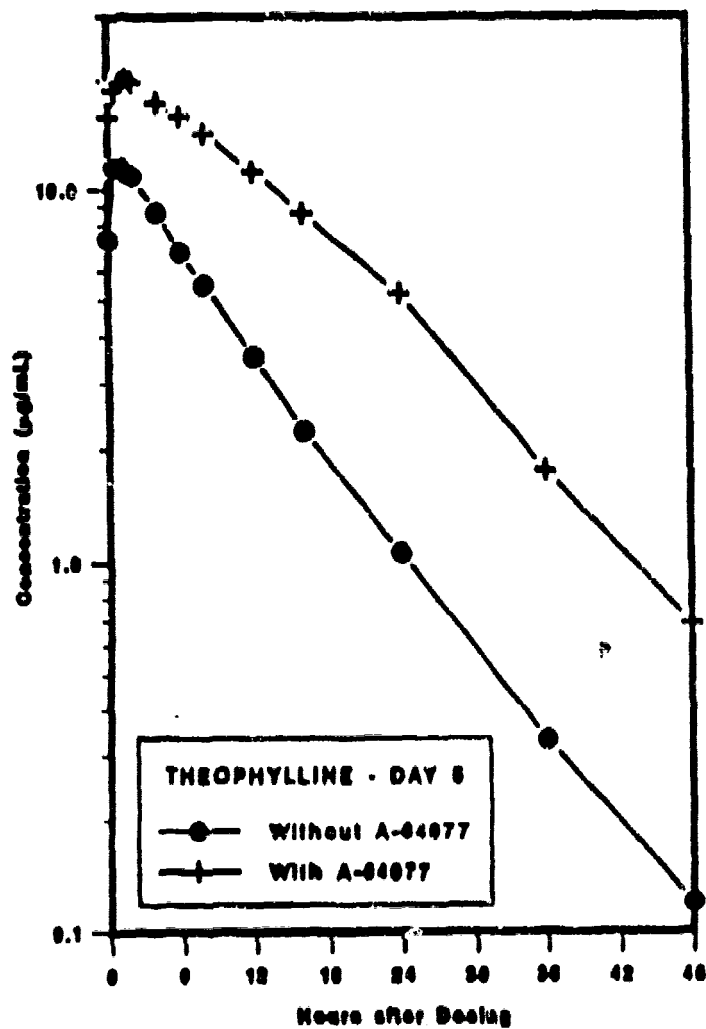


FIGURE 22

Figure 2. Mean Plasma Concentrations of Propranolol after Single Oral 80-mg Dose of Propranolol Administered Alone and with 600 mg of Zileuton Four-Times Daily

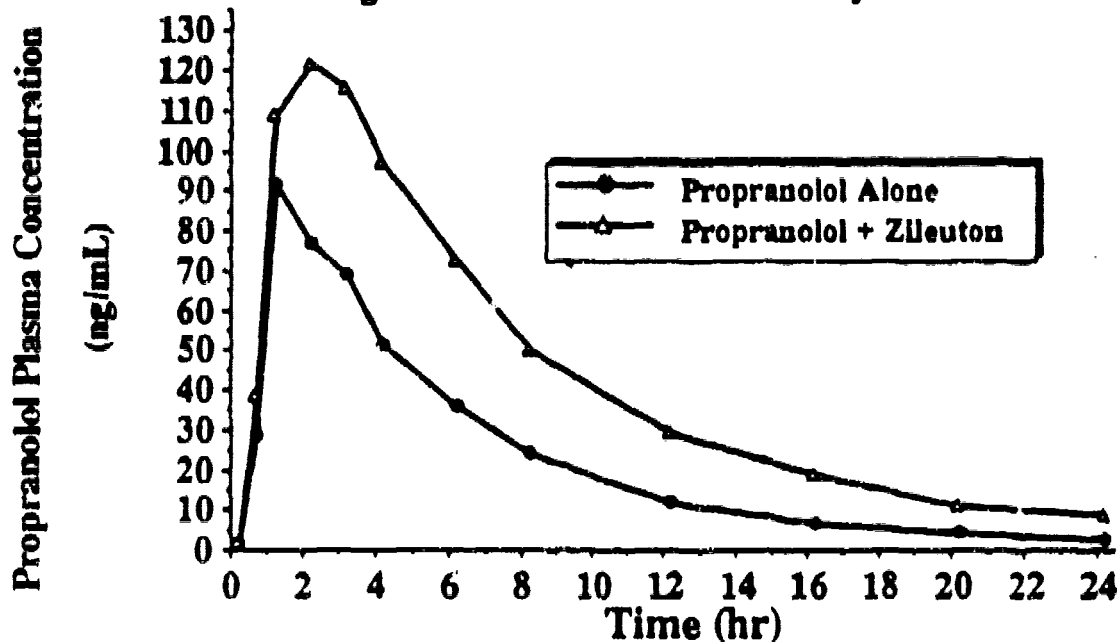


Figure 3. Mean Plasma Concentrations of 4-Hydroxy-Propranolol after Single Oral 80-mg Dose of Propranolol Administered Alone and with 600 mg of Zileuton Four-Times Daily

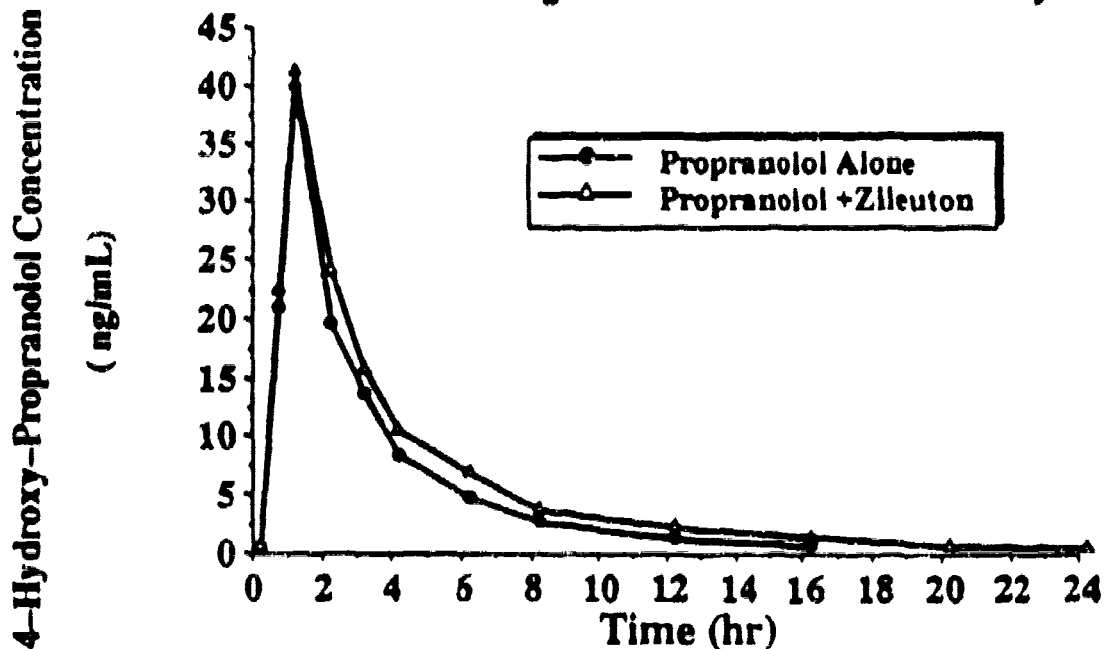


FIGURE 23

Figure 1. Mean Plasma Concentration of Phenytoin after Concomitant Administration of a 300 mg Single Dose of Phenytoin with Placebo or Zileuton in Study M92-820

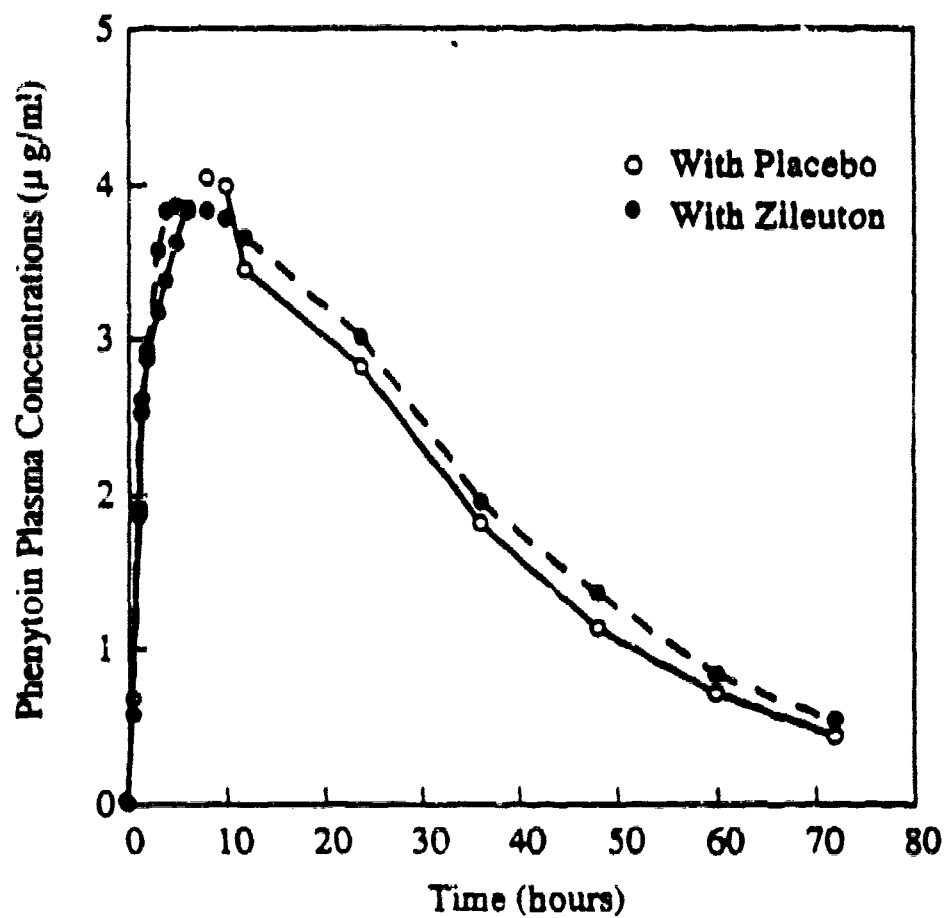


FIGURE 24

Figure 1. Mean Steady-State Plasma Concentrations of Digoxin With Concomitant Administration of Placebo or Zileuton

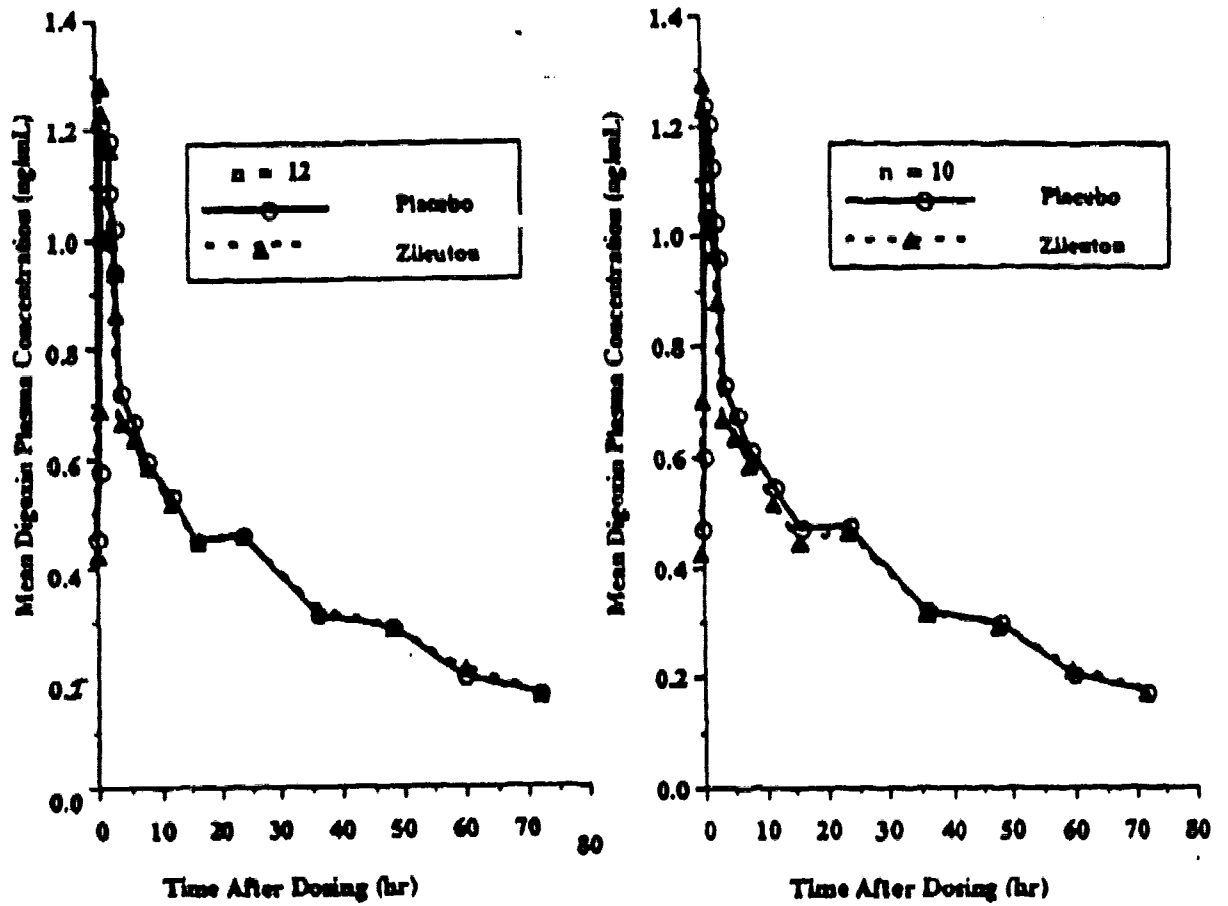


FIGURE 25

Figure 1. Mean (+/- SD) Plasma Concentration Versus Time Profile of Terfenadine After Oral 60 Mg q12h Dosing of Terfenadine Alone (Day 5) and with 600 Mg q6h Zileuton (Day 10)

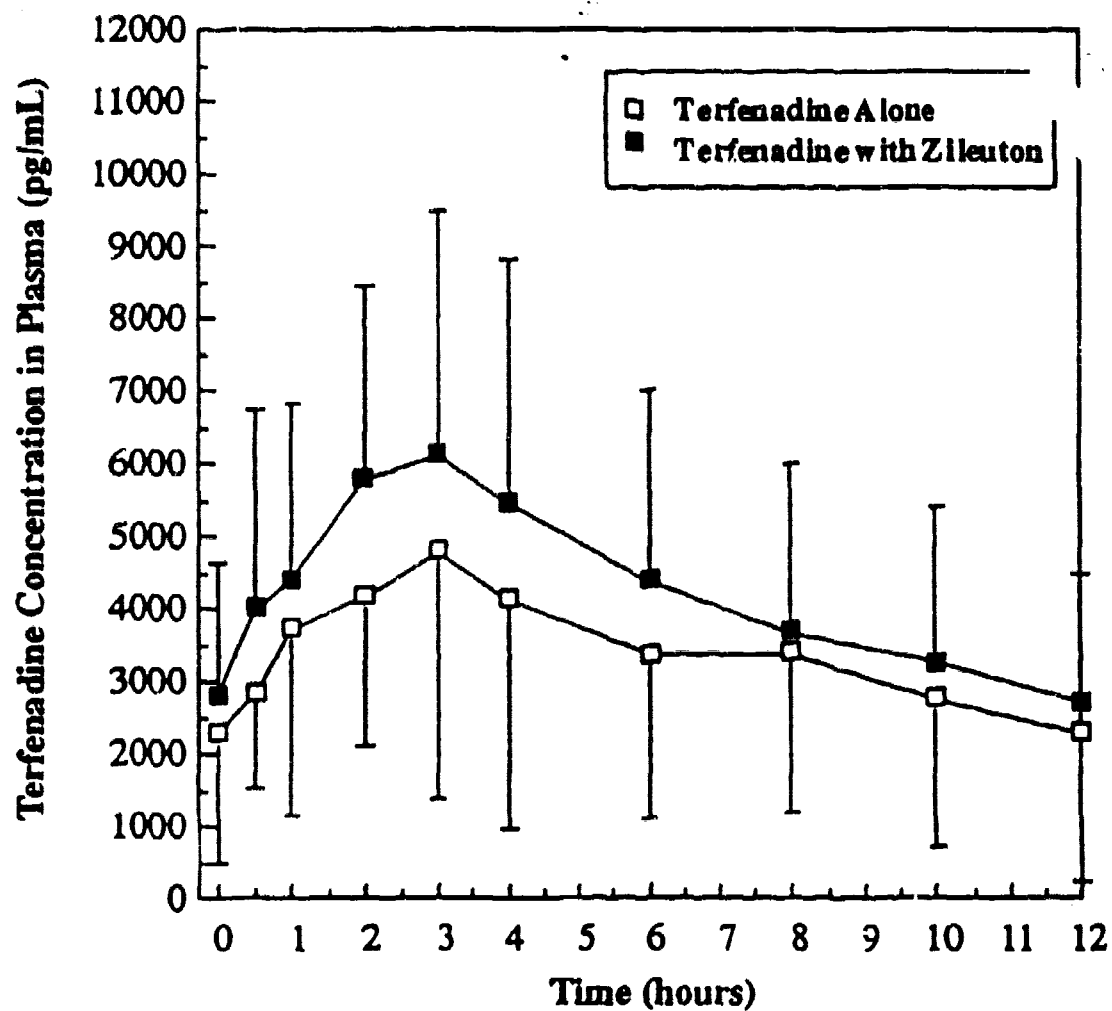
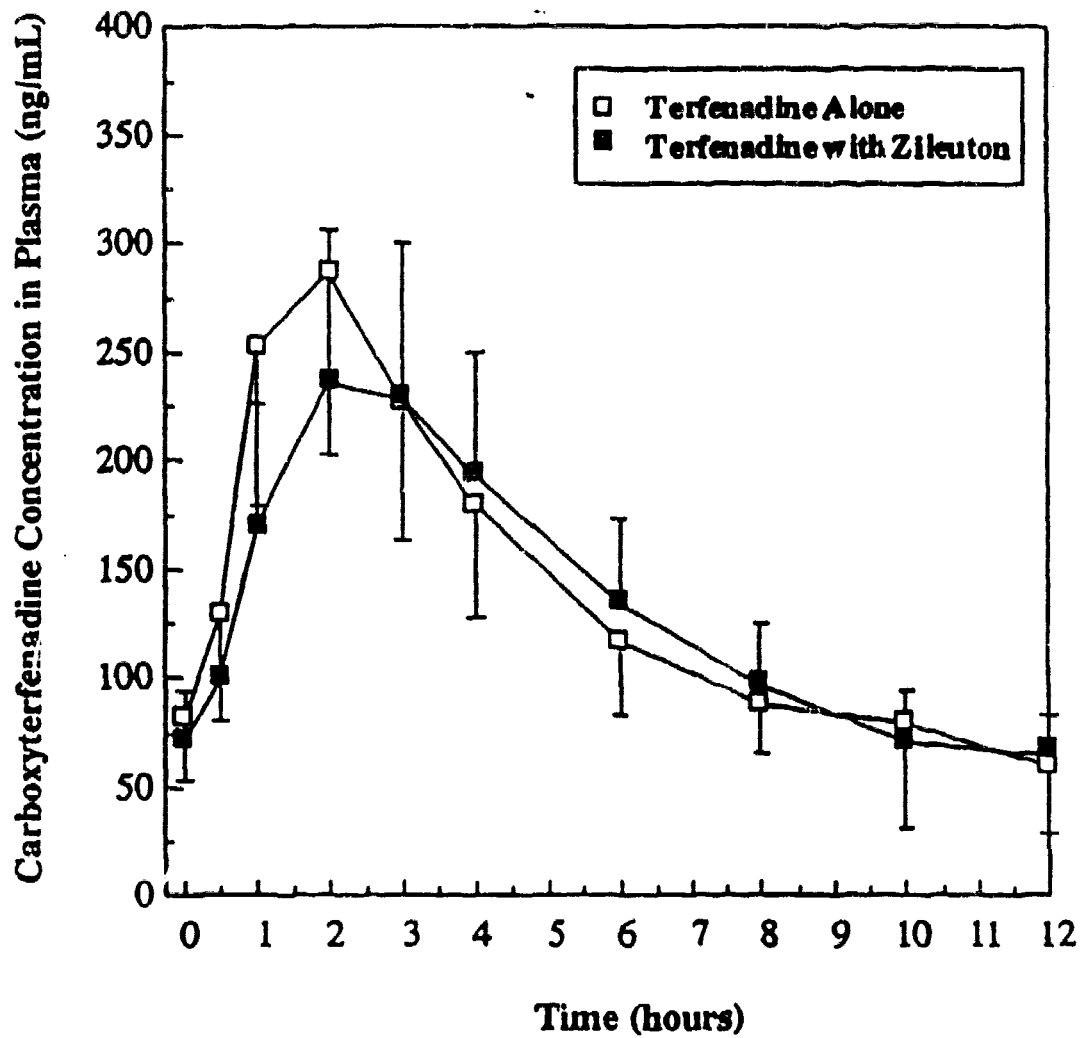


FIGURE 26

Figure 2. Mean (+/- SD) Plasma Concentration Versus Time Profile of Carboxyterfenadine After Oral 60 Mg q12h Dosing of Terfenadine Alone (Day 5) and with 600 Mg q6h Zileuton (Day 10)



ATTACHMENT 1

MAR 9 1994

IND #
Drug: Zileuton/Abbott-64077
February 16, 1994/revised March 8, 1994...
Medical Officer's Consultation

Reviewed: Ser. No. 218 12/27/9
Final Report for
Study MA2-800

Zileuton is a member of the leukotriene family; specifically it is a potent 5-lipoxygenase inhibitor. 5 lipoxygenase is the first dedicated enzyme in the leukotriene synthesis pathways. Zileuton is being developed for the chronic and presumably long term treatment of bronchial asthma

The concern is that men treated chronically and long term will have changes in reproductive potential.

An earlier consultation by this division for HFD-155 made suggestions regarding necessary male reproductive toxicity studies in the development of this leukotriene for clinical use. Prostate and testicular atrophy had been observed as the only abnormalities in animal studies. These abnormalities were seen in 6 to 9 month old male dogs when doses of 9 - 20 times the human doses were used. As a result of these findings the following was suggested:

- Evaluation of semen parameters over a period of at least 84 days to 6 months using standard WHO or CONRAD criteria;
- Evaluation of prostate and testicular changes with treatment using ultrasound and Prostate Specific Antigen (PSA); and
- Identification of drug secretion and accumulation in the semen.

The sponsor (Abbott) has completed a study in which they have evaluated the effect of Zileuton on the following:

- PROSTATE
- TESTES
- SEMEN
- REPRODUCTIVE HORMONES - TESTOSTERONE, LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE AND DIHYDROTESTOSTERONE.
- SERUM AND SEMEN DRUG CONCENTRATIONS.

The study provided by the sponsor included 43 healthy sexually mature, adult male volunteers, between the ages of

18 and 52, enrolled at two different sites. Forty subjects completed the entire study. 30 were randomized to active drug - zileuton and thirteen to placebo. The men were given a single tablet of Zileuton 4 times a day with 240 ml of water. Each tablet is 600 mg. They were dosed for a period of 18 weeks.

The evaluations were done at baseline, mid-treatment and end of treatment. WHO criteria were used as recommended. Contraception techniques were used by the subjects during the study and for 90 days following discontinuation of study medication. The men were non-smokers and were on no other medication. A urine drug and alcohol screen was conducted at screening weeks 8 and 18. The prostate was examined by rectal exam and testicular size determined by ultrasound.

Results of the study:

- PROSTATE - no statistical or clinical changes in prostate size..
- PSA - no statistical or clinical changes in levels of PSA.
- TESTES - no statistical or clinical changes in testicular size. There was no evidence of testicular decrease in size with drug therapy.
- SEMEN - a few small, but statistically significant changes in spermatazoan structure and function were observed. These changes in semen characteristics are probably not clinically significant in this short evaluation. The changes noted were increased motility (day 85,127 and final evaluation) and sperm concentration (day 29) from baseline in the placebo group and increases in cytoplasmic droplets in the treated group at all time points compared with placebo group. At all times the cytoplasmic droplets were well within normal and less than 1%.
- REPRODUCTIVE HORMONES - TESTOSTERONE, LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE AND DIHYDROTESTOSTERONE. Only DHT demonstrated a significant difference but was within clinical parameters.(zileuton group 41.8 vs 52.8 ng/dL)
- SERUM AND SEMEN DRUG CONCENTRATIONS.
Concentration of plasma and semen zileuton
The mean peak zileuton concentration of 5 to 7 ug/mL are consistent with peak zileuton

concentrations in other studies at this dose. The semen zileuton concentrations are approximately 1 ug/mL without evidence of accumulation. This would appear to be at the limit of ability to assay.

The levels of zileuton concentration in seminal fluid ranged to approximately 15% of the plasma zileuton concentrations. There did not appear to be an accumulation of zileuton in the seminal fluid. The dosing appeared to mimic the recommended dosing.

The sponsor has identified all patients who deviated or were withdrawn from the study:

- subject 114 - positive drug test for cannabinoids.
- subject 303 - abnormal baseline
- subject 106 - inadequate abstinence period.

The sponsors believe that there were no clinical significant or statistical differences between Zileuton and placebo groups on all of the observed in any of the endpoints. The sponsor concludes that there are no statistical or clinical differences from treatment for 18 weeks with 600 ml q.d in normal men.

Reviewer's summary:

The sponsor has provided an 18 week study in 40 men, 30 of whom were on active drug. The sponsor found no clinically significant reproductive changes and demonstrated that only very small amounts of drug are secreted into the semen. This should give us some confidence that the drug is not toxic in short term use to developing and maturation of sperm and the current available measurements.

There is no evidence that this drug clinically alters any of the parameters measured, e.g., reproductive hormones, spermatogenesis or sperm function as measured by accepted techniques. It is accepted that wide variations in sperm parameters within the accepted normal range may not alter fertility potential. In no other mammal is there such heterogeneity and variability in the end product of procreation. All of the semen parameters were maintained well above clinically normal measurements. The increase in the presence of cytoplasmic droplets in the treated arm remained small and the clinical significance is unknown. The cytoplasmic droplet is a small mass of cytoplasm which is retained by spermatozoa as they are released from the seminiferous epithelium; it is a transient structure which is displaced during the transit of sperm through the epididymis and shed from the sperm tail. It is not uncommon to find a sizeable fusiform mass of cytoplasm surrounding the neck and

contiguous portions of the nucleus and middle piece. In these studies it is important to note that there is no change in motility.

The study provided is short and evaluates little more than one cycle of spermatogenesis; therefore, it will be important to monitor any changes with chronic use. This should be considered in any post-approval commitments since only long term surveillance will identify small effects of drugs.

When men, active sexually or at the peak of reproductive potential, are on drugs it is important to remind them of possible drug effects on reproduction. A recent report suggests that investigators should consider including a discussion of developmental toxicity in the informed consent process for male subjects who may be exposed to developmental toxicants in the course of a clinical study. (Mattison, 1994) This will be important in any drug used chronically.

Recommendations:

- The 18 week study submitted by Abbot does not demonstrate any clinical reproductive changes in the treated men. It is encouraging that zileuton does not appear to be secreted and concentrated in the seminal fluid.
- There is no evidence that the drug is toxic to developing or post-maturation sperm using current criteria.
- Post approval commitment should include continued surveillance of possible reproductive changes with chronic use of zileuton including semen evaluation after 3 to 4 cycles of treatment.

Jean L. Fourcroy
Jean L. Fourcroy, M.D., PhD
Medical Officer

cc: HFD-510/JFourcroy/2/28/94./3/9/94
HFD-510/Consults (+cons. req.)
HFD-155/Jenkins/Schumaker/Buccine
HFD-155/DF
Arch.IND

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**Table 7. Zileuton Semen to Plasma Concentration Ratios (%)
After Oral Administration of 600 Mg Zileuton
Q.I.D.**

1-10
6-21
10-51
17-84

Subject ID	Weeks of Dosing		
	4	12	18

high 13-1670
CV.

Mean	16.38	13.13	13.41
SD	15.19	11.44	15.63
%CV	92.7	86.0	112.3

NS = No Semen or Plasma Samples or Both
Calculations were done prior to rounding.

Figure 2. Mean ($\pm$ SD) Zileuton and Abbott-66193 Plasma Concentrations After Oral Administration of 600 Mg Zileuton Q.I.D.

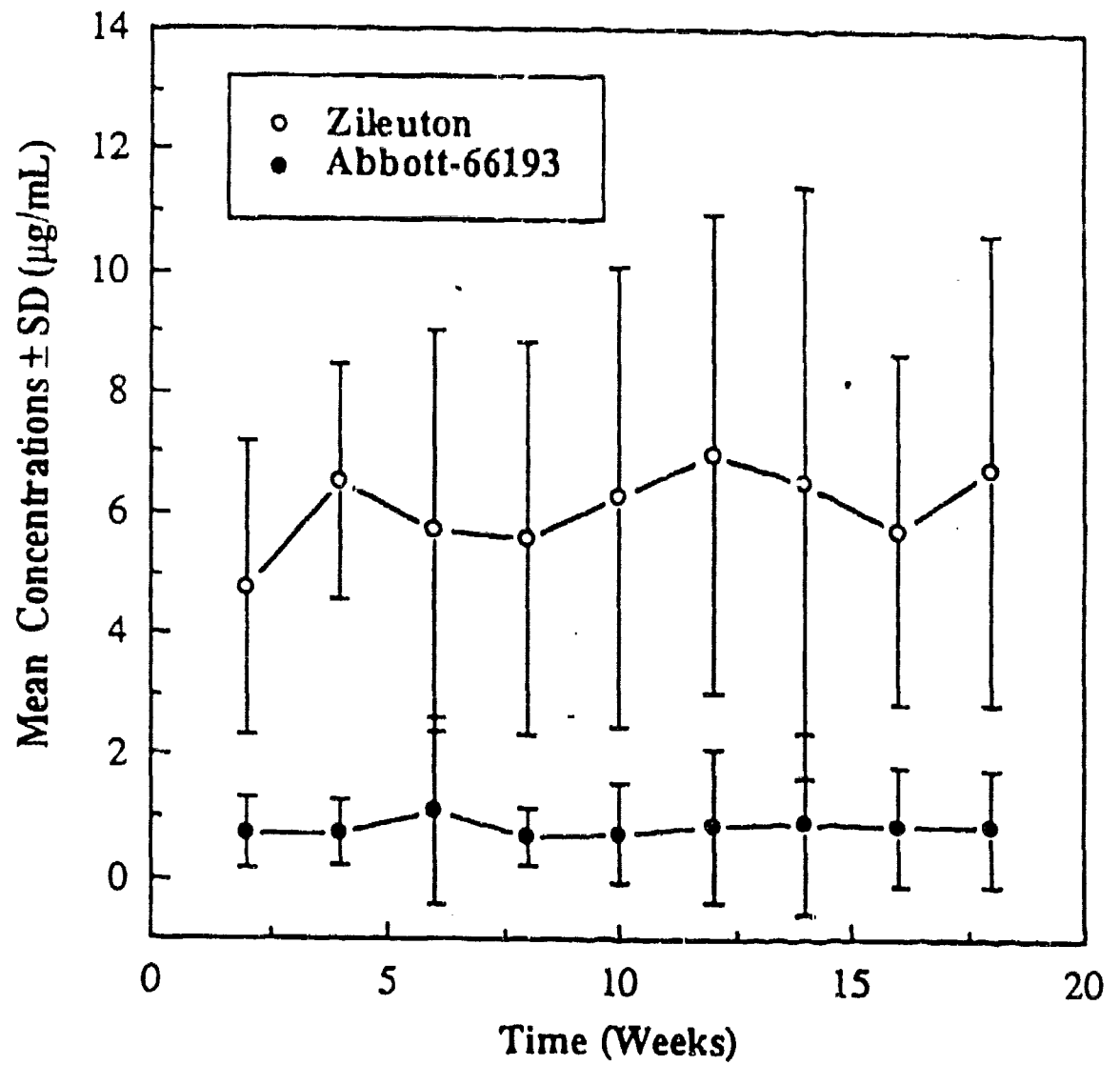
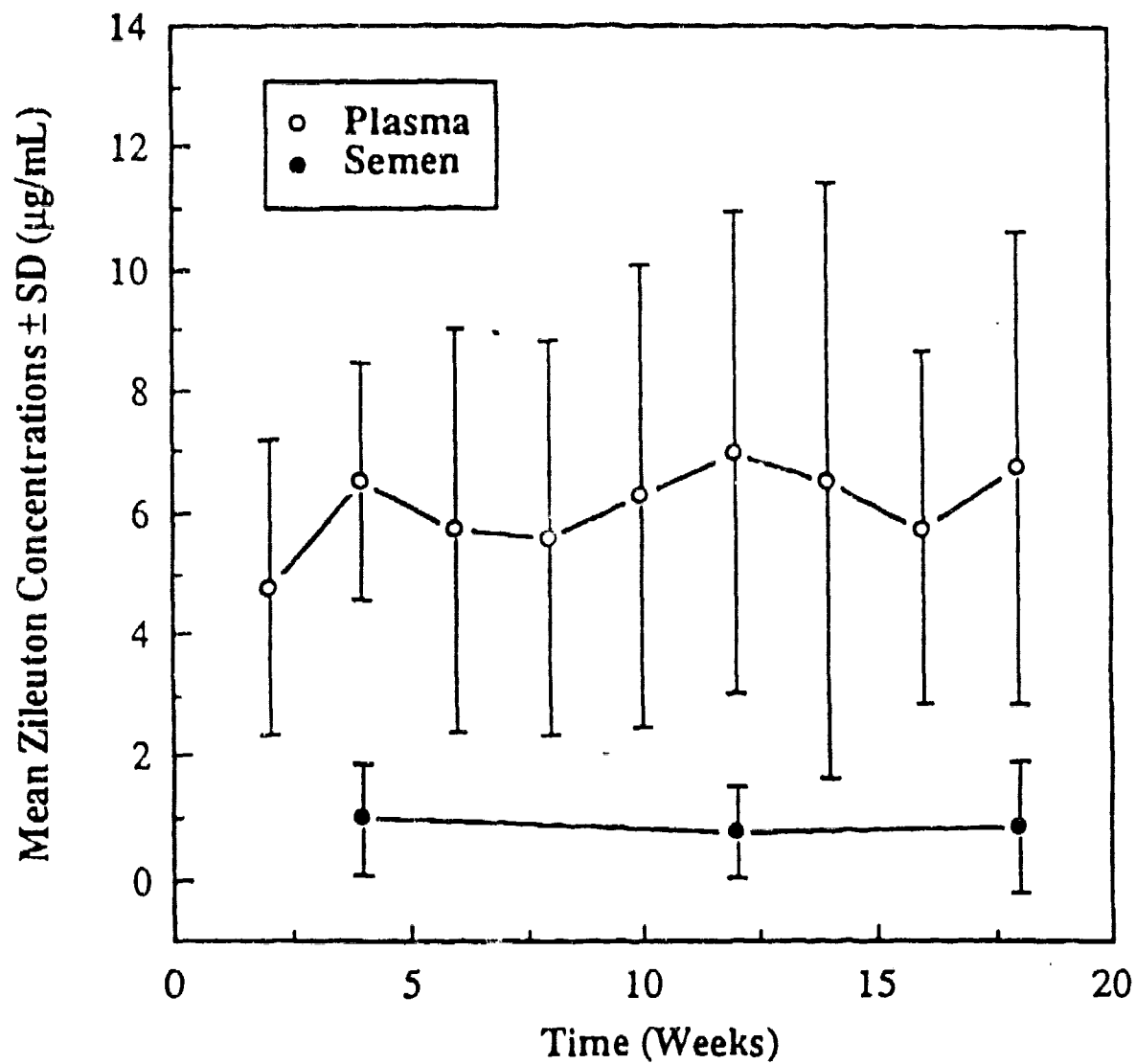


Figure 3. Mean ($\pm$ SD) Zileuton Plasma and Semen Concentrations After Oral Administration of 600 Mg Zileuton Q.I.D.



AUG 1 1994

MEDICAL OFFICER REVIEW

21-Day Clinical Review

NDA	20-471	SUBMITTED	18 July 1994
REVIEWER	R. F. Anthracite, M.D.	STAMP DATE	19 July 1994
SPONSOR	Abbott	M.O. RECEIVED	22 July 1994
DRUG	(Zileuton)	REVIEWED	17 Aug. 1994
MECHANISM	5'-lipoxygenase inhibitor		
INDICATION	Asthma		

1.0 INTRODUCTION

This represents a new class of drugs for the treatment of asthma, it is a 5'-lipoxygenase inhibitor. By blocking arachidonic acid metabolism, a host of leukotriene inflammatory mediators are reduced. This is an oral drug that has been undergoing clinical trials for the last 7 years, many of which have been guided by FDA input.

1.1 INDICATIONS

is indicated for the prevention and control of symptoms associated with reversible bronchospasm and for maintenance therapy in the management of asthma patients with mild, moderate or severe airway obstruction." [1:86]

1.2 PROPOSED DOSAGE

"...one 600 mg tablet (or two 300 mg tablets) four times a day for a total dose of 2400 mg. While maximal improvement...was produced by the 600 mg QID regimen, some patients may show clinical benefit at a dosage regimen of QID." [1:93]

1.3 FORMULATIONS

Three strengths of scored tablet are planned for marketing, 300, 600 mg. These are said to be of the same composition, differing only in compressional weight and tablet tooling design. Although close to a dozen formulations and strengths have been used in clinical pharmacology studies, only two have been employed in asthma clinical trials. Moreover, the majority of these have been with the to-be-marketed formulation, designated "FF" in the Table below showing studies, strengths and formulations [1:120]:

INVESTIGATIONAL FORMULATIONS USED IN ASTHMA CLINICAL TRIALS		
STUDY	FORMULATION	LOT. NO.
M91-685	600 mg Tablet, FF	62-027-AR, 60-777-AR 62-028-AR, 60-778-AR

INVESTIGATIONAL FORMULATIONS USED IN ASTHMA CLINICAL TRIALS		
STUDY	FORMULATION	LOT. NO.
M92-720	600 mg Tablet, FF	62-027-AR 62-028-AR
M91-615	600 mg Tablet, FF	60-778-AR
M92-724	600 mg Tablet, FF	60-778-AR
M92-744	600 mg Tablet, FF	66-352-AR, 79-362-AR
M90-460	200 mg Capsule II	33-779-AR
M90-545	200 mg Capsule II	40-393-AR

Since the beginning of clinical trials in 1987, two types of solid dosage formulations have been used, capsules and tablets. Capsules were used in earlier clinical studies. Nine bioavailability studies link the two capsule formulations, I and II, which were manufactured in the U.K. and U.S., respectively, with the three "FF" tablets strengths. These same bioequivalence studies link the two manufacturing sites for the tablets, in Abbott Park and in Puerto Rico.

All bioavailability studies, except the definitive bioavailability study for the final, to-be-marketed 600 mg strength of zileuton tablet, F92-219, used the 200 mg Capsule II formulation manufactured in North Chicago as the reference formulation. The F92-219 definitive bioavailability study used the previously tested 300 mg strength final tablet manufactured in Abbott Park, Illinois as the reference formulation. [1:113-20, 2:280-98]

2.0 MARKETING HISTORY

This drug is not commercially marketed in any country. [1:107]

3.0 CLINICAL DATABASE

A total of 42 clinical pharmacology and 14 safety or asthma-efficacy studies have been performed. Of these latter, 13 were placebo-controlled and 1 had an active control. Additionally, there are 15 on-going trials, 5 are placebo-controlled, 6 have active controls and 4 are uncontrolled.

3.1 CLINICAL PHARMACOLOGY

A total of 42 in vivo studies have examined various aspects of the clinical pharmacology of this drug. These include the following:

- a. 15 biopharmaceutical studies of bioavailability/bioequivalence [1:257-9, 2:48-

- 9]
- b. 10 single- and multiple-dose PK studies in healthy subjects [1:49-50]
 - c. 11 trials of interaction/metabolism which includes sulfasalazine, naproxen, theophylline, antipyrine, prednisone, warfarin, propranolol, phenytoin, digoxin, terfenadine and contraceptives [1:50-1]
 - d. 3 studies of "special" populations which includes trials of age, gender, renal and hepatic impairment [1:51]
 - e. 3 other trials examined the male reproductive tract, cardiovascular and respiratory parameters in normal volunteers [1:52]

3.2 EFFICACY/SAFETY CLINICAL STUDIES

A total of 14 completed, controlled clinical trials for asthma were submitted. All but one were placebo-controlled, the last using an active control. Ten of the trials were done in the U.S. Four domestic, uncontrolled trials are ongoing. A total of 11 controlled trials are ongoing: 5 are domestic and placebo-controlled, 1 is domestic and uses active-control. The remaining 5 are foreign and employ active controls. The following Table categorizes all of the asthma studies [1:52-5, 165:3-9B]:

		CONTROLLED TRIALS		
		Placebo	Active	None
COMPLETED	Domestic	10	1	
	Foreign	3		
ON-GOING	Domestic	5	1	4
	Foreign		5	

In addition, zileuton has been studied in several other diseases and safety data from those studies is available [388:119]:

A total of 697 patients received zileuton in Phase I trials and 1994 in Phase II/III studies, most of which were in asthma patients, 1015 [388:120-1]. A summary of the controlled asthma trials follows [165:3-9B]:

STUDY NUMBER	DESCRIPTION	PATIENTS	LENGTH	END-POINTS
M88-147	prevention of cold air-induced constriction; cross-over; 800 mg v. placebo control	13	1 day (single dose)	spirogram
M90-460	800 mg BID v. 600 mg QID v. placebo control in moderate asthma; parallel	146	4 weeks	spirogram, D/N Sx scores, rescue med. use
M90-545	prevention of ASA-induced constriction; cross-over; 600 mg QID v. placebo	8	1 week	spirogram, Sx score
M91-597	5-LO products in BAL fluid; cross-over; 600 mg QID v. placebo control	5	1 day	
M91-615	prevention of exercise-induced constriction; cross-over; 600 mg QID v. placebo control	26	6 days	spirogram
M91-654	effect on cell infiltrates after local bronchoscopic antigen challenge; cross-over; 600 mg QID v. placebo control	14	8 days	
M91-685	600 mg QID v. 400 mg QID v. placebo control; moderate asthma; parallel	401	13 weeks	PEFR, spirogram, D/N Sx & QOL scores, worsenings, rescue med. use
M92-720	600 mg QID v. placebo control; moderate asthma; parallel	372	26 weeks	PEFR, spirogram, D/N Sx & QOL scores, worsenings, rescue med. use
M92-724	prevention of nocturnal asthma; cross-over; 600 mg QID v. placebo control	18	1 week	
M92-785	effect on bronchial hyper-responsiveness; parallel; 600 mg QID v. placebo control	118	13 weeks	

STUDY NUMBER	DESCRIPTION	PATIENTS	LENGTH	END-POINTS
M92-797 *****	compared to beclomethasone; moderate asthma; parallel; 600 mg QID v. beclomethasone (84 mcg QID)	370	13 weeks	PEFR, spirogram, Sx scores
M89-386C	different dose intervals; moderate asthma; 40-800 mg TID v. 200-600 mg QID v. placebo control	6	8 weeks	
M90-416	moderate asthma; 600 mg QID v. placebo control	77	5 weeks	
M88-175	prevention of allergen-induced constriction; cross-over; 800 mg v. placebo control	12	1 day (single dose)	spirogram, LTE excretion

***** Studies to be reviewed

4.0 EFFICACY/SAFETY ISSUES

4.1 SAFETY

Zileuton causes elevated liver enzymes in human subjects, 2% with ALT elevations $\geq 3XULN$ and 0.5% $\geq 8XULN$ [414:18]. This compares with 0.1% of placebo treated patients with ALT $\geq 3XULN$ and none $\geq 8XULN$ [414:25]. The total number of patients with ALT's $> ULN$ is not stated in the integrated summary of benefits and risks. In about 70% of patients who will experience elevated LFT's, the elevations occur within about 2 months of starting the drug [414:18]. Spontaneous resolution occurs in about 30% during continued treatment with zileuton, but one patient did develop severe liver injury and jaundice.

Drug Safety evaluation of zileuton revealed prostatic and testicular atrophy in dogs associated with high (>100 mg/Kg) daily doses [1:137, 144]. No testicular atrophy was found during the recovery period, and most of the prostatic atrophy had reversed [414:37]. Zileuton racemate, and its enantiomers, bound with weak affinity *in vitro* to soluble androgen receptor from prostate glands of dogs, rats, monkeys and man. The highest affinity was found in dog prostate. Androgen receptor affinity was 91-fold weaker in humans than in dogs, which is thought to protect humans from the prostatic and testicular atrophy that was found in canines [1:137].

A statistically significant increase in hepatic adenomas and combined adenomas/carcinomas was observed among female mice administered 450 mg/Kg/day [1:151, 414:31]. Although not statistically significant, an increased incidence of kidney tubular adenomas and adenocarcinomas was also observed for female mice, at the same dose and in rats, of both sexes, at 170 mg/Kg/day [1:151, 414:32]. Mostly ovarian hemangiosarcomas were also observed in female mice, at a dose of 450 mg/Kg/day [1:151, 414:35]. A statistically significant, dosage-related increase in benign Leydig cell

tumors was observed in the testes of male rats [1:152].

One drug interaction study was done with terfenadine and showed no interactive effects. A reviewer noted that the mechanism of action of zileuton, cytochrome inhibition, made it likely that it might cause Torsades if given with terfenadine. This brought closer scrutiny of the study and it was noted that the ECG's were sampled only at a time that represented the trough effect of zileuton. The company was encouraged to redo the study with ECG's sampled at more appropriate intervals. By phone conversation with CSO Buccine, the company estimates that this second study will be complete and submitted to this agency by November of this year.

5.0 LABELLING ISSUES

- 1.
2. Should it be approved for both "prevention" and for "control" of asthma?
3. How realistic is the LFT surveillance of once per month, for 3 months and then periodically?
4. What to do if LFT elevations occur and how high must they be to trigger some clinical change in patient care?
5. How are the drug interactions to be handled in the labelling?
6. What kind of labelling will be required for the neoplasms associated with early animal studies?
7. Do the data support approval of the

6.0 PROPOSED STUDIES FOR AUDITING

Studies chosen for audit were selected from two IND submissions of the two Phase III pivotal trials. Trial M91-685 was reported, in full, as Serial #246 submitted 2 June 1994. Appendix B.4.13 lists each investigator, the number of patients processed under each arm of the study and the mean percent change from baseline for that arm [193:77-9]. Two investigators with the greatest effect on the cumulative experimental result were chosen for DSI audit. These are Lazarus and Pleskow. Trial M92-720 was also fully reported, as Serial #250 submitted 17 June 1994. Appendix B.4.12 has a comparable listing [249:71-3]. For this pivotal trial, the two investigators selected for audit are Fink and Seltzer.

7.0 PROPOSED REVIEW PLAN

The review will begin with a careful look at the Integrated Summary of Safety and then proceed to the analysis of individual experiments. From the start, this NDA review will be assembled as a set of notes on the computer which will be, finally, crafted into the final report.

The studies that are marked with asterisks in the Table under 3.2 above will be most thoroughly scrutinized. The two pivotal Phase III trials, M91-685 and M92-720, will be reviewed first. They both use doses of 600 mg QID and are placebo controlled. They differ primarily because the former is a 13-week study and the latter lasts for 26 weeks. Next, M90-460 will be examined. It is a short (1 month) study and employs a longer dosing interval (800 mg BID) compared with 600 mg QID and placebo.

The next trials to be reviewed will be those that examine the local lung anti-inflammatory action that is attributed to zileuton, M91-597 and M91-654. Next will come reviews of specific

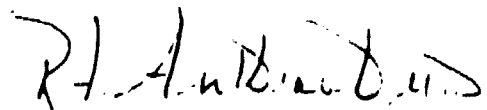
diagnostic entity treatment trials, M91-615 for exercise-induced bronchoconstriction and M92-724 for use in the prevention of nocturnal asthma. Last, from this group, will be a review of M92-797 which compares zileuton to inhaled beclomethasone.

Zileuton is similar to theophylline in the number of drug-drug interactions that it displays. A group of studies have systematically examined these interactions and all will be reviewed last. These include the following study designations and the interacting drugs [1:50-1]

<u>STUDY DESIGNATION</u>	<u>INTERACTING DRUG</u>
M88-265	theophylline
M90-462	antipyrine
M90-541	warfarin
M90-543	oral contraceptives
M91-617	prednisone
M92-789	propranolol
M92-820	phenytoin
M92-821	digoxin
M93-054	terfenadine

8.0 CONCLUSION

All of the elements necessary to perform a complete review are either present or promised. Therefore, there is no contraindication to filing this NDA.



Raymond F. Anthracite, M.D.
Medical Review Officer

cc
NDA #20-471
HFD-150\Division Files
HFD-150\Group Leader\Jenkins
HFD-150\Reviewer\Anthracite
HFD-150\Biopharmacologist\Sista
HFD-150\Pharmacologist\Sun
HFD-150\Chemist\Leak
HFD-150\CSO\Buccine

Statistical Review and Evaluation

NDA #: 20471
Applicant: Abbott Laboratories
Name of Drug: (zileuton) Tablets
Indication: Treatment of Asthma
Documents Reviewed: Volumes 1.1, 1.435-1.549, 1.617-1.619 dated July 19, 1994, an unnumbered volume (including data files) dated September 9, 1994, and an unnumbered volume dated January 5, 1995

This review pertains to the review of two Phase III studies of 600 mg Zileuton tablets compared to placebo in the treatment of asthmatics.

The medical officer for this submission is R. Anthracite, M.D., HFD-150, with whom this review was discussed.

I. Background

Zileuton is a 5-lipoxygenase inhibitor. 5-lipoxygenase is the first dedicated enzyme in the leukotriene synthesis pathway. Leukotrienes, the end products of this pathway, have been implicated in the pathophysiology of numerous disease processes, including asthma.

This reviewer requested data files for these two studies in a telephone conversation with the sponsor. The data files were supplied in the sponsor's September 9, 1994 submission.

This reviewer requested that the sponsor provide for each study a table comparing treatment groups using a representative set of analyses to assess how baseline severity affected treatment comparisons. The sponsor had provided some analyses in their submission for the three severity subgroups [mild airway obstruction ($FEV_1 > 70\%$), moderate airway obstruction ($50\% \leq FEV_1 \leq 70\%$) and severe airway obstruction ($FEV_1 < 50\%$)] and these tables gave the impression that most efficacy was occurring in the severe subgroup. This reviewer requested a table to help better decide whether efficacy was being shown in all subgroups, only the most severe, or whether results were mixed. The sponsor provided these tables in their January 5, 1995 submission.

II. Study Design and Method of AnalysisA. Study M91-685

This was a multi-center, randomized, parallel group study with a 13 week treatment period and a 10 day placebo lead-in period comparing 600 mg zileuton, zileuton and placebo QID in patients with mild to severe asthma (FEV₁ of 40 to 80% of predicted normal). To enter the study patients had to exhibit a 15% increase in FEV₁ after inhaled beta-agonist use. Patients on stable inhaled nasal steroids could continue their inhaled nasal steroid if the dose had remained stable for greater than or equal to 6 weeks. Patients on stable doses of allergen immunotherapy (allergy shots) could be entered into the study.

To be eligible for entry into the double-blind phase of the trial the patients had to have taken 75% of their study medication during the lead-in phase and completed the patient diary in an acceptable manner. They had to have an FEV₁ of 40 to 80% of predicted normal, had a total symptom score on the Daily Symptom Assessment scale of at least 10, but no greater than 25 during the 10 day lead-in period, and must have used the supplied beta-agonist less than 20 times during this period.

Randomization was done within a center by blocked randomization with a block size of 6, every treatment represented twice.

Clinic visits were scheduled at the first week of treatment and every two weeks after that.

PFTs were performed prior to the morning dose and serially over the six-hour period at Double-Blind Days 1, 8, 36, 64 and 92. These serial PFTs were prior to dosing and at 30, 60, 120, 180, 240, 300 and 360 minutes post-dosing.

On Double-Blind Days 22 and 50 study medication was taken by the patient before coming to the clinic visit. On these dates only one PFT was performed. [These results will not be discussed here because they are not a trough assessment.]

On Single-Blind Day 1 and Double-Blind Day 78 the investigator performed a response to beta-agonist Inhalation Test. On Single-Blind Day 1, PFTs were performed prior to inhalation of the beta-agonist and at 30, 60, 120, 180 and 240 minutes post beta-agonist inhalation. On Double-Blind Day 78, a PFT was performed immediately prior to study drug administration. Then two hours after dosing the study medication, a second series of PFTs were performed, prior to beta-agonist administration and at 30, 60, 120, 180 and 240 minutes post beta-agonist administration.

Ventolin MDIs were provided to all patients to be used as needed. Patients were instructed to refrain from using Ventolin at least 8 hours prior to each study visit.

The patients used daily diaries to list their PEFr values (taken

by Mini-Wright peak flow meters) and made Daily and Nocturnal Symptom Assessments. The PEFs were performed twice daily. The first (AM PEF) was prior to the first dose and the evening PEF was performed two hours after the third dose of the study medication. Thus the AM PEF is a trough assessment whereas the evening PEF is a peak assessment.

The Daily Symptom Assessment was made in the evening and was an assessment reflective over the waking hours. It used a 4-point scale:

0=No symptoms, unrestricted activity.

1=Symptoms occurred but with little or no discomfort, unrestricted activities.

2=Symptoms occurred, were somewhat annoying or affecting routine activities.

3=Symptoms severe-very little improvement after inhaler use and requires the use of additional medication and/or doctor's visit.

The Nocturnal Symptom Assessment was made in the morning and used a 4-point scale:

0=Slept well-no asthma symptoms.

1=Spent restless night, and may have awakened, because of asthma symptoms. May have used inhaler.

2=Awakened more than once because of symptoms; Inhaler used.

3=Awake all night because of symptoms. Inhaler used.

The patients also assessed wheezing, shortness of breath, cough and nasal congestion every evening using a 4-point scale:

0=Absent -No symptoms.

1=Mild -Symptom is frequently troublesome, but not sufficient to interfere with normal daily activity or sleep.

2=Moderate -Symptom is sufficiently troublesome to interfere with normal daily activity or sleep.

3=Severe -Symptom is so severe as to prevent normal activity and/or sleep.

The patients also recorded in their diary their beta-agonist usage (number of puffs). The investigator recorded whether the patient had an acute exacerbation of asthma since the previous visit.

Compliance with study medication was monitored by tablet count for all patients and plasma concentration of zileuton for some patients.

An Asthma Quality of Life Questionnaire, developed by Juniper et al, was given during the screening visit and at Double-Blind Days 36 and 92. It had 32 questions within 4 domains: activity limitations (11 items), symptoms (12 items), emotional function (5 items) and exposure to environmental stimuli (4 items). Responses were on a 7 point Likert Scale. A 0.5 difference from baseline in a domain average is considered to be a clinically significant

difference.

The non-diary baseline values were determined at the end of placebo lead-in just prior to first dose of study medication. The diary baseline were values obtained by averaging over the 10 day lead-in period.

The analyses were done using a two-way Analysis of Variance with effects of treatment, investigators and treatment by investigator interaction. The sponsor used SAS type III sums of squares to test treatments which gives equal weight to all centers.

The sponsor states that the primary analysis would be an analysis of percent change from baseline to the pre-dosing measurement at the patient's "final" treatment visit. Centers not having two patients on each treatment group were pooled for this analysis. This pooling was used for all the other analyses.

B. Study M92-720

This study was the same as Study M91-685 with the following exceptions: It had a 25 week double-blind period rather than a 13 week period. Double-blind clinic visits were at Double-Blind Day 8 and every 28 days after that. Serial PFTs were done throughout 6 hours on Double-Blind Days 1, 92 and 176. PFTs were performed prior to dosing at Double-Blind Days 8, 36, 64, 120 and 148. The PFTs performed to evaluate how zileuton affects beta-agonist use were done at Single-Blind Day 1 and Double-blind Day 148. No quality of life assessments were solicited.

III. Results

There were 488 patients entered into Study M91-685 of whom 401 entered the double-blind portion of the study (132 on 600 mg zileuton, 134 on 300 mg zileuton and 135 on placebo). There were 36 centers with the largest center having 26 patients (21 in the double-blind portion). Eighty-one (20.2%) of the patients who entered the double-blind portion discontinued prematurely from the study. There were 26 patients (4 on 600 mg zileuton, 7 on 300 mg zileuton and 15 on placebo) who dropped out for lack of efficacy or for lack of efficacy and adverse reaction. There were 40 patients (12 on 600 mg zileuton, 12 on 300 mg zileuton and 16 on placebo) who dropped out for adverse events or for adverse events and lack of efficacy. Only 4 patients (1 on each of the zileuton treatments and 2 on placebo) dropped out only for lack of efficacy.

The treatment groups were comparable in demographic variables and baseline efficacy variables in Study M91-685 with the exceptions of weight where the placebo group was heavier than the other two groups (mean weights were 162.6 lbs, 158.8 lbs and 169.1 lbs for zileuton 600 mg, zileuton 300 mg and placebo, respectively) and of

baseline FEV₁, where the mean values were 2.35 liters, 2.12 liters and 2.26 liters for 600 mg zileuton, zileuton and placebo, respectively. The difference in weight is unlikely to have much effect on efficacy. The difference in baseline FEV₁, could affect efficacy.

There were 456 patients entered into Study M92-720 of whom 373 entered the double-blind portion of the study (125 on 600 mg zileuton, 126 on zileuton and 122 on placebo). There were 34 centers with the largest center having 26 patients (18 in the double-blind portion). One hundred and one (27.1%) of the patients who entered the double-blind portion discontinued prematurely from the study. There were 25 patients (7 on 600 mg zileuton, 3 mg zileuton and 15 on placebo) who dropped out for lack of efficacy or for lack of efficacy and adverse reaction. There were 44 patients (13 on 600 mg zileuton, 15 zileuton and 16 on placebo) who dropped out for adverse events or for adverse events and lack of efficacy. Only 7 patients (4 on 600 mg zileuton and 3 on placebo) dropped out only for lack of efficacy.

The treatment groups were comparable in demographic variables and baseline efficacy variables in Study M92-720 with the exceptions of Daily and Nocturnal Symptom Assessments. The mean of the baseline Daily Symptom Assessments were 1.38, 1.44 and 1.30 for 600 mg zileuton, zileuton and placebo, respectively. The mean of the baseline Nocturnal Symptom Assessment were 0.94, 0.97 and 0.78 for 600 mg zileuton, zileuton and placebo, respectively.

The compliance, as measured by tablet counts, was good in these studies. The compliance rate was about 75% in both studies.

The sponsor only provided analysis of variance tables for the primary efficacy analysis, final percent change in FEV₁, in which the treatment by center was provided. There was no indication of treatment by center interaction in this analysis in either study. With the large number of centers in these studies, no individual center can influence the efficacy assessments to a great extent.

Table 4.1 presents the results of the analyses of percent changes from baseline to pre-dose values of FEV₁ (trough values) at the days of clinic visits (except Double-Blind Day 1 to be discussed later) in both studies. In Study M91-685 significantly more improvement in FEV₁, than placebo was seen for the 600 mg dose of zileuton at Double-Blind Days 8, 92 and the final (endpoint) analysis. The results at Day 36 were only nearly significant (0.05<p<0.10). The results at Days 64 and 78 were not significant. In Study M92-720 significantly more improvement in percent changes in FEV₁, than placebo was seen for the 600 mg dose of zileuton at Double-Blind Days 8, 36, 92, 120, 148 and 176 and final (endpoint) analysis. The results at Day 64 were only nearly significant (0.05<p<0.10). In particular the comparison of 600 mg zileuton and

placebo for final (endpoint), which had been stated to be the primary efficacy analysis, was significant in both studies. The only significant difference in improvement in percent changes in FEV₁ from placebo for the dose of zileuton was seen at Double-Blind Day 8 in Study M92-720 although the comparisons with placebo at some of the days were nearly significant (0.05<p<0.10). The results for the analyses of changes from baseline in FEV₁ were similar to those of the percent changes from baseline. The analyses for FVC (not presented here) only showed sporadic significant results.

The sponsor provided results from an analysis of covariance of trough percent change in FEV₁ using baseline FEV₁ as covariate and got essentially the same results as Table 4.1. Therefore, the baseline differences in FEV₁ in Study M91-685 were not the cause of the significance seen in percent changes in FEV₁.

Table 5.1 presents the p-values comparing treatments for the serial FEV₁ measurements at the clinic visits (except for Double-Blind Day 1 to be discussed later) when these serial measurements were taken. Zileuton 600 mg was significantly better than placebo for the average of the 2-4 hour assessments for all days in Study M91-685 except Double-Blind Day 64 and for Double-Blind Day 92 in Study M92-720 (the results on Double-Blind Day 176 were only nearly significant). Some significant differences from placebo were seen at 30 and 60 minutes for the 600 mg dose of zileuton in both studies. Only a few sporadic significant differences from placebo were seen for the

Table 7.1 presents the results of the analyses of the serial FEV₁ values at Double-Blind Day 1 for both studies. The 600 mg dose of zileuton was significantly different from placebo for the averages of 2-4 hour percent changes from baseline of FEV₁ in both studies. was showing significant differences from placebo also for the average of 2-4 hours in Study M92-720.

Tables 8.1 and 8.2 contain biweekly means (Study M91-685) and monthly means (Study M92-720) and p-values comparing treatments for changes from baseline for AM and PM PEFrs. Significant differences from placebo were seen for most time intervals for both AM and PM PEFr for the 600 mg dose. The only significant difference observed for th was for PM PEFr at Double-Blind Days 1-15 in Study M91-685.

Table 10 presents the results for the analyses of Daily and Nocturnal Symptom Assessments. The 600 mg dose of zileuton was significantly better than placebo for the Daily Symptom Assessment for almost all time intervals in both studies. The 600 mg dose of zileuton was significantly better than placebo for the Nocturnal Symptom Assessment for all time intervals in Study M92-720 and about half the time intervals in Study M91-685. dose of

zileuton was significantly better than placebo for Daily and Nocturnal Symptom Assessments for almost all the time intervals in Study M92-720 but none in Study M91-685.

Significant differences from placebo were seen in most of the time intervals for both doses of zileuton for shortness of breath and wheezing and Daily Number of Puffs of Ventolin in Study M92-720. In Study M91-685 only the 600 mg dose was significantly better than placebo (about half the time intervals) for shortness of breath and Daily Number of Puffs of Ventolin.

No significant differences from placebo were seen in the PFT assessments done out to 240 minutes after taking beta-agonist (done two hours after they took their double-blind medication). Therefore, zileuton has not affected the benefits gotten by beta-agonist usage in the whole study population. Its effect if needed as rescue medication was not addressed in these studies.

Although the 600 mg dose of zileuton showed significant differences from placebo for the 4 domains of the quality of life assessments (Study M91-685), the difference was larger than 0.5 only for the emotions domain. The 0.5 mean difference would correspond to a clinically significant difference (minimally important difference) between treatments.

Efficacy was seen in both genders for the 600 mg Zileuton Q.I.D. dose. Numerically, males tended to show more efficacy than females in the pulmonary parameters whereas the females showed more efficacy than the males in symptom parameters.

Table 1 contains the mean changes from baseline for each of the important efficacy variables in Studies M91-685 and M92-720 for baseline & Predicted FEV₁ categories corresponding to mild, moderate and severe disease. Although much of the efficacy of Zileuton was seen in the severe patients, there was always some variables in which efficacy was suggested in the mild and moderately diseased patients. Sample sizes are not adequate to have sufficient power to detect statistically significant differences between treatments in severity subgroups.

IV. Reviewer's Comments

Although there are two zileuton treatments in these studies, the sponsor did not adjust the p-values for the multiple comparisons. The sponsor stated that the 600 mg zileuton vs placebo was primary and thus inferred that no adjustment was necessary. If a Bonferroni adjustment was made for multiple comparisons, the p-values for the 600 mg zileuton and placebo comparisons would usually still be significant. zileuton vs placebo comparisons were usually not significant. Therefore, the 600 mg zileuton QID dose is

the effective dose.

V. Overall Conclusions

The 600 mg zileuton dose QID was significantly more effective than placebo in trough FEV₁ at endpoint (final visit) in both studies (the primary efficacy analyses) ($p < 0.03$ in both studies) and at most days of clinic visits. This efficacy was also seen in AM and PM PEFR and in Daily and Nocturnal Symptom Assessments at most of the pre-assigned time intervals. Efficacy was also demonstrated for shortness of breath assessment and number of puffs of back-up ventolin.

Adequate evidence of efficacy was not demonstrated for dose of zileuton QID.



James R. Gebert, Ph.D.
Mathematical Statistician HFD-713

Concur: Dr. Wilson *SEW' 2/22/95*

Dr. Dubey *6 2-22-95*

This review contains 8 pages of text and 22 pages of tables.

cc:

Orig NDA 20-471

HFD-150

HFD-150/Dr. Anthracite / *JENKINS*

HFD-150/Mr. Buccine

HFD-344/Dr. Lisook

HFD-713/Dr. Dubey [File 1.3.2 NDA]

HFD-713/Dr. Gebert

HFD-713/Dr. Wilson

BIO Review

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 20,471

Submission Date: March 22, 1996

Drug Name, Dose and Formulation: Zilutrol (Zileuton) tablets, 300 and 600 mg strengths

Sponsor: Abbott Laboratories, Abbott Park, Illinois - 60064

Reviewer: Venkata Ramana S. Uppoor, Ph.D.

Type of Submission: Response to comments in non-approval letter

ISSUE: In this submission, the sponsor responded to several comments and deficiencies that were sent to them through the non-approval letter. Three comments were sent by the Biopharmaceutics' reviewer. The sponsor has responded to a) all these 3 comments along with b) the suggested labeling changes. The 3 comments (in bold), sponsor's response (in italics) and the reviewer's evaluation (in plain font) are discussed below.

a. RESPONSE TO COMMENTS IN NON-APPROVAL LETTER

1. The tablets obtained upon rework are not bioequivalent to non-reworked tablets. Therefore, the rework procedure is unacceptable.

It is agreed that the rework procedure is unacceptable. Zileuton tablets will not be reworked at this time. Should an acceptable rework procedure be developed in the future, the requisite information would be submitted for prior approval using the appropriate regulatory mechanism, e.g., "supplement to an approved application."

Reviewer's comment: Sponsor's response acceptable. Current rework procedure will not be used by the sponsor.

2. The dissolution method is acceptable. However, the dissolution medium is not physiological. Data indicate that there is poor in-vivo/in-vitro correlation and that the in-vitro dissolution does not accurately predict the pharmacokinetics (AUC and C_{max}) in humans. Hence, the proposed dissolution method is useful only for quality control purposes. Based on the dissolution data provided, the proposed dissolution specification is unacceptable and should be set at not less than Q in 45 minutes.

The specification for the dissolution of zileuton tablets was established based on results obtained from the definitive bioavailability study (Study F92-219, see NDA Volume 84 and NDA Volume 117, NDA 20-471, July 18, 1994) and label claim stability data. Lot 59-592-AR, used in this biostudy, would not meet SI acceptance criteria at the proposed specification of Q- in 45 minutes. Twelve run data are summarized in the following table (not shown in this review, refer to original NDA review). Results from the bioavailability study indicate acceptable performance for this lot, therefore, the current proposed specification of Q in 45 minutes is justified.

Reviewer's comment: Sponsor's response acceptable. Re-evaluation of the dissolution data from the biobatch and stability batches indicates that Q₁₀ would be adequate and is not likely to compromise the quality of the product. The mean % zileuton dissolved in 45 minutes (biobatch) Based on this data, setting a specification of Q₁₀ may prove to be quite difficult to meet. Hence, the sponsor proposed dissolution specification of Q₁₀ in 45 minutes is acceptable for Zileuton immediate release tablets.

3. The proposed marketed product is a bisected tablet. No comparative dissolution data on the 2 portions of the tablet are provided in the NDA.

Dissolution on two portions of a bisected tablet was performed and compared to the whole tablet. Dissolution profile data are shown in the table below. Results of each of the tablet strengths for half tablets compared to whole tablets are similar at all time points with less than 10% difference in the mean release at all time points except the 10 minute sampling time. Therefore, two half tablets are equivalent in performance to the whole tablet.

Tablet strength	% label claim dissolved (standard deviation)				
	10 min	20 min	30 min	45 min	60 min
300 mg (whole)					
300 mg (half)					
600 mg (whole)					
600 mg (half)					

Reviewer's comment and discussion: The sponsor's response is acceptable. Since this is a scored tablet, dissolution data on the 2 portions of the bisected tablet is required. As expected, due to increase in surface area, the dissolution rate is faster on the half tablets than whole tablets. However, this increase is less than 10% (of label claim dissolved). This data also meets the dissolution specifications set for the whole tablet.

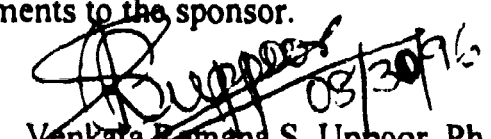
Comparison of the dissolution profiles of the whole tablet and the half tablets using the F₂ equation, to test the similarity of 2 curves, indicates that the dissolution profiles of whole tablet and the half tablets are similar (reviewer computed values). This is indicated by the F₂ metric being greater than 50 (see calculations below). F₂ values of 50 and greater shows that the dissolution is similar for whole and half tablets. Hence, this information submitted by the sponsor is acceptable.

Page
Purged

2. Delete figure 1 on page 3, since the graph showing zileuton plasma concentration and LTB₄ inhibition does not provide any additional useful information. There was no direct relationship established between LTB₄ and FEV₁. Hence, this figure does not provide useful information to the prescribing physician.
3. On page 3, in the first paragraph under Pharmacokinetics, remove the word 'hepatic' from the sentence 'elimination of zileuton is via hepatic metabolism'. The metabolism could be either due to hepatic or gut metabolism. It is not known whether zileuton elimination is via hepatic metabolism alone.
4. Under absorption, on page 3, remove 'essentially completely absorbed' from the sentence 'Zileuton is rapidly and essentially completely absorbed after oral administration'. This sentence should be changed to 'Zileuton is rapidly absorbed after oral administration'. Since an absolute bioavailability study has not been conducted, the phrase, essentially completely absorbed is misleading.
5. On page 4, remove the sentence, 'Different dose strengths of the drug are therapeutically bioequivalent'. This is not accurate.
6. On page 5, under hepatic insufficiency, include information to indicate that zileuton unbound plasma concentrations increased 4 fold in mild to moderate hepatic impairment.
7. Please include the dosing recommendations for hepatically impaired patients in the DOSAGE AND ADMINISTRATION section.
8. Under drug interactions with Prednisone, the dose of prednisone must be corrected to be 40 mg single dose.

RECOMMENDATION

This submission has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics. The sponsor's responses to the Biopharmaceutics' comments in the non-approval letter are acceptable. Please forward the labeling comments to the sponsor.


Venkata Ramana S. Uppoor, Ph.D.
Division of Pharmaceutical Evaluation II

FT Initialed by Dale Conner, Pharm.D. AKC 5/5/96

CC list:

HFD-570: NDA 20,471; HFD-570: Division file; HFD-570: CSO\Betty Kuzmik;
HFD-570: Medical Reviewer\Ray Anthracite; HFD-570: Chemist\John Leak;
HFD-570: Pharmacologist\Joe Sun; HFD-870: Dale Conner; HFD-870: John Hunt;
HFD-870: ChenMe; HFD-850: Biopharm\Lesko; HFD-870: Chron; HFD-870: Drug;
HFD-870: Venkata Ramana S. Uppoor; HFD-340: Viswanathan; HFD-205: FOI.

DEC 7 1995

OFFICE OF CLINICAL PHARMACOLOGY & BIOPHARMACEUTICS REVIEW

NDA 20,471

Submission Date: JUNE 16, 1995

Drug Name, Dose and Formulation: Leutrol[®] (Zileuton) Tablets, Zileuton 300 and 600 mg tablet strengths

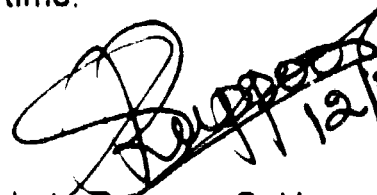
Sponsor: Abbott Laboratories, Abbott Park, Illinois

Reviewer: Venkata Ramana S. Uppoor

Type of Submission: Revised labeling

The NDA for zileuton immediate release tablet was reviewed by Division of Biopharmaceutics and was found to be acceptable. Several review and labeling comments were made in the review. In response to our request to reformat the labeling to conform to the suggested format issued by the Division, the sponsor sent in the revised labeling in this submission.

On October 19, 1995, the sponsor was sent a non-approval letter for this NDA. Hence this labeling submission will not be reviewed at this time. Revised label has to be reviewed when the sponsor submits a response to non-approvable letter along with any other studies that may be provided at that time.


12/07/95

Venkata Ramana S. Uppoor, Ph.D.
Division of Pharmaceutical Evaluation - II

Initialed by Dale P. Conner, Pharm. D.  12/7/95

CC list: HFD-570: NDA 20,471; HFD-570: Division file; HFD-570: CSO Joe Buccine; HFD-570: Medical Reviewer\Ray Anthracite; HFD-870: Biopharm\Dale Conner; HFD-870: Biopharm\John Hunt; HFD-870: Biopharm\ChenMe; HFD-860: Biopharm\Malinowski; HFD-880: Biopharm\FleischerN; HFD-850: Biopharm\Lesko; HFD-850: Biopharm\Chron; HFD-850: Biopharm\Drug; HFD-850: Biopharm\Venkata Ramana S. Uppoor; HFD-340: Viswanathan; HFD-205: FOI

BIOPHARMACEUTICS / PHARMACOKINETICS' REVIEW

NDA 20-471 amendment

Submission Date: June 16, 1995

Drug Name, Dose and Formulation:

(Zileuton) Tablets, Zileuton 300 and 600 mg tablet strengths

Sponsor: Abbott Laboratories, Abbott Park, Illinois

Reviewers: Venkata Ramana K. Sista, Ph.D

Type of Submission: Response to FDA request for information

*This comment requires
transmittal. It
was rec'd after
NA ltr 4/17/95*

BACKGROUND:

Zileuton is a leukotriene antagonist developed by the sponsor as an anti-inflammatory bronchodilator agent for chronic use in bronchial asthma to be administered four times a day. The NDA is currently under review. Based on the dissolution data from biobatches in the NDA, the Division of Biopharmaceutics has set dissolution specifications for this product. The dissolution

CONCERN OF CHEMISTS:

Dissolution data from production batches manufactured in 1992/1993 seems to be faster than those manufactured prior to that which have been included in the NDA. Hence this was consulted to Biopharmaceutics to evaluate the data and comment on the need for tightening the dissolution specifications.

RESULTS:

Following table shows the dissolution data from the 1992/1993 batch and the ones used in setting the dissolution specifications:

Time in minutes	Mean % dissolved			
	300 mg current batch (lot # 76-296-AR)	300 mg biobatches	600 mg current batch (lot # 66-433-AR)	600 mg biobatches
10				
20				
30				
45				
60				

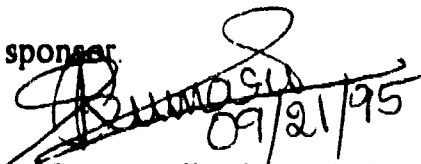
COMMENTS:

1. We agree that dissolution rate of tablets manufactured in 1992/1993 appears to be faster than those submitted in the NDA. However, this has occurred only in the 300 mg tablet strength and not the 600 mg strength.
2. Based on these results, no changes in the dissolution specifications are necessary at this time. The specifications should remain as Q = in 45 minutes.
3. Results (faster dissolution) from one batch of each strength (1992/1993 batch) are not sufficient to re-evaluate the dissolution specifications. Since the dissolution data for this product did not correlate with the in vivo performance of the product, the dissolution method is useful only for quality control purposes. Data from the new production batches indicate faster dissolution. Hence we request the sponsor to provide the agency with dissolution data from their next 3 production batches for both the tablet strengths. We will reevaluate the dissolution data for the ~~adequacy~~ ^{adequacy} of the dissolution specifications set at this time.

RECOMMENDATION:

The Division of Biopharmaceutics has reviewed the dissolution data from this submission. The dissolution specifications set by the agency are Q in 45 minutes. The sponsor must ~~also~~ provide dissolution data from their next 3 production batches for both tablet strengths to the agency for evaluation.

Please forward this recommendation and comments to the sponsor.


09/21/95
Venkata Ramana K. Sista, Ph.D.
Pharmacokinetic Evaluation Branch I

FT initialed by Mehul U. Mehta, Ph.D.

mm 9/21/95

cc HFD-155: NDA 20-471
 HFD-155: Division file
 HFD-155: JBuccine
 HFD-155: RAnthracite
 HFD-155: JLeak
 HFD-155: Chong Ho Kim
 HFD-426: NFleischer
 HFD-426: MMehta
 HFD-426: MChen
 HFD-426: Chron
 HFD-426: Drug; HFD-426: Reviewer (VRKSista); HFD-340: Viswanathan
 HFD-19: FOI

AUG 23 1995

BIOPHARMACEUTICS / PHARMACOKINETICS' REVIEW

NDA 20-471

Submission Date: July 18, 1994; October 21, 1994; March 29, 1995

Drug Name, Dose and Formulation: (Zileuton) Tablets, Zileuton 300, and 600 mg tablet strengths

Sponsor: Abbott Laboratories, Abbott Park, Illinois

Reviewers: Venkata Ramana K. Sista, Ph.D., Bradley Gillespie, Pharm.D., Ene Ette, Ph.D.

Type of Submission: New Drug Application, NME 1S

SYNOPSIS: Zileuton, the first of the new class of 5-lipoxygenase inhibitors is developed by the sponsor as an bronchodilator agent for chronic use in bronchial asthma to be administered four times a day.

The NDA contains several pharmacokinetic and clinical studies. The pharmacokinetics section of this NDA is exhaustive with several studies, some that may not have been necessary if properly planned.

28 in-vivo studies and 9 in-vitro studies have been reviewed. Several studies have not been reviewed either because the studies were done with formulations that are not to be marketed or because they will not be manufactured or sold in United States or were not providing any additional information than those studies reviewed. 12 drug interactions studies were provided in this NDA. Of these a total of 10 have been reviewed (antipyrine and 1 of 2 terfenadine interaction studies were not reviewed).

The sponsor has adequately validated the assay for zileuton and its metabolites. The sponsor also adequately characterized the pharmacokinetics (single and multiple dose) of zileuton in healthy volunteers, hepatically impaired patients and renally impaired patients. Pharmacokinetics were also studied in elderly subjects, and in asthmatic patients (both adult and pediatric patients). Effect of gender and race has been studied. The metabolite profile and metabolic enzymes (cytochrome P450 isozymes) responsible for zileuton metabolism have been identified. About 95% of the zileuton dose administered is excreted as metabolites in urine and about 2% in feces. Practically no unchanged drug is found in urine. About 80% of dose is excreted as glucuronides. Zileuton is metabolized primarily to glucuronides by glucuronyl transferases. Minor metabolic pathway is mediated by cytochrome P-450 isozymes 3A4, 1A2 and 2C9. Zileuton is practically insoluble in water, hence absolute bioavailability information on the tablets is not available. Bioavailability of tablets relative to suspension is about 94%. Zileuton is absorbed rapidly with a t_{max} of about 2 hours. The drug has a short half-life of about 2.5 hours. It is highly protein bound

(92-94%) and binds to both albumin and α_1 -acid glycoprotein. The dispositional pharmacokinetics of zileuton are dose-proportional over a dose range of 200 - 800 mg. Dose-proportionality is clear with respect to AUC but a slight non-linearity was seen with respect to C_{max} . Food increased C_{max} of zileuton by 27% and AUC by 1.4%. Age, gender and race do not seem to effect the pharmacokinetics of zileuton. Weight adjusted pharmacokinetic parameters in pediatric asthmatic patients were similar to the pharmacokinetic parameters determined in adults. Zileuton pharmacokinetics exhibit a diurnal variation with higher C_{min} values in the morning (after night dosing). In mild and moderate hepatic impairment, zileuton unbound AUC increased by 2 and 4-fold following administration of 400 mg t.i.d. In renal impairment, there was no change in zileuton pharmacokinetics, however, accumulation of glucuronides in plasma was observed. Pharmacokinetics of zileuton were similar in asthmatic patients and normal volunteers. The pharmacokinetics of zileuton have been correlated to the pharmacodynamic surrogate marker (leukotriene B₄ (LTB₄) inhibition) using a sigmoid E_{max} model.

Several formulations were used during zileuton drug development and the ones used in pharmacokinetic studies and clinical trials have been linked via bioequivalence studies. 200 mg capsule I and II are bioequivalent to each other (C.I. on AUC = 0.943-1.091, C.I. on C_{max} = 0.925-1.164). 600 mg final tablets manufactured at 2 manufacturing sites when compared to 300 mg final tablet in a bioequivalence study showed a C.I. on AUC of 1.029-1.141 (for site Abbott Park (AP)) and 0.949-1.052 (for site Puerto Rico (PR)), while the C.I. on C_{max} was 0.996-1.300 for AP and 0.964-1.258 for PR. 400 mg final tablets manufactured at AP and PR sites are bioequivalent to 200 mg capsule II and to each other. The 300 mg final tablets AP and PR when compared to 200 mg capsule II showed a C.I. on AUC of 0.932-1.025 for AP and 0.963-1.059 for PR, while the C.I. on C_{max} was 0.970-1.212 for AP and 1.030-1.287 for PR. Although C_{max} C.I. in some cases exceeded the current bioequivalence criteria, it should be noted that the 600 mg tablets have been studied in pivotal clinical trials.

When administered concomitantly with zileuton, a 50% decrease in steady state clearance of theophylline was observed. Administration of zileuton with terfenadine resulted in a reduction in clearance of terfenadine by 22%. Coadministration of zileuton with warfarin resulted in a 15% decrease in clearance of R-warfarin and no effect on S-warfarin. Coadministration of zileuton with propranolol resulted in decrease of propranolol clearance of 42%. There was no drug interaction between zileuton and oral contraceptives, digoxin, prednisone, sulfasalazine, phenytoin and naproxen.

RECOMMENDATION: The present submission (NDA 20-471) has been reviewed by the Division of Biopharmaceutics. The submission is approvable provided that labeling comments # 1-8 and comment # 2 and 9 are adequately addressed by the sponsor. The sponsor is also encouraged to consider and incorporate the information suggested in comments # 1-6 in their future submissions. The tablets which are obtained upon rework are not bioequivalent and hence rework procedure is not acceptable as indicated in comment # 7. The dissolution specifications set by the agency as provided in comment # 8 should be used. Please forward comments 2, 7, 8 and 9 for sponsor's response and all other comments for their information.

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I. BACKGROUND

Leutrol[®] tablets contain zileuton which is a benzothiophene N-hydroxyurea derivative with potent and selective 5-lipoxygenase inhibitory activity. 5-lipoxygenase is an enzyme that converts arachidonic acid to 5-hydroperoxy-eicosa-6,8,11,14-tetraenoic acid (5-HPETE) in the pathway leading to production of leukotrienes. Inhibition of leukotriene synthesis has potential benefits in conditions in which leukotriene synthesis is elevated such as asthma,

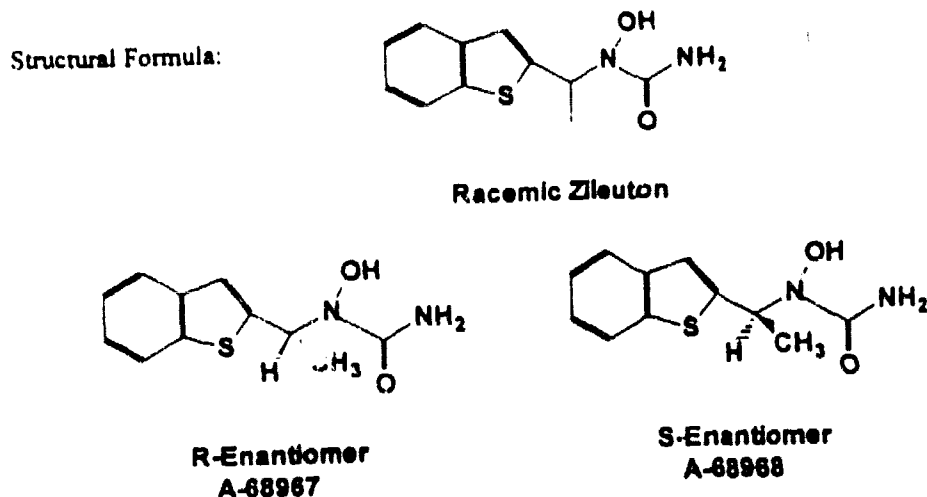
The sponsor has proposed to market the zileuton tablets in three dose strengths of 300, and 600 mg. All three proposed final tablet strengths are compressed from a single granulation and therefore are of the same composition, differing only in compressional weight.

PROPOSED INDICATIONS for Leutrol are for the prevention and control of symptoms associated with reversible bronchospasm and for maintenance therapy in the management of asthma patients with mild, moderate or severe airway obstruction.

PROPOSED DOSAGE: The recommended dosage of LEUTROL for the symptomatic treatment of patients with asthma is one 600 mg tablet (or two 300 mg tablets) four times a day for a total dose of 2400 mg. While maximal improvement in pulmonary function was produced by the 600 mg QID regimen, some patients may show clinical benefit at a dosage regimen of

STRUCTURE OF DRUG ENTITY: Zileuton is chemically N-(1-benzo[b]thien-2-ylethyl)-N-hydroxyurea with a molecular weight of 236.3. Zileuton contains a single chiral center and is administered as a racemic mixture (50:50) of the R(+) and S(-) enantiomers (structure shown in figure below).

FIGURE ①



SOLUBILITY CHARACTERISTICS: Zileuton is practically insoluble in water, the solubility in various aqueous media (at 37°C) is $172 \pm 1 \mu\text{g/ml}$ (water), $173 \pm 3 \mu\text{g/ml}$ (0.1N hydrochloric acid, pH 1), $176 \pm 4 \mu\text{g/ml}$ (0.1M acetate buffer, pH 5), $161 \pm 5 \mu\text{g/ml}$ (0.05M phosphate buffer, pH 6.8), and $162 \pm 2 \mu\text{g/ml}$ (0.05M phosphate buffer, pH 7.5).

II. FORMULATION: During the course of drug development, the sponsor changed formulations several times. In their initial studies, they utilized two different capsules (capsule I and II), then switched to tablets with several different experimental formulations of tablets before they finally arrived with the final to-be marketed tablet formulation. The details of the final tablet formulation and of the capsule I and II formulation are given below. It should be noted that the different strengths of final tablet formulation are compositionally proportional. Tablets are compressed from a single granulation and differ only in compressional weight.

a. **ZILEUTON FINAL TABLET FORMULATIONS (300, 600 MG TABLET STRENGTHS):** All the 3 (to-be marketed) strengths are compositionally proportional and only differ in tablet weight.

Table ①

INGREDIENT	SCALE (%)

b. COMPOSITION OF ZILEUTON 200 MG CAPSULE FORMULATIONS: Capsule I and II used during drug development (not to-be marketed).

Table 2

INGREDIENT	% OF COMPOSITION	
	CAPSULE I	CAPSULE II

III. Several studies have been submitted as part of the NDA, however, studies that are pivotal will be included in this review. List of studies reviewed and those not reviewed with reasons are provided below.

STUDIES REVIEWED: IN-VIVO STUDIES

Bioequivalence:

M89-315: Single dose bioequivalence study; absorption characteristics of zileuton from 200 mg tablet, capsule I and II, and suspension.

F92-216: Single dose bioequivalence study; evaluation of bioavailability of 300 mg FF tablets vs. 200 mg capsule II.

F92-218: Single dose bioequivalence study: evaluation of bioavailability of 7FF tablets vs. 200 mg capsule II.

F92-219: Single dose bioequivalence study: evaluation of bioavailability of 600 mg FF tablets vs. 300 mg FF tablet.

F92-226: Single dose bioequivalence study; evaluation of bioavailability of rework FF tablet FF tablet.

Food effect:

F92-222: Food effect study; evaluation of the effect of food on zileuton bioavailability from 600 mg FF tablet.

Single and multiple dosing:

M87-068: Single dose safety, PK and leukotriene B₄ inhibition of 200, 400, 600 and 800 mg doses of zileuton.

M88-173: Multiple dose 14 day; PK of zileuton following 200 QID, 400 TID and 800 BID administration.

M89-346: Multiple dose 14 day; PK of zileuton following 600 TID doses.

M89-366: Multiple dose 14 day; PK of zileuton following 600 QID doses.

F93-233: Single dose, dose-proportionality study; evaluation of dose-proportionality in PK of zileuton (200, 400, 600 and 800 mg) in normal healthy volunteers.

M83-034: Evaluation of the diurnal variation in PK of zileuton in normal volunteers following 600 mg FF tablet single dose administration.

M89-336: Metabolism and disposition of ¹⁴C zileuton in humans following a single oral dose.

M92-724: Zileuton concentration in human plasma and bronchoalveolar lavage fluid.

Special populations:

M90-529: Comparison of PK of zileuton between elderly and younger subjects.

M90-512: Evaluation of PK following single and multiple doses to subjects with varying degrees of renal function.

M90-503: Evaluation of PK following single and multiple doses to subjects with varying degrees of hepatic function.

M90-460: PK-PD of zileuton (600 mg QID and 800 mg BID for 28 days) in patients with moderate asthma.

M91-685: PK-PD of zileuton (600 mg QID for 3 months) in a phase III study in moderate asthma.

Drug interaction:

M88-218: Multiple dose Zileuton-sulfasalazine interaction study.

M88-291: Multiple dose zileuton-naproxen interaction study.

M88-265: Multiple dose zileuton-theophylline interaction study.

M91-617: Multiple dose zileuton-single dose prednisone interaction study.

M90-541: Multiple dose zileuton-warfarin interaction study.

M92-789: Multiple dose zileuton-single dose propranolol interaction study.

M92-820: Multiple dose zileuton-single dose phenytoin interaction study.

M92-821: Multiple dose zileuton-single dose digoxin interaction study.

M94-166: Multiple dose zileuton-terfenadine interaction study.

M90-543: Multiple dose zileuton-oral contraceptives study.

IN-VITRO STUDIES AND ANALYSIS

V87-043: In-vitro plasma protein binding of ¹⁴C-zileuton.

V90-022: Protein binding interactions between ¹⁴C-zileuton and selected other drugs in human plasma.

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 9
Submission date: 07/19/94

V90-036: In-vitro protein binding of [R]- and [S]- enantiomers of ¹⁴C-zileuton.

V93-021: In-vitro human red blood cell binding of ¹⁴C-zileuton and its N-dehydroxylated metabolite.

Rpt.#110: Identification of human Cytochrome P-450 enzymes involved in the metabolism of zileuton and its N-dehydroxylated metabolite.

V94-003: Effect of Terfenadine on the in-vitro protein binding of ¹⁴C-zileuton and effect of zileuton on the in-vitro protein binding of ³H-terfenadine in human plasma.

Rpt.#108: Meta-analysis of the PD relationships between the ex-vivo inhibition of LTB₄ in whole blood and zileuton plasma concentration from Phase I studies^o.

Report: Dissolution methods, profiles, and dissolution method validation studies.

Report: Analytical method development and validation studies.

Report: Population analysis and meta-analysis.

The following two studies F90-179 and F90-195 were previously reviewed by Dr. Hisham Abdallah (see Biopharmaceutics' review dated September 8, 1992).

F90-179: Single dose bioequivalence study between 600 mg tablet F1, F2 and 200 mg capsule II; this are not included here as the formulations used here are not the final to-be marketed forms and have not been used in any clinical studies.

F90-195: Single dose bioequivalence study between 300 mg tablet F1, F2, F2; 600 mg tablet F3, F4 and 200 mg capsule II; this was not included here as the formulations used here are not the final to-be marketed forms and have not been used in any clinical studies.

STUDIES THAT WERE NOT REVIEWED:

IV. PHARMACOKINETICS:

The summary of pharmacokinetics of the drug obtained from both capsules and tablets is provided here.

a. ABSOLUTE BIOAVAILABILITY (and RELATIVE BIOAVAILABILITY): Pharmacokinetics of the drug have not been determined following intravenous route of administration. As per the sponsor, zileuton could not be given by intravenous route because of low aqueous solubility. Hence, information on absolute bioavailability is not available. However, information from mass balance study where radiolabeled drug was administered indicates that approximately 95% of administered drug crossed the gastrointestinal barrier. Information on relative bioavailability can be obtained from bioequivalence studies where a suspension arm was included. It was found that bioavailability of the tablets relative to suspension was >94% based on AUC (study # F92-219). Another bioequivalence study (#M89-315) indicated the bioavailability of capsules relative to suspension to be about 88% based on AUC.

b. ABSORPTION: Following oral doses up to 800 mg (both via single and multiple administration) in healthy volunteers, zileuton was absorbed rapidly. The mean ($\pm$ SD) T_{max} across all formulations was 1.72 ± 0.94 (range of 0.5 - 3 hours following single dose) hours. C_{max} normalized to 100 mg dose across formulations was 0.83 ± 0.33 μ g/ml (range of 0.5 - 1.0 μ g/ml/100 mg zileuton dose). Mean $\pm$ SD of zileuton T_{max} and C_{max} at steady state after administration of the recommended dose of

600 mg QID to normal healthy volunteers are 2.3 ± 0.6 hours and 6.0 ± 1.0 $\mu\text{g/ml}$, respectively. The systemic exposure, AUC normalized to dose is approximately 3.2 $\mu\text{g}\cdot\text{hr/ml}$ per 100 mg single dose. Absolute bioavailability determination could not be made due to problems stated above. Relative to suspension, the estimated bioavailability of the 600 mg zileuton final tablet formulation is $>94\%$.

c. **DISTRIBUTION:** Apparent volume of distribution of zileuton (V_d/F) after administration of multiple dose administration of zileuton (600 mg QID) final tablet formulation was found to be 93 ± 19 L. Higher (almost two-fold) V_d/F was obtained from studies with capsules. However, this could be because of terminal phase being confounded by absorption, due to protracted absorption following capsule formulations. V_d/F obtained from studies after administration of suspension formulation was 94 L.

Plasma protein binding: Zileuton is 92-94% bound to plasma proteins as shown by determination of protein binding in-vitro by ultrafiltration technique at concentrations of 0.1 - 100 $\mu\text{g/ml}$. Zileuton binds to both albumin and α_1 -acid glycoprotein. At physiological concentrations of the proteins, zileuton is 88% bound to human serum albumin (40 mg/ml) and 34% bound to α_1 -acid glycoprotein (1 mg/ml). Zileuton has about 2.6 fold greater affinity to albumin than α_1 -acid glycoprotein.

Tissue distribution: There is no information on tissue distribution of drug in humans.

Distribution in bronchoalveolar lavage fluid (BALF): Both zileuton and its metabolite (Abbott-66193) appeared in BALF (highly variable levels). The mean ($\pm$ SD) concentration of zileuton were 0.142 ± 0.079 and 0.134 ± 0.113 $\mu\text{g/ml}$ in asthmatic patients and healthy normal volunteers. A statistically significant relationship between zileuton concentration in plasma and BALF was observed (slope = 0.029, $p < 0.005$, r-squared = 0.405). Although there seems to some relationship between concentrations of drug in plasma vs. BALF, it should be noted that the slope is quite different from asthmatics (0.017) to that of normal subjects (0.0521). However, this data can give preliminary information of drug availability in the pulmonary region.

d. **ELIMINATION (METABOLISM AND EXCRETION):**

Terminal phase half-life: Half-life of zileuton is approximately 2.5 hours (obtained from tablet data and suspension data). Data (terminal portion of the plasma concentration-time profile) from capsules was confounded by prolonged absorption.

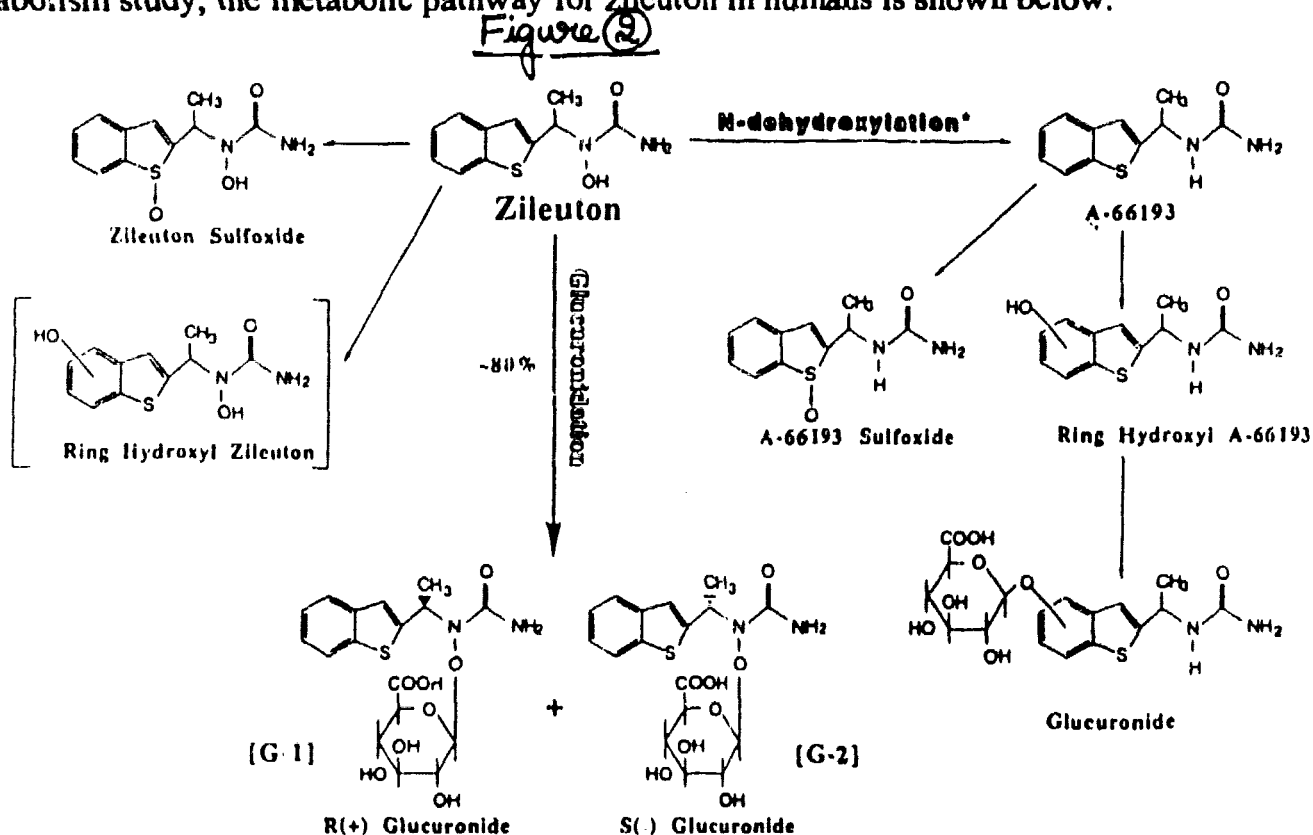
Metabolism: Zileuton is highly metabolized and is subject to first pass metabolism. Practically no unchanged drug ($<0.5\%$) is found in urine upon oral administration. Major metabolites of zileuton found in human urine are about equal amounts of R(+) and S(-) zileuton glucuronides (about 80% of zileuton dose), also called as G-1 and G-2 glucuronides. N-dehydroxylated derivative of zileuton, Abbott-66193 accounted for $<0.5\%$ of the dose (N-dehydroxylation is shown in rats to be caused by intestinal bacteria). Polar materials and other metabolites (were not identified) represented about 8 and 0.4% of the dose, respectively.

Glucuronosyl transferase(s) responsible for conjugation of zileuton have not been identified. In-vitro studies were conducted to identify the hepatic cytochrome P450 systems responsible for metabolism of zileuton and N-dehydroxylated metabolite (Abbott-66193). The two compounds are metabolized by parallel pathways to ring-hydroxylated and sulfoxide (diastereomeric) metabolites.

CYP3A was identified to be the major P450 isoform involved in sulfoxidation of both zileuton and Abbott-66193. CYP2C9 may also contribute to sulfoxidation of Abbott-66193. CYP1A2 was identified as the major isoform mediating the hydroxylation of zileuton and Abbott-66193, although CYP2C9 seems to be playing a minor role especially in Abbott-66193 metabolism. Involvement of CYP2D6 in metabolism of zileuton and Abbott-66193 is not clear.

The N-dehydroxylated metabolite and the zileuton glucuronides have no lipoxygenase inhibitory activity.

Based on the results from the radiolabeled study (mass-balance study) and the in-vitro metabolism study, the metabolic pathway for zileuton in humans is shown below:



Excretion: Upon administration of a single oral dose of radiolabeled zileuton, an average of 94.5% and 2.2% of administered radioactivity was found in urine and feces, respectively. Practically no unchanged drug was found in urine. This indicates that most of zileuton is renally excreted as metabolites.

e. DOSE PROPORTIONALITY: When studied at four dose levels of 200, 400, 600 and 800 mg zileuton administered as 200 mg capsules, the dispositional pharmacokinetics are linear. AUCs were dose-proportional (5.31, 10.98, 16.78 and 22.30 $\mu\text{g}\cdot\text{hr}/\text{ml}$ at 200, 400, 600 and 800 mg dose levels respectively). This data indicates that total plasma clearance and bioavailability (or at least C₁/F) are dose-independent. When C_{max} was considered, it was dose-proportional between 200 and 400 mg; and 600 and 800 mg dose levels (1.44, 2.90, 3.62, 4.43 $\mu\text{g}/\text{ml}$). This could be due to dissolution limited absorption (flip-flop). However, this non-linearity may not be seen in the final tablet

formulation (where no flip-flop was seen, where terminal slope of plasma concentration-time data from tablet and solution did not indicate any flip-flop).

f. DIURNAL VARIATION: The C_{min} value (at steady state) of zileuton at time '0' before morning dose after multiple dose administration was found to be higher (2-3 fold) than the C_{min} values (at end of morning dosing intervals) during the day. The reason for this is not known. Possibility of slow absorption and reduced hepatic metabolism because of subjects' inactivity and recumbence during night is speculated. To take advantage of higher steady state C_{min} levels before morning dose, phase II/III trials were conducted with dose administration with unequal intervals i.e. at 7 a.m., 1 p.m., 6 p.m. and 11 p.m. This should be reflected on the label of the product. Once dosing interval is adjusted (unequal dosing intervals instead of Q6hours), the overall impact of diurnal variation is minimal (pharmacokinetic profiles were similar).

g. ACTIVITY OF THE TWO ENANTIOMERS OF ZILEUTON AND THEIR PHARMACOKINETICS: Biochemical assays and animal pharmacology studies indicated that R(+) enantiomer is more active (2-fold) than S(-) form as indicated by the IC_{50} s in biochemical assays. In study of calcium ionophore stimulated LTB_4 formation in human whole blood, mean IC_{50} s were 0.8 μ M and 1.8 μ M for R(+) and S(-) enantiomers, while it was 1.0 μ M for racemic zileuton. Unbound fraction of R(+) form is 2-3 times greater than S(-) form in human plasma, suggesting that the potencies of unbound enantiomers are similar. Zileuton and its individual enantiomers have similar toxicity profiles. From the dog studies, no chiral interconversion of enantiomers was observed. In humans, the major urinary metabolites of zileuton are approximately equal amounts of R(+) and S(-) zileuton, and hence interconversion does not seem to be occurring to a great extent.

Plasma clearance of S(-) form ($Cl/F = 1276 \pm 397$ ml/min) is approximately twice as large as that of R(+) form ($Cl/F = 566 \pm 164$ ml/min). Pharmacokinetics of the two enantiomers are independent of formulation.

h. FOOD EFFECT: Food increased zileuton C_{max} by 27% (statistically significant difference) and AUC by only 1.4% when zileuton 600 mg final tablet formulation was administered within 0.5 hours after breakfast (this was not a high fat breakfast, fat content was less). On an older 300 mg formulation, mean C_{max} was higher by 31% and AUC by 18% when administered within 30 minutes after high fat breakfast. Although food appears to have an effect on C_{max} of zileuton, the difference may not be clinically significant.

i. SPECIAL POPULATIONS:

Age: Upon comparison of pharmacokinetics of zileuton between healthy young (20-40 years) and healthy elderly (65-81 years), it was found that mean apparent clearance after single 600 mg dose was statistically significantly lower (<20%) in elderly subjects. However, no statistically significant difference was found after multiple dose administration. Other PK parameters were not statistically significant both after single dose and multiple dose.

Pediatric population: Pharmacokinetics of zileuton in children (age 8-12 years) were found to be similar to adults after adjustment for body weight. Differences in body weight and surface area

appear to account for differences between adults and children in mean dose-normalized C_{max} and AUC, apparent clearance and volume of distribution. Although only 12 subjects participated in this study, slight difference in clearance was seen between males and females. Average apparent clearance of zileuton was 288 ml/min in females and 317 ml/min in males.

Gender: Statistically significant differences were observed in C/F after single dosing, and in β and Vd/F after multiple dosing. However, once normalized to body weight, none of the weight-normalized parameters were statistically significantly different with respect to gender.

Population pharmacokinetic analysis in patients also indicated that there is no gender effect on pharmacokinetics of zileuton. Differences in body weight but not gender helped explain some of the variability in apparent clearance of zileuton.

Race: Pharmacokinetics of zileuton appear to be independent of race as indicated from the population pharmacokinetic analysis of zileuton in Phase II/III studies.

Hepatic impairment: Pharmacokinetics of zileuton was studied after administration of 300 mg t.i.d. to subjects with normal hepatic function, mild hepatic insufficiency and moderate hepatic insufficiency. Mean apparent plasma clearance of total zileuton in hepatic impaired subjects was half that of normal subjects. This was similar in subjects with mild and moderate impairment. However, the fraction of zileuton bound to plasma proteins was lower in subjects with moderate hepatic impairment than that of mild hepatic impairment and healthy subjects. Therefore, the mean AUC of unbound zileuton increased 2-fold in subjects with mild hepatic impairment and 4-fold in subjects with moderate hepatic impairment as compared to that of healthy subjects. Dose-adjustment is therefore necessary in hepatically impaired subjects for zileuton. The increase in AUC in hepatic impairment could be more pronounced with the recommended dose of 600 mg q.i.d. administration.

Renal impairment: This study was carried out in subjects with normal renal function ($Cl_{cr} > 90$ ml/min), subjects with mild renal impairment (Cl_{cr} 60-90 ml/min), subjects with moderate renal impairment (Cl_{cr} 30-59 ml/min), subjects with severe renal impairment ($Cl_{cr} < 30$ ml/min), and in subjects with renal failure requiring hemodialysis. The pharmacokinetics of zileuton were similar in all the above subjects. Although glucuronides accumulated in plasma in renally impaired subjects, since glucuronides do not have any activity, this is of little concern (also, no toxic effects have been attributed to glucuronides). In subjects with renal failure requiring hemodialysis, PK of zileuton was not altered by hemodialysis. Therefore, dose adjustment in renal insufficiency does not appear to be necessary.

PK in patients: Pharmacokinetics of zileuton in healthy volunteers were found to be similar in patients with mild to moderate asthma.

j. BIOEQUIVALENCE BETWEEN FORMULATIONS:

600 MG FINAL TABLET FORMULATION: 600 mg tablets manufactured at the 2 different manufacturing sites (Abbott Park (AP), Illinois and Puerto Rico (PR)) are bioequivalent to each other. Based on the results obtained, the 90% CI's for AUC, but not C_{max} (C.I. upper limit is 1.30 for AP and 1.258 for PR), of both tablet formulations A (600 mg FF formulation manufactured at Abbott Park, IL) and B (600 mg FF formulation manufactured at Puerto Rico) lie within acceptable limits compared to that of C (300 mg FF tablet manufactured at Abbott Park, IL) as reference; therefore,

both tablet formulations could NOT be considered bioequivalent to the 300 mg tablet formulation. Relative bioavailability of the tablets to the suspension is > 94% based on AUC. The enantiomeric ratios of C_{max} and AUC after dosing with all the four formulations were similar to each other. It should be noted that the 600 mg final tablet formulations have been studied in pivotal Phase III clinical trials. Hence, the bioequivalence comparison is not very critical (also this is not a typical bioequivalence study since different number of units for test and reference are being compared, and in such a situation this type of results (wider C.I.) are expected). It is also important to note that E_{max} of concentration-response curve with respect to LTB_4 inhibition occurs at zileuton concentrations of 2 $\mu\text{g/ml}$ and higher. Hence, increases in C_{max} (as observed with higher C.I. of 1.30) may not have any impact on efficacy of the drug assuming that LTB_4 inhibition is a good surrogate marker for efficacy.

300 MG FINAL TABLET FORMULATION: 300 mg FF tablets manufactured at the 2 different manufacturing sites are bioequivalent to each other. The 300 mg FF tablets manufactured at Abbott Park is bioequivalent to capsule II (reference). However, the 300 mg FF tablets manufactured at Puerto Rico cannot be declared bioequivalent to the reference, since the criterion on C_{max} was not met (upper limit of C.I. was 1.287). The enantiomeric ratios of C_{max} and AUC after dosing with all the three formulations were similar to each other (no statistically significant difference between formulations). All the 3 final formulation strengths are compositionally proportional and hence, bioequivalence study comparing the highest strength (600 mg FF) to that of reference capsule would have been sufficient. All other strengths could be compared with in-vitro dissolution, and the current study would not have been necessary. The 300 mg strength has not been evaluated in pivotal clinical studies. However, the higher C_{max} obtained with this strength may not be critical in the context that higher C_{max} does not effect efficacy as E_{max} for % inhibition of LTB_4 is achieved at C_{max} of 2 $\mu\text{g/ml}$. With respect to safety, it was observed in study # F92-219 (BE study comparing 600 mg tablet vs. 300 mg tablet) that the C_{max} following 600 mg tablet formulation was higher than that of 300 mg tablet formulation. The 600 mg formulation has been studied in Phase III clinical trials and has been found to be safe.

200 MG CAPSULE I AND II: Capsule I and capsule II are bioequivalent to each other. Relative bioavailability of the capsules to the suspension is about 88% based on AUC and 58-60% based on C_{max} .

V. DISSOLUTION: The proposed dissolution method for the zileuton tablet formulation is USP

not accurately predict what happens in vivo.

VI. PHARMACODYNAMICS:

- a. **PK-PD:** Plasma concentration of zileuton were correlated with that of percent inhibition of leukotriene B₄ (LTB₄) using a sigmoid E_{max} model. A counterclockwise hysteresis loop was observed between concentration of zileuton in plasma and the improvement in FEV₁.
- b. **Meta-analysis:** Meta-analysis of the pharmacodynamic relationship between inhibition of leukotriene B₄ biosynthesis in whole blood and plasma concentrations of zileuton obtained from five Phase I studies showed that the data could be fitted to a sigmoid E_{max} model. IC₅₀ obtained was 0.46 µg/ml with % CV of 68.1%. Data indicates that zileuton is a strong inhibitor of LTB₄ biosynthesis in whole blood with population estimates of 0.46 µg/ml and 0.87 for IC₅₀ and γ, respectively.
- c. **Population pharmacokinetics:** Population analyses of zileuton plasma concentration versus time and of pharmacodynamic measure LTB₄ was done in two Phase II/III clinical trials. A one compartment model with first order absorption was the model describing pharmacokinetics of zileuton. Body weight was found to be an important covariate that explains some variability in PK of zileuton. PD modeling using FEV₁ as end point was not carried out.

VII. DRUG INTERACTIONS:

a. **In-vitro protein binding interaction studies** in human plasma between zileuton and several drugs was studied. Addition of theophylline, terfenadine, prednisone, and salbutamol had no effect on protein binding of zileuton. Addition of naproxen, salicylate, ibuprofen and sulfasalazine produced small decreases in binding of zileuton (1.4, 2, 0.7 and 0.3% respectively). Zileuton did not affect the protein binding of warfarin and any of these above mentioned compounds. These results indicate that clinically significant interactions due to protein binding may be quite unlikely. Effect of warfarin on zileuton binding was not determined.

b. In-vivo drug interaction studies:

Theophylline: When given concomitantly with zileuton (800 mg every 12 hours) a significant decrease (approximately 50%) in the steady-state clearance of theophylline (CYP1A2 involvement) was observed. C_{max} and AUC increased by 73% and 92%, respectively. Elimination t_{1/2} was increased by 24%.

Terfenadine: Coadministration of zileuton (600 mg every 6 hours) with terfenadine (CYP3A4)

reduced the clearance of terfenadine by 22% resulting in increases in C_{max} and AUC of 35% and 36%, respectively. However, no significant QTc interval changes were observed.

Warfarin: Concomitant administration of zileuton (600 mg every 6 hours) and warfarin (titrated to desired PT of 14-18 seconds) was accompanied by a 15% decrease in R-warfarin (CYP1A2) clearance and an increase in AUC of 22%. The pharmacokinetics of S-enantiomer were not affected. These pharmacokinetic changes were accompanied by a significant increase in PT (~ 12%). However, stratification of subjects by PT suggested a trend correlating higher PT to greater prolongation of clotting time. Since subjects whose PT exceeded 20 seconds were discontinued from the study, the potential of this interaction may be underestimated.

Propranolol: Administration of a single 80 mg dose of propranolol (CYP2D6, CYP2Cmp, glucuronide conjugation) in normal subjects also receiving zileuton (600 mg every 6 hours) resulted in a decrease of propranolol clearance of 42%. C_{max} and AUC were increased by 52% and 104%, respectively. Elimination $t_{1/2}$ was prolonged by 25%.

Oral contraceptives: Coadministration of zileuton (600 mg every 6 hours) and oral contraceptives for two menstrual cycles had no significant pharmacokinetic effect on either ethinylestradiol or progestogen in healthy females. Further, no clinically significant pharmacodynamic effect was observed with respect to the suppression of luteinizing hormone, follicle stimulating hormone or progesterone.

Digoxin, prednisone, sulfasalazine, phenytoin and naproxen: No significant interactions were observed between zileuton and digoxin (0.250 mg qd for 11 days), prednisone (40 mg single dose), sulfasalazine (1 gm every 12 hours for 7 days), phenytoin (300 mg single dose), or naproxen (500 mg every 12 hours for 9 days).

COMMENTS:

This is an NDA which is practically complete in terms of number and types of studies, and in meeting the requirements, however, in few cases as presented in the comments it would have been beneficial if such information was thought of before the study was carried out. Some studies although carried out did not give all the information that would have otherwise been obtainable if those studies were properly planned.

1. The sponsor has done an excellent job in characterizing the cytochrome P450 isozymes involved in metabolism of zileuton. The approach taken by the sponsor to study the P450 mediated metabolism of zileuton is thoughtful and consistent with contemporary thinking regarding ways to determine isoforms responsible for metabolism. However, since this is only a minor pathway, the sponsor should instead have spent their resources in carrying out in vitro inhibition studies. These types of studies would have helped in identification of possible drug-drug interactions.

2. There is no information on whether the elevation of liver enzymes observed in some of the patients in clinical trials is caused by zileuton or its metabolites.

3. It would have been very helpful if the sponsor provided information on side effects or liver

enzyme level as a measure of toxicity in the PK-PD analyses. This could help enhance understanding of the drug's concentration-toxic effect relationship.

4. The sponsor should provide information on batch size of the lot used in each study. In this NDA, even though descriptive information was provided in few cases (such as production size lots), actual batch size was not provided in the same study report. For studies in which batch size was an important consideration, the reviewer had difficulty in getting this information as such information was only found in the summary of dissolution data. It is recommended that such information be presented along with the lot # in every study report in future.

5. The sponsor should in future include in the Human pharmacokinetics and Biopharmaceutics' section the summary of adverse events that occurred in each study.

6. In future PK-PD submissions, the sponsor should attempt PK-PD model using the efficacy end-point as the PD measure. The sponsor should also test the predictive performance of the population models developed.

7. The tablets which are obtained upon rework are not bioequivalent and hence rework procedure is not acceptable.

8. The dissolution method is acceptable, however, the dissolution medium is not physiological and the data shows that in this case, in vitro dissolution does not accurately predict what happens in vivo. Based on the dissolution data provided, the specification should be set at not less than in 45 minutes.

9. Since this is a bisected tablet, the sponsor should provide comparative dissolution data on the two portions of the tablet.

LABELING COMMENTS:

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 19
Submission date: 07/19/94

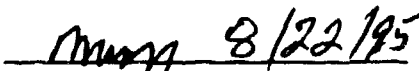

08/17/95
Venkata Ramana K. Sista, Ph.D.
Pharmacokinetic Evaluation Branch I


8/17/95
Bradley Gillespie, Pharm.D.
Pharmacokinetic Evaluation Branch I


8/18/95
Eric Ette, Ph.D.

Biopharm. Day 3/27/95 (Collins, Ludden, Malinowski, Fleischer, A.Hussain, W.Gillespie, Mehta).

FT initialed by Mehul U. Mehta, Ph.D.


8/22/95

cc: HFD-155: NDA 20-471
HFD-155: Division file
HFD-155: JBuccine
HFD-155: RAr.thracite
HFD-155: JLeak
HFD-155: JSun
HFD-426: NFleischer
HFD-426: MMehta
HFD-426: MChen
HFD-426: Chron
HFD-426: Drug; HFD-426: Reviewer (VRKSista); HFD-340: Viswanathan
HFD-19: FOI

NDA 020471

FIRM: ABBOTT LABS

4 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

APPENDIX I

① STUDY M89-336:

METABOLISM AND DISPOSITION OF ZILEUTON-¹⁴C IN HUMANS FOLLOWING A SINGLE ORAL DOSE:

Reference: Volume 88

Investigator:

Study Location:

Objective:

To determine the metabolic fate and routes of excretion of racemic Zileuton-¹⁴C in adult human subjects after oral administration of a single dose.

Study Design:

Four normal, healthy non-smoking adult male volunteers (77-84 kg) participated in this study. Each subject was given a single oral dose of radiolabeled Zileuton, containing 100 μ Ci of carbon-14 in a soft gelatin capsule. Each subject was given 200 ml of water to drink after ingestion of the capsule. Breakfast and lunch were served after 3 and 6 hours respectively. Blood samples (10 ml) were collected at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, 16, 20, 24, 36, 48, 72, 96, 120, 144 and 168 hours after drug administration. All urine and feces excreted by each subject during the seven day period after drug administration were collected. Urine samples were collected as voided, however, each subject attempted to empty his bladder at 0, 4, 8, 12, 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Plasma, urine and fecal homogenates were radioassayed for determining the total carbon-14 activity.

All metabolic pattern analyses on plasma and urine samples (after suitable extraction procedures) were performed by reversed phase gradient HPLC system. The radioactivity in the column effluent was monitored with a radioactive flow detector. The instrument was also equipped with an ultraviolet detector to allow simultaneous monitoring of UV and radioactivity traces. Since this method did not resolve Zileuton and N-dehydroxylated metabolite (Abbott-66193), plasma levels of Abbott-66193 were determined using an isocratic system with UV detection designed to separate these components.

The diastereomeric glucuronides of Zileuton (G-1 and G-2) were isolated from pooled urine (0-12 and 0-24 hour) from each of the four subjects. These compounds were identified by mass spectral analysis.

Results:

Total radioactivity in plasma: The levels of radioactivity in the plasma of the subjects given oral dose of Zileuton-¹⁴C are presented in table 3.

Peak levels of radioactivity in all of the subjects occurred within 4 hours, suggesting relatively rapid absorption from the gastrointestinal tract.

TABLE 3
LEVELS OF RADIOACTIVITY IN THE PLASMA OF SUBJECTS GIVEN
ORAL DOSE OF Zileuton-¹⁴C

Hrs. after dose	Mean $\pm$ Standard deviation Microgram equivalents per milliliter
0.25	
0.50	
0.75	
1.0	
1.5	
2.0	
3.0	
4.0	
6.0	
8.0	
12	
16	
20	
24	
36	
48	
72	

Excretion: The excretion data from subjects given a oral dose of radioactive zileuton are presented in table 4. An average of 71% of the ¹⁴C-dose was excreted in the urine during the first 12 hours after drug administration, and 87% of the dose had been eliminated by that route within 24 hours, suggesting good absorption. In the 7-day study period, a total of 94.48 $\pm$ 6.35% of the ¹⁴C dose was recovered in the urine. Fecal excretion averaged about 2.21 $\pm$ 0.89% after 7 days. Total recoveries in the four subjects ranged from 88.96 to 102.33% and averaged 96.7 $\pm$ 5.6%.

Distribution of Radioactive metabolites: Zileuton glucuronides were the major circulating metabolites

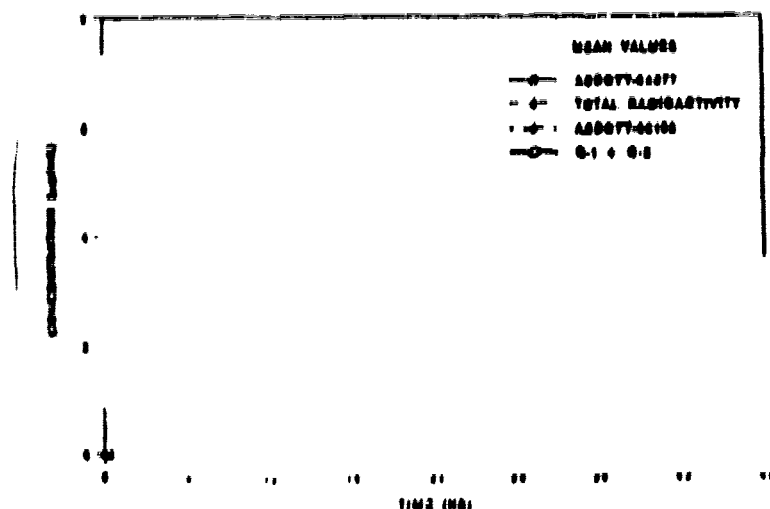
at all time points. From 1.5 to 8 hours after dosing, plasma levels of G-1 + G-2 were 1.4-2 fold greater than those of zileuton, suggesting a significant first pass effect. Plasma concentrations of Zileuton, Abbott-66193 and the glucuronides are shown in figure 8.

Zileuton was rapidly absorbed from gastro-intestinal tract. Average peak plasma levels (2.26 µg/ml) were found at 3 hours and declined monoexponentially with a $t_{1/2}$ of 3.8 hours (2.1-5.6 hours - range). Plasma concentrations of Abbott-66193 exhibited considerable inter-subject variability. Mean peak levels did not occur until 12 hours.

TABLE 4
EXCRETION OF RADIOACTIVITY BY SUBJECTS GIVEN A ORAL DOSE
OF Zileuton-¹⁴C

Hours after dose	Mean percent Carbon-14 Dose	
	Urine	Feces
0-4		
4-8		
9-12		
12-24		
24-48		
48-72		
72-96		
96-120		
120-144		
144-168		
SUM		
TOTAL		

FIGURE 3



The levels of Zileuton, Abbott-66193, and the G-1 and G-2 glucuronides accounted for essentially all of the plasma radioactivity in the human subjects.

Distribution of urinary metabolites: Table 5 shows the distribution of metabolites in 0-24 hours pooled human urine. The absence of free parent drug in any of the samples indicated extensive metabolism, primarily by Phase II reactions. The major metabolites were the G-1 and G-2 diastereomeric glucuronides. Polar material (possibly further metabolite of Abbott-66193) and other metabolites comprised only 8.0 and 0.4% of the dose. Collectively, the G-1 and G-2 glucuronides accounted for 90% of the radioactivity in the 0-24 hour urine.

TABLE 5

DISTRIBUTION OF METABOLITES IN THE 0-24 HOUR URINE OF SUBJECTS
GIVEN A ORAL DOSE OF Zileuton-¹⁴C

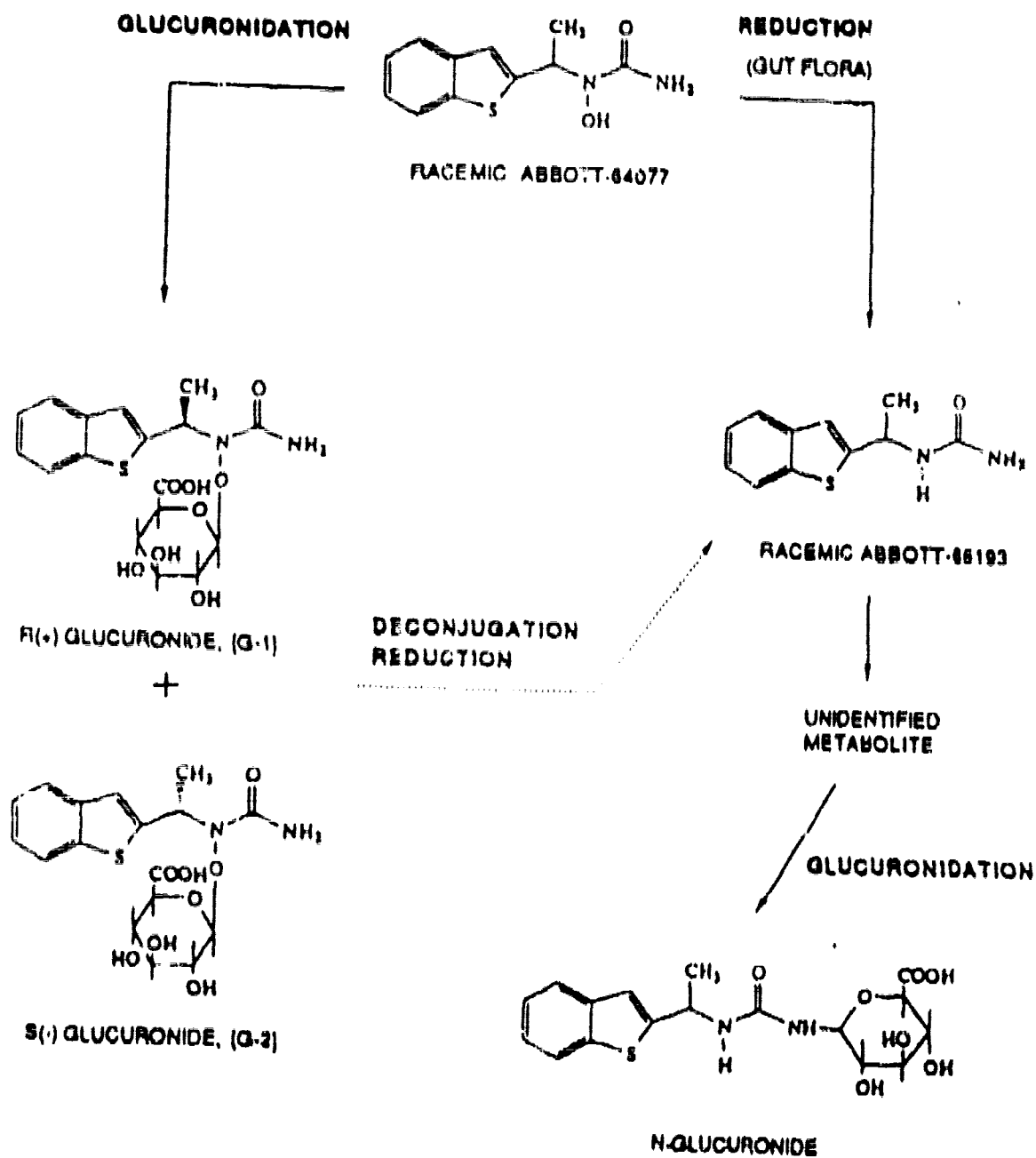
Subject	% of the ¹⁴ C Dose				% Dose excreted
	Polar	(G-1)	(G-2)	Others	
1					
2					
3					
4					
Mean	8.2	35.3	43.2	0.40	87.0

A proposed metabolic pathway for zileuton is shown in figure 4. The primary metabolic route for zileuton appeared to be the formation of (R)- and (S)-Zileuton O-glucuronides, which occurred rapidly in the liver at lower doses of parent drug. At higher doses, the unabsorbed fraction of the dose may travel through the gastrointestinal tract to the large intestine where it is reduced by gut microflora to Abbott-66193. Following re-absorption, Abbott-66193 may be further transformed in the liver to N-glucuronide of Abbott-66193.

COMMENTS: The assay validation data for this study has not been provided.

FIGURE 4

Proposed Metabolic Pathway for ABBOTT-64077



② **IDENTIFICATION OF THE HUMAN CYTOCHROME P-450 ENZYMES INVOLVED IN THE METABOLISM OF ZILEUTON (ZILEUTON) AND ITS N-DEHYDROXYLATED METABOLITE, ABBOTT-66193**

Study ID: R&D/94/194

Objective:

1. To define the metabolism of zileuton and Abbott-66193 in a panel of human liver microsomes, and
2. to identify the hepatic Cytochrome P-450 (CYP) isoforms involved in the oxidative metabolism of ¹⁴C-zileuton and its N-dehydroxylated metabolite, ¹⁴C-Abbott-66193, by human liver microsomes.

IN VITRO METABOLISM OF ¹⁴C-ZILEUTON AND ¹⁴C-ABBOTT 66193: These two materials (zileuton concentrations: 5 - 120 μM and Abbott-66193 concentrations: 2.5 - 60 μM) were incubated with human liver microsomes (0.5 - 2 mg/ml microsomal protein) at 37°C. This mixture also contained potassium phosphate buffer, magnesium chloride, β-NADP⁺, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase. The reactions were allowed to proceed for about 0.5 - 3 hours and then stopped by addition of an equal volume of chilled methanol to precipitate the protein. The samples were centrifuged and analyzed by HPLC with radioactivity flow detection. K_m and V_{max} parameters for metabolism of ¹⁴C-zileuton and ¹⁴C-Abbott 66193 were then determined from this data.

Involvement of CYP system was assessed by omission of NADPH generating system and addition of clotrimazole (potent CYP inhibitor). K_m and V_{max} parameters for zileuton and Abbott-66193 metabolism were determined using liver microsomes. Zileuton at concentrations of 5 - 120 μM was incubated with microsomes at protein concentration of 2 mg/ml for 3 hours, while Abbott-66193 at concentrations of 2.5-60 μM was incubated with microsomes at protein concentration of 0.5 mg/ml for 0.5 hours. Under these conditions, reactions were linear with respect to protein concentration and incubation time.

Specific cytochrome P450 enzymes responsible for metabolism of zileuton and Abbott-66193 were identified by correlation analysis, chemical inhibition studies and by incubation with purified CYP forms and cDNA-expressed CYP systems.

CORRELATION OF ZILEUTON AND ABBOTT-66193 METABOLISM WITH THE ACTIVITY OF CYP SPECIFIC PROBE SUBSTRATES: The rates of zileuton and Abbott-66193 metabolism were determined in a panel of 11 human hepatic microsomes at a final drug concentration of 50 μM. These rates were compared with those of selective CYP substrates (7-ethoxyresorufin O-deethylase for CYP1A2, coumarin 7-hydroxylase for CYP2A6, tolbutamide methyl hydroxylase for CYP2C9, dextromethorphan O-demethylase for CYP2D6, N,N-dimethylnitrosamine N-demethylase for CYP2E1 and erythromycin A N-demethylase for CYP3A) to detect significant relationships between zileuton and Abbott-66193 metabolism and specific CYP forms.

CYTOCHROME P-450 SELECTIVE INHIBITORS: A series of inhibitors were incubated

with 50 μ M of zileuton and Abbott-66193 to determine which compounds could inhibit the metabolism of either or both compounds. The inhibitors selected were α -naphthoflavone (10 μ M) and furafylline (40 μ M) for CYP1A2; coumarin (200 μ M) for CYP2A6; sulfaphenazole (2 μ M) for CYP2C9; quinidine (5 μ M) for CYP2D6; 4-methylpyrazole (50 μ M) for CYP2E1; ketoconazole (2 μ M), troleandomycin (100 μ M) and erythromycin A (200 μ M) for CYP3A.

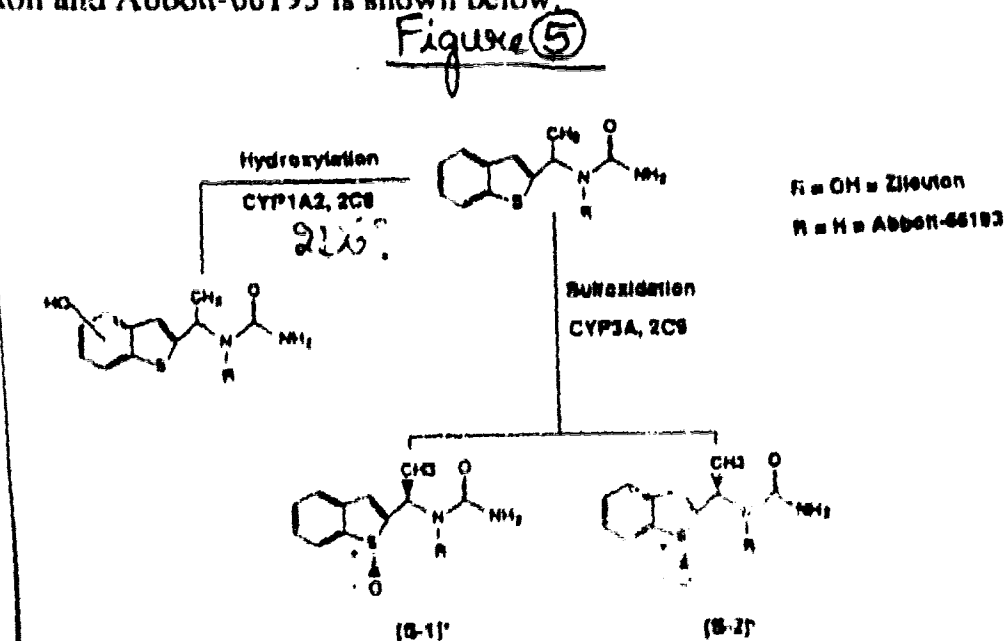
PURIFIED CYP FORMS AND cDNA-EXPRESSED CYP ACTIVITIES: Zileuton and Abbott-66193 at 20 μ M concentrations were incubated with human β -lymphoblastoid cells containing cDNA-expressed CYP1A2, CYP2A6, CYP2B6, CYP2D6 and CYP2E1 for 4 hours. The ability of CYP2C9 to metabolize zileuton or Abbott-66193 was determined by incubation with the purified enzyme isolated from human liver microsomes (incubation for 1 hour). Involvement of CYP3A4 in zileuton and Abbott-66193 metabolism was determined using a purified recombinant fusion protein containing human CYP3A4 and rat NADPH-P-450 reductase (incubation for 1 hour).

EFFECT OF ZILEUTON AND ABBOTT-66193 ON METABOLISM OF OTHER CYP SELECTIVE SUBSTRATES: Effect of zileuton and Abbott-66193 (1 - 200 μ M) on dextromethorphan O-demethylase (CYP2D6), tolbutamide methyl hydroxylase (CYP2C9) and erythromycin N-demethylase (CYP3A) activity was investigated in liver microsomes. Concentration of dextromethorphan, erythromycin and tolbutamide was 20 μ M, 0.5 mM and 0.1 mM, respectively. Their effect on CYP1A2 substrates was not determined. Experiments were also carried out with CYP selective inhibitors, quinidine (CYP2D6, 5 μ M), ketoconazole (CYP3A, 2.0 μ M) and sulfaphenazole (CYP2C9, 2.0 μ M).

All samples were analyzed by HPLC attached to radioactivity detector. Metabolites from human liver microsomal incubations were subjected to mass spectral analysis for identification.

Results:

Based on the metabolites identified in this study, proposed scheme for oxidative metabolism of zileuton and Abbott-66193 is shown below:



Data shown below in the table indicates that the oxidative metabolism of zileuton and Abbott-66193 is NADPH-dependent and is mediated to a large extent by cytochrome P-450 enzyme.

Table 6

Parameter	Subject	% Control Activity			
		Zileuton		Abbott-66193	
		Sulfoxidation (S-1 + S-2)	Hydroxylation	Sulfoxidation (S-1 + S-2)	Hydroxylation
Minus NADPH					
Clotrimazole (50 µM)					
Thiobenzamide (200 µM)					
	Mean	89.8			
	SD	3.0			

Kinetic parameters for hydroxylation and sulfoxidation of zileuton and Abbott-66193 is shown below. Hydroxylation and sulfoxidation of zileuton is 13 and 37-fold less than corresponding rates of Abbott-66193. According to the information from the sponsor, the sulfoxidation of zileuton and Abbott-66193 is not reversible.

Table 7

Parameter	Zileuton		Abbott-66193	
	Hydroxylation	Sulfoxidation (S-1)*	Hydroxylation	Sulfoxidation (S-1 + S-2)**
K _m (µM)	173	463	193	230
V _{max} (pmol/min/mg)	19.6	34.4	727	430
V _{max} /K _m (nL/min/mg)	0.11	0.074	3.72	1.87

S-1 and S-2 = sulfoxide diastereomers
* K_m and V_{max} not determined for S-2 due to very low rates of sulfoxidation
** Sulfoxide diastereomers could not be resolved by HPLC

Results from correlation studies of zileuton hydroxylation and sulfoxidation with that of Abbott-66193 indicated that hydroxylation and sulfoxidation of both drugs are mediated by same CYP isozyme, but the isozymes involved in hydroxylation are different from those involved in sulfoxidation.

Identification of the CYP isoforms:

1. Correlation of the rates of zileuton and Abbott-66193 metabolism with those of classical CYP selective markers in human liver microsomes.
2. Inhibitor studies (ketoconazole and troleandomycin for CYP3A; sulphaphenazole for CYP2C9; quinidine for CYP2D6; flavones for CYP1A2; and coumarin for CYP2A6).
3. Metabolism by cDNA-expressed CYP isoforms.

Correlation studies indicated the following: (see table below)

Zileuton and Abbott-66193 hydroxylation = CYP1A2 and CYP2C9 and CYP2D6

.....correlated with ethoxyresorufin O-deethylase activity (CYP 1A2)

.....correlated with tolbutamide methyl hydroxylase (CYP2C9)

.....correlated with dextromethorphan O-deethylase (CYP2D6)

Zileuton and Abbott-66193 sulfoxidation = CYP3A and CYP2A6 (2A6 effect not clear)

.....correlated with erythromycin N-demethylase (CYP3A)

.....correlated with coumarine hydroxylase (CYP2A6)

Inhibitor studies.

Sulfoxidation: Ketoconazole and troleandomycin inhibited the sulfoxidation of both zileuton and Abbott-66193 to a greater extent as compared to sulfaphenazole. This indicates that sulfoxidation of both the compounds is primarily mediated by CYP3A4 and only to a lesser extent by CYP2C9.

Table 8

Correlation of Various Cytochrome P-450 (CYP) Form-Selective Monooxygenase Activities with the Sulfoxidation and Hydroxylation of Zileuton and Abbott 66193 in a Panel of Human Liver Microsomes

Reaction ^b	CYP Form	Correlation Coefficient (r) ^a				
		Zileuton		Abbott-66193		
		Hydroxylation	Sulfoxidation (S-1)	Sulfoxidation (S-2)	Hydroxylation	Sulfoxidation (S-1 + S-2)
ERODase	1A2	0.762**	0.212	0.268	0.653**	0.228
DEHase	2D6	0.835**	0.470	0.527	0.801**	0.521
DMNase	2E1	0.126	0.148	0.130	0.222	0.158
ERNase	3A4	0.207	0.815**	0.794**	0.439	0.836***
COHase	2A6	0.130	0.777**	0.781**	0.130	0.687*
TOLase	2C9	0.863**	0.509	0.530	0.935***	0.678
Total CYPs		0.416	0.938**	0.941**	0.453	0.877***
Zileuton						
S-1		0.305		0.993**	0.395	0.951***
S-2		0.336			0.411	0.950***
Hydroxylation					0.968***	0.403
Abbott-66193						
S-1 + S-2					0.508	

^a The number of liver samples in the panel was eleven, except for TOLase activity (N = 10). The statistical significance of the correlation is denoted by p < 0.05*, p < 0.01** and p < 0.001***

^b The various activities are: ERODase = 7 ethoxyresorufin O-deethylase; DEHase = dextromethorphan O-demethylase; DMNase = N,N-dimethylnitrosamine N-demethylase; ERNase = erythromycin N-demethylase; COHase = coumarin hydroxylase; TOLase = tolbutamide methyl hydroxylase

Table 9

Inhibition of Zileuton Metabolism in Human Liver
Microsomes by Cytochrome P-450 (CYP) Selective
Inhibitors

Inhibitor	CYP	% Activity Remaining*					
		Sulfoxidation (S-1)		Sulfoxidation (S-2)		Hydroxylation	
		Mean	SD	Mean	SD	Mean	SD
Furafylline	1A2	105	12	138	9	57.5	3.3
α -Naphthoflavone	1A2	132	9	197	36	73.8	8.6
Troleandomycin	3A	18.0	19.3	43.4	21.7	97.1	0.8
Ketoconazole	3A	66.6	18.5	68.4	10.5	103	3
Sulfaphenazole	2C9	99.6	13.9	120	20	90.6	8.9
Quinidine	2D6	119	9	114	24	104	6
4-Methylpyrazole	2E1	106	8	91.6	3.9	88.5	10.3
Coumarin	2A6	106	15	101	21	103	21

* Data represent the mean $\pm$ SD and are expressed as the activity remaining as a percentage of the control (solvent alone). Experiments were done with microsomes from three different livers (GC4476, GF2060 and F1W309).

Table 10

Inhibition of Abbott-66193 Metabolism in Human
Liver Microsomes by Cytochrome P-450 (CYP)
Selective Inhibitors

Inhibitor	CYP	% Activity Remaining*			
		Sulfoxidation**		Hydroxylation	
		Mean	SD	Mean	SD
Furafylline	1A2	106	9	59.2	18.1
α -Naphthoflavone	1A2	555	102	49.2	15.8
Troleandomycin	3A	32.6	8.6	80.0	16.7
Ketoconazole	3A	40.2	12.8	75.6	0.6
Sulfaphenazole	2C9	79.5	16.9	44.2	11.7
Quinidine	2D6	115	15	109	11
Methylpyrazole	2E1	93.4	7.2	91.0	9.2
Coumarin	2A6	109	3	84.1	7.2

* Data represent the mean $\pm$ SD and are expressed as the activity remaining as a percentage of the control (solvent alone). Experiments were done with microsomes from three different livers (GC4476, GF2060 and F1W309).

** S-1 + S-2

Hydroxylation: Furofylline and α -naphthoflavone inhibited the sulfoxidation of both zileuton and Abbott-66193 to a greater extent as compared to sulfaphenazole (see table above). This indicates that hydroxylation of both the compounds is primarily mediated by CYP1A2 and only to a lesser extent by CYP2C9. Implication of CYP3A, CYP2A6 and CYP2E1 in hydroxylation of Abbott-66193 and CYP2E1 in zileuton hydroxylation was also indicated.

Metabolism by cDNA-expressed isoforms and purified enzymes: Results are shown in table below.

Table (11)

CYP Form	Specific Activity ^a				
	Zileuton			Abbott-66193	
	Hydroxylation	Sulfoxidation (S-1)	Sulfoxidation (S-2)	Hydroxylation	Sulfoxidation (S-1 + S-2) ^b
1A2 ^a	2.14 ± 0.27	nd	nd	6.42 ± 0.12	nd
2A6 ^a	nd	nd	nd	nd	nd
2B6 ^a	nd	nd	nd	nd	≤0.08
2E1 ^a	1.19 ± 0.0	nd	nd	nd	nd
2D6 ^a	0.18 ± 0.01	nd	0.13 ± 0.04	0.29 ± 0.02	0.06 ± 0.01
Control ^{a*}	nd	nd	nd	nd	nd
2C9 [#]	0.08	nd	nd	2.69	0.91
3A4 [§]	nd	0.49	0.25	nd	4.3

^a nmoles of product formed/nl/minute CYP, where indicated, the data represent the mean ± SD of duplicate determinations

^{a*} Naive β -lymphoblast cell microsomes which do not contain cDNA expressed CYP proteins

^a Incubations were carried out in the presence of β -lymphoblastoid microsomes containing various expressed CYP

^b The sulfonide diastereomers were not resolved in the HPLC system

[#] The drugs were added to a conventionally reconstituted system containing purified human CYP2C9

[§] The drugs were added to a reconstituted system containing a purified recombinant fusion protein consisting of human CYP3A4 and rat NADPH-P-450 inducible

nd = not detected

Table below shows the inhibition of CYP selective activities by zileuton and Abbott-66193.

Table (12)

CYP Activity [#]	CYP Isoform	% Activity Remaining ^a	
		Zileuton (200 μ M)	Abbott-66193 (200 μ M)
ERNDase	3A	55.1 ± 0.3	85.3 ± 2.9
TOLase	2C9	66.0 ± 4.5	58.6 ± 6.2
DEXase	2D6	78.4 ± 1.6	99.3 ± 8.1

^a Data are expressed as the mean ± SD of duplicate determinations and represent the activity remaining as a percent of the control (solvent alone)

[#] ERNDase = erythromycin N-demethylase; TOLase = tolbutamide methyl hydroxylase; DEXase = dextromethorphan O-demethylase

Conclusion: Results from this study demonstrates that metabolism of zileuton and Abbott-66193 occur by parallel pathways involving ring hydroxylation and sulfoxidation. V_{max} of hydroxylation and sulfoxidation of Abbott-66193 is higher than that of zileuton. CYP3A is the principal isozyme involved in sulfoxidation of both these compounds. CYP2C9 is also involved in sulfoxidation of Abbott-66193 but not of zileuton. Involvement of CYP2A6 is not clear. CYP1A2 is the major isozyme involved in hydroxylation of both the compounds, although CYP2C9 seems to play a minor role. Involvement of CYP2D6 is also not clear.

COMMENTS: 1. Incubation of zileuton with hepatic microsomes for 3 hours is not normally done in in vitro studies with hepatic microsomes. Hence, the statement "in order to normalize differences in the rates of metabolism, zileuton was incubated with the microsomes at a protein concentration of 2 mg/ml for 3 hours, while Abbott-66193 was incubated at protein concentration of 0.5 mg/ml for 0.5 hours" appears to be strange. However, it is interesting to note the sponsor's statement that under these conditions, reactions were linear with respect to protein concentration and incubation time.

2. The approach taken by Abbott Labs to study the P-450 mediated metabolism of zileuton is thoughtful and consistent with contemporary thinking regarding ways to determine isoforms responsible for metabolism. In many aspects, it would be a model for a general approach to NMEs.

3. It is rather puzzling that zileuton's inhibition of P450-1A2 was specifically not studied, since clinical effects on theophylline concentrations have already been noted.

③ **Single and multiple dose pharmacokinetics**

Although several studies to determine the single and multiple dose pharmacokinetics were carried out, only studies M89-346 and M89-366 are included in this review because M89-366 utilized the proposed dose and dosing regimen. M89-346 is included to compare the same dose but different dosing schedule.

PHARMACOKINETICS OF ZILEUTON AFTER SINGLE AND MULTIPLE 600 MG DOSES OF ZILEUTON IN HEALTHY VOLUNTEERS

Study ID: M89-346 and M89-366
Investigators: M89-346: ;
M89-366: ;
Clinical Site: M89-346: ;
M89-366: ;

Objective:

To evaluate the safety and pharmacokinetics of zileuton after single and multiple 600 mg Q8hours (study M89-346) and Q6hours (study M89-366) doses of zileuton in healthy volunteers.

Drug Dosage Forms:

Study M89-346: 200 mg capsule I, Bulk lot # 22-003-VII
Study M89-366: 200 mg capsule I, Bulk lot # 22-028-VII

Study Design:

Both studies were randomized double-blind, parallel, placebo-controlled trials. In study M89-346, six subjects received zileuton 600 mg Q8hours and 3 received placebo. In study M89-366, seven subjects received zileuton 600 mg Q6hours and 3 received placebo. All the subjects in these studies were healthy nonsmoking, adult, caucasian males. The demographics of subjects who received zileuton are as follows:

Table ⑬

Study	Mean ± SD (Range)		
	Age (yr)	Height (cm)	Weight (kg)
M89-346	28 ± 5 (23-35)	178 ± 7 (166-187)	75 ± 7 (64-82)
M89-366	26 ± 8 (19-44)	178 ± 6 (170-186)	69 ± 10 (59-88)

In study M89-346 three 200 mg capsules were administered Q8hours on days 1-14, however, only the a.m. dose was administered on day 1 and day 14. In study M89-366, dosing was done the same way except three 200 mg capsules were administered Q6hours instead of 8 hours.

After an overnight fast, each subject received the dose of zileuton with 200 ml of water. Breakfast, lunch, dinner and an evening snack were served 1, 4, 10 and 16 hr after morning dose. No food or beverage was allowed other than the meals provided in the study and water to quench

After an overnight fast, each subject received the dose of zileuton with 200 ml of water. Breakfast, lunch, dinner and an evening snack were served 1, 4, 10 and 16 hr after morning dose. No food or beverage was allowed other than the meals provided in the study and water to quench thirst during confinement period (from 24 hours before 1st dose until day 15). No breakfast was served on days 1, 4 and 14.

Study M89-346: Blood samples were collected into evacuated blood collection tubes before (0), and at 1.5, 3, 4.5, 6, 8, 10, 12, 16 and 24 hr after dosing on day 1, and at 0, 1.5, 3, 4.5, 6, 8, 9.5, 11, 12.5, 14 and 16 hours on day 4, at 0 and 3 hours on day 10 and at 0, 1.5, 3, 4.5, 6, 8, 12, 16, 20 and 24 hours on day 14. Blood samples were centrifuged and kept at -10EC until assayed.

Study M89-366: Blood samples were collected into evacuated blood collection tubes before (0), and at 1.5, 3, 4.5, 6, 8, 10, 12, 16, 20 and 24 hr after dosing on day 1, at 3 hours on day 2, and at 0, 1.5, 3, 4.5, 6, 7.5, 9, 10.5, and 12 hours on day 4, at 0 and 3 hours on day 10 and at 0, 1.5, 3, 4.5, 6, 8, 12, 16, 20 and 24 hours on day 14. Blood samples were centrifuged and kept at -10EC until assayed.

Blood samples were analyzed for zileuton, Abbott-66193 and R(+) and S(-) enantiomers of zileuton using validated HPLC method. Pharmacokinetics parameters were estimated from the data by non-compartmental methods. Non-parametric statistical methods of analyses were performed for both studies to test if the median ratio of selected PK parameters was equal to 1.0 or if difference was equal to 0. Wilcoxon Signed Rank test (matched pair) was used for this purpose to compare PK parameters on days 14 vs. day 1. Wilcoxon Rank Sum test (2 samples) was used to compare PK parameters between Q8hours and Q6 hour dosing regimens.

Study M89-346: Blood samples for LTB₄ assay were drawn at 0, 3 hours on day 1; at 3, 8, 9.5, 11, 12.5, 16 hours on day 4; 0, 3 hours on day 10 and at 3 hours on day 14.

Study M89-366: Blood samples for LTB₄ assay were drawn at 0, 3 hours on day 1; at 3 hours on day 2; at 3, 6, 7.5, 9, 10.5, 12 hours on day 4; 0, 3 hours on day 10 and at 0, 3 hours on day 14.

PK modeling: The individual plasma concentration-time curves of each enantiomer were modeled with PCNONLIN according to a one-compartment model with first-order absorption.

PD modeling: The observed percent inhibition of LTB₄ biosynthesis in blood versus the measured total zileuton concentrations were modeled with PCNONLIN according to a sigmoidal E_{max} model.

PK-PD simulations: These were carried out based on predicted plasma concentrations which were used to predict LTB₄ inhibition. This was plotted along with observed data (LTB₄ and predicted LTB₄ obtained from observed plasma concentrations).

Results:

The plasma concentrations of zileuton increased rapidly after administration of drug with a t_{max} of about 2 hours, followed by a monoexponential decline. Measurable levels of Abbott-66193 were observed in plasma from several hours to more than a day after drug administration with a t_{max} of 12-24 hours. The concentrations of Abbott-66193 were quite variable.

The mean pharmacokinetic parameters of zileuton in each study after first dose and at steady state (day 14) are shown below.

Table (14)

PK parameters (mean $\pm$ st.dev.) of zileuton

Parameters	Study M89-346 600 mg Q8hrs	Study M89-366 600 mg Q6hrs
DAY 1 (single dose)		
T_{max} (hr)	2.0 $\pm$ 0.8	3.0 $\pm$ 0.9
C_{max} (μ g/ml)	2.07 $\pm$ 0.28	2.29 $\pm$ 0.68
AUC_{0-24} (μ g.hr/ml)	11.10 $\pm$ 2.10	13.92 $\pm$ 4.08
Cl/F (ml/min)	931 $\pm$ 197	770 $\pm$ 216
$t_{1/2}$ (hr)	3.93 $\pm$ 3.22	3.56 $\pm$ 0.93
Vd/F (L)	293 $\pm$ 206	231 $\pm$ 64
DAY 14 (steady state)		
T_{max} (hr)	1.5 $\pm$ 0.9	1.5 $\pm$ 0.9
C_{max} (μ g/ml)	3.07 $\pm$ 1.13	4.37 $\pm$ 1.02
$C_{min 0}$ (μ g/ml)	1.24 $\pm$ 0.67	3.40 $\pm$ 0.70
$C_{min \tau}$ (μ g/ml)	0.43 $\pm$ 0.14	1.43 $\pm$ 0.36
$AUC_{0-\tau}$ (μ g.hr/ml)	13.43 $\pm$ 3.42	18.33 $\pm$ 4.67
Cl/F (ml/min)	793 $\pm$ 233	579 $\pm$ 162
$t_{1/2}$ (hr)	2.54 $\pm$ 1.48	2.37 $\pm$ 0.59
Vd/F	179 $\pm$ 126	115 $\pm$ 29
Accumulation ratio	1.53 $\pm$ 0.31	2.10 $\pm$ 0.29

Mean plasma concentration-time profiles of zileuton and Abbott-66193 following 600 mg Q8hour administration of zileuton on days 1, 4 and 14 are shown below.

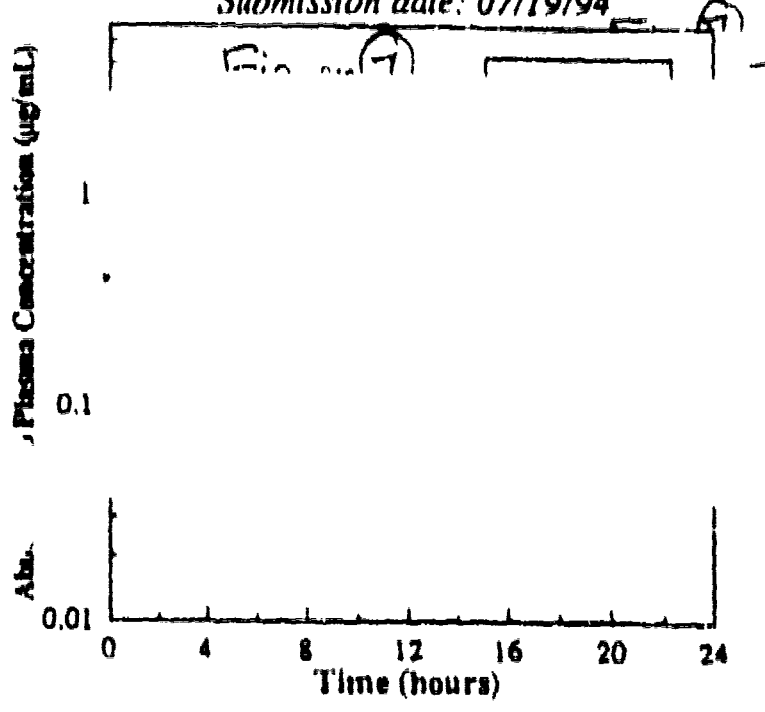
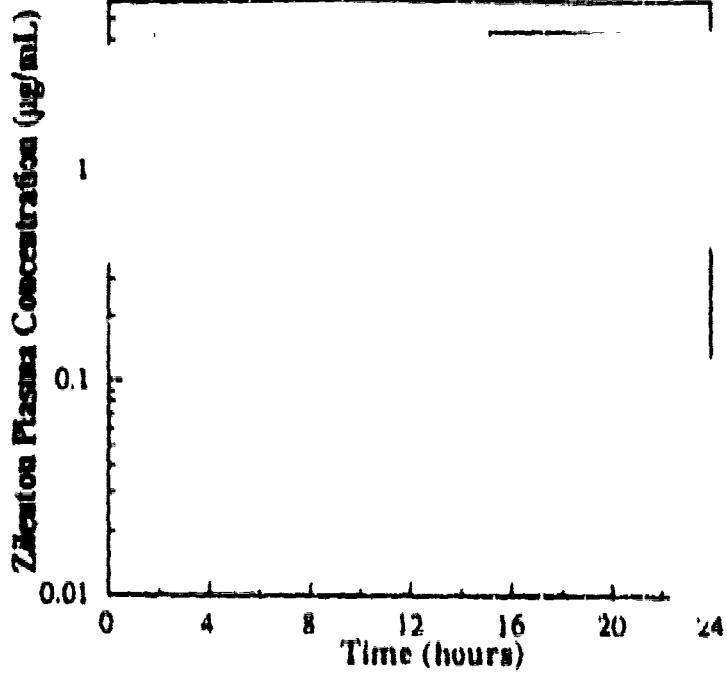
NDA 20,471

Abbott 64077 (Zileuton) Leulrol tablets

Pharmacokinetic section, 34

Submission date: 07/19/94

Fig. 6



Mean plasma concentration-time profiles of zileuton and Abbott-66193 following 600 mg Q6hour administration of zileuton on days 1, 4 and 14 are shown below.

Fig. 8

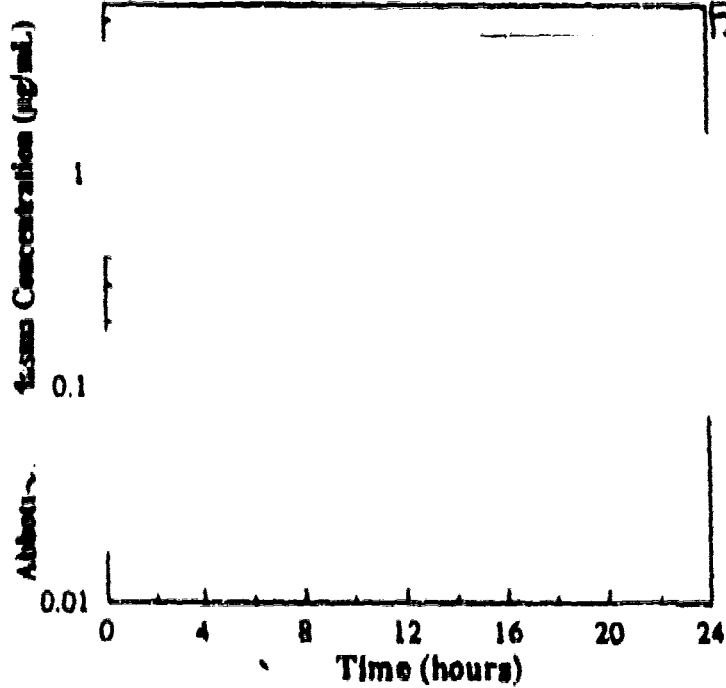
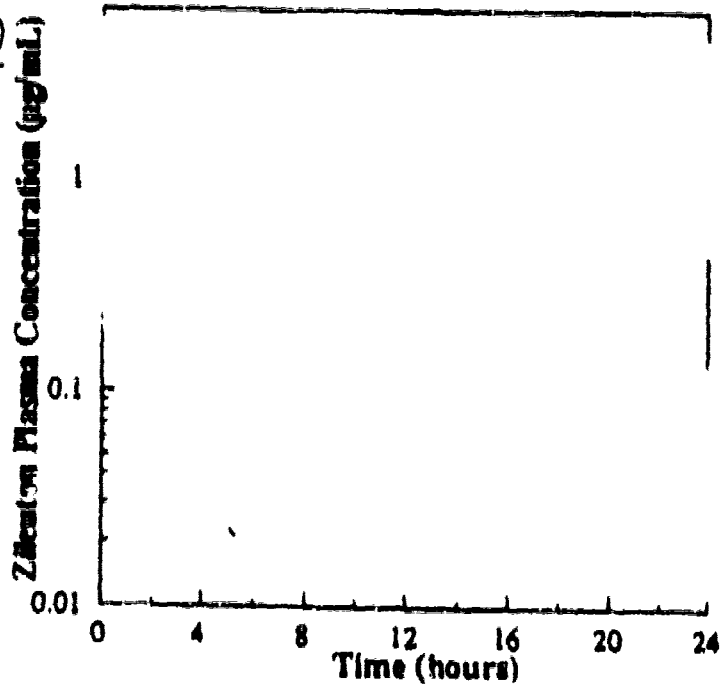


Fig. 9

Steady state was attained by day 4 as indicated by no statistically significant difference between C_{max} , t_{max} , C_{minD} , C_{minE} and CVF of zileuton following morning dose on day 4 vs. that of day 14.

Diurnal variation seems to exist as seen in higher C_{min} values before morning dose (C_{minD}) as compared to C_{min} values during the day (C_{minE}).

600 mg Q6hours was selected for clinical studies since this produced a C_{min} levels of $>1\mu\text{g/ml}$ which corresponded to at least 50% inhibition of LTB_4 biosynthesis.

Pharmacokinetics of R(+) and S(-) zileuton:

The noncompartmental PK parameters of R(+) and S(-) zileuton following administration of 600 mg Q8hours and 600 mg Q6hours of racemic zileuton are shown below.

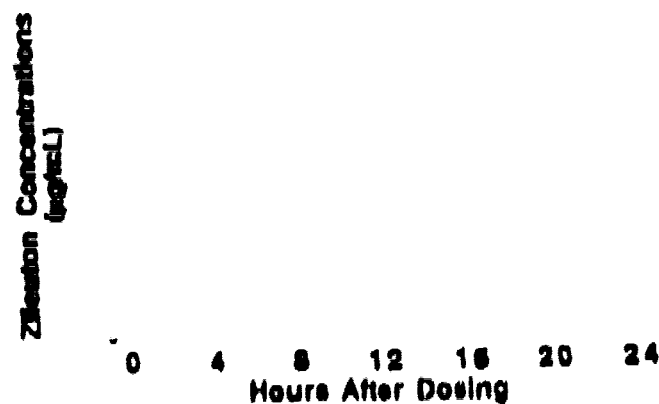
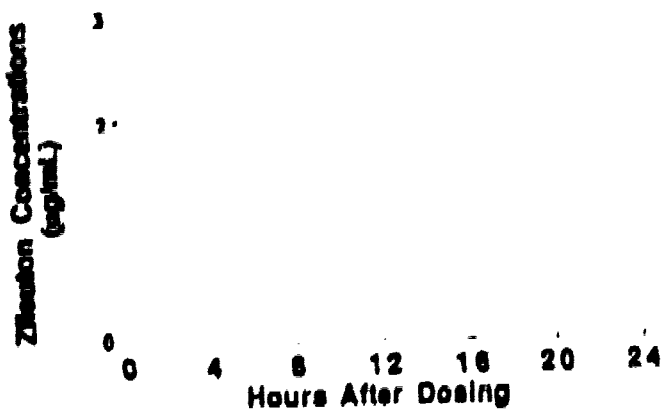
Table (15)

Parameters	Study M89-346 600 mg Q8hrs		Study M89-366 600 mg Q6hrs	
	R(+)	S(-)	R(+)	S(-)
DAY 1 (single dose)				
T _{max} (hr)	2.5 ± 1.2	1.5 ± 0.0	3.0 ± 0.9	2.1 ± 0.8
C _{max} (µg/ml)	1.14 ± 0.17	1.03 ± 0.19	1.29 ± 0.37	0.99 ± 0.34
AUC ₀₋₂₄ (µg.hr/ml)	7.28 ± 1.77	3.34 ± 0.51	9.44 ± 2.54	4.26 ± 1.30
CV/F(ml/min)	721 ± 173	1524 ± 219	566 ± 164	1276 ± 397
t _{1/2} (hr) (avg.)	2.83	1.82	4.52	2.14
DAY 14 (steady state)				
T _{max} (hr)	1.5 ± 0.9	2.0 ± 1.2	1.5 ± 0.9	1.7 ± 1.0
C _{max} (µg/ml)	2.02 ± 0.68	1.07 ± 0.44	3.15 ± 0.62	1.33 ± 0.40
C _{min 0} (µg/ml)	1.01 ± 0.50	0.24 ± 0.18	2.64 ± 0.64	0.87 ± 0.26
C _{min τ} (µg/ml)	0.37 ± 0.13	0.05 ± 0.02	1.21 ± 0.25	0.18 ± 0.10
AUC _{0-τ} (µg.hr/ml)	9.69 ± 2.49	3.75 ± 1.05	13.86 ± 3.32	4.65 ± 1.15
CV/F (ml/min)	549 ± 158	1446 ± 482	383 ± 109	1133 ± 273
t _{1/2} (hr) (avg.)	1.90	1.54	2.21	2.14

Mean plasma concentration-time profiles of R(+) and S(-) zileuton are shown below.

Fig. (10)

Fig. (11)



Fitted and secondary parameters of R(+) and S(-) zileuton are summarized below:

Table 16

Parameters	Study M89-346 600 mg Q8hrs		Study M89-366 600 mg Q6hrs	
	R(+)	S(-)	R(+)	S(-)
K_e (hr^{-1})	0.779 ± 0.384	1.951 ± 1.232	0.503 ± 0.371	0.863 ± 0.462
V/F (L)	145 ± 74	185 ± 118	112 ± 28	173 ± 78
Cl/F (ml/min)	680 ± 132	1366 ± 290	553 ± 145	1254 ± 339
K_e (hr^{-1})	0.325 ± 0.119	0.560 ± 0.322	0.298 ± 0.036	0.498 ± 0.198
$t_{1/2}$ (hr)	2.45 ± 1.10	1.56 ± 0.79	2.36 ± 0.32	1.64 ± 0.75
T_{max} (hr)	1.5 ± 0.2	1.0 ± 0.3	0.5 ± 0.5	0.9 ± 0.4
C_{max} ($\mu g/ml$)	1.80 ± 0.46	1.19 ± 0.37	3.04 ± 0.70	1.35 ± 0.31
C_{min} ($\mu g/ml$)	1.06 ± 0.50	0.25 ± 0.18	2.81 ± 0.82	0.88 ± 0.29
C_{min} τ ($\mu g/ml$)	0.47 ± 0.17	0.09 ± 0.04	1.27 ± 0.34	0.33 ± 0.15

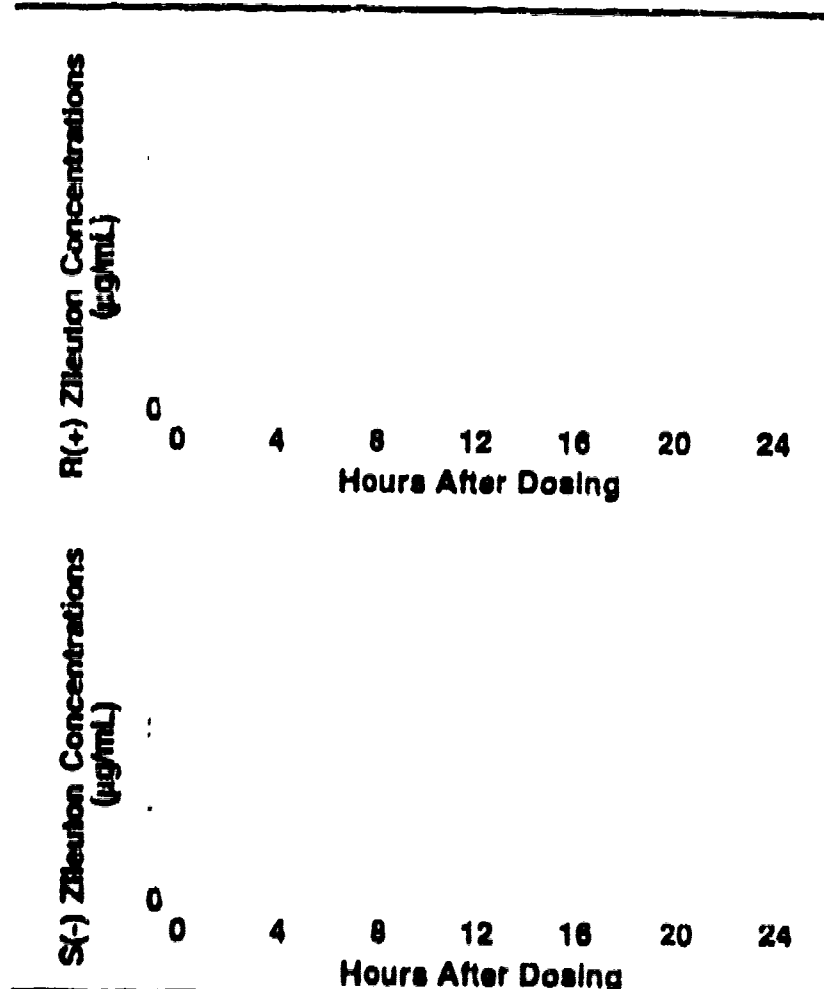
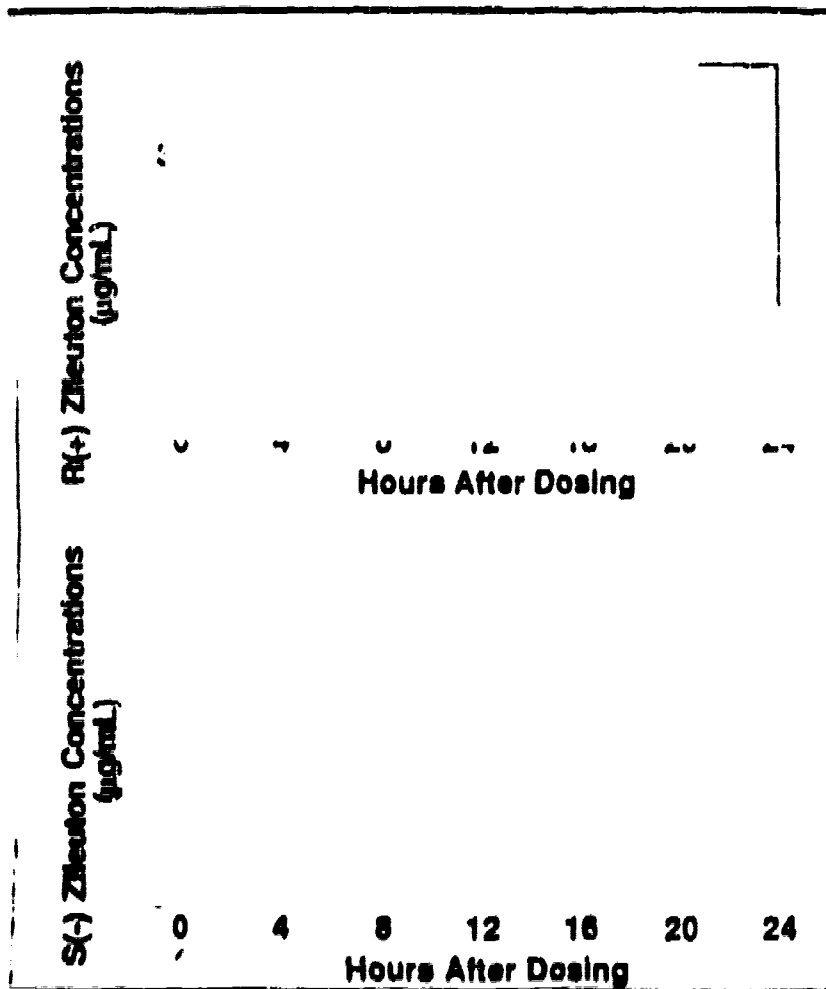
Mean observed and predicted plasma concentration-time profiles of the two enantiomers in both studies are shown in figures below.

Figure 12

Mean Observed and Predicted Plasma Concentrations of R(+) and S(-) Zileuton After Single and Q8H Dosing With Racemic Zileuton

Figure 13

Mean Observed and Predicted Plasma Concentrations of R(+) and S(-) Zileuton After Single and Q6H Dosing With Racemic Zileuton



It was observed that the clearance of S(-) zileuton is 2-fold higher than that of R(+) zileuton.

The PD parameter means of LTB₄ inhibition are shown below.

Table (17)

PD parameters	600 mg Q8hours	600 mg Q6hours
I _{max} (%)	100 (fixed)	100 (fixed)
IC ₅₀ (µg/ml)	0.58 ± 0.31	0.75 ± 0.39
γ	0.82 ± 0.31	1.74 ± 0.98

Observed vs. predicted % LTB₄ inhibition are shown below.

Fig. (14)

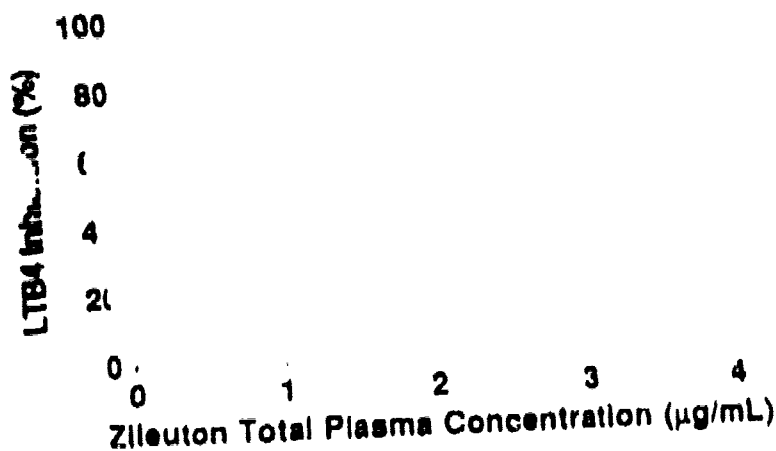
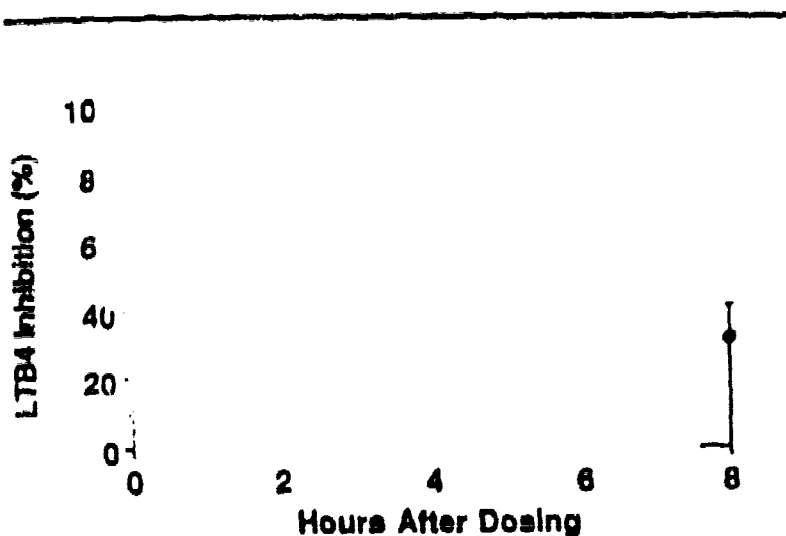


Fig. (15)



From the above figures, it can be seen that inhibition of LTB₄ with 600 mg Q6hours was greater than 600 mg Q8hours. Assuming correlation of LTB₄ to efficacy, 600 mg Q6hours appears to be superior to that of 600 mg Q8hours.

④ **EVALUATION OF THE DOSE-PROPORTIONALITY IN THE PHARMACOKINETICS OF ZILEUTON (ZILEUTON) IN NORMAL HEALTHY VOLUNTEERS**

Study ID:
Investigators:
Clinical Site:

Objective:

To evaluate the dose-proportionality in the pharmacokinetics of zileuton at four different dose levels (200 mg, 400 mg, 600 mg and 800 mg) administered under fasting conditions.

Drug Dosage Forms:

Zileuton capsules (capsule II), bulk NPRO 4942R, Lot 44-827-AR, 200 mg per capsule.

Study Design:

Sixteen healthy non-smoking adult male volunteers, age 20-51 yr, body weight 65.3-97.5 kg, participated in this single-dose, randomized, four-period, open-label, complete-crossover study. Each subject received four doses of zileuton on four successive occasions separated by one-week washout periods. The 16 subjects were randomly assigned to receive a sequence of Regimens A, B, C and D so that one-fourth of the subjects received each of the regimens during the first study period. Each subject should have received all four regimens upon completion of the study. The regimens are defined as follows:

- Regimen 'A': A single dose of 200 mg zileuton (one 200 mg capsule).
Regimen 'B': A single dose of 400 mg zileuton (two 200 mg capsules).
Regimen 'C': A single dose of 600 mg zileuton (three 200 mg capsules).
Regimen 'D': A single dose of 800 mg zileuton (four 200 mg capsules).

After an overnight fast, each subject received the dose of zileuton with 180 ml of water. Breakfast, lunch and dinner were served 3, 5 and 10 hr after dosing. No food or beverage was allowed other than the meals provided in the study and water to quench thirst starting from 12 hr before until after the 24 hour blood collection in each period. Blood samples were collected into evacuated blood collection tubes before (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hr after dosing. Blood samples were centrifuged within 2 hr and kept at -10EC until assayed. Urine was collected at 0 (before dosing), 0-4, 4-8, 8-12, and 12-24 hours postdosing.

Assay Methodology:

The method used in this study measures zileuton, its inactive N-dehydroxylated metabolite, Abbott-66193, the R and S enantiomers of zileuton and the two diastereomeric glucuronides.

Achiral assay for zileuton:
Analytical Site:

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 39
Submission date: 07/19/94

Procedure:

This was carried out by the same procedure as described in the bioequivalence studies.

Chiral assay for R(+) and S(-) zileuton:

Analytical Site: Abbott Laboratories, Abbott Park, IL.

Procedure:

Data Analysis:

Model-independent methods were used to analyze concentration-time data. ANOVA was performed for C_{max} and AUC for dose-normalized values, with the sources of variation being sequence, subject nested with sequence, period and dose. All statistical tests performed on C_{max} and AUC utilized dose-normalized values.

Results:

Mean pharmacokinetic parameters after administration of each dose are given below in table #18. Plot of mean plasma concentrations of zileuton following administrations of various formulations is provided in figure #16. Plots of C_{max} vs. dose and AUC vs. dose are provided in figures #17 and #18 respectively.

Table # 18

Parameter	Zileuton PK parameters			
	200 mg	400 mg	600 mg	800 mg
I. Zileuton:				
T_{max} , hr	1.7 ± 1.2	1.5 ± 0.6	1.9 ± 0.9	1.7 ± 1.0
C_{max} , µg/ml	1.44 ± 0.40	2.90 ± 0.64	3.62 ± 1.34	4.43 ± 1.45
AUC, µg.hr/ml	5.31 ± 1.16	10.98 ± 2.14	16.78 ± 3.99	22.30 ± 5.74
$t_{1/2}$, hr	1.85 ± 0.48	2.26 ± 0.76	2.28 ± 0.80	3.51 ± 3.03
TBC/F, ml/min	653 ± 129	627 ± 117	630 ± 156	630 ± 140
$Vd\beta/F$, L	102 ± 25	120 ± 38	123 ± 52	183 ± 138
II. Metabolite:				
C_{max} , µg/ml	0.04 ± 0.06	0.09 ± 0.07	0.22 ± 0.31	0.46 ± 0.47
T_{max} , hr	9.0 ± 2.8	13.7 ± 5.0	17.1 ± 6.9	17.7 ± 6.2
AUC(0-24), µg.hr/ml	0.36 ± 0.52	1.11 ± 0.88	2.42 ± 3.58	5.13 ± 5.73

FIGURE # 16 Mean ($\pm$ SD) Zileuton Plasma Concentration Versus Time Plots after Single Oral Doses of 200, 400, 600, and 800 Mg in Healthy Volunteers

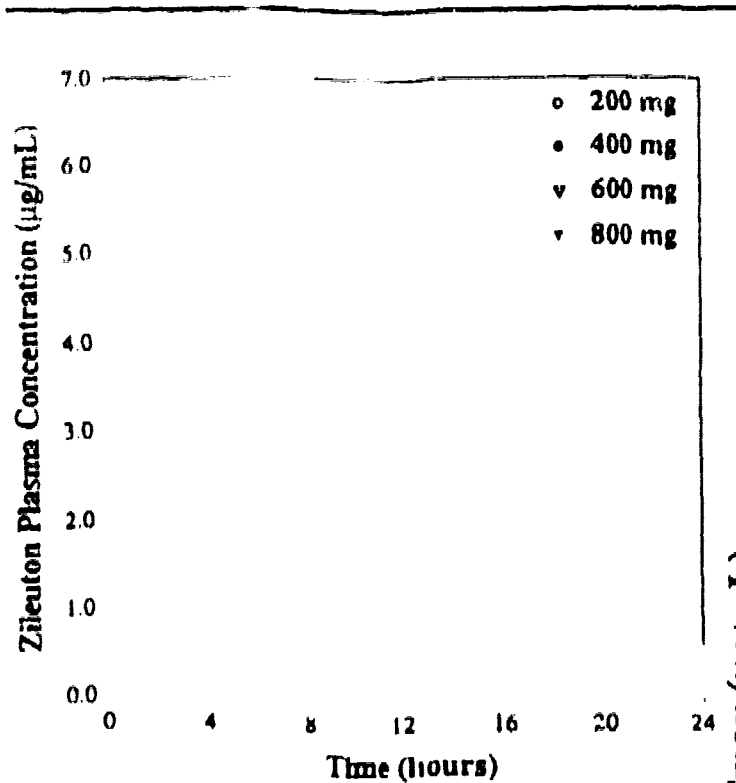


FIGURE # 17

Zileuton C_{max} Mean ($\pm$ SD) after Single Oral Doses of 200, 400, 600, and 800 Mg in Healthy Volunteers

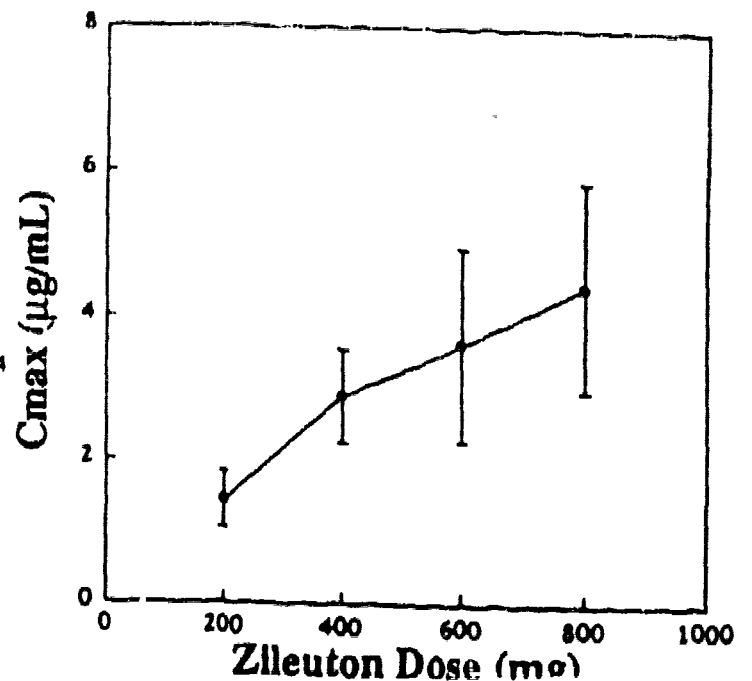


FIGURE #18

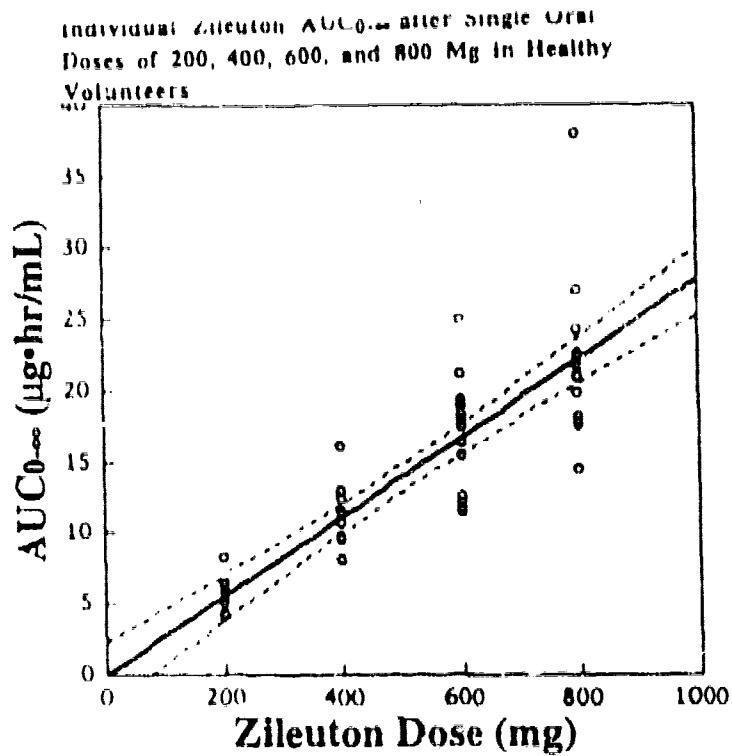


Table #19 provides the mean $\pm$ SD pharmacokinetic parameters of the two enantiomers of zileuton, and zileuton glucuronides.

Table #19

Parameter	R(+) Zileuton		S(-) Zileuton	
	200 mg	600 mg	200 mg	600 mg
T_{max} , hr	2.3 $\pm$ 1.0	2.3 $\pm$ 1.0	1.4 $\pm$ 1.0	1.6 $\pm$ 0.7
C_{max} , μ g/ml	0.83 $\pm$ 0.19	1.80 $\pm$ 0.47	0.88 $\pm$ 0.27	1.78 $\pm$ 0.71
AUC, μ g.hr/ml	3.49 $\pm$ 0.56	10.75 $\pm$ 2.07	2.41 $\pm$ 0.71	6.55 $\pm$ 2.26
$t_{1/2}$, hr	1.88 $\pm$ 0.57	2.49 $\pm$ 0.90	1.59 $\pm$ 0.80	2.66 $\pm$ 1.20
TBC/F, ml/min	492 $\pm$ 99	481 $\pm$ 96	735 $\pm$ 175	846 $\pm$ 295
Vdb/F, L	79 $\pm$ 23	102 $\pm$ 39	101 $\pm$ 63	194 $\pm$ 132
Parameter	R(+)/S(-) Zileuton			
	200 mg		600 mg	
C_{max} ratio	1.00 $\pm$ 0.27		1.08 $\pm$ 0.21	
AUC ratio	1.49 $\pm$ 0.25		1.72 $\pm$ 0.26	

Parameter	G-1 glucuronide		G-2 glucuronide	
	200 mg	600 mg	200 mg	600 mg
T_{max} , hr	2.7 ± 1.0	2.4 ± 0.9	2.4 ± 0.9	1.8 ± 0.7
C_{max} , µg/ml	2.42 ± 0.67	6.64 ± 2.37	3.09 ± 0.62	8.05 ± 2.05
AUC, µg hr/ml	12.11 ± 2.09	45.09 ± 10.32	10.98 ± 1.28	40.23 ± 13.70
$t_{1/2}$, hr	2.51 ± 0.95	3.41 ± 1.27	1.61 ± 0.86	3.11 ± 1.24
CL_R , ml/min	192.5 ± 42.9	144.7 ± 24.6	237.3 ± 37.6	171.8 ± 46.6

CONCLUSION:

The dose-normalized AUC is independent of dose. Hence, the pharmacokinetics appear to be linear. Apparent total body clearance was found to be similar throughout the dose range studied. C_{max} did not proportionally increase with dose. Considering the solubility characteristics of zileuton, the less than proportional increase of C_{max} with dose indicates that absorption rate from capsules may be dissolution rate limited at higher doses.

Mean dose-normalized AUC and C_{max} of R(+) and S(-) zileuton were similar. AUC and C_{max} of Abbott-66193 increased more than proportionally with the dose of zileuton administered.

The concentration time profiles of the glucuronides and the zileuton enantiomers were parallel to each other, suggesting that elimination of glucuronides is formation-rate limited. Renal clearances of the glucuronides exceeded GFR. This could be due to renal tubular secretion in addition to filtration.

COMMENT:

Dose-proportionality in PK of zileuton is demonstrated. However, at 800 mg dose, non-linearity is seen as suggested by C_{max} information.

⑤ **EVALUATION OF DIURNAL VARIATION IN PHARMACOKINETICS OF ZILEUTON IN NORMAL HEALTHY VOLUNTEERS**

Study ID: M93-034

Investigators:

Clinical Site:

n.

Objective:

To evaluate the diurnal variation in the pharmacokinetics of zileuton in healthy normal volunteers.

Drug Dosage Forms:

Zileuton 600 mg tablets FF, bulk NPRO 5634N, Lot 60-778-AR.

Study Design: This was a single and multiple-dose, fasting (except during multiple-dosing), three-period, open-label, randomized, crossover study (cross-over in first 2 periods) utilizing 12 healthy adult male subjects (age 18-48 years). The subjects were divided into two groups and randomly assigned to receive either Regimen A or B in Period 1 of the study. In Period 2, the 2 groups were crossed over to receive the other single-dose regimen. In Period 3, all 12 subjects were administered Regimen C, consisting of the QID dosing for five days.

A schematic of the study design follows:

Table ②①

	Regimens		
	Period 1	Period 2	Period 3
I	A	B	C
II	B	A	C

Regimen A: A single 7:00 a.m. Dose of 600 mg zileuton (one tablet)

Regimen B: A single 11:00 p.m. Dose of 600 mg zileuton (one tablet)

Regimen C: Multiple Doses of 600 mg zileuton (one tablet) QID for 5 days
(7:00 a.m., 1:00, 6:00 and 11:00 p.m.)

Methods: Blood samples were collected on Days 1 and 2 in Periods 1 and 2 (at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24 hours) and on Days 5 and 6 of Period 3 (on day 5 at 0, 1, 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 14, 16, 17, 18, 19, 20, 22 and 24 hours). All plasma samples were analyzed for zileuton and its N-dehydroxylated metabolite by validated HPLC method.

Results: The pharmacokinetic parameters of zileuton (mean $\pm$ SD) after single-dose administration at 7:00 a.m. or 11:00 p.m. and at steady-state (Day 5 of QID dosing) are summarized below:

Table (21)

Single Dose		
Zileuton Pharmacokinetic Parameters		
	A M Dosing	P M Dosing
T _{max} (hr)	2.25 ± 1.48	2.08 ± 1.16
C _{max} (µg/mL)	5.08 ± 2.26	5.43 ± 1.72
AUC _{0-∞} (µg•hr/mL)	20.10 ± 6.12	21.59 ± 5.94
Cl/F (mL/min)	545 ± 179	495 ± 132
C _{ss} (µg/mL)	3.35 ± 1.02	3.60 ± 0.99
t _{1/2} (hr)	2.36 ± 0.67	2.28 ± 0.29
β (hr ⁻¹)	0.314 ± 0.083	0.310 ± 0.053
Vd/F (L)	113 ± 59	96 ± 23

Table (22)

Steady State					
Zileuton Pharmacokinetic Parameters	7:00 AM Dosing 0-6 Hours	1:00 PM Dosing 6-11 Hours	6:00 PM Dosing 11-16 Hours	11:00 PM Dosing 16-24 Hours	Entire 24 Hours
T _{max} (hr)	2.33 ± 0.49	1.50 ± 0.67	2.17 ± 0.72	3.08 ± 1.68	2.27 ± 0.55*
C _{max} (µg/mL)	6.01 ± 1.89	6.19 ± 1.38	6.04 ± 1.62	5.63 ± 1.95	5.97 ± 0.99*
C _{min0} (µg/mL)	2.15 ± 1.34	1.63 ± 0.52	1.58 ± 0.43	1.82 ± 0.53	1.80 ± 0.56*
C _{min1} (µg/mL)	1.63 ± 0.52	1.58 ± 0.43	1.82 ± 0.53	1.46 ± 0.80	
AUC ₀₋₁ (µg•hr/mL)	21.69 ± 5.37	18.78 ± 3.43	18.58 ± 3.84	23.98 ± 5.34	20.76 ± 3.38*
Cl/F (mL/min)	496 ± 168	548 ± 96	561 ± 126	435 ± 92	493 ± 79
C _{ss} (µg/mL)	3.62 ± 0.90	3.76 ± 0.69	3.72 ± 0.77	3.00 ± 0.67	3.46 ± 0.56
t _{1/2} (hr)				2.51 ± 0.33	
β (hr ⁻¹)				0.280 ± 0.036	
Vd/F (L)				93 ± 19	

* Average of values of the four dosing intervals

Fig. 19
Mean (+/- SD) Zileuton Plasma Concentration-Time Curve After A.M. or P.M.
Single 600 Mg Dose of Zileuton (Study M93-034)

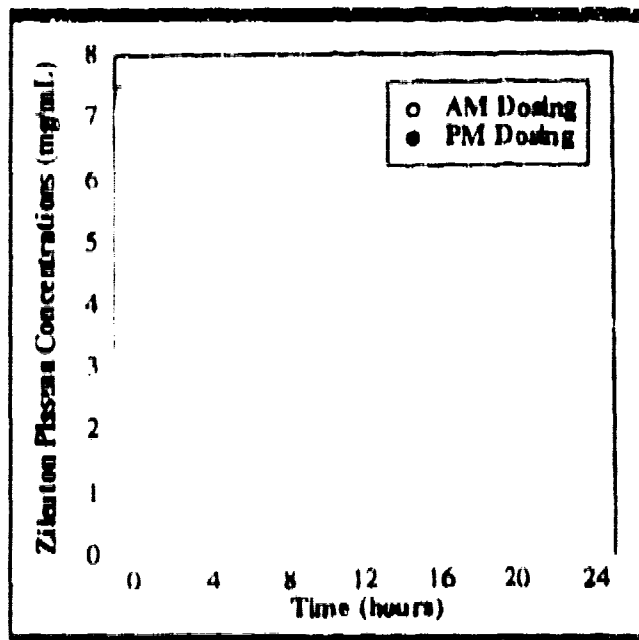
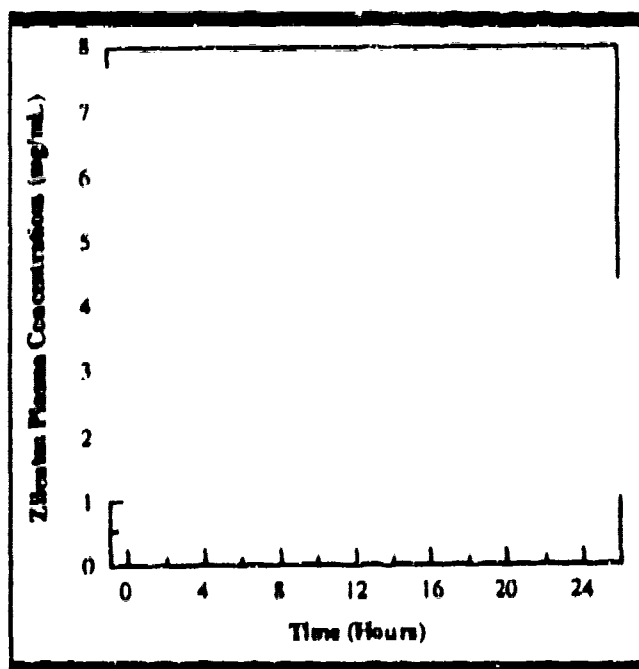


Figure 20
Mean (+SD) Zileuton Plasma Concentration-Time Curve on Day 5 of 600 Mg QID Dosing
of Zileuton (Study M93-034)



The results of this study indicate that there was no significant diurnal variation in the pharmacokinetics of zileuton after single-dose administration. The mean plasma concentration-time profiles of zileuton across dose intervals at steady state are similar. At steady state, the mean AUC value after the 7:00 A.M. morning dose was not statistically significantly different from the

average of the three AUC means for the three P.M. doses of zileuton. Diurnal variation in zileuton trough concentrations was observed since the 7:00 A.M. mean trough concentrations was statistically significantly higher than the average of the three subsequent P.M. mean trough concentrations. This demonstrates that an 11:00 P.M. dose provides adequate zileuton plasma concentration prior to the 7:00 A.M. dose. The mechanism for the higher A.M. trough concentration is unknown. The pharmacokinetics of zileuton after multiple doses were similar to that after single-dose administration.

After the A.M. and P.M. single-dose administration of zileuton, measurable plasma concentrations of the N-dehydroxylated metabolite, Abbott-66193, did not appear until approximately 10 hours after administration of the zileuton dose. The plasma concentrations of Abbott-66193 at steady state were relatively constant throughout the day within a dosing interval, but quite variable among subjects.

Table 23
Single-Dose (mean ± SD)

Abbott-66193 Pharmacokinetic Parameters	A.M. Dosing	P.M. Dosing
T _{max} (hr)	11.00 ± 7.06	10.33 ± 7.71
C _{max} (µg/mL)	0.13 ± 0.16	0.03 ± 0.02
AUC ₀₋₂₄ (µg·hr/mL)	1.44 ± 1.75	0.25 ± 0.23

Table 24 Steady-State

Abbott-66193 Pharmacokinetic Parameters	7:00 AM Dosing (0-6 hrs)	1:00 PM Dosing (6-11 hrs)	6:00 PM Dosing (11-16 hrs)	11:00 PM Dosing (16-24 hrs)
T _{max} (hr)	4.33 ± 1.56	3.67 ± 1.97	1.67 ± 1.92	1.33 ± 2.42
C _{max} (µg/mL)	0.85 ± 0.58	1.04 ± 0.71	1.11 ± 0.75	1.03 ± 0.71
AUC ₀₋₁ (µg·hr/mL)	4.27 ± 2.46	4.57 ± 3.17	5.04 ± 3.52	6.78 ± 4.58
C _{min} (µg/mL)	0.71 ± 0.41	0.91 ± 0.63	1.01 ± 0.70	0.85 ± 0.57

Conclusions: Diurnal variation in the pharmacokinetics of zileuton was not observed after single-dose administration. Diurnal variation was observed in zileuton trough levels since at steady state, the morning mean trough concentration was statistically significantly higher than the average of the three subsequent P.M. mean trough concentrations. The P.M. mean trough concentrations of zileuton exceeded 1 µg/mL and concentrations > 1 µg/mL corresponded to greater than 50% inhibition of *ex vivo* blood LTB₄ synthesis.

Comments: It is very interesting to note that when you compare on day 5, mean C_{min} at 24 hours (1.46 ± 0.80) is less than that of C_{min} at 0 hours (2.15 ± 1.34). However, this could be due to variability in the subjects. Hence, based on this data there may not be any diurnal variation; after all, especially when administered QID at unequal intervals.

BIOEQUIVALENCE STUDIES

6. ABSORPTION CHARACTERISTICS OF ZILEUTON, FROM CAPSULE, TABLET AND SUSPENSION FORMULATIONS

Study ID: M89-315

Investigators:

Clinical Site:

Objective:

To determine the relative bioavailabilities of 800 mg doses of zileuton administered as A) four 200 mg tablet (not final formulation); B) four 200 mg capsules (capsule I); C) four 200 mg capsules (capsule II) and D) 800 mg/50 ml suspension.

Drug Dosage Forms:

FORMULATIONS	BULK NPRO	BULK LOT
A: Tablet	4572N	26-083-AR
B: Capsule I	4525N	24-923-AR
C: Capsule II	4158	14-999-AR
D: Suspension	4584N	26-109-AR
Suspension vehicle	4585N	26-110-AR

Study Design:

Sixteen healthy adult male volunteers, age 18-40 yr, body weight 66.2-86.9 kg, participated in this single-dose, randomized, four-period, open-label, complete-crossover study. Each subject received 800 mg of zileuton on four successive occasions separated by one-week washout periods. After an overnight fast, each subject received the dose of zileuton with 180 ml of water in case of tablet or capsule. The suspension formulation was extemporaneously prepared by adding 50 ml of vehicle to the unit-dose container with concentrated powder, shaken and given to the subjects along with 2 rinses of the container (volume of rinse 20 ml) and total amount of 180 ml of water. Breakfast, lunch and dinner were served 3, 5 and 10 hr after dosing. No food or beverage was allowed other than the meals provided in the study and water to quench thirst starting from 12 hr before until after the 24 hour blood collection in each period. Blood samples were collected into evacuated blood collection tubes before (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hr after dosing. Blood samples were centrifuged within 2 hr and kept at -10°C until assayed.

Assay Methodology:

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 48
Submission date: 07/19/94

Data Analysis:

Model-independent methods were used to analyze concentration-time data. $AUC(0-24)$ was considered equivalent to the total AUC .

ANOVA was performed for each concentration point, T_{max} , C_{max} and AUC , with the sources of variation being sequence, subject nested with sequence, period and formulation. Pairwise comparisons were made between each two formulations. Relative bioavailability was assessed using the two one-sided test procedure.

Results:

Mean pharmacokinetic parameters after administration of each formulation are given below in table #25. Plot of mean plasma concentrations of zileuton and its metabolite following administrations of various formulations is provided in figure #21. Plots of C_{max} , $t_{1/2}$, AUC and apparent clearance are provided in figures #22 and 23.

Table # 25

Parameter	TREATMENT			
	Tablet	Capsule I	Capsule II	Suspension
I. Zileuton:				
T_{max} , hr	2.2 ± 1.2	2.7 ± 1.2	2.1 ± 1.2	0.9 ± 0.7
C_{max} , µg/ml	4.51 ± 1.52	4.34 ± 1.79	4.00 ± 1.02	7.05 ± 2.20
AUC, µg.hr/ml	25.90 ± 7.03	26.00 ± 8.93	25.08 ± 5.71	28.60 ± 7.33
$t_{1/2}$, hr	4.11 ± 1.44	3.67 ± 1.48	4.09 ± 2.11	2.28 ± 0.64
TBC/F, ml/min	552 ± 151	572 ± 194	568 ± 150	496 ± 129
$Vd\beta/F$, L	193 ± 75	173 ± 63	189 ± 79	94 ± 22
II. Metabolite:				
C_{max} , µg/ml	0.68 ± 0.49	0.74 ± 0.68	0.74 ± 0.54	0.21 ± 0.16
T_{max} , hr	18.8 ± 6.2	16.4 ± 7.1	17.1 ± 6.0	10.3 ± 6.6
AUC, µg.hr/ml	7.10 ± 6.39	8.09 ± 7.98	7.69 ± 6.41	2.39 ± 2.15
AUCm/AUCp	0.31 ± 0.29	0.37 ± 0.39	0.35 ± 0.31	0.09 ± 0.08

FIGURE # 21

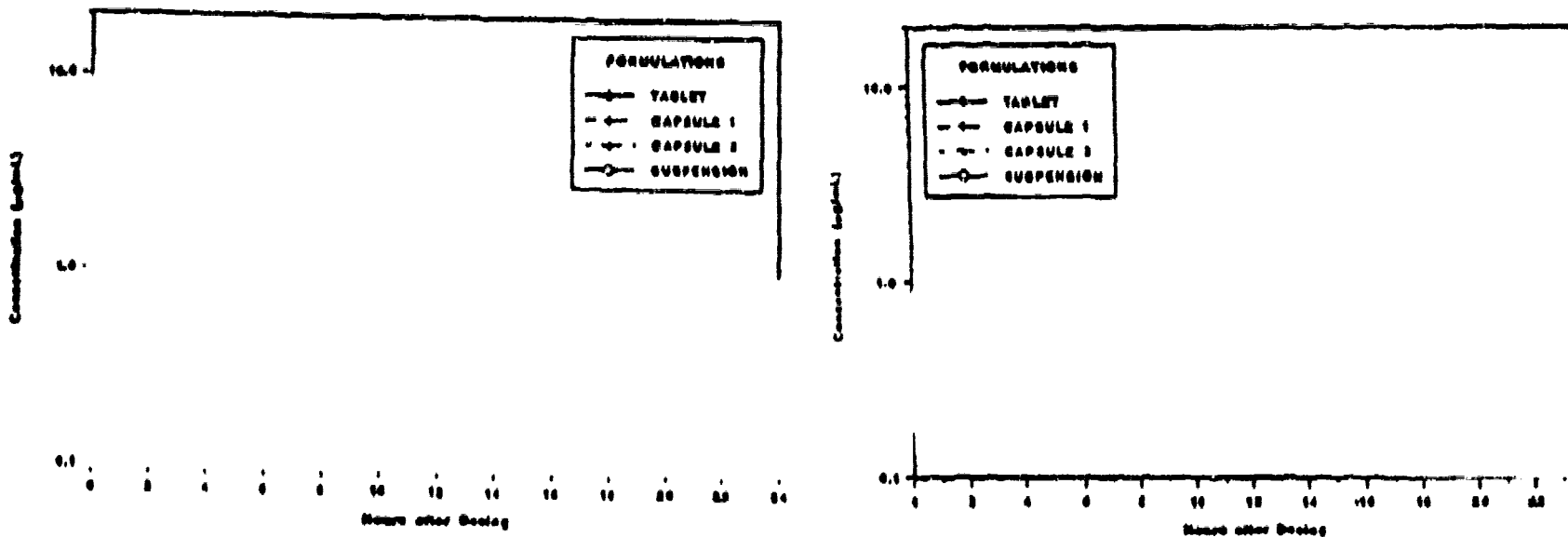


FIGURE #22 Comparison of the Mean Pharmacokinetic Parameters of ABBOTT-64077
Calculated after Single Oral Administration of
Four ABBOTT-64077 Formulations

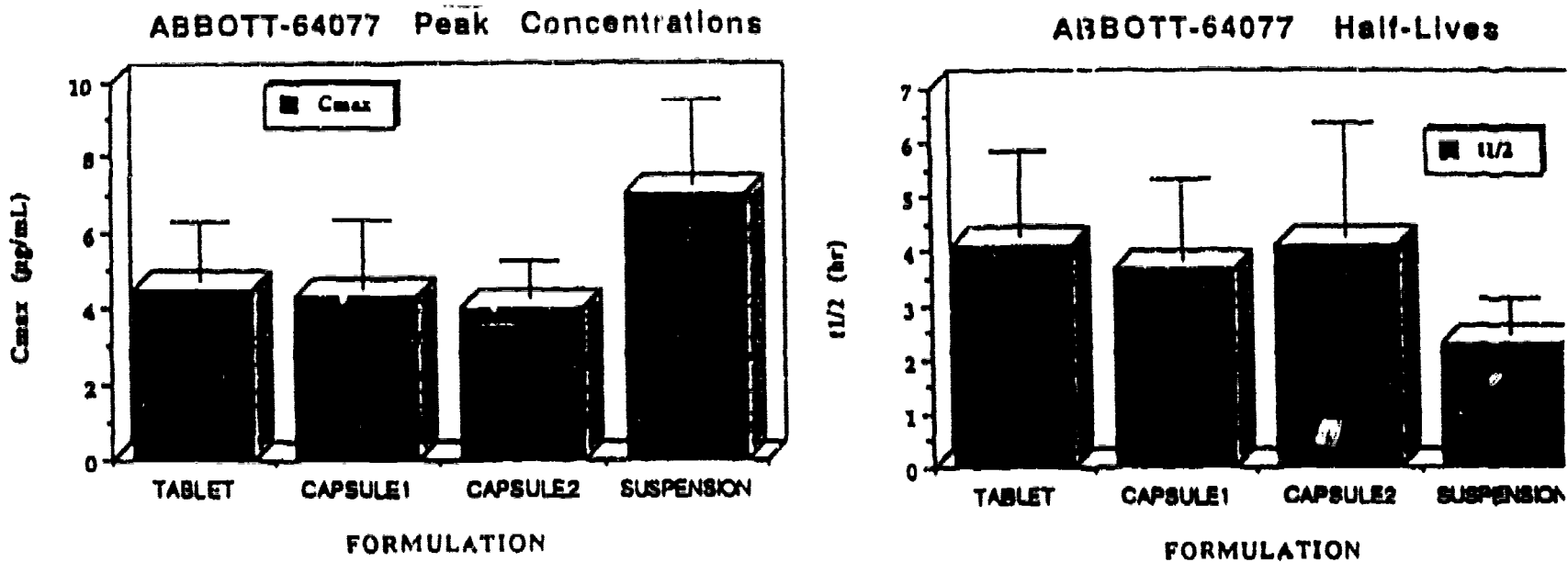


FIGURE #23

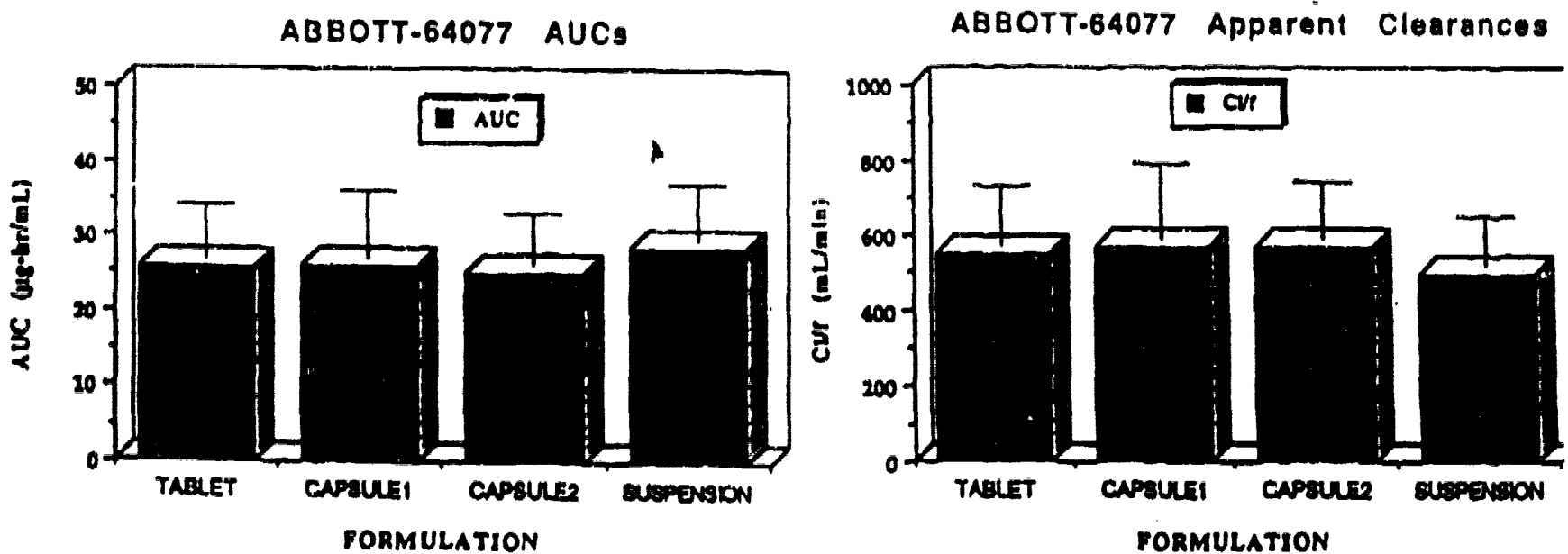


Table #~~26~~ provides confidence intervals obtained upon calculation using two one-sided tests procedure on log-transformed AUC and C_{max} .

Table #26

Comparisons for Zileuton	Point estimate	90% confidence interval
AUC		
Tablet vs. suspension	0.902	0.839 - 0.970
<i>Capsule I vs. suspension</i>	<i>0.888</i>	<i>0.825 - 0.954</i>
<i>Capsule II vs. suspension</i>	<i>0.875</i>	<i>0.814 - 0.941</i>
Tablet vs. capsule II	1.031	0.959 - 1.109
<i>Capsule I vs. capsule II</i>	<i>1.014</i>	<i>0.943 - 1.091</i>
Tablet vs. capsule I	1.017	0.945 - 1.093
C_{max}		
Tablet vs. suspension	0.636	0.567 - 0.713
<i>Capsule I vs. suspension</i>	<i>0.596</i>	<i>0.532 - 0.669</i>
<i>Capsule II vs. suspension</i>	<i>0.575</i>	<i>0.512 - 0.644</i>
Tablet vs. capsule II	1.106	0.987 - 1.241
<i>Capsule I vs. capsule II</i>	<i>1.038</i>	<i>0.925 - 1.164</i>
Tablet vs. capsule I	1.066	0.951 - 1.196

Calculation of C.I. for AUC and C_{max} of capsule I vs. capsule II:

AUC (capsule I vs. capsule II)

ERROR MEAN SQUARE . . . 0149305
REFERENCE MEAN 3.1888
TEST MEAN 3.2028
NUMBER OF SUBJECTS . . 16
DEGREES OF FREEDOM . . 14
NUMBER OF TREATMENTS . 2
DELTA 2

POWER ANALYSIS

POWER FOR .2 M(r)= 97.15111 %
POWER FOR -.2 M(r)= 99.54159 %

DETECTABLE DIFFERENCE:13.90054 %

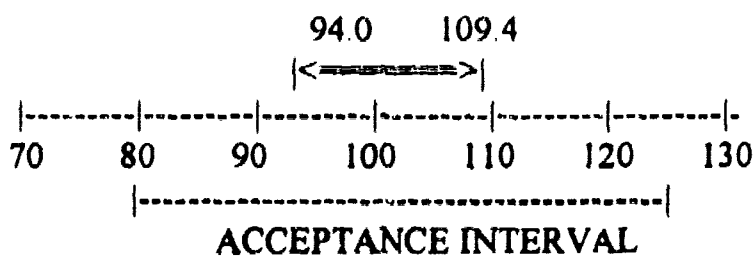
10 SUBJECTS NEEDED FOR A
19.07597 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

LOWER CI (% OF REF MEAN): 93.97986
UPPER CI (% OF REF MEAN): 109.4272
CONCLUSION: PASS

P VALUES OF TWO ONE-SIDED TEST

p< 80 % REF MEAN: 0.00004
p> 120 % REF MEAN: 0.00081
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 94.0% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 109.4% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +0.44% OF THE REFERENCE MEAN.

C_{max} (capsule I vs. capsule II)

ERROR MEAN SQUARE . . 3.713466E-02
REFERENCE MEAN 1.3554
TEST MEAN 1.3925
NUMBER OF SUBJECTS . . 16
DEGREES OF FREEDOM . . 14
NUMBER OF TREATMENTS . 2
DELTA 2

POWER ANALYSIS

POWER FOR .2 M(r)= 69.81419 %
POWER FOR -.2 M(r)= 86.11633 %

DETECTABLE DIFFERENCE:22.78504 %

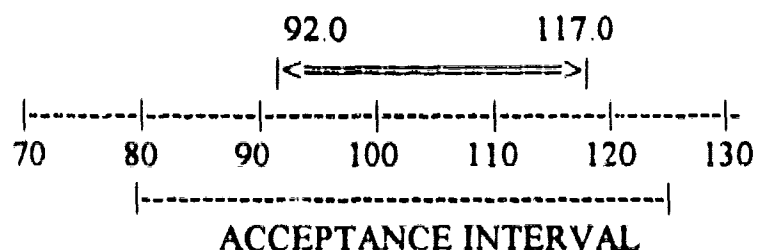
20 SUBJECTS NEEDED FOR A
19.78806 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

LOWER CI (% OF REF MEAN): 92.0444
UPPER CI (% OF REF MEAN): 117.0112
CONCLUSION: PASS

P VALUES OF TWO ONE-SIDED TEST

p < 80 % REF MEAN: 0.00094
p > 120 % REF MEAN: 0.02571
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 92.0% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 117.0% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +2.74% OF THE REFERENCE MEAN.

Since the formulations of interest are capsule I and II, it was suggested that confidence interval computation be carried out using data only from relevant treatments and treatment data in other arms as missing. Such calculation is shown on pages 52 a and b. This type of computation did not lead to different conclusion since the variances across the 4 treatments of the study were similar.

CONCLUSION:

Capsule I and capsule II are bioequivalent. Relative bioavailability of the capsules to the suspension is about 88% based on AUC and 58-60% based on C_{max} .

COMMENT:

It should be noted that the formulations of interest in this NDA are capsule I (treatment B), capsule II (treatment C) and suspension (treatment D). Although these two capsules are not to-be marketed formulations, bioequivalence between capsule I and II is important since these have been used in some important clinical trials. Comparison to suspension data is important since, in this NDA, study to determine absolute bioavailability has not been carried out due to solubility constraints of the drug. Since we do not have any I.V. data or oral solution data, the next alternative to get some indication of oral bioavailability of the zileuton capsule dosage form is comparing it to the suspension.

Calculations based on MSE obtained
only from relevant treatments Capsule I

CAPSBECMAX

POWER ANALYSIS

vs.
II

ERROR MEAN SQUARE . . . 4.076598E-02
REFERENCE MEAN 1.355444
TEST MEAN 1.392482
NUMBER OF SUBJECTS . . . 16
DEGREES OF FREEDOM . . . 14
NUMBER OF TREATMENTS . . 2
DELTA 2

POWER FOR .2 M(r)= 65.58392 %
POWER FOR -.2 M(r)= 82.82962 %

DETECTABLE DIFFERENCE: 23.99452 %

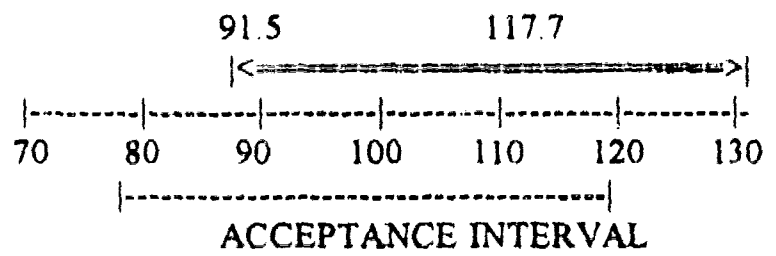
22 SUBJECTS NEEDED FOR A
19.64315 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

P VALUES OF TWO ONE-SIDED TEST

LOWER CI (% OF REF MEAN): 91.51276
UPPER CI (% OF REF MEAN): 117.6763
CONCLUSION: PASS

p < 80 % REF MEAN: 0.00133
p > 120 % REF MEAN: 0.03071
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 91.5% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 117.7% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +2.73% OF THE REFERENCE MEAN.

C.I. new = 0.915 - 1.177
(Previous C.I. = 0.925 - 1.164)

CAPSBEAUC

ERROR MEAN SQUARE 0197457
REFERENCE MEAN 3.188757
TEST MEAN 3.202697
NUMBER OF SUBJECTS . . . 16
DEGREES OF FREEDOM . . . 14
NUMBER OF TREATMENTS . . 2
DELTA 2

POWER ANALYSIS

POWER FOR .2 M(r)= 92.51018 %
POWER FOR .2 M(r)= 98.28584 %

DETECTABLE DIFFERENCE: 16.14616 %

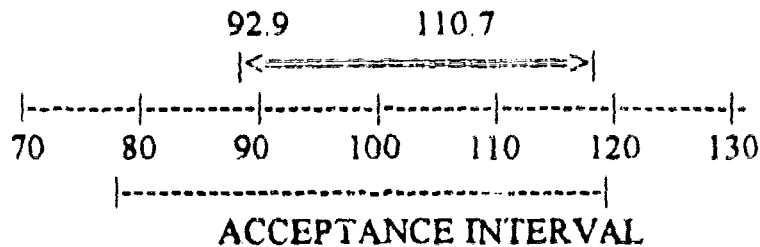
12 SUBJECTS NEEDED FOR A
19.51174 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

LOWER CI (% OF REF MEAN): 92.9077
UPPER CI (% OF REF MEAN): 110.6767
CONCLUSION: PASS

P VALUES OF TWO ONE-SIDED TEST

p < 80 % REF MEAN: 0.00015
p > 120 % REF MEAN: 0.00221
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 92.9% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 110.7% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +0.44% OF THE REFERENCE MEAN.

C.I. new = 0.929 - 1.107
(Previous C.I. = 0.943 - 1.091)



EVALUATION OF THE BIOAVAILABILITY OF 600 MG TABLET AND SUSPENSION FORMULATIONS OF ZILEUTON

Study ID: F92-219

Investigators:

Clinical Site:

Objective:

1. To assess the bioequivalence of a final 600 mg zileuton tablet manufactured a (formulation B) to a final 600 mg zileuton tablet manufactured at Abbott Park, IL (formulation A).
2. To assess the bioequivalence of a final 600 mg zileuton tablet manufactured at (B) and that at Abbott Park, IL (A), to a 300 mg final zileuton tablet (formulation C), manufactured at Abbott Park, IL.
3. To assess the bioavailability of each final 600 mg tablet and 300 mg tablet relative to a 600 mg zileuton suspension (formulation D).

Drug Dosage Forms:

FORMULATIONS	BULK NPRO	BULK LOT	FINISHED LOT #
A: Tablet (600 MG FF AP)	5590N	50-592-AR	64-178-AR
B: Tablet (600 MG FF PR)	5619N	60-699-AR	64-179-AR
C: Tablet (300 MG FF AP)	5592N	59-594-AR	64-180-AR
D: Suspension (600 mg)	5689N	62-993-AR	64-181-AR

Each batch of tablet was a full scale production batch.

Study Design:

Twenty four healthy adult male volunteers, age 24-54 yr, body weight 63.5-92.5 kg, participated in this single-dose, randomized, four-period, open-label, complete-crossover study (of whom 23 completed the study). Each subject received 600 mg of zileuton on four successive occasions separated by one-week washout periods. After an overnight fast, each subject received the dose of zileuton with 180 ml of water in case of tablet or capsule. The suspension formulation was extemporaneously prepared by adding 50 ml of vehicle to the unit-dose container with concentrated powder, shaken and given to the subject: followed by 180 ml of water. Breakfast, lunch and dinner were served 3, 5 and 10 hr after dosing. No food or beverage was allowed other than the meals provided in the study and water to quench thirst starting from 12 hr before until after the 24 hour blood collection in each period. Blood samples were collected into evacuated blood collection tubes before (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hr after dosing. Blood samples were centrifuged within 2 hr and kept at -10°C until assayed.

Assay Methodology:

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 54
Submission date: 07/19/94

Analytical Site: Drug Analysis Section, Abbott Laboratories, Abbott Park, IL.
Procedure:

Data Analysis:

Model-independent methods were used to analyze concentration-time data. ANOVA was performed for each concentration point, T_{max} , C_{max} and $AUC_{0-\infty}$ for total zileuton and also individual enantiomers, with the sources of variation being sequence, subject nested with sequence, period and formulation. Pairwise comparisons were made between each of two formulations by least significant difference procedure, with an alpha level of 0.05. Bioequivalence between the two final 600 mg tablets and the 300 mg final tablet were assessed using the two one-sided test procedure via 90% confidence intervals from analysis on natural logarithms of C_{max} and AUC of total zileuton.

Results:

Mean pharmacokinetic parameters of total zileuton and individual enantiomers after administration of each formulation are given below in table #27. Plot of mean plasma concentrations of zileuton and its enantiomers following administrations of various formulations is provided in figures #24 and #25.

Table # 27

Parameter	FORMULATIONS			
	A: 600 mg FF Tablet AP	B: 600 mg FF Tablet PR	C: 2 x 300 mg FF Tablet AP	D: 600 mg Suspension
I. Zileuton:				
T_{max} , hr	1.78 ± 1.06	1.96 ± 1.11	1.61 ± 1.08	1.65 ± 0.87
C_{max} , µg/ml	5.56 ± 1.73	5.39 ± 1.37	4.98 ± 1.92	6.28 ± 1.63
AUC, µg.hr/ml	23.19 ± 5.77	21.36 ± 5.04	21.45 ± 5.24	22.60 ± 4.99
$t_{1/2}$, hr	2.83 ± 1.26	3.00 ± 1.10	3.09 ± 1.39	2.67 ± 1.27
II. R(+) Zileuton:				
T_{max} , hr	1.9 ± 1.1	1.9 ± 0.90	1.8 ± 1.0	1.9 ± 0.8
C_{max} , µg/ml	2.58 ± 0.69	2.75 ± 0.88	2.35 ± 0.61	3.21 ± 0.78
AUC, µg.hr/ml	14.02 ± 3.60	12.86 ± 3.18	13.24 ± 3.19	14.30 ± 3.38
$t_{1/2}$, hr	2.90 ± 1.26	3.81 ± 1.45	4.05 ± 2.69	3.17 ± 0.88
III. S(-) Zileuton:				
T_{max} , hr	1.4 ± 1.1	1.5 ± 1.1	1.4 ± 1.4	1.2 ± 0.6
C_{max} , µg/ml	2.33 ± 0.84	2.54 ± 1.16	2.15 ± 0.77	3.01 ± 0.93
AUC, µg.hr/ml	7.17 ± 2.10	6.27 ± 1.73	6.28 ± 1.58	6.90 ± 1.65
$t_{1/2}$, hr	1.31 ± 0.73	1.29 ± 0.25	1.41 ± 0.48	1.03 ± 0.48
IV. R(+)/S(-) Zileuton:				
C_{max} Ratio	1.17 ± 0.29	1.13 ± 0.18	1.16 ± 0.35	1.10 ± 0.20
AUC Ratio	2.01 ± 0.33	2.07 ± 0.20	2.14 ± 0.35	2.09 ± 0.24

FIGURE # 24

Mean Plasma Concentrations of Zileuton for
Formulations A, B, C, and D After a Single Oral
600 Mg Zileuton Dose (Study F92-219)

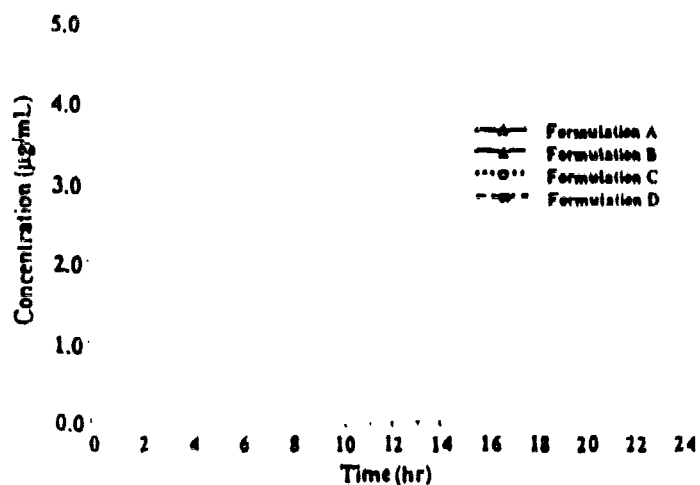


FIGURE #95

Mean R(+)/S(-) Zileuton C_{max} and AUC Ratios for Various Formulations After a Single Oral 600 Mg Zileuton Dose (8 Subjects From Study F92-219)

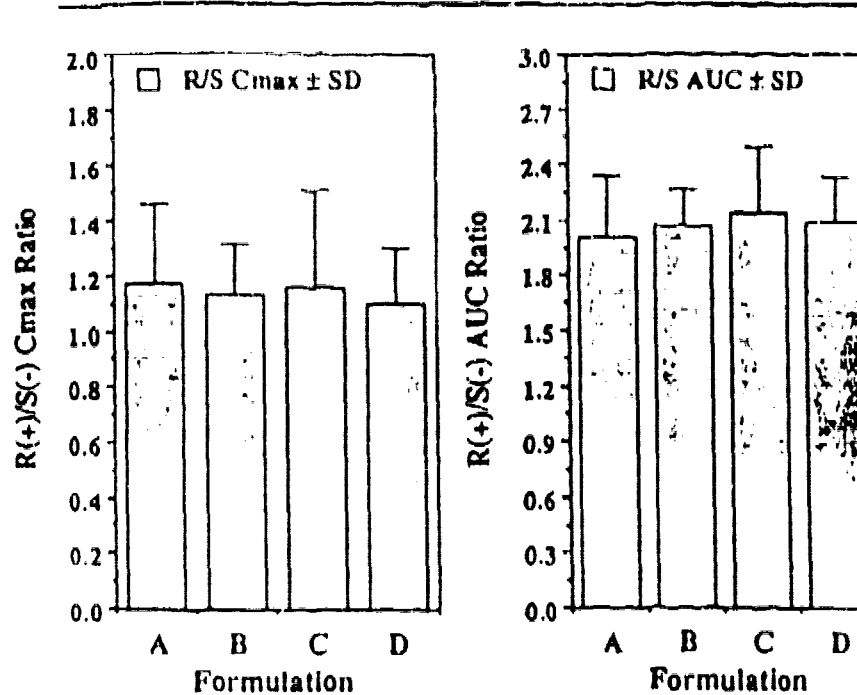


Table # ⁹⁸ provides confidence intervals obtained upon calculation using two one-sided tests procedure on log-transformed AUC and C_{max}.

Table # 28

Comparisons for Zileuton	Point estimate	90% confidence interval
AUC		
A vs. C (600 mg AP vs. 300 mg AP)	1.084	1.029 - 1.141
B vs. C (600 mg PR vs. 300 mg AP)	1.000	0.949 - 1.052
B vs. A (600 mg PR vs. 600 mg AP)	0.922	0.876 - 0.971
C_{max}		
A vs. C (600 mg AP vs. 300 mg AP)	1.138	0.996 - 1.300
B vs. C (600 mg PR vs. 300 mg AP)	1.101	0.964 - 1.258
B vs. A (600 mg PR vs. 600 mg AP)	0.968	0.847 - 1.106

Calculation of C.I. for AUC and C_{max} of A vs. C:

AUC (600 mg AP tablet vs. 300 mg AP)

ERROR MEAN SQUARE ... 1.086988E-02
REFERENCE MEAN ... 3.0342
TEST MEAN ... 3.1145
NUMBER OF SUBJECTS ... 24
DEGREES OF FREEDOM ... 22
NUMBER OF TREATMENTS ... 2
DELTA ... 2

POWER ANALYSIS

POWER FOR .2 M(r)= > 99.99855 %
POWER FOR -.2 M(r)= > 99.97 %

DETECTABLE DIFFERENCE: 9.225141 %

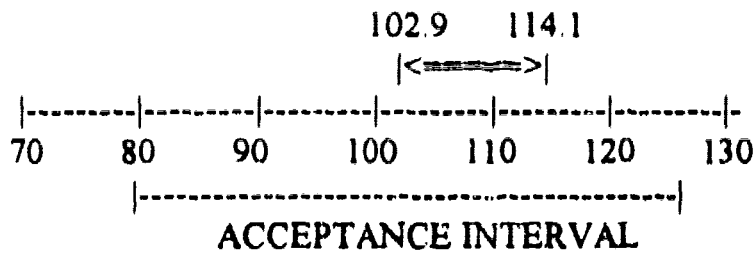
8 SUBJECTS NEEDED FOR A
19.09895 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

LOWER CI (% OF REF MEAN): 102.9034
UPPER CI (% OF REF MEAN): 114.1085
CONCLUSION: PASS

P VALUES OF TWO ONE-SIDED TEST

p < 80 % REF MEAN: < 0.00030
p > 120 % REF MEAN: 0.00132
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 102.9% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 114.1% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +2.65% OF THE REFERENCE MEAN.

C_{max} (600 mg AP tablet vs. 300 mg AP)

ERROR MEAN SQUARE ... 7.291306E-02
REFERENCE MEAN ... 1.535
TEST MEAN ... 1.6641
NUMBER OF SUBJECTS ... 24
DEGREES OF FREEDOM ... 22
NUMBER OF TREATMENTS ... 2
DELTA ... 2

POWER ANALYSIS

POWER FOR .2 M(r)= 60.35641 %
POWER FOR -.2 M(r)= 78.0922 %

DETECTABLE DIFFERENCE: 25.67631 %

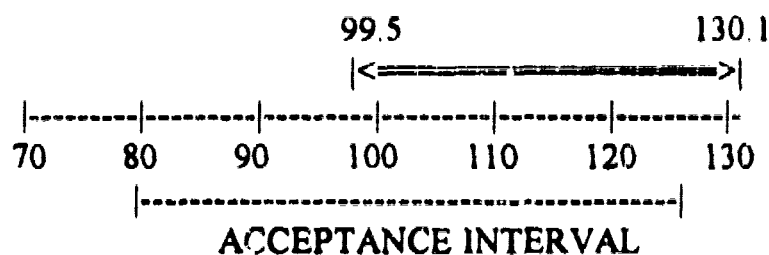
38 SUBJECTS NEEDED FOR A
19.53075 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

LOWER CI (% OF REF MEAN): 99.52647
UPPER CI (% OF REF MEAN): 130.0758
CONCLUSION: FAIL

P VALUES OF TWO ONE-SIDED TEST

p< 80 % REF MEAN: <0.00030
p> 120 % REF MEAN: 0.25100
CONCLUSION: FAIL



EQUIVALENCE WOULD BE DECLARED ($\alpha = .05$) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 99.5% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 130.1% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +8.41% OF THE REFERENCE MEAN.

CONCLUSION:

1. 600 mg tablets manufactured at the 2 different manufacturing sites are bioequivalent to each other.
2. Based on the results obtained, the 90% CI's for AUC, but not C_{max} of both tablet formulations A and B lie within acceptable limits compared to that of C as reference; therefore, both tablet formulations could NOT be considered bioequivalent to the 300 mg tablet formulation.
3. Relative bioavailability of the tablets to the suspension is > 94% based on AUC.
4. The enantiomeric ratios of C_{max} and AUC after dosing with all the four formulations were similar to each other.

COMMENT:

It should be noted that the 600 mg final tablet formulations have been studied in pivotal Phase III clinical trials. Hence, the bioequivalence comparison is not very critical. It is also important to note that E_{max} of concentration-response curve with respect to LTB₄ inhibition occurs at zileuton concentrations of 2 $\mu\text{g/ml}$ and higher. Hence, small increases in C_{max} may not have impact on efficacy of the drug. However, effect on safety needs to be looked into.

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 59
Submission date: 07/19/94

5 Pages

Purged



EVALUATION OF THE BIOAVAILABILITY OF 300 MG TABLET FORMULATIONS OF ZILEUTON

Study ID: F92-216

Investigators:

Clinical Site:

Objective:

To evaluate the relative bioavailabilities of two 300 mg tablet formulations of the final zileuton tablet formula manufactured at different production sites (AP and PR) to that of the 200 mg capsule formulation, used in phase I and phase II/III trials, as reference (capsule II).

Drug Dosage Forms:

- A: Zileuton tablet FF (AP), bulk NPRO 5592N, finished Lot 61-847-AR, 300 mg
- B: Zileuton tablet FF (PR), bulk NPRO 5617N, finished Lot 61-848-AR, 300 mg
- C: Zileuton capsules (capsule II), bulk NPRO 4942N, finished Lot 61-849-AR, 200 mg

Study Design:

Twenty four, non-smoking healthy male volunteers, age 27-52 yrs., body weight 63-101 kg, participated in this single-dose (600 mg), randomized, fasting, three-period, open-label, complete-crossover study. Subjects were randomly assigned to three groups as follows:

Table (32)

Group #	Subjects	Formulations		
		Period 1	Period 2	Period 3
I	8	A	B	C
II	8	B	C	A
III	8	C	A	B

After an overnight fast, each subject received the assigned dose of zileuton with 180 ml of water. Breakfast, lunch and dinner were served 3, 6 and 10 hr after dosing. No food or beverage was allowed, other than the meals provided in the study and water to quench thirst starting from 12 hr before to 10 hr after dosing. Blood samples were collected into heparinized, chilled collection tubes before (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hr after dosing. Blood samples were centrifuged within 2 hr and kept at -10°C until assayed. Subjects stayed in the clinic until 24 hr post-dose. Treatments were separated by 8 day washout periods.

Assay Methodology:

Data Analysis:

Model-independent methods were used to analyze concentration-time data. ANOVA was performed for each concentration point, T_{max} , C_{max} and $AUC_{0-\infty}$ for total zileuton and also individual enantiomers, with the sources of variation being sequence, subject nested with sequence, period and formulation. Pairwise comparisons were made between each of two formulations by least significant difference procedure, with an alpha level of 0.05. The zileuton bioavailability of a test formulation versus that of a reference formulation (A, B vs. C; B vs. A) was assessed by the two one-sided tests procedure. Bioequivalence between a test and reference formulation was concluded if the 90% confidence interval on test:reference ratio on the analysis of $\ln(AUC)$ and $\ln(C_{max})$ were within 0.80 - 1.25 range.

Results:

Mean pharmacokinetic parameters of total zileuton and individual enantiomers after administration of each formulation are given below in table #33. Plot of mean plasma concentrations of zileuton and its enantiomers following administrations of various formulations is provided in figures #35 and #36. The cumulative percent zileuton dissolved from the capsule and tablets is shown in table #34.

Table # 33

Parameter	FORMULATIONS		
	A: 2 x 300 mg FF Tablet AP	B: 2 x 300 mg FF Tablet PR	C: 3 x 200 mg Capsule II
I. Zileuton: T_{max} , hr C_{max} , $\mu\text{g/ml}$ AUC , $\mu\text{g}\cdot\text{hr/ml}$ CL/F , ml/min	1.7 ± 0.8 4.28 ± 1.24 17.33 ± 2.87 592 ± 98	1.7 ± 1.0 4.55 ± 1.39 17.93 ± 3.24 574 ± 93	2.0 ± 1.0 4.03 ± 1.52 17.84 ± 3.69 582 ± 110
II. R(+)-Zileuton: T_{max} , hr C_{max} , $\mu\text{g/ml}$ AUC , $\mu\text{g}\cdot\text{hr/ml}$ CL/F , ml/min	2.4 ± 1.2 2.27 ± 0.41 11.92 ± 1.16 423 ± 42	2.8 ± 1.3 2.29 ± 0.40 11.82 ± 1.13 426 ± 38	2.3 ± 0.7 2.09 ± 0.47 12.50 ± 1.21 403 ± 38
III. S(-)-Zileuton: T_{max} , hr C_{max} , $\mu\text{g/ml}$ AUC , $\mu\text{g}\cdot\text{hr/ml}$ CL/F , ml/min	1.3 ± 0.8 2.00 ± 0.79 5.24 ± 0.60 966 ± 114	1.8 ± 1.0 1.61 ± 0.51 4.70 ± 0.62 1079 ± 143	1.6 ± 0.7 1.68 ± 0.50 5.29 ± 0.85 966 ± 149
IV. R(+)/S(-) Zileuton: C_{max} Ratio AUC Ratio	1.22 ± 0.32 2.29 ± 0.28	1.49 ± 0.34 2.54 ± 0.33	1.31 ± 0.31 2.42 ± 0.47

FIGURE # 28

Figure 28

Mean Plasma Concentrations of Zileuton for Various Formulations after a Single Oral 600 Mg Zileuton Dose (Study F92-216)

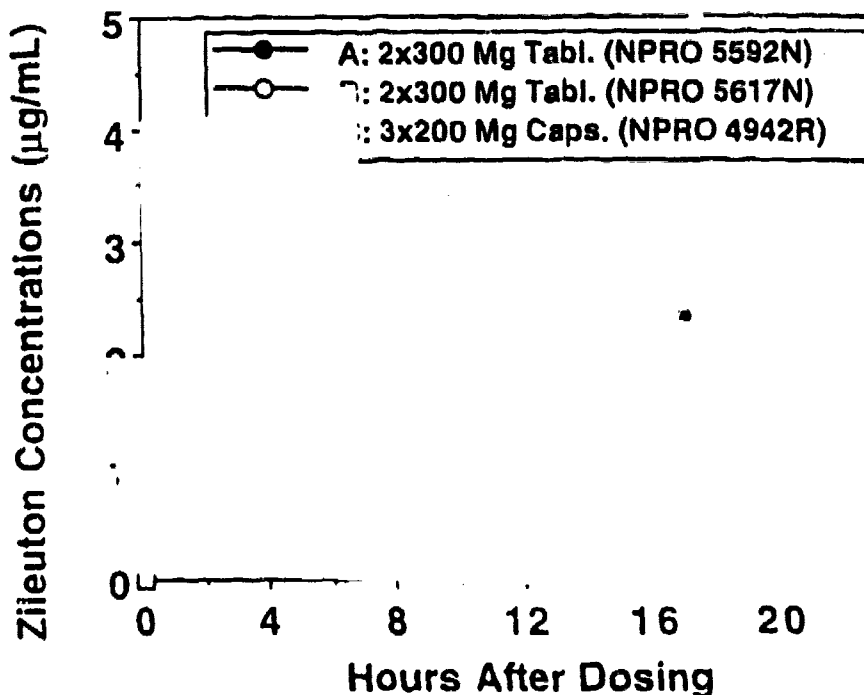


FIGURE # 29

Figure 29

Mean C_{max} and AUC Ratios R(+) over S(-) Zileuton for Various Formulations after a Single Oral 600 Mg Zileuton Dose (8 Subjects from Study F92-216)

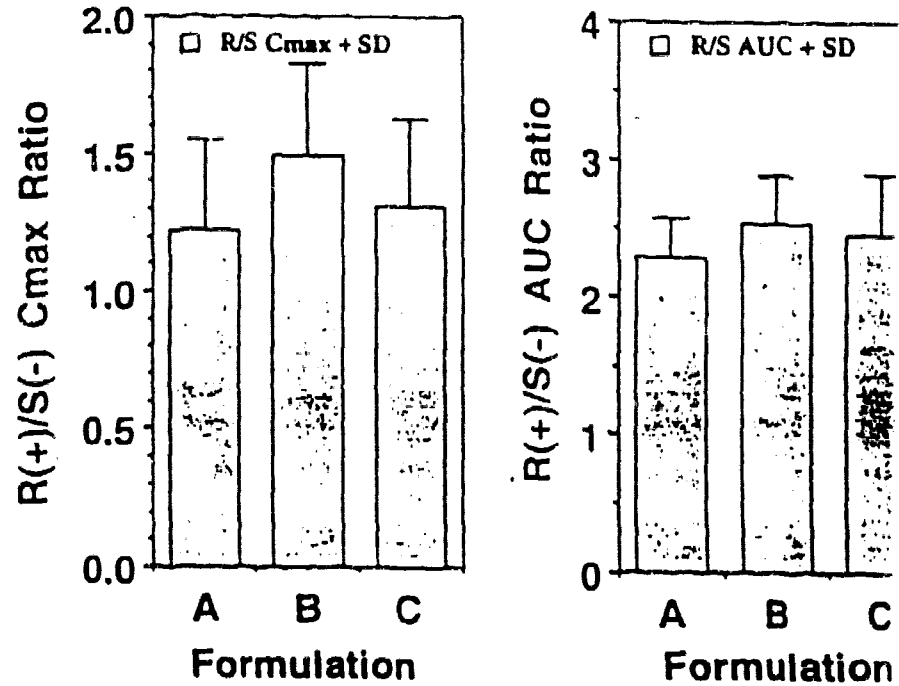


TABLE # 34

IN VITRO DISSOLUTION
STUDY F92-216

Apparatus:
Agitation:
Medium:
Assay:

Time min	Percent Dissolved, Mean ± SD (n=12)		
	Cap II (n=6) 200 mg	Tab AP 300 mg	Tab PR 300 mg
10			
20			
30			
45			
60			

Table # ³⁵ provides confidence intervals obtained upon calculation using two one-sided tests procedure on natural log-transformed AUC and C_{max}.

Table # 35

Comparisons for Zileuton	Point estimate	90% confidence interval
<i>AUC</i>		
<i>A vs. C (300 mg AP vs. 200 mg capsule II)</i>	<i>0.978</i>	<i>0.932 - 1.025</i>
<i>B vs. C (300 mg PR vs. 200 mg capsule II)</i>	<i>1.010</i>	<i>0.963 - 1.059</i>
<i>B vs. A (300 mg PR vs. 300 mg AP)</i>	<i>1.033</i>	<i>0.985 - 1.083</i>
<i>C_{max}</i>		
<i>A vs. C (300 mg AP vs. 200 mg capsule II)</i>	<i>1.085</i>	<i>0.970 - 1.212</i>
<i>B vs. C (300 mg PR vs. 200 mg capsule II)</i>	<i>1.152</i>	<i>1.030 - 1.287</i>
<i>B vs. A (300 mg PR vs. 300 mg AP)</i>	<i>1.062</i>	<i>0.950 - 1.187</i>

Calculation of C.I. for AUC and C_{max} of A vs. C:

AUC (300 mg FF AP tablet vs. 200 mg capsule II)

 ERROR MEAN SQUARE . . . 9.562259E-03
 REFERENCE MEAN 2.8621
 TEST MEAN 2.8395
 NUMBER OF SUBJECTS . . . 24
 DEGREES OF FREEDOM . . . 22
 NUMBER OF TREATMENTS . . 2
 DELTA 2

90% CONFIDENCE INTERVAL

 LOWER CI (% OF REF MEAN): 93.13956
 UPPER CI (% OF REF MEAN): 102.6209
 CONCLUSION: PASS

POWER ANALYSIS

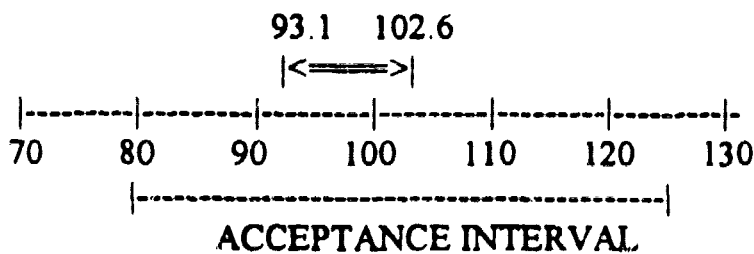
 POWER FOR .2 M(r)= > 99.97 %
 POWER FOR -.2 M(r)= > 99.97 %

DETECTABLE DIFFERENCE: 8.628488 %

8 SUBJECTS NEEDED FOR A
 17.81373 % DETECTABLE DIFFERENCE

P VALUES OF TWO ONE-SIDED TEST

 p < 80 % REF MEAN: <0.00030
 p > 120 % REF MEAN: <0.00030
 CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 93.1% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 102.6% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS -0.79% OF THE REFERENCE MEAN.

C_{max} (300 mg FF AP tablet vs. 200 mg capsule II)

POWER ANALYSIS

ERROR MEAN SQUARE . . . 5.263129E-02
REFERENCE MEAN 1.334
TEST MEAN 1.4151
NUMBER OF SUBJECTS . . . 24
DEGREES OF FREEDOM . . . 22
NUMBER OF TREATMENTS . . 2
DELTA 2

POWER FOR .2 M(r)= 74.81583 %
POWER FOR -.2 M(r)= 89.59884 %

DETECTABLE DIFFERENCE: 21.43018 %

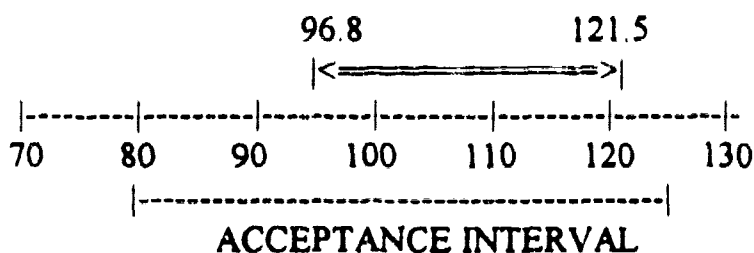
28 SUBJECTS NEEDED FOR A
19.54409 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

P VALUES OF TWO ONE-SIDED TEST

LOWER CI (% OF REF MEAN): 96.79087
UPPER CI (% OF REF MEAN): 121.5089
CONCLUSION: FAIL

p< 80 % REF MEAN: <0.00030
p> 120 % REF MEAN: 0.07039
CONCLUSION: FAIL



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 96.8% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 121.5% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +6.08% OF THE REFERENCE MEAN.

CONCLUSION:

1. 300 mg FF tablets manufactured at the 2 different manufacturing sites are bioequivalent to each other.
2. The 300 mg FF tablets manufactured at Abbott Park is bioequivalent to capsule II

(reference).

3. However, the 300 mg FF tablets manufactured at Puerto Rico cannot be declared bioequivalent to the reference, since the criterion on C_{max} was not met.

3. The enantiomeric ratios of C_{max} and AUC after dosing with all the three formulations were similar to each other (no statistically significant difference between formulations).

COMMENT:

1. All the 3 final formulation strengths are compositionally proportional and hence, bioequivalence study comparing the highest strength (600 mg FF) to that of reference capsule would have been sufficient. All other strengths could be compared with in-vitro dissolution, and the current study would not have been necessary.

2. The 300 mg strength has not been evaluated in pivotal clinical studies. However, the higher C_{max} obtained with this strength may not be critical in the context that higher C_{max} does not effect efficacy as E_{max} for % inhibition of LTB_4 is achieved at C_{max} of 2 μ g/ml.

3. With respect to safety, it was observed in study # F92-219 (BE study comparing 600 mg tablet vs. 300 mg tablet) that the C_{max} following 600 mg tablet formulation was higher than that of 300 mg tablet formulation. The 600 mg formulation has been studied in Phase III clinical trials and has been found to be safe.



**EVALUATION OF THE BIOAVAILABILITY OF
ZILEUTON**

REWORK TABLET FORMULATIONS OF

Study ID: F92-226

Investigators:

Clinical Site:

Objective:

To compare the bioavailability of a reworked zileuton tablet to that of reference
The purpose of the rework procedure was to improve the dissolution profile for
tablets which have failed the specification due to overgranulation.

Drug Dosage Forms:

A: Zileuton tablet (Reworked formulation), bulk NPRO 5885R, Lot 70-915-AR,

B: Zileuton tablet FF, bulk NPRO 5633R, finished 62-027-AR,

Study Design:

Twenty four, non-smoking healthy male volunteers, age 19-50 yrs., body weight 63-93 kg,
participated in this single-dose randomized, fasting, two-period, open-label, complete-
crossover study. Subjects were randomly assigned to two groups.

After an overnight fast, each subject received the assigned dose of zileuton with 180 ml of water.
Breakfast, lunch and dinner were served 3, 6 and 10 hr after dosing. No food or beverage was
allowed, other than the meals provided in the study and water to quench thirst starting from 12 hr
before to 10 hr after dosing. Blood samples were collected into heparinized, chilled collection
tubes before (0), and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14 and 24 hr after dosing. Blood samples
were centrifuged within 2 hr and kept at -10°C until assayed. Subjects stayed in the clinic until 24
hr post-dose. Treatments were separated by 8 day washout periods.

Methodology:

Data Analysis:

Model-independent methods were used to analyze concentration-time data. ANOVA was
performed for each concentration point, T_{max} , C_{max} and $AUC_{0-\infty}$ for zileuton with the sources of
variation being sequence, subject nested with sequence, period and formulation. The zileuton
bioavailability of a test formulation versus that of a reference formulation (A vs. B) was assessed
by the two one-sided tests procedure. Bioequivalence between a test and reference formulation
was concluded if the 90% confidence interval on test:reference ratio on the analysis of $\ln(AUC)$

and $\ln(C_{max})$ were within 0.80 - 1.25 range.

Results:

Mean pharmacokinetic parameters of total zileuton and individual enantiomers after administration of each formulation are given below in table #36. Plot of mean plasma concentrations of zileuton following administration of various formulations is provided in figure #30. Plot of dissolution profiles of the two tablet groups is provided in figure #31 and the data is provided in table #37.

Table # 36

Parameter	FORMULATIONS	
<u>I. Zileuton:</u>		
T_{max} , hr	2.3 ± 0.9	1.6 ± 0.7
C_{max} , µg/ml	2.4 ± 0.8	3.6 ± 1.2
AUC, µg.hr/ml	11.1 ± 1.9	12.3 ± 2.7
$t_{1/2}$, hr	2.3 ± 1.1	1.7 ± 0.5

FIGURE # 30

Mean Plasma Concentrations of Zileuton for the Two Formulations after a Single Oral Zileuton Dose (Study F92-226)

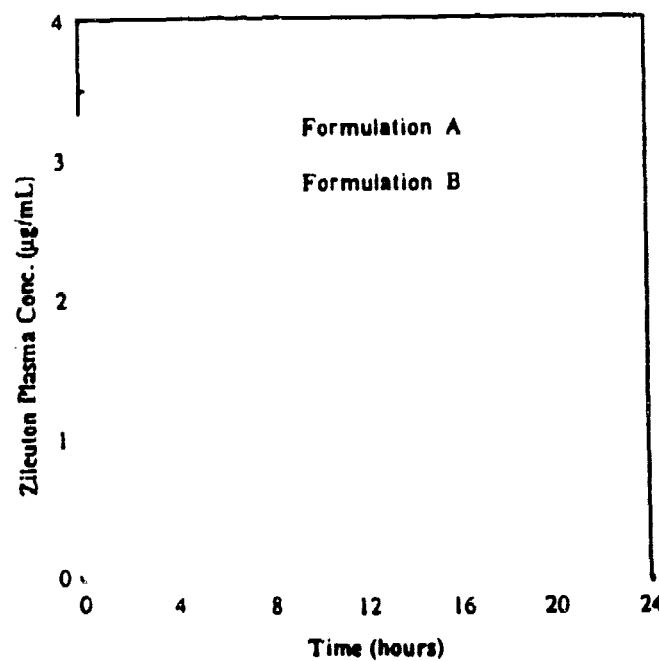
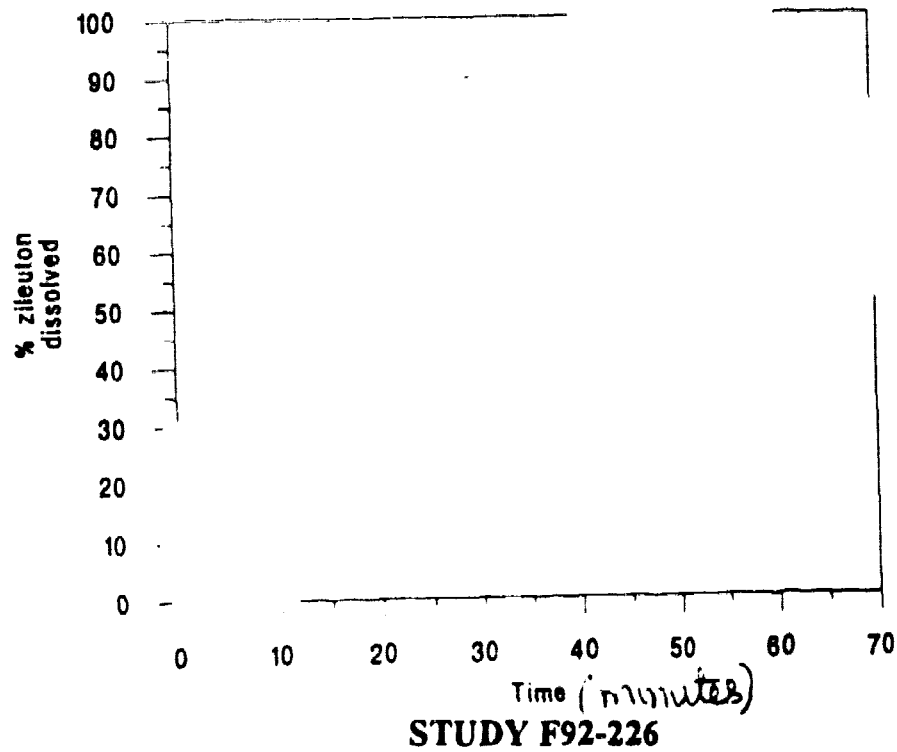


FIGURE # 31
Dissolution of rework & FF blet



Apparatus:
Agitation:
Medium:
Assay:

Table 37

Time min	% Released (mean $\pm$ SD) (n = 6)
10	
20	
30	
45	
60	

Table # ³⁸ provides confidence intervals obtained upon calculation using two one-sided tests procedure on natural log-transformed AUC and C_{max}.

Table # 38

Comparisons for Zileuton	Point estimate (Test to ref.)	90% confidence interval
AUC	0.907	0.854 - 0.962
C _{max}	0.669	0.581 - 0.770

CONCLUSION:

1. The rework tablet was equivalent to the final tablet formulation with respect to AUC but not with C_{max}. Hence, the rework procedure does not result in development of tablets that are bioequivalent. Thus, this rework procedure is not acceptable.
2. Differences in initial dissolution rate although did not have much effect on AUC, had a larger effect on C_{max}. This shows that initial dissolution rate is very important.

II **STUDY F92-222: A STUDY EVALUATING THE EFFECT OF FOOD ON ZILEUTON BIOAVAILABILITY FROM LEUTROL® 600 MG TABLETS (FINAL FORMULATION): TWO-WAY CROSSOVER DESIGN**

Reference: Volume 87

Investigator:

Study Location:

Objective:

To characterize the effect of a food on the relative bioavailability of zileuton when administered orally as a single dose of the 600 mg tablet (final formulation).

Formulation: 600 mg tablet; drug: zileuton; bulk NPRO 5590N; Bulk lot number 59-592-AR; finished under NPRO 5746; Lot number 64-158-AR.

Study Design:

Eighteen healthy adult male volunteers (age 28-42 years, body weight 61.2-105.7kg, height 170-191 cm) participated in this randomized, open-label, single dose, two-way cross-over study. Subjects were randomly assigned to groups, defined by regimen sequence as follows:

Table (39)

Sequence group	Number of subjects	Regimen	
		Period I	Period II
I	9	A	B
II	9	B	A

Treatment A: One 600 mg Tablet, administered half hour after breakfast (non-fasting) (details of breakfast in table #40&41 below).

Treatment B: One 600 mg Tablet, administered 3 hours before breakfast (fasting).

Table # Breakfast with 8 oz. skim milk

40

Item	Amount	Carbo- hydrate (gm)	Protein (gm)	Fat (gm)	Calories
Orange juice	4 oz.	15	0	0	60
Scrambled eggs (medium eggs made with milk and butter)	2	2.5	10.5	12	162.5
Bacon (strip)	2	0	0	10	90
Toast (slice)	2	30	6	trace	160
Margarine (pat)	2	0	0	10	90
Jelly (teaspoons)	4	13	trace	trace	67
Skim milk	8 oz	12	8	trace	90
Total		72.5	24.5	32	719.5

Table # Breakfast with 8 oz. coffee with milk and sugar

41

Item	Amount	Carbo- hydrate (gm)	Protein (gm)	Fat (gm)	Calories
Orange juice	4 oz.	15	0	0	60
Scrambled eggs (medium eggs made with milk and butter)	2	2.5	10.5	12	162.5
Bacon (strip)	2	0	0	10	90
Toast (slice)	2	30	6	trace	160
Margarine (pat)	2	0	0	10	90
Jelly (teaspoons)	4	13	trace	trace	67
Coffee	8 oz	trace	trace	trace	2.6
Sugar (for coffee)		6	0	0	23
Milk (for coffee)		1.5	1	trace	11.25
Total		68	17.5	32	666.35

An eight day washout period separated the two periods of the cross-over. Blood samples were drawn immediately prior to drug administration (0 hour) and at 0.5, 1.0, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hours after dosing. Plasma concentrations of zileuton were determined by the validated HPLC procedure. The key zileuton parameters C_{max} , T_{max} , $AUC_{0-\infty}$, terminal elimination rate constant β and $t_{1/2}$ were calculated by noncompartmental methods. ANOVAs were performed for concentrations at each sampling time, C_{max} , T_{max} , $AUC_{0-\infty}$, and $t_{1/2}$ of zileuton. The sources of variation included in model were sequence, subjects nested within sequence, period and regimen. For AUC and C_{max} , a 95% confidence interval for the ratio of nonfasting mean to fasting mean was obtained by the sponsor.

Results:

Mean plasma concentration-time curves of zileuton following Treatments A (fed) and B (fasted) are shown in figure 32.

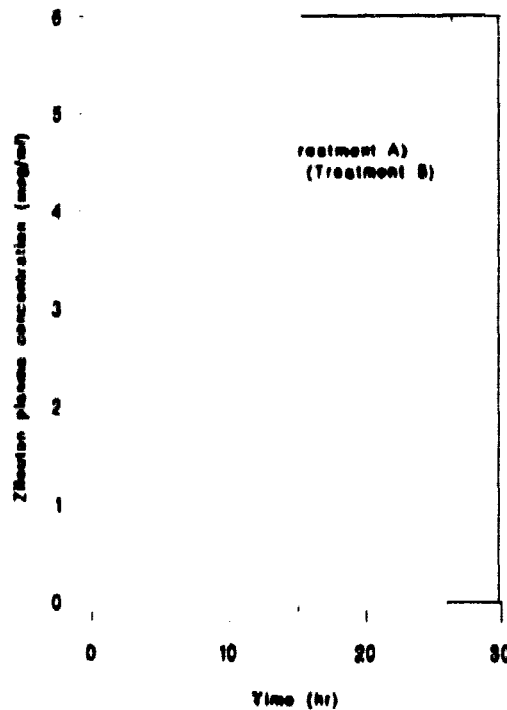
Mean pharmacokinetic parameters of zileuton are as follows (n = 18) (Table #):

Table # 42

PARAMETER	UNIT	MEAN (% CV)	
		A (FED)	B (FASTED)
C_{max}	$\mu\text{g/ml}$	6.97 (33)	5.48 (35)
T_{max}	hr	1.8 (65)	1.3 (67)
$AUC_{0-\infty}$	$\mu\text{g}\cdot\text{hr/ml}$	21.61 (28)	21.31 (29)
$t_{1/2}$	hr	1.78 (8)	2.06 (31)

$AUC_{0-\infty}$: Area under the plasma concentration-time curve from time zero to infinity.

Figure 32



Statistical analyses (including 90% confidence intervals calculated based on two one-sided t-tests at $\alpha = 0.05$) of the ratios of C_{max} and $AUC_{0-\infty}$ for Treatment A (fed) relative to Treatment B (fasted) are shown in table 43.

Table 43

PARAMETER	RATIO (A/B) %		
	RATIO OF MEANS	90% C.I.*	95% C.I.
C_{max}	127.2	106.3-148.1	101.6-159.1
$AUC_{0-\infty}$	101.4	94-108.9	92.5-110.9

* calculated by the reviewer

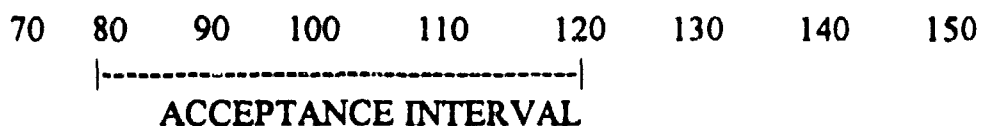
Conclusion: Concomitant administration of the 600 mg formulation with food resulted in slight delay in the rate of absorption (T_{max} prolonged from 1.3 hours to 1.8 hours), and an increase in albuterol C_{max} (27%) and AUC (1.4%), as compared to fasted state.

Comments:

1. Food delayed the absorption of zileuton from the 600 mg Leutrol Tablet, however, the increase in T_{max} was not found to be significant.
2. Food increased the mean C_{max} and AUC(tf) of albuterol by 27.2% and 1.4% respectively. This increase in AUC was found to be insignificant. The issue of clinical relevance of this increase in C_{max} with respect to safety and efficacy will be referred to the Medical Reviewer. However, it is suggested that the food effect on C_{max} be reflected on the label.
3. It should be noted here that the breakfast administered here is not the standard high fat breakfast suggested by FDA and it has lower fat content. In an earlier study (study # F91-201) conducted by the sponsor to study the effect of food on an earlier zileuton 300 mg tablet formulation, it was found that after a high-fat breakfast, C_{max} and AUC were statistically significantly higher by 31% and 18%. In that study, effect of both low fat and high fat breakfast were studied. Although the ratio of means and confidence intervals obtained after drug administration with low fat vs. high fat breakfast were within the bioequivalence criteria, there was a trend towards increase in C_{max} and AUC with high fat breakfast. The breakfast administered in the current study is in between the low fat and high fat breakfast with respect to its contents.
Based on the above information, if zileuton is administered along with a high fat breakfast, it could result in higher C_{max} and AUC than what has been observed in the current study.

Calculation of 90% confidence intervals:

Food effect on C_{max} (zil)	POWER ANALYSIS
-----	-----
ERROR MEAN SQUARE . . . 3.88	
REFERENCE MEAN 5.479	POWER = 33.0046 %
TEST MEAN 6.968	
NUMBER OF SUBJECTS . . 18	DETECTABLE DIFFERENCE: 35.77034 %
DEGREES OF FREEDOM . . 16	
NUMBER OF TREATMENTS . 2	54 SUBJECTS NEEDED FOR A
DELTA 2	19.75534 % DETECTABLE DIFFERENCE
90% CONFIDENCE INTERVAL	P VALUES OF TWO ONE-SIDED TEST
-----	-----
LOWER CI (% OF REF MEAN): 106.254	p< 80 % REF MEAN: 0.00060
UPPER CI (% OF REF MEAN): 148.0989	p> 120 % REF MEAN: 0.72117
CONCLUSION: FAIL	CONCLUSION: FAIL
106.3	148.1
<----->	



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 106.3% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 148.1% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +27.18% OF THE REFERENCE MEAN.

AUC of zileuton (food effect)

POWER ANALYSIS

ERROR MEAN SQUARE . . . 7.445324
REFERENCE MEAN 21.312
TEST MEAN 21.612
NUMBER OF SUBJECTS . . 18
DEGREES OF FREEDOM . . 16
NUMBER OF TREATMENTS . 2
DELTA 2

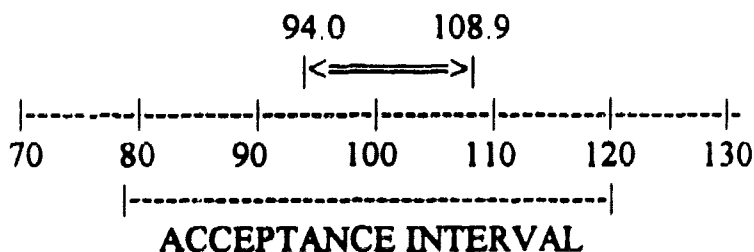
POWER = 98.96118 %
DETECTABLE DIFFERENCE: 12.73873 %
10 SUBJECTS NEEDED FOR A
18.29378 % DETECTABLE DIFFERENCE

90% CONFIDENCE INTERVAL

P VALUES OF TWO ONE-SIDED TEST

LOWER CI (% OF REF MEAN): 93.95664
UPPER CI (% OF REF MEAN): 108.8587
CONCLUSION: PASS

p < 80 % REF MEAN: 0.00007
p > 120 % REF MEAN: 0.00025
CONCLUSION: PASS



EQUIVALENCE WOULD BE DECLARED (ALPHA = .05) IF IT IS ACCEPTABLE FOR THE RATIO OF THESE PARAMETER MEANS TO BE AS LOW AS 94.0% OF THE OBSERVED REFERENCE MEAN, AND IT IS ACCEPTABLE FOR THE RATIO OF THEIR MEANS TO BE AS HIGH AS 108.9% OF THE OBSERVED REFERENCE MEAN. THE OBSERVED DIFFERENCE BETWEEN THE TEST AND REFERENCE MEANS IS +1.41% OF THE REFERENCE MEAN.

Assay Methodology for zileuton used in this study:

NDA 20,471
Abbott 64077(Zileuton)Leutrol tablets

Pharmacokinetic section, 82
Submission date: 07/19/94

Analytical Site:
Procedure:

1

12 **STUDY M90-529: A STUDY COMPARING THE PHARMACOKINETICS OF ZILEUTON BETWEEN HEALTHY ELDERLY AND YOUNGER SUBJECTS**

Reference: Volume 94

Investigator:

Study Location:

Objective:

1. To characterize and compare the single and multiple dose pharmacokinetics of zileuton between healthy young male and female volunteers and healthy elderly male and female volunteers.
2. To assess the differences in liver blood flow between young and elderly subjects.

Formulation: 200 mg capsules; drug: zileuton; bulk NPRO 4525R; finished under NPRO 5197; Lot number 47-301-AR. Cardiogreen vials; containing 50 mg indocyanine green (ICG), USP, lot # 14

Study Design:

5 males and 4 females in the young group and 9 males and 9 females in elderly group participated in this open, parallel, comparative design to evaluate the differences in the zileuton pharmacokinetics. The demographic information including serum albumin concentrations and creatinine clearances are provided in the following table.

Table 45

Variables	Young		Elderly	
	Males (N=5)	Females (N=4)	Males (N = 9)	Females (N=9)
Age (yr)	28.4 ± 5.9	29.0 ± 9.6	68.0 ± 2.3	71.2 ± 6.3
Age Range (yr)	23 - 37	20 - 40	65 - 72	65 - 81
Height (cm) [Ⓢ]	177.3 ± 4.5	166.1 ± 3.8	177.1 ± 6.7	163.0 ± 9.5
Weight (kg) [Ⓢ]	80.1 ± 6.1	63.2 ± 9.0	77.5 ± 7.7	71.0 ± 9.4
BSA (m ²) [Ⓢ]	1.98 ± 0.08	1.70 ± 0.08	1.94 ± 0.11	1.77 ± 0.17
LBM (%) [Ⓢ]	83.1 ± 3.0	73.2 ± 9.4	75.9 ± 4.1	64.1 ± 3.0
Albumin (g/dl) [*]	4.5 ± 0.1	4.5 ± 0.2	4.3 ± 0.2	4.3 ± 0.1
Cl _{cr} (ml/min)	112.0 ± 31.3	74.7 ± 19.2	71.1 ± 27.8	75.2 ± 19.9

* Age has a statistically significant effect on these variables

Ⓢ Gender has a statistically significant effect on these variables.

In this study, zileuton was administered as single and multiple oral doses of 600 mg q6 hours to healthy young and elderly volunteers of both genders. On day 0 (i.e. a day before start of the drug administration), an intravenous dose of 0.5 mg/kg indocyanine green (ICG) was administered over a 15 second period to all subjects. On day 1, all subjects received a single oral dose of 600 mg of zileuton (three 200 mg capsules). From day 3-7, all subjects received an oral 600 mg zileuton dose every 6 hours (q6h).

Blood samples were drawn from all subjects on days 1 and 7 prior to dosing (0 hour), and at

0.5, 1.5, 3, 4.5, 6, 8, 12, 16, 20, 24, 36, and 48 hours postdosing for determinations of plasma concentrations of zileuton and its N-dehydroxylated metabolite. For measurement of protein binding of zileuton, a blood sample was collected prior to dosing on day 1. For indocyanine green, blood samples were collected from an arm vein on the site contralateral to ICG injection prior to dosing (0 hour) and at 1, 3, 5, 7, 10, 15, 20 and 25 minutes after dosing.

Designated plasma samples were assayed for zileuton, Abbott-66193 and indocyanine green using validated HPLC methods. Protein binding of zileuton was measured with radioassay after ultrafiltration.

Pharmacokinetic parameters were calculated using standard non-compartmental methods. ICG blood clearance, as an indicator of hepatic blood flow, was calculated as:

$Cl_{\text{blood}} = Cl_{\text{plasma}} / (1 - \text{Hct})$. Anthropometric data and pharmacokinetic parameters were evaluated with analyses of variance (ANOVAs). P-values less than or equal to 0.05 were considered statistically significant.

Results:

The group means of the ICG blood clearances (ml/min/kg) are shown below:

Young males: 21.80 ± 4.51 ml/min/kg

Young females: 17.16 ± 2.13 ml/min/kg

Elderly males: 23.97 ± 7.58 ml/min/kg

Elderly females: 19.59 ± 12.24 ml/min/kg

(No statistically significant difference between different groups (young vs. elderly, and males vs. females) were observed).

The mean values of pharmacokinetic parameters of zileuton following single dose and at steady state are provided below:

Table 46

PK parameters (mean ± st.dev.) after single 600 mg dose of zileuton

Parameters	Young		Elderly	
	Males (N=5)	Females (N=4)	Males (N = 9)	Females (N=9)
T _{max} (hr)	2.4 ± 0.8	1.9 ± 0.8	1.8 ± 0.7	1.8 ± 0.7
C _{max} (µg/ml)	4.25 ± 1.25	5.15 ± 0.31	4.82 ± 1.54	5.69 ± 1.22
AUC (µg.hr/ml)*	19.10 ± 3.65	21.28 ± 1.95	23.23 ± 5.63	26.34 ± 4.55
Cl/F (ml/min)*	538 ± 96	473 ± 40	447 ± 79	391 ± 74
t _{1/2} (hr)	2.20 ± 0.53	1.85 ± 0.47	2.34 ± 0.86	2.12 ± 0.44
Vd _f /F (L)	103 ± 34	75 ± 18	89 ± 38	72 ± 22
Free (%)	6.98 ± 0.68	7.08 ± 0.17	6.70 ± 0.33	7.23 ± 0.54
AUC _f (µg.hr/ml)*	1.32 ± 0.16	1.51 ± 0.11	1.56 ± 0.36	1.91 ± 0.41
Cl _f /F (ml/min)* [⊗]	7681 ± 955	6667 ± 441	6678 ± 1213	5454 ± 1216

* Age has a statistically significant effect on these variables.

⊗ Gender has a statistically significant effect on Cl_f/F, but not on Cl_f/F normalized to body weight.

Mean plasma concentration-time profiles following single dose administration are shown in the following figure:

Figure 33

Mean Plasma Concentrations of Zileuton in Young and Elderly Subjects After Single Doses of Zileuton

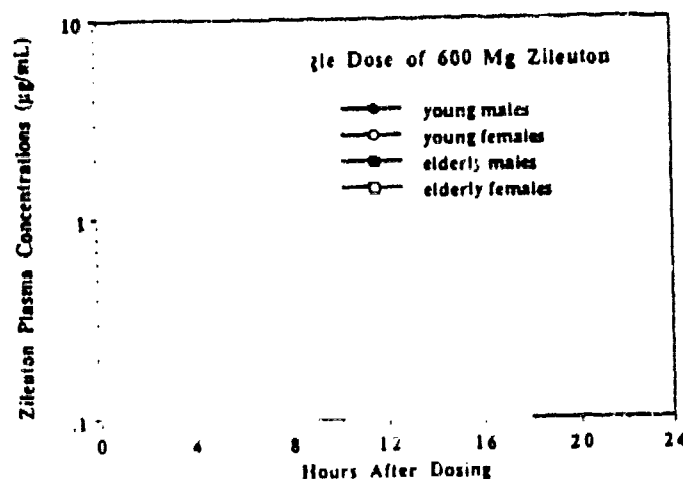


Table 47

Steady state PK parameters (mean $\pm$ st.dev.) after 600 mg dose of zileuton Q6hrs

Parameters	Young		Elderly	
	Males (N=5)	Females (N=4)	Males (N = 9)	Females (N=9)
T_{max} (hr)	2.1 $\pm$ 0.8	1.9 $\pm$ 0.8	1.4 $\pm$ 0.7	2.0 $\pm$ 0.8
$C_{max,SS}$ (μ g/ml)	7.13 $\pm$ 2.32	7.55 $\pm$ 1.98	7.47 $\pm$ 1.58	7.63 $\pm$ 1.65
C_{min,SS_m} (μ g/ml)	4.91 $\pm$ 2.53	3.91 $\pm$ 1.20	3.19 $\pm$ 1.14	3.58 $\pm$ 0.76
C_{min,SS_d} (μ g/ml)	1.80 $\pm$ 0.41	1.83 $\pm$ 0.29	1.80 $\pm$ 0.69	1.77 $\pm$ 0.58
AUC (μ g.hr/ml)	28.03 $\pm$ 7.65	29.09 $\pm$ 7.82	26.51 $\pm$ 7.39	30.18 $\pm$ 4.87
Cl/F (ml/min)	382 $\pm$ 120	361 $\pm$ 86	402 $\pm$ 105	339 $\pm$ 57
$t_{1/2}$ (hr)	2.20 $\pm$ 0.15	1.77 $\pm$ 0.21	2.21 $\pm$ 0.36	2.12 $\pm$ 0.34
Vd_b/F (L) [ⓐ]	73 $\pm$ 25	54 $\pm$ 9	75 $\pm$ 18	62 $\pm$ 16
Free (%)	7.01 $\pm$ 0.80	7.04 $\pm$ 0.09	6.73 $\pm$ 0.36	7.32 $\pm$ 0.57
AUC_r (μ g hr/ml)	1.94 $\pm$ 0.48	2.04 $\pm$ 0.54	1.79 $\pm$ 0.52	2.22 $\pm$ 0.47
Cl _r /F (ml/min)	5457 $\pm$ 1636	5125 $\pm$ 1200	6028 $\pm$ 1800	4692 $\pm$ 1028

C_{min,SS_m} (μ g/ml) = morning steady state trough level (8:00 a.m.)

C_{min,SS_d} (μ g/ml) = day steady state trough level (2:00 p.m.)

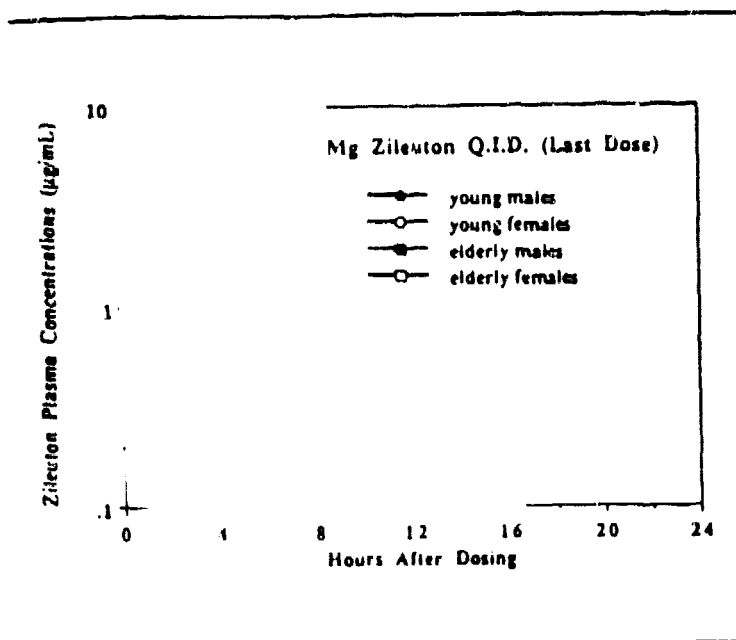
Note: Age has no statistically significant effect on any of these parameters.

[ⓐ] Gender has a statistically significant effect on β and Vd_b/F , but not on Vd_b/F normalized to body weight.

Mean plasma concentration-time profiles following multiple dose administration are shown in the following figure:

Figure 34

Mean Plasma Concentrations of Zileuton in Young and Elderly Subjects After Multiple Doses of Zileuton



Abbott-66193 levels: Following administration of single dose of zileuton, no Abbott-66193 levels were measurable in several subjects. However following multiple dose administration, at steady state, the mean steady state AUC ratios of Abbott-66193 over parent drug (AUC_m/AUC_p) were 0.21 ± 0.12 in young males, 0.30 ± 0.25 in young females, 0.52 ± 0.37 in elderly males and 0.38 ± 0.29 in elderly females. Because of large variability, the differences in ratios between young and elderly, and between males and females, were not statistically significant.

Conclusion: Upon comparison of pharmacokinetics of zileuton between healthy young and elderly, it was found that mean apparent clearance of zileuton after single 600 mg dose was statistically significantly lower (<20%) in elderly subjects. However, no statistically significant difference was found after multiple dose administration.

Although not an objective of this study, gender does not appear to have any significant effect on body weight normalized parameters. Hence, it appears that body weight and not gender can effect the PK parameters of zileuton.

13 **STUDY M92-752: PHARMACOKINETICS AND PHARMACODYNAMICS OF ZILEUTON IN MILD ASTHMATIC CHILDREN (AGE 8-12 YEARS)**

Investigator:

Study Location:

Objective:

1. To determine the pharmacokinetics and pharmacodynamics of a single dose of zileuton in asthmatic children ages 8 through 12 years.
2. To document any improvement in pulmonary function in these patients.

Formulation: 300 mg zileuton tablet: bulk lot # 76-297-AR
Placebo for 300 mg: bulk lot # 60-781-AR
150 mg zileuton (above 300 mg tablet bisected): bulk lot # 81-434-AR
Placebo for 150 mg: bulk lot # 81-435-AR

Study Design:

This was a randomized, single dose, crossover study in mild asthmatic children of age 8 - 12 years. 13 children were randomly assigned to sequences of single dose of placebo, 150 mg zileuton and 300 mg zileuton in three treatment periods. A one week washout period separated the study. 12 children (age 9-12 years, weight 28.6-67.3 kg) completed the study.

On each dosing day, study drug was administered with 180 ml of water. On the dosing day, breakfast, lunch and dinner were served at 2, 6 and 10 hours following dosing. Blood samples were drawn from all subjects prior to dosing (0 hour), and at 0.5, 1, 2, 3, 4, 6, 8, and 12 hours postdosing for determinations of plasma concentrations of zileuton. From some children, a 24 hours sample was also collected. Whole blood was also obtained at 0, 1, 2, 3, 4, 6, 12 and 24 hours for LTB₄ determination. Pulmonary function tests were performed (consisting of FEV₁, FVC and FEF_{25-75%}) at 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours postdosing.

Pharmacokinetic parameters were calculated using standard non-compartmental methods. ANOVA was performed for dose-normalized C_{max}, C_{1/F}, T_{max} and β. Relationship for C_{1/F}, dose-normalized C_{max} and Vd/F with body weight was explored by performing an analysis of covariance with body weight as the covariate and with classification by gender.

Pharmacodynamic analysis was carried out as % inhibition of LTB₄. Relationship between LTB₄ and zileuton concentrations was studied by carrying out population PD analysis.

ASSAY: For zileuton concentrations, is acceptable.

Results:

The mean values of pharmacokinetic parameters of zileuton following 150 and 300 mg doses in children are provided below:

Table (48)

Parameters	150 mg zileuton	300 mg zileuton
T _{max} (hr)	1.6 ± 0.9	1.8 ± 0.9
C _{max} (µg/ml)	2.78 ± 1.07	5.73 ± 2.07
AUC (µg.hr/ml)	8.37 ± 2.47	20.29 ± 7.61
Cl/F (ml/min)	324 ± 102	276 ± 92
Cl/F (ml/min/kg)	8.29 ± 2.19	7.08 ± 2.15
t _{1/2} (hr)	2.04 ± 0.60	2.00 ± 0.41
Vd _B /F (L)	56.4 ± 24.8	47.3 ± 15.0
Vd _B /F (L/kg)	1.42 ± 0.32	1.21 ± 0.38

Pharmacokinetics of zileuton in children (mild asthmatics) and adults (normal volunteers) are compared in the following table:

Table (49)

Parameters	Children 300 mg zileuton	Adults 600 mg zileuton
Body weight (kg)	39.9 ± 11.6	75.7 ± 7.3
T _{max} (hr)	1.8 ± 0.9	2.25 ± 1.48
C _{max} (µg/ml)	5.73 ± 2.07	5.08 ± 2.26
AUC (µg.hr/ml)	20.29 ± 7.61	20.10 ± 6.12
t _{1/2} (hr)	2.00 ± 0.41	2.36 ± 0.67
Cl/F (ml/min/kg)	7.08 ± 2.15	7.17 ± 2.19
Vd _B /F (L/kg)	1.21 ± 0.38	1.50 ± 0.83

Mean plasma concentration-time profiles following single dose administration (150 and 300 mg in children and another plot comparing 300 mg in children and 600 mg in adults) are shown in the following figures:

Figure 35

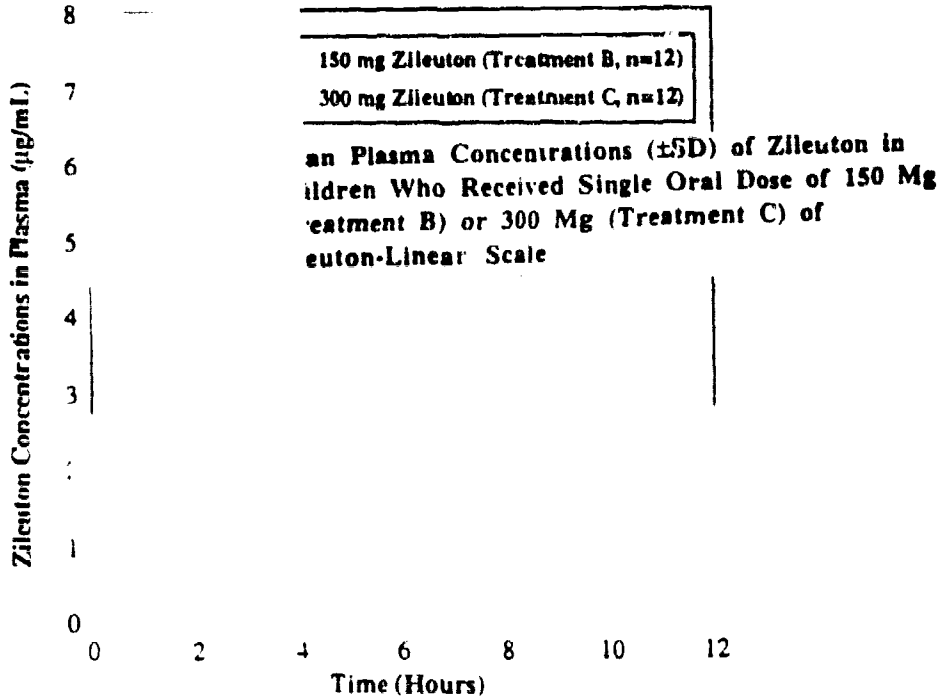
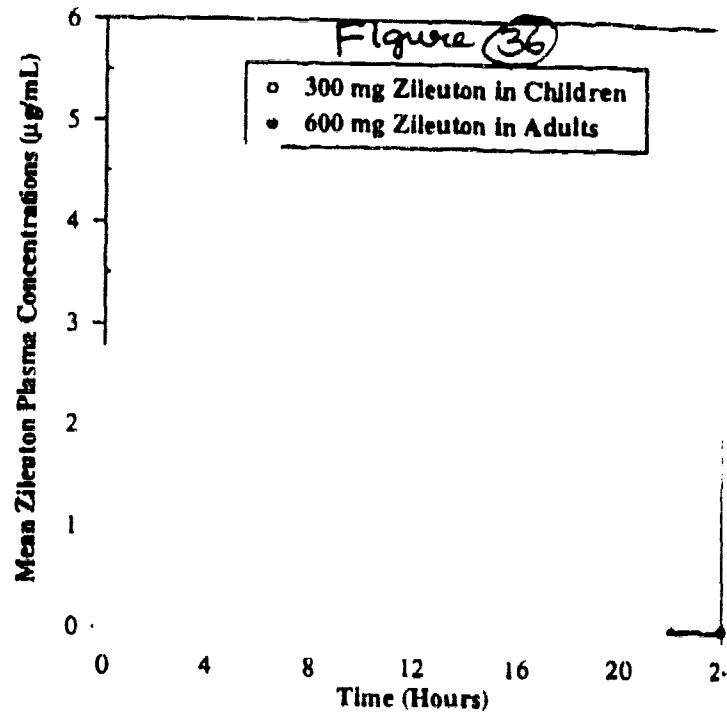
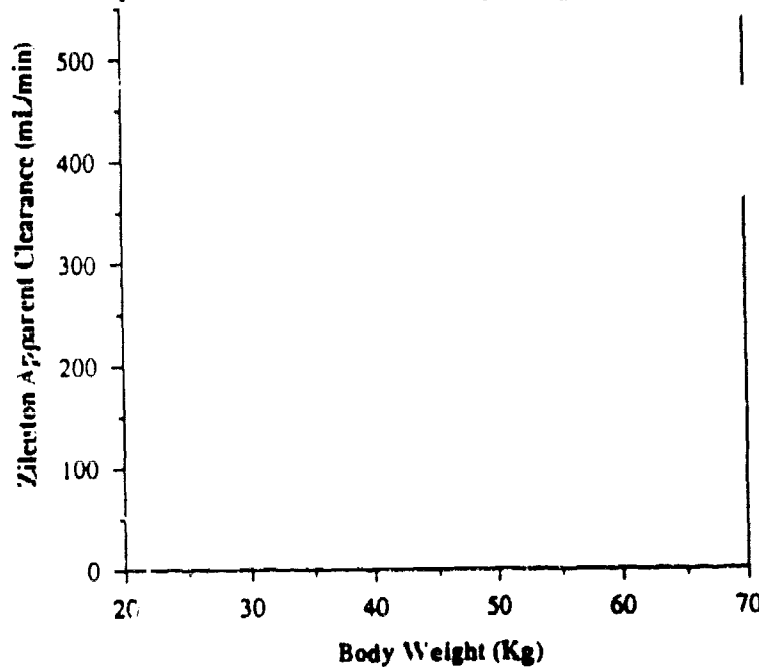


Figure 36



Relationship between total plasma clearance and body weight is shown in the following figure:

Figure 37



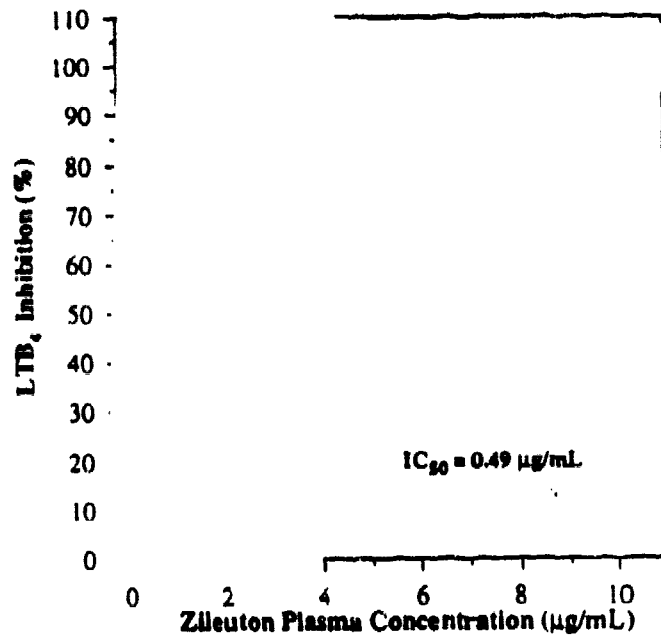
... Differences in body weight between adults and children appear to account for the differences in the mean dose-normalized values of C_{max} between the 2 groups. Weight was found to be a statistically significant factor in explaining the variability between children with a decrease in C_{max} with increasing weight.

... Gender was a marginally significant factor in determining the variability in zileuton dose-normalized C_{max} in children ($p=0.08$). Mean zileuton C_{max} was $1.96 \mu\text{g/ml}/100 \text{ mg}$ zileuton dose for females and $1.76 \mu\text{g/ml}/100 \text{ mg}$ zileuton dose for males, an 11% difference. After weight is accounted for, C_{max} was 33% higher for females than males.

The relationship of LTB₄ inhibition to zileuton concentrations was similar to that in adults as shown in figure below:

Figure 38

Observed and Predicted Percent LTB₄ Inhibition as a Function of Zileuton Plasma Concentrations After Single Doses of Placebo and 150 Mg and 300 Mg Zileuton



Although the pulmonary function measurements were made, the sponsor has not attempted to examine relationships between the PFTs and zileuton concentrations. Further, any analyses carried out, as per the sponsor will be provided separately in a clinical summary.

Conclusion: After adjustment of body weight, PK parameters of zileuton in children and adults were similar. Differences in body weight or surface area help explain differences in PK parameters between children and adults. Marginal gender effect was found in this study. Population estimate of IC_{50} in children agreed with that of adult studies where LTB₄ inhibition and zileuton concentrations were measured. Zileuton weight adjusted PK and PD (LTB₄ inhibition only and not FEV₁) in asthmatic children of ages 9-12 years were similar to that of adults.

14 STUDY IN RENALLY IMPAIRED PATIENTS:

**EVALUATION OF THE PHARMACOKINETICS OF ZILEUTON (ABBOTT-64077)
FOLLOWING ADMINISTRATION OF SINGLE AND MULTIPLE DOSES TO SUBJECTS WITH
VARYING DEGREES OF RENAL FUNCTION**

Study ID: M90-512

Investigators:

Clinical Site:

Objective:

... To evaluate the pharmacokinetics of zileuton following administration of single and multiple oral doses to subjects with varying degrees of renal function.

Drug Dosage Forms:

A: Zileuton capsules, NPRO 4525R, Lot 38-184-AR, 200 mg capsule II

B: Zileuton tablets, NPRO 5633N, Lot 60-777-AR,

Study Design:

... Five subjects with normal renal function (creatinine clearance > 90 ml/min), six subjects with mild renal impairment (Clcr between 60-90 ml/min), eight subjects with moderate renal impairment (Clcr between 30-59 ml/min), and six subjects with severe renal impairment (Clcr < 30 ml/min) were enrolled in Group I. An additional group of five subjects with renal failure requiring hemodialysis were enrolled in Group II.

Table 50

Group # (N completed study)		Clearance (ml/min)
I	Healthy (N=5)	>90
	Mild(N=5)	60-90
	Moderate (N=7)	30-59
	Severe (N=5)	<30
II (N=5)		Hemodialysis

... For group I, the study was a single and multiple dose, one-period open label design. On day 1 and 7 (8 a.m.), subjects were given a single dose of _____ and on days 3 - 6, _____ (8 a.m., 3 p.m., and 10 p.m.) zileuton was given.

... For group II, study was a single dose, 2-period, open label design. In period I, subjects were given a single _____ dose of zileuton 3 hours before hemodialysis. Following a 7-day washout period, the subjects received a single _____ zileuton dose after hemodialysis in a dialysis-free period.

... All but 4 subjects received capsules, 4 subjects with severe renal impairment received tablet. 2 subjects with mild renal impairment, 3 with moderate renal impairment and 2 with severe

renal impairment were dosed with 600 mg zileuton (only glucuronide data from these subjects were normalized to to use in analysis).

For group I, blood samples were collected into heparinized, chilled collection tubes on days 1 and 7, at 5 minutes before (predose), and at 0.5, 1.5, 3, 4.5, 6, 8, 12, 16, 24 and 48 hr after dosing. On day 1 and day 6, 24 hour urine was collected. For subjects in group II, venous blood samples were collected prior to dosing (0 hour) and at 0.5, 1.5, 3, 4.5, 6, 8, 12, 16, 24 and 48 hr after dosing in periods I and II. In period I, both arterial and venous samples were collected at the start of dialysis, 30 minutes thereafter, and hourly after the start until the completion of dialysis (dialysis time = 3 hours).

Blood, urine and dialysate samples were collected for determination of concentrations of zileuton and its glucuronide metabolites by validated HPLC method. Plasma protein binding of ¹⁴C-zileuton was determined by ultrafiltration. Cl_{cr} was determined over the same time interval.

PK parameters were determined by standard non-compartmental method.

Data Analysis:

In group I, the relationship between PK parameters and degree of renal insufficiency (24 hours creatinine clearance) was analyzed by regression. In group II, paired t-tests were used to compare PK parameters when dialysis was begun 3 hours postdose to those when dialysis was completed predose.

Results:

The mean pharmacokinetic parameters of zileuton, for each group following 400 mg dose after both single dose and at steady state are provided below:

Table (51)

GROUP I: PK parameters (mean ± st.dev.) of zileuton

Parameters	Normal (N=5)	Mild (N=5)	Moderate (N=5)	Severe (N=4)
DAY 1 (single dose)				
T _{max} (hr)	1.6 ± 0.9	2.1 ± 1.3	2.1 ± 0.82	1.9 ± 0.8
C _{max} (µg/ml)	3.11 ± 1.42	3.38 ± 0.87	3.33 ± 1.64	3.31 ± 0.65
AUC _{0-∞} (µg.hr/ml)	10.83 ± 3.19	15.67 ± 4.13	14.20 ± 7.98	12.67 ± 4.76
Cl/F (ml/min)	662 ± 202	452 ± 125	587 ± 268	571 ± 157
t _{1/2} (hr)	1.82 ± 0.35	2.60 ± 1.09	2.17 ± 0.69	1.41 ± 0.36
Vd _β /F (L)	102 ± 23	95 ± 20	98 ± 24	67 ± 20
Fraction bound (%)	93.66 ± 0.41	91.53 ± 1.50	92.48 ± 1.55	91.69 ± 0.72
Urinary recovery as glucuronides (% of dose)	81.2 ± 7.6	83.6 ± 4.6	71.7 ± 8.2	62.5 ± 11.5

DAY 7 (t.i.d.)				
T_{max} (hr)	1.2 ± 1.1	1.9 ± 1.5	2.4 ± 1.3	2.0 ± 1.2
C_{max} (µg/ml)	4.31 ± 1.68	4.87 ± 1.58	3.42 ± 1.41	4.10 ± 0.73
C_{min} AM (µg/ml)	0.78 ± 0.34	0.95 ± 0.33	0.75 ± 0.54	0.94 ± 0.95
C_{min} PM (µg/ml)	0.34 ± 0.10	0.69 ± 0.36	0.39 ± 0.20	0.39 ± 0.38
AUC ₀₋₈ (µg.hr/ml)	14.44 ± 3.29	18.06 ± 5.47	13.01 ± 3.81	15.95 ± 6.20
Cl/F (ml/min)	484 ± 125	396 ± 112	552 ± 170	455 ± 128
$t_{1/2}$ (hr)	2.34 ± 1.01	2.35 ± 0.78	2.39 ± 1.01	1.43 ± 0.41
Vd _f /F (L)	94 ± 32	75 ± 12	104 ± 26	53 ± 8
Accumulation ratio	1.46 ± 0.13	1.40 ± 0.30	1.16 ± 0.37	1.31 ± 0.05
Urinary recovery as glucuronides (% of dose)	93.1 ± 12.5	83.4 ± 10.8	82.2 ± 10.0	66.4 ± 17.2

Mean plasma concentration-time profiles on day 7 following zileuton administration to group I are shown in the following figure:

Figure (39)

Mean (+SD) Zileuton Plasma Concentrations from Normal Healthy Subjects and Subjects with Mild, Moderate and Severe Renal Impairment on Day 7

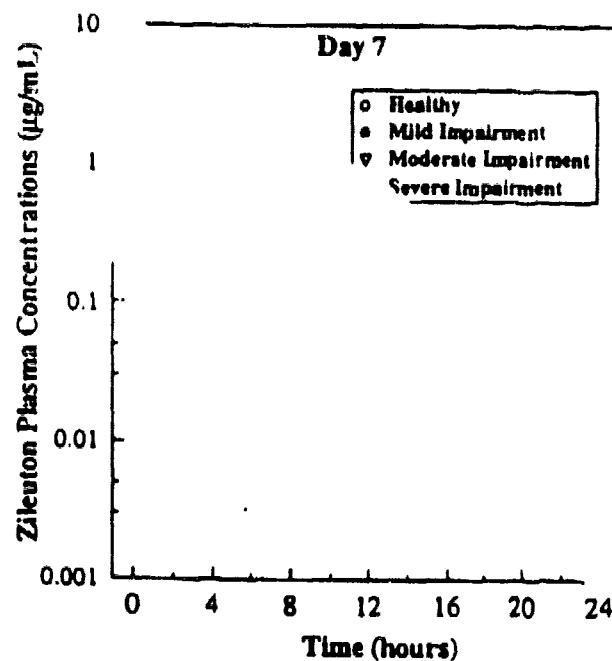


Table 52

GROUP II: PK parameters (mean ± st.dev.) of zileuton in subjects with renal failure requiring hemodialysis

Parameters	Period I Dialysis day	Period II Dialysis-free day
T _{max} (hr)	2.4 ± 0.8	2.1 ± 0.8
C _{max} (µg/ml)	2.13 ± 0.79	2.72 ± 1.68
AUC _{0-4h} (µg.hr/ml)	10.25 ± 3.67	11.67 ± 4.57
Cl/F (ml/min)	795 ± 512	721 ± 494
Cl _{HD} (ml/min)	9.8 ± 6.9	
Zileuton recovered in dialysate (% of dose)	0.30 ± 0.22	
G-1(glucuronide) recovered in dialysate (% of dose)	17.29 ± 3.92	
G-2(glucuronide) recovered in dialysate (% of dose)	22.83 ± 5.08	
Fraction bound (%)	87.72 ± 3.70	90.01 ± 1.19

The mean pharmacokinetic parameters of zileuton glucuronides for each group following 400 mg dose after both single dose and at steady state are provided below:

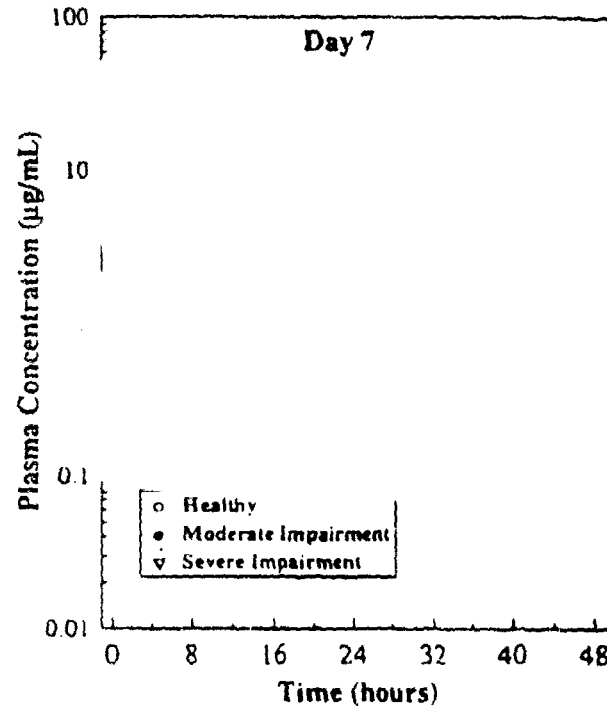
Table 53

GROUP I: PK parameters (mean ± st.dev.) of zileuton G-1 glucuronide

Parameters	Normal (N=3)	Moderate (N=3)	Severe (N=5)
DAY 1 (single dose)			
T _{max} (hr)	3.5 ± 0.9	5.7 ± 2.5	5.6 ± 2.5
C _{max} (µg/ml)	2.4 ± 0.24	5.16 ± 0.97	10.57 ± 1.43
AUC _{0-∞} (µg.hr/ml)	15.42 ± 4.27	54.94 ± 19.81	169.24 ± 41.16
Cl _R (ml/min)	179 ± 33	47 ± 15	15 ± 5
t _{1/2} (hr)	2.67 ± 0.66	4.13 ± 0.80	8.31 ± 1.67
M/P ratio	1.43 ± 0.29	3.79 ± 0.20	13.86 ± 4.48
DAY 7 (t.i.d.)			
T _{max} (hr)	2.0 ± 0.9	2.0 ± 1.7	3.6 ± 1.3
C _{max} (µg/ml)	4.09 ± 0.87	8.70 ± 3.81	30.32 ± 7.73
C _{min} AM (µg/ml)	2.04 ± 0.52	7.68 ± 2.56	24.00 ± 7.45
C _{min} PM (µg/ml)	1.25 ± 0.50	6.54 ± 2.57	24.84 ± 6.49
AUC ₀₋₈ (µg.hr/ml)	23.15 ± 5.52	61.64 ± 27.82	217.86 ± 58.53
Cl _R (ml/min)	131 ± 24	52 ± 24	11 ± 4
t _{1/2} (hr)	2.66 ± 0.82	5.80 ± 1.63	8.94 ± 2.58
M/P ratio	1.65 ± 0.35	5.05 ± 1.80	14.37 ± 4.20
Accumulation ratio	1.94 ± 0.30	2.27 ± 0.38	3.69 ± 1.47

Mean plasma concentration-time profiles of G-1 glucuronide on day 7 following zileuton administration to group I are shown in the following figure:

Figure 40



The relationship between creatinine clearance and renal clearance of G-1 glucuronide is shown in figure below:

Figure 41

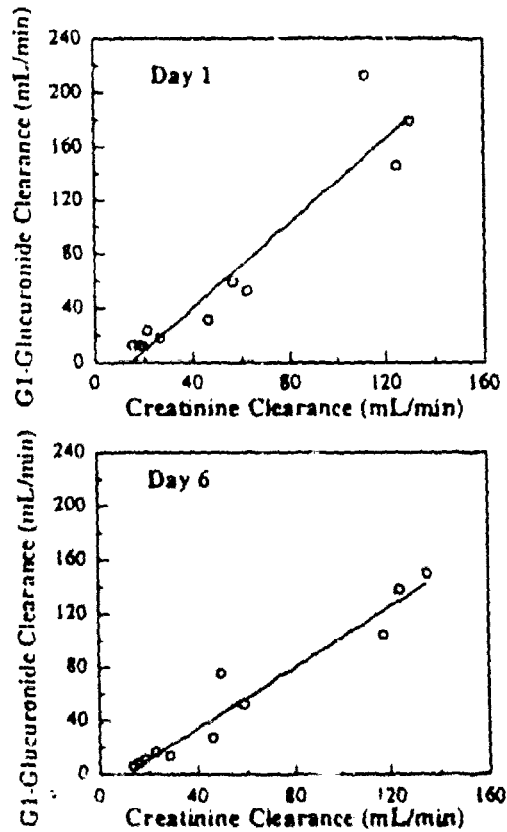


Table 54

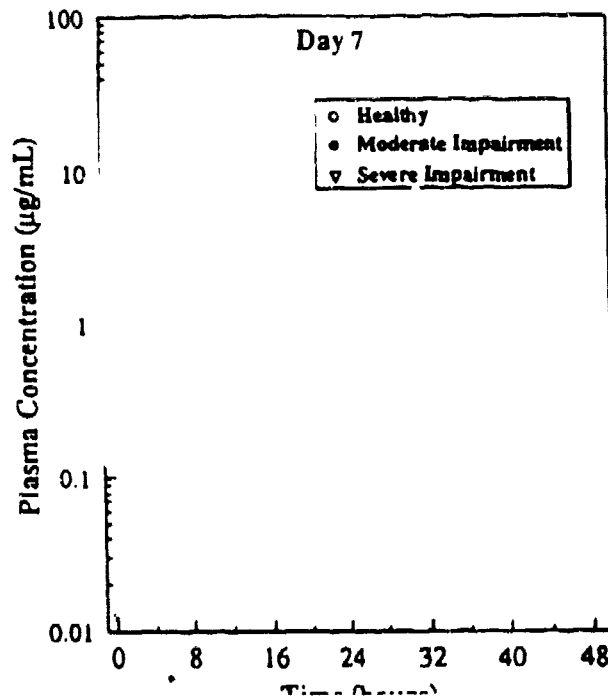
GROUP I: PK parameters (mean ± st.dev.) of zileuton G-2 glucuronide

Parameters	Normal (N=3)	Moderate (N=3)	Severe (N=5)
DAY 1 (single dose)			
T _{max} (hr)	2.0 ± 0.9	4.2 ± 3.4	3.0 ± 0.0
C _{max} (µg/ml)	2.68 ± 0.52	6.33 ± 2.39	15.08 ± 2.49
AUC _{0-∞} (µg.hr/ml)	11.32 ± 3.21	41.58 ± 15.08	136.57 ± 35.44
Cl _R (ml/min)	271 ± 61	64 ± 21	19 ± 6
t _{1/2} (hr)	2.15 ± 0.49	2.73 ± 0.95	6.33 ± 2.22
M/P ratio	1.05 ± 0.23	2.87 ± 0.16	11.18 ± 3.84
DAY 7 (t.i.d.)			
T _{max} (hr)	2.0 ± 0.87	3.0 ± 1.5	2.7 ± 0.7
C _{max} (µg/ml)	3.80 ± 1.36	7.80 ± 3.62	27.99 ± 5.85
C _{min} AM (µg/ml)	0.89 ± 0.41	4.39 ± 1.51	18.51 ± 6.00
C _{min} PM (µg/ml)	0.38 ± 0.32	3.70 ± 1.62	16.26 ± 4.01
AUC ₀₋₈ (µg.hr/ml)	14.28 ± 5.00	44.71 ± 19.33	178.34 ± 42.29
Cl _R (ml/min)	227 ± 62	68 ± 27	13 ± 4
t _{1/2} (hr)	2.10 ± 1.31	2.95 ± 0.81	6.35 ± 1.88
M/P ratio	1.00 ± 0.27	3.61 ± 0.94	11.68 ± 1.88
Accumulation ratio	1.38 ± 0.20	1.54 ± 0.15	2.38 ± 0.69

Mean plasma concentration-time profiles of G-2 glucuronide on day 7 following zileuton administration to groups of subjects are shown in the following figure:

Figure 42

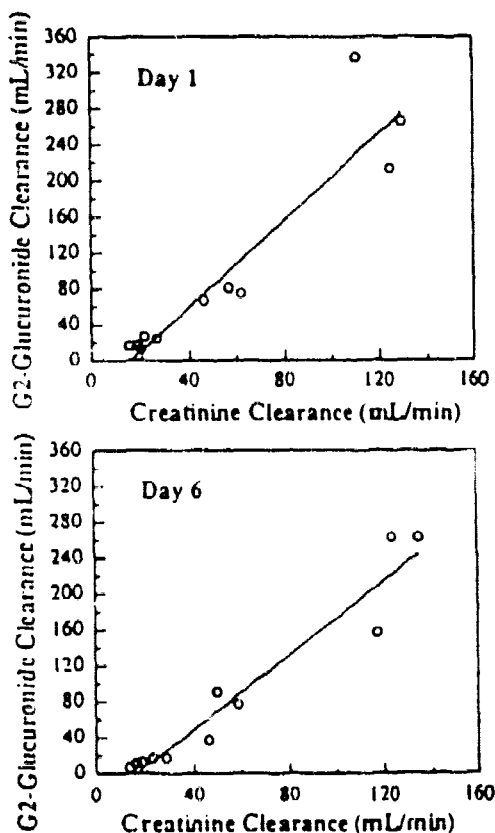
Mean (+SD) G-2 Glucuronide Plasma Concentrations from Normal Healthy Subjects and Subjects with Moderate and Severe Renal Impairment on Day 7



The relationship between creatinine clearance and renal clearance of G-2 glucuronide is shown in figure below:

Figure 43

The Relationship Between Subject's Creatinine Clearance and the Renal Clearance of G-2 Glucuronide



Conclusion: The pharmacokinetics of zileuton are unchanged in renal impairment. Glucuronide metabolites of zileuton accumulate in plasma in this condition.

In subjects with renal failure requiring hemodialysis, PK of zileuton was not altered by hemodialysis and only about 0.5% of administered zileuton dose was removed by hemodialysis. The hemodialysis procedure was effective in removing the zileuton glucuronides from the patient's blood.

Since the glucuronide metabolites are inactive and unlikely to be toxic (as per the sponsor) and since zileuton was well tolerated in the renally impaired patients, dosage adjustment of zileuton in patients with renal insufficiency may not be necessary.

Comments:

It is known that the metabolites of zileuton are not active. Toxicity of the zileuton glucuronides is not known. It would have been beneficial if the sponsor provided the side effects that the subjects experienced in this study, in order to obtain more meaningful information from this study. The sponsor should in future provide a table of side effects observed in each study even in the PK section.

15 STUDY IN HEPATICALLY IMPAIRED PATIENTS:

EVALUATION OF THE PHARMACOKINETIC PROFILE OF ZILEUTON (ABBOTT-64077) FOLLOWING ADMINISTRATION OF SINGLE AND MULTIPLE DOSES TO SUBJECTS WITH VARYING DEGREES OF HEPATIC FUNCTION

Study ID: M90-503

Investigators:

Clinical Site:

Objective:

To evaluate the pharmacokinetic profile of zileuton when administered as single mg and multiple t.i.d. oral doses to subjects with varying degrees of chronic hepatic insufficiency.

Drug Dosage Forms:

Zileuton capsules 200 mg, bulk lot # 38-242-AR, finished lot # 45-971-AR

Study Design:

Seven healthy subjects (31-66 years) (of which 6 completed the study, however data from one subject is not used because dose administered was 600 mg instead of 400 mg) and 4 subjects (42-59 years) with mild hepatic disease (child-pugh category 'A'), 4 subjects (37-53 years) with moderate hepatic disease (child-pugh category 'B') participated in this open-label, single and multiple dose study.

The stabilized cirrhotic subjects were graded by severity of liver disease by 5 criteria in accordance with the following child-pugh classification.

Table 55

Grading of severity of liver disease in accordance with Child-Pugh classification

Measurement	Numerical score for increasing abnormality		
	1	2	3
Ascitis	None	Slight	Moderate to severe
Encephalopathy	None	Slight to moderate	Moderate to severe
Bilirubin (mg/dl)	<2	2 - 3	>3
Albumin (g/l)	>35	28 - 35	<28
Prothrombin time (%) (indicates severity of coagulation disorders)	>70	40 - 70	<40

Addition of above scores for five criteria gives the risk grade by which a subject was classified. Classes A (mild), B (moderate) and C (severe) were defined by the ranges 5-6, 7-9, and 10-15, respectively for the sum of scores.

In this study, subjects with no evidence of encephalopathy were selected. Liver cirrhosis was determined by liver biopsies previously obtained in these patients or by other appropriate tests.

... Zileuton was administered as a single dose on day 1 TID on days 2 through 5, and a single dose on day 6. Following evaluation of data from these subjects, decision was made not to administer zileuton to subjects with severe hepatic impairment (as subjects with mild and moderate hepatic impairment showed significant changes in pharmacokinetics of zileuton).

... Blood samples were collected for determination of zileuton concentrations at 0, 1.5, 3, 4.5, 6, 8, 12, 16 and 24 hours postdosing on days 1 and 6. Urine was collected on days 1 and 6 at 0 hrs, 0-4 hours, 4-8 hours, 8-12 hours and 12-24 hours after drug administration.

... Plasma samples were analyzed for zileuton using validated HPLC method. Plasma samples from 3 subjects in each group and all urine samples were analyzed for zileuton glucuronides using validated HPLC method. Plasma protein binding was determined in zero hour samples on days 1 and 6 by an ultrafiltration technique.

... Pharmacokinetic parameters of zileuton and its glucuronides were estimated by non-compartmental methods. Effect of stages of hepatic impairment on PK profile of zileuton and its glucuronides was examined using one-way analysis of variance. Pairwise comparisons of the 3 categories were conducted utilizing the least significant difference procedure.

Results:

... The pharmacokinetic parameters of zileuton and zileuton G-1 and G-2 glucuronides for each group of subjects are shown in tables below:

Table 56
PK parameters of zileuton

	Healthy (N = 5)	Mild (N = 4)	Moderate (N = 4)
Day 1 (Single dose)			
T _{max} (hr)	2.4 ± 0.8	2.3 ± 0.9	3.4 ± 0.8
C _{max} (mg/ml)	1.98 ± 0.59	3.87 ± 1.00*	3.57 ± 1.28*
AUC _{0-∞} (µg.hr/ml)	7.93 ± 1.86	17.47 ± 6.35*	20.80 ± 6.33*
Cl/F (mL/min)	872 ± 168	416 ± 128*	344 ± 104*
t _{1/2} (hr)	1.70 ± 0.41	2.52 ± 0.59	3.40 ± 0.99*
Vd/F (L)	125 ± 28	86 ± 16	100 ± 44
Percent bound	93.66 ± 0.94	92.13 ± 2.04	87.82 ± 2.69* [Ⓢ]
AUC _{free} (mean) (µg.hr/ml)	0.502	1.375	2.53

Day 6 (			
T _{max} (hr)	1.5 ± 0.0	3.3 ± 1.4*	1.5 ± 0.0 [@]
C _{max} (µg/ml)	3.07 ± 0.25	4.84 ± 1.33*	4.34 ± 1.02
C _{min} AM (µg/ml)	0.46 ± 0.26	1.18 ± 0.80	1.16 ± 0.74
C _{min} PM (µg/ml)	0.33 ± 0.27	0.84 ± 0.29*	1.06 ± 0.39*
AUC ₀₋₈ (µg.hr/ml)	10.03 ± 0.99	20.72 ± 7.50*	20.85 ± 5.60*
Cl/F (mL/min)	670 ± 66	355 ± 123*	337 ± 88*
t _{1/2} (hr)	1.82 ± 0.40	2.58 ± 0.74	3.78 ± 0.41* [@]
Vd/F (L)	105 ± 20	76 ± 20	111 ± 37
Accumulation ratio	1.40 ± 0.19	1.41 ± 0.43	1.29 ± 0.17
Percent bound	93.58 ± 0.51	92.89 ± 1.63	88.09 ± 2.44* [@]

* statistically significant when compared to healthy, [@] statistically significant compared to mild.

Table 57
PK PARAMETERS OF ZILEUTON G-1 GLUCURONIDE

	Healthy (N = 3)	Mild (N = 3)	Moderate (N = 3)
Day 1 (Single dose)			
T _{max} (hr)	3.0 ± 0.0	3.0 ± 0.0	3.5 ± 0.9
C _{max} (µg/ml)	2.34 ± 0.85	2.10 ± 0.28	1.24 ± 0.15*
AUC _{0-∞} (µg.hr/ml)	11.91 ± 0.95	17.24 ± 0.82*	13.07 ± 3.17 [@]
Cl _R (mL/min)	188 ± 25	173 ± 22	286 ± 39* [@]
t _{1/2} (hr)	2.68 ± 0.79	3.87 ± 1.25	6.47 ± 2.18*
M/P Ratio	1.51 ± 0.45	1.02 ± 0.37	0.61 ± 0.21*
Day 6 (t.i.d.)			
T _{max} (hr)	1.5 ± 0.0	3.9 ± 0.8*	3.0 ± 0.0*
C _{max} (µg/ml)	3.31 ± 0.80	3.63 ± 0.34	1.77 ± 0.06* [@]
C _{min} AM (µg/ml)	0.98 ± 0.21	1.75 ± 0.23*	0.96 ± 0.09 [@]
C _{min} PM (µg/ml)	0.82 ± 0.15	1.47 ± 0.20*	1.11 ± 0.03 [@]
AUC ₀₋₈ (µg.hr/ml)	15.69 ± 3.17	20.30 ± 0.39*	11.33 ± 0.35* [@]
Cl _R (mL/min)	162 ± 30	145 ± 17	276 ± 34* [@]
t _{1/2} (hr)	2.88 ± 0.86	3.17 ± 0.14	5.74 ± 1.67* [@]
M/P Ratio	1.64 ± 0.51	0.98 ± 0.39	0.56 ± 0.17*
Accumulation ratio	1.61 ± 0.03	1.83 ± 0.07*	1.78 ± 0.16

* statistically significant when compared to healthy, [@] statistically significant compared to mild.

Table (58)

PK PARAMETERS OF ZILEUTON G-2 GLUCURONIDE

	Healthy (N = 3)	Mild (N = 3)	Moderate (N = 3)
Day 1 (Single dose)			
T _{max} (hr)	2.5 ± 0.9	2.5 ± 0.9	3.0 ± 0.0
C _{max} (µg/ml)	2.30 ± 0.67	3.07 ± 0.25	2.12 ± 0.52
AUC _{0-∞} (µg.hr/ml)	9.24 ± 0.59	12.29 ± 1.14*	9.28 ± 1.5 [~] @
Cl _R (mL/min)	285 ± 47	249 ± 32	397 ± 61* [@]
t _{1/2} (hr)	1.81 ± 0.84	1.60 ± 0.30	2.06 ± 0.67
M/P Ratio	1.15 ± 0.25	0.71 ± 0.21	0.44 ± 0.13*
Day 6 (t.i.d.)			
T _{max} (hr)	1.5 ± 0.0	2.9 ± 1.4	3.0 ± 0.0
C _{max} (µg/ml)	3.33 ± 1.0	3.47 ± 0.38	2.07 ± 0.13* [@]
C _{min} AM (µg/ml)	0.35 ± 0.16	0.56 ± 0.19	0.36 ± 0.10
C _{min} PM (µg/ml)	0.35 ± 0.26	0.36 ± 0.07	0.30 ± 0.05
AUC ₀₋₈ (µg.hr/ml)	10.39 ± 2.56	13.73 ± 0.99*	9.22 ± 0.51 [@]
Cl _R (mL/min)	215 ± 39	206 ± 19	348 ± 67* [@]
t _{1/2} (hr)	1.49 ± 0.13	1.66 ± 0.22	1.92 ± 0.48
M/P Ratio	1.09 ± 0.38	0.66 ± 0.28	0.46 ± 0.18*
Accumulation ratio	1.23 ± 0.23	1.20 ± 0.15	1.15 ± 0.16

* statistically significant when compared to healthy, @ statistically significant compared to mild.

Following figures show the mean plasma concentration-time profiles of total zileuton on days 1 and 6 in the 3 groups of subjects.

Mean (+SD) Total Zileuton Plasma Concentrations from Healthy Subjects and Subjects with Mild and Moderate Hepatic Impairment on Day 1

Mean (+SD) Total Zileuton Plasma Concentrations from Healthy Subjects and Subjects with Mild and Moderate Hepatic Impairment on Day 6

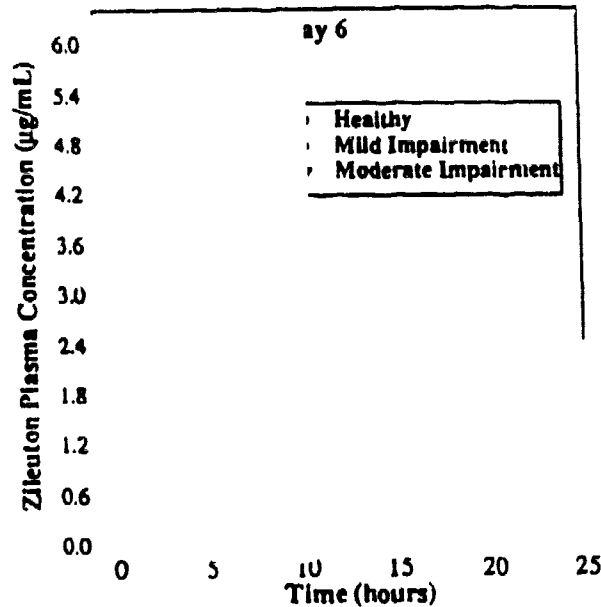
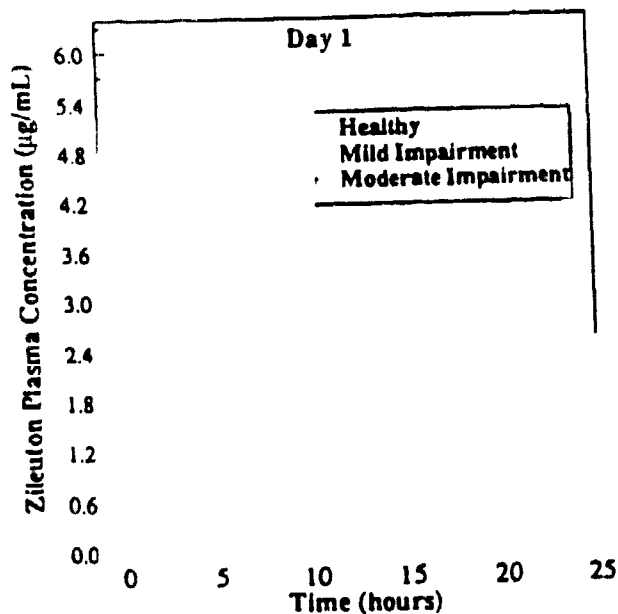


Figure (A4)

Figure (A5)

Following figures show the mean plasma concentration-time profiles of unbound zileuton on days 1 and 6 the 3 groups of subjects.

Figure 46

Mean (+SD) Unbound Zileuton Concentrations in Plasma from Healthy Subjects and Subjects with Mild and Moderate Hepatic Impairment on Day 1

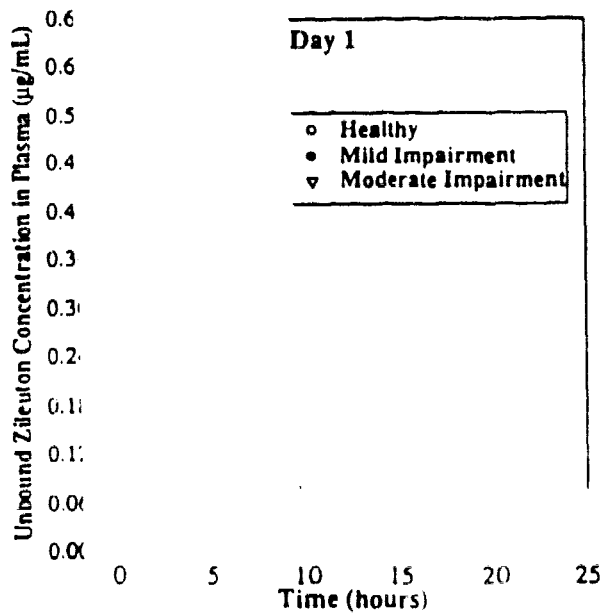
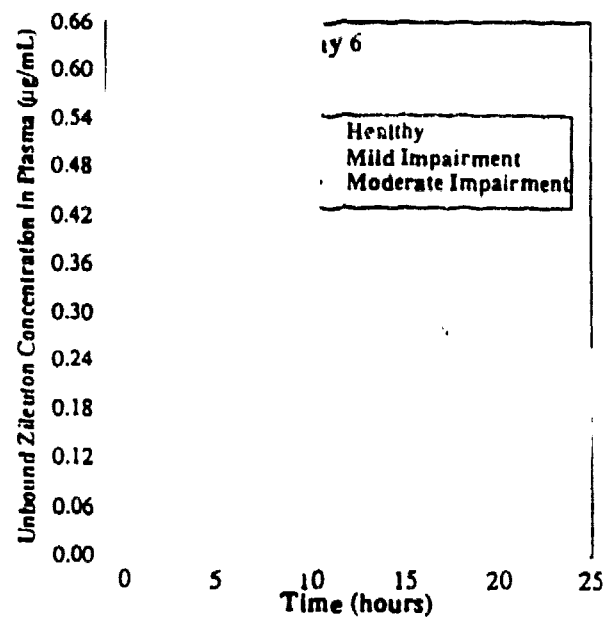


Figure 47

Mean (+SD) Unbound Zileuton Concentrations in Plasma from Healthy Subjects and Subjects with Mild and Moderate Hepatic Impairment on Day 6



The relationship between the concentration of albumin in plasma and percent of zileuton bound to plasma proteins is shown below.

The Relationship Between the Concentration of Albumin in Plasma and the Percent of Zileuton Bound to Plasma Proteins

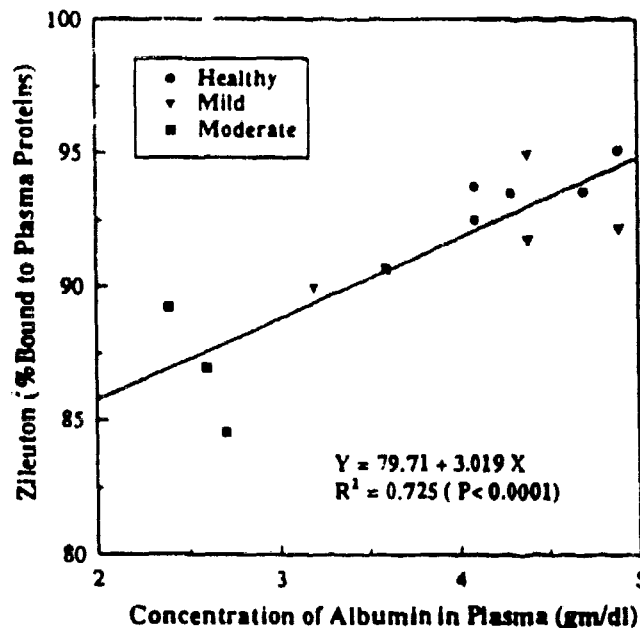


Figure 48

Conclusion: Results indicate accumulation of zileuton in hepatic impairment. Mean apparent plasma clearance of total zileuton in subjects with hepatic impairment was approximately half the value of healthy subjects. Unbound clearance decreased 2-fold in subjects with mild hepatic impairment and 4-fold in subjects with moderate hepatic impairment. Hence, zileuton dose adjustment is necessary in patients with hepatic insufficiency. However, zileuton was not used at the proposed dose and dosing regimen. At the proposed (recommended) dose, zileuton exposure is likely to be greater than seen in this study and thus in case of hepatic insufficiency, the dose should be reduced more than reflected by the study.

Comment: Although based on data provided the assay method used in this study is adequate, there are some data points, both in calibration curves and also in quality control samples, that have not been provided and have been removed as outliers. What is the basis for this removal? Was there an assay problem?

16 PROTEIN BINDING:

IN VITRO PLASMA PROTEIN BINDING OF ZILEUTON-¹⁴C

Study ID: V87-043

Objective:

To determine the in vitro protein binding of Zileuton-¹⁴C in rat, dog, monkey and human plasma.

Concentration range of drug selected for this study was 0.1-100 µg/ml, so as to accommodate the anticipated therapeutic plasma levels and those seen in subchronic toxicity studies in rat and dog.

Study Design:

Plasma from human (adult male and female volunteers), rats, dogs and monkeys was obtained. Radiolabeled zileuton (¹⁴C) stock solution was added to plasma to obtain final concentration of 0.1, 1.0, 10 and 100 µg/ml. Similar solutions were also obtained in physiological saline.

Protein binding was determined using an ultrafiltration technique. The saline solutions were also subjected to ultrafiltration to determine the adsorption to the device. The plasma, saline and ultrafiltrate samples were then radioassayed directly in Insta-Gel scintillation fluid and counted in a Liquid Scintillation Spectrometer. Percent binding was then determined from these results.

Results: At concentrations ranging from 0.1-100 µg/ml, the protein binding of Zileuton-¹⁴C averaged 94-92% in human plasma, 92-88% in rat plasma, 90-87% in monkey plasma, and 89-84% in dog plasma. Protein binding appears to be similar in both males and females and also appears to be saturable at higher concentrations of drug.

Table 59

% protein binding in human plasma

	<u>0.1 µg/ml</u>	<u>1 µg/ml</u>	<u>10 µg/ml</u>	<u>100 µg/ml</u>
Male	94.1	93.4	93.6	91.3
Females	93.5	93.4	93.8	91.8
Mean	93.8	93.4	93.7	91.6

17 **IN VITRO PLASMA PROTEIN BINDING INTERACTIONS BETWEEN ZILEUTON-¹⁴C AND
SELECTED OTHER DRUGS IN HUMAN PLASMA**

Study ID: V90-022

Objective:

1. To determine possible protein binding interactions between zileuton and salicylate, theophylline, naproxen, ibuprofen, prednisone, sulfasalazine and salbutamol (effect of these drugs on protein binding of zileuton).
2. To determine whether zileuton affects the protein binding of warfarin, salicylate, theophylline, naproxen, ibuprofen and prednisone.
3. To determine the binding affinity of zileuton toward human albumin and α_1 -acid glycoprotein using an equilibrium dialysis technique.

Concentration of zileuton selected for this study was 5 $\mu\text{g/ml}$, representing the C_{max} of this drug. The concentration of other drugs studied was at a single concentration which was near the upper end of its therapeutic range, or at a concentration obtained from clinical interaction studies with zileuton.

Study Design:

Plasma from human (adult male (2) and female (2) volunteers) was obtained. An aliquot of radiolabeled drug (¹⁴C) stock solutions was added to plasma to obtain final concentration as shown in tables below. Plasma was then divided into 1 ml portions, and an aliquot of water, 50% aqueous ethanol or one of the stock solutions of an unlabeled drug was added to give desired concentrations.

... Protein binding was determined using an ultrafiltration technique. The ultrafiltrate samples were then radioassayed directly in Insta-Gel scintillation fluid and counted in a Liquid Scintillation Spectrometer. Percent binding was then determined from these results.

... Human albumin and α_1 -acid glycoprotein binding: The binding affinity of the radiolabeled zileuton was determined in an Equilibrium dialysis system. The cells were filled with either α_1 -acid glycoprotein or human albumin on the left side and phosphate buffer containing zileuton on right side. Duplicate samples of each protein and buffer solution were radioassayed and counted in a Liquid Scintillation Spectrometer. Protein binding was calculated from this data.

Results:

... Addition of theophylline (20 $\mu\text{g/ml}$), prednisone (50 $\mu\text{g/ml}$) and salbutamol (0.005 $\mu\text{g/ml}$) had no effect on protein binding of zileuton.

... Addition of naproxen (100 $\mu\text{g/ml}$), salicylate (250 $\mu\text{g/ml}$), ibuprofen (50 $\mu\text{g/ml}$), and sulfasalazine (50 $\mu\text{g/ml}$) produced small, but statistically significant reductions in the binding of zileuton, leading to net decreases of 1.4, 2.0, 0.7 and 0.3% respectively (see table below).

Zileuton (5 µg/ml) did not affect the protein binding of warfarin (10 µg/ml), salicylate (250 µg/ml), theophylline (20 µg/ml), naproxen (100 µg/ml), ibuprofen (50 µg/ml) and prednisone (50 µg/ml) (see table below).

Table 60

Effects of Salicylate, Theophylline, Naproxen, Ibuprofen, Sulfasalazine, Prednisone and Salbutamol on the Protein Binding of [¹⁴C]Zileuton in Human Plasma

Compound	Concentration (µg/ml)	% [¹⁴ C]Zileuton Bound				
		Female	Female	Male	Male	Mean±S.D.
[¹⁴C]Zileuton						
• Water	5	93.0	92.7	93.9	93.3	93.480.39
• 50% EtOH	5	92.4	92.0	93.0	93.4	92.780.62
• Salicylate	250	90.8	90.4	92.0	92.2	91.480.85*
• Theophylline	20	92.8	92.3	93.8	93.8	93.280.68
• Naproxen	100	91.0	90.2	92.1	92.0	91.380.90**
• Ibuprofen	50	91.4	91.0	92.6	92.9	92.080.91**
• Prednisone	50	92.2	91.8	93.1	93.0	92.380.71
• Sulfasalazine	50	91.6	91.6	93.0	93.2	92.480.87**
• Salbutamol	0.005	92.4	92.1	93.2	93.3	92.880.59

** Significantly (P<0.05) different from the 50% ethanol control.
* Significantly (P<0.05) different from the water control.

Table 61

Effect of Zileuton on the Protein Binding of Other Selected Radiolabeled Drugs in Human Plasma

Compound	Concentration (µg/ml)	% Radiolabeled Drug Bound				
		Female	Female	Male	Male	Mean±S.D.
[¹⁴C]Warfarin						
• Zileuton	5	98.4	98.3	98.3	98.3	98.3±0.08
[¹⁴C]Salicylate						
• Zileuton	5	79.2	78.6	77.6	81.8	79.3±1.79
[¹⁴C]Theophylline						
• Zileuton	5	51.4	50.6	49.3	49.1	50.6±1.43
[³H]Naproxen						
• Zileuton	5	98.6	98.3	98.8	98.6	98.6±0.12
[¹⁴C]Ibuprofen						
• Zileuton	5	99.0	98.2	99.0	99.0	98.8±0.40
[³H]Prednisone						
• Zileuton	5	48.8	33.0	47.0	60.8	52.4±6.14

Zileuton binds to both human albumin and α₁-acid glycoprotein, but the affinity to albumin appears to be 2.6 times greater. At physiological concentrations of the two proteins, the binding of zileuton was 87.5 ± 0.17% with human albumin (40 mg/ml) and 33.9 ± 3.4% with α₁-acid glycoprotein (1 mg/ml).

18 *IN VITRO PLASMA PROTEIN BINDING INTERACTIONS BETWEEN ZILEUTON-¹⁴C AND ³H-TERFENADINE IN HUMAN PLASMA*

Study ID: V94-003

Objective:

To determine the effect of terfenadine on protein binding of ¹⁴C-zileuton, and the effect of zileuton on protein binding of ³H-terfenadine.

Concentrations of zileuton selected for this study were 0.5, 5 and 10 µg/ml, representing the range of zileuton concentrations seen in humans. Terfenadine was selected at concentrations of 0.005, 0.01 and 0.05 µg/ml.

Study Design:

Plasma from human (adult, male (2) and female (2) volunteers) was obtained. An aliquot of radiolabeled drug (¹⁴C) stock solutions was added to plasma to obtain final concentration as shown in tables below. Plasma was then divided into 1 ml portions, and an aliquot of water, 50% aqueous ethanol or one of the stock solutions of an unlabeled drug was added to give desired concentrations.

Protein binding was determined using an ultrafiltration technique. The ultrafiltrate samples were then radioassayed directly in Insta-Gel scintillation fluid and counted in a Liquid Scintillation Spectrometer. Percent binding was then determined from these results.

Results:

At zileuton concentrations of 0.5, 5 and 10 µg/ml in absence of terfenadine, zileuton plasma protein binding ranged from 94.39 - 93.21%. In presence of terfenadine (0.01 µg/ml), zileuton binding ranged from 93.98 - 92.42% as shown in the table below.

Table (62)

The Effect of Terfenadine on [¹⁴C]Zileuton Protein Binding in Human Plasma

Compound	Concentration (µg/mL)	% Protein Bound						
		Female	Female	Male	Male	Mean ± SD (M)	Mean ± SD (F)	Mean ± SD (M + F)
¹⁴ C]Zileuton + Terfenadine	0.5	93.34	93.50	93.98	94.28	94.13 ± 0.21	93.42 ± 0.11	93.78 ± 0.50
	0.01	92.79	93.19	93.85	93.97	93.91 ± 0.09	92.99 ± 0.28	93.45 ± 0.65
¹⁴ C]Zileuton + Terfenadine	5	93.42	93.44	93.82	94.35	94.09 ± 0.38	93.43 ± 0.02	93.76 ± 0.47
	0.01	92.70	93.01	93.96	93.88	93.92 ± 0.05	92.85 ± 0.22	93.39 ± 0.75
¹⁴ C]Zileuton + Terfenadine	10	93.21	93.37	93.75	94.39	94.07 ± 0.45	93.29 ± 0.12	93.68 ± 0.55
	0.01	92.42	93.36	93.73	93.98	93.86 ± 0.18	92.89 ± 0.67	93.37 ± 0.68

SD = standard deviation

At terfenadine concentrations of 0.005, 0.01 and 0.05 µg/ml in absence of zileuton, terfenadine plasma protein binding ranged from 99.90 - 99.73%. In presence of zileuton (5 µg/ml), terfenadine binding ranged from 99.90 - 99.82%(see table below).

Table (63)

The Effect of Zileuton on [³H]Terfenadine Protein Binding in Human Plasma

Compound	Concentration (µg/mL)	% Protein Bound						
		Female	Female	Male	Male	Mean ± SD (M)	Mean ± SD (F)	Mean ± SD (M + F)
³ H]Terfenadine + Zileuton	0.005	99.86	99.82	99.73	99.81	99.77 ± 0.06	99.84 ± 0.03	99.81 ± 0.05
	5	99.86	99.82	99.82	99.86	99.84 ± 0.03	99.84 ± 0.03	99.84 ± 0.001
³ H]Terfenadine + Zileuton	0.01	99.90	99.83	99.85	99.87	99.86 ± 0.02	99.86 ± 0.05	99.86 ± 0.000
	5	99.87	99.83	99.86	99.86	99.86 ± 0.005	99.85 ± 0.03	99.85 ± 0.007
³ H]Terfenadine + Zileuton	0.05	99.88	99.85	99.85	99.89	99.87 ± 0.03	99.87 ± 0.02	99.87 ± 0.001
	5	99.88	99.86	99.87	99.90	99.89 ± 0.02	99.87 ± 0.02	99.88 ± 0.009

SD = standard deviation

Conclusion: At the concentrations tested, neither zileuton nor terfenadine appear to interfere with the plasma protein binding of the other drug.

19 IN VITRO PLASMA PROTEIN BINDING OF [R]- AND [S]- ENANTIOMERS OF ABBOTT-64077 (ZILEUTON)-¹⁴C

Study ID: V90-036

Objective: To determine the in vitro protein binding of [R]- and [S]- enantiomers of Zileuton-¹⁴C in rat, dog, monkey and human plasma.

Concentration range of enantiomers selected for this study was 0.1-50 µg/ml.

Study Design: Plasma from human (adult male and female volunteers), rats, dogs and monkeys was obtained. Radiolabeled [R]- and [S]- enantiomers of zileuton (¹⁴C) stock solution was added to plasma to obtain final concentration of 0.1, 1.0, 10 and 50 µg/ml. Similar solutions were also obtained in physiological saline.

Protein binding was determined using an ultrafiltration technique. The saline solutions were also subjected to ultrafiltration to determine the adsorption to the device. The plasma, saline and ultrafiltrate samples were then radioassayed directly in Insta-Gel scintillation fluid and counted in a Liquid Scintillation Spectrometer. Percent binding was then determined from these results.

Results: At concentrations ranging from 0.1-50 µg/ml, the protein binding of [R]- and [S]- enantiomers of Zileuton-¹⁴C was extensive (84-96%), independent of drug concentration. The mean unbound (free) fraction of the R(+) enantiomer was about 3.3 fold greater than that of S(-) enantiomer in human plasma. Mean plasma protein binding data are summarized below:

Table 64

SPECIES	ENANTIOMER	% BOUND	% UNBOUND
Rat	R(+)	91.26	8.74
	S(-)	86.41	13.59
	R/S	1.06	0.64
Dog	R(+)	88.79	11.21
	S(-)	89.11	10.90
	R/S	1.00	1.03
Monkey	R(+)	84.04	15.96
	S(-)	93.35	6.65
	R/S	0.90	2.40
Human	R(+)	87.79	12.21
	S(-)	96.31	3.69
	R/S	0.91	3.31

20 IN VITRO HUMAN RED BLOOD CELL BINDING OF ABBOTT-64077-¹⁴C (ZILEUTON) AND
¹⁴C ABBOTT-66193

Study ID: V93-021

Objective:

To determine the in vitro red blood cell binding of Zileuton-¹⁴C and its N-dehydroxylated metabolite in human whole blood.

Concentration range of drug selected for this study was 0.5-5 µg/ml, so as to encompass anticipated therapeutic plasma levels.

Study Design:

Blood obtained from 4 adult male and 4 adult female human volunteers was used for this study. Hemotacrit was measured in these samples. Appropriate aliquots of stock solutions were added to whole blood aliquots to give initial ¹⁴C-zileuton or ¹⁴C-Abbott 66193 concentrations of approximately 0.5 and 5.0 µg/ml. These spiked whole blood samples were then incubated at 37°C for 1 hour. Both the whole blood samples and plasma separated from these samples were radioassayed for determination of concentration of zileuton or Abbott-66193. Erythrocyte binding was then determined based on the results.

Results: The results of in vitro red blood cell binding studies with ¹⁴C-zileuton in human blood are presented below.

Table 65

Subject	Sex	Fraction Bound to Red Blood Cells*	
		0.5 µg/mL	5.0 µg/mL
1	Male	0.160	0.132
3	Male	0.133	0.115
5	Male	0.198	0.221
7	Male	0.162	0.141
	Mean	0.163	0.152
	SD	0.027	0.047
2	Female	0.130	0.115
4	Female	0.123	0.107
6	Female	0.160	0.051
8	Female	0.161	0.180
	Mean	0.144	0.113
	SD	0.020	0.053

* Fraction of [¹⁴C]zileuton in whole blood bound to red blood cells (f_{bc}) after 1 hour in vitro incubation.
The average over the concentration range was 0.158 ± 0.008 for males and 0.129 ± 0.022 for females. Male plus female average was 0.144 ± 0.021.
SD = Standard Deviation

In the concentration range studied, minor concentration dependent differences in RBC binding were observed in male subjects. However, in females, both a gender-based difference and a concentration-dependent difference was observed. The fraction of ¹⁴C-zileuton bound to RBC was higher in males (0.158 ± 0.008) than for the females (0.129 ± 0.022). This lower binding in females correlated with a lower hematocrit (average hematocrit was 40.7% in 4 females and 44.1% in 4 males).

The fraction of zileuton and Abbott-66193 bound to RBC and ratios of C_{rbc}/C_p and C_{blood}/C_p are shown in the following table.

Table (66)

Comparison of *In Vitro* Red Blood Cell Binding, Red Blood Cell to Plasma Concentration Ratios and Blood to Plasma Concentration Ratios for [¹⁴C]Zileuton and [¹⁴C]Abbott-66193 in Human Whole Blood

Parameter	Sex	Zileuton	Abbos-66193
f_{rbc}^a	Male	0.158 ± 0.008	0.125 ± 0.019
	Female	0.129 ± 0.022	0.111 ± 0.009
	Mean	0.144 ± 0.021	0.118 ± 0.010
C_{rbc}/C_p^b	Male	0.243 ± 0.012	0.185 ± 0.034
	Female	0.216 ± 0.042	0.182 ± 0.015
	Mean	0.230 ± 0.019	0.184 ± 0.007
C_{blood}/C_p^c	Male	0.666 ± 0.005	0.641 ± 0.015
	Female	0.682 ± 0.016	0.667 ± 0.007
	Mean	0.674 ± 0.011	0.654 ± 0.018

^aFraction of [¹⁴C]Zileuton or [¹⁴C]Abbott-66193 in whole blood bound to red blood cells after 1 hour *in vitro* incubations.

^bRatio of the concentration of [¹⁴C]Zileuton or [¹⁴C]Abbott-66193 in red blood cells to the concentration in plasma after 1 hour *in vitro* incubations.

^cRatio of the concentration of [¹⁴C]Zileuton or [¹⁴C]Abbott-66193 in blood to the concentration in plasma after 1 hour *in vitro* incubations.

All data represent mean ± standard deviation.

21 TABULATION OF ZILEUTON CONCENTRATIONS IN HUMAN PLASMA AND
BRONCHOALVEOLAR LAVAGE FLUID (BALF)

Study ID: M92-724

Objective:

The study objective was to determine whether the use of zileuton, 600 mg QID might improve reactivity to methacholine in patients with nocturnal asthma. A secondary objective was to explore the effects of zileuton and those of placebo on pulmonary function in patients with nocturnal asthma with a comparison of these effects to normal subjects

Study Design:

This study was a randomized, double-blind crossover, placebo-controlled single-center study in patients with nocturnal asthma compared to normal subjects. Normal subjects and patients were selected in a 2-week single blind period. On study days 15, patients and subjects selected were randomized to zileuton 600 mg QID or placebo for 1 week. On the 7th day of treatment in both periods (day 21 and day 36 of study), patients were admitted to sleep laboratory for pulmonary function tests, methacholine challenge and a bronchoscopy with bronchoalveolar lavage. A 1-week washout period was included between the 2 treatment periods.

Plasma samples for determination of zileuton were obtained on study days 0, 22 and 36 from each patient prior to pulmonary function test. On study day 8 at 4:00 p.m. and on study days 22 and 35 at 4:00 a.m., bronchoscopies were performed immediately following methacholine challenge. At this time, warm saline was instilled through bronchoscope and removed by gentle aspiration. The samples were centrifuged to separate fluid from cells. The aliquots of lavage fluid (BALF) were used for determination of concentration of zileuton and its N-dehydroxylated metabolite. Samples were analyzed for determination of zileuton and its metabolite in plasma and BALF by HPLC.

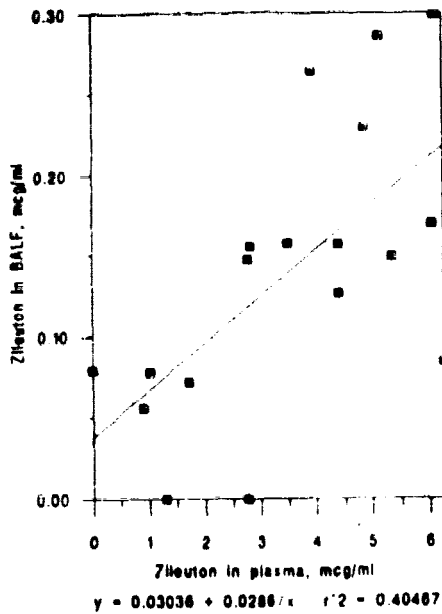
Results:

The plasma concentrations of zileuton in normal subjects (n=6) were 3.227 ± 2.113 $\mu\text{g/ml}$ (range 1.173 - 6.396 $\mu\text{g/ml}$). In asthmatic subjects (n=12), zileuton plasma concentrations were 4.081 ± 1.917 $\mu\text{g/ml}$ (range 0.269 - 6.523 $\mu\text{g/ml}$).

BALF contained measurable levels of zileuton in 5 of 6 normals and 11 of 12 asthmatics. The BALF concentrations of zileuton in normal subjects (n=5) were 0.134 ± 0.113 $\mu\text{g/ml}$ (range 0.056 - 0.299 $\mu\text{g/ml}$). In asthmatic subjects (n=11), zileuton BALF concentrations were 0.142 ± 0.079 $\mu\text{g/ml}$ (range 0.078 - 0.286 $\mu\text{g/ml}$).

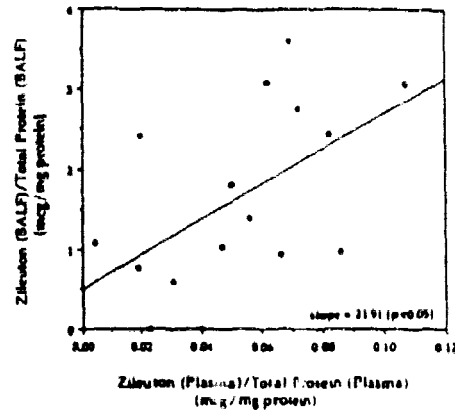
The sponsor explored the relationship between BALF and plasma concentrations of zileuton, as shown in the 2 figures below. A statistically significant relationship (slope 0.029, $p < 0.005$) was noted between the concentration of zileuton in the 2 fluids.

Fig. 49



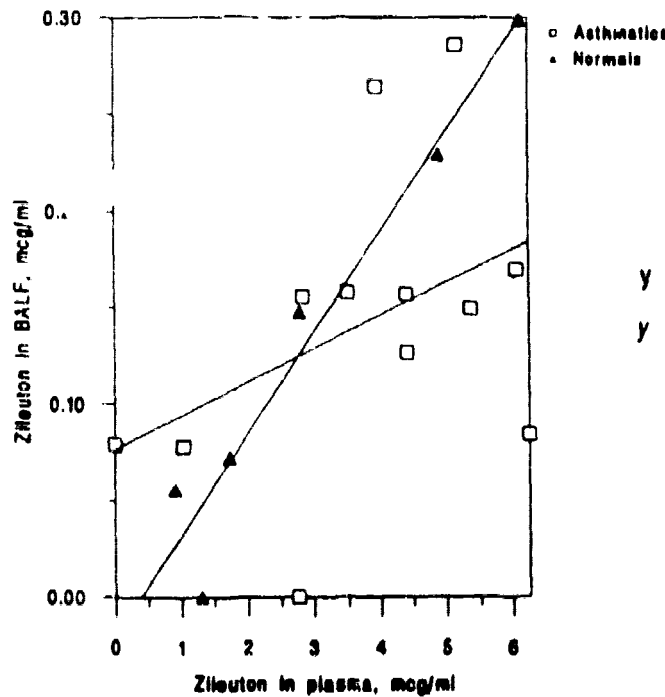
Relationship between Protein Normalized Zileuton Concentrations in Plasma versus BALF Samples (M92-734)

Fig. 50



It should be noted that upon separation of data from normals vs. patients, it was observed (by the reviewer) that the slopes are different in both groups, as shown in figure below.

Fig. 51



$$y = 0.07212 + 0.01710x \quad r^2 = 0.17174 \text{ (ASTHMATICS)}$$

$$y = -0.03442 + 0.05214x \quad r^2 = 0.94367 \text{ (NORMALS)}$$

Comments:

The time at which the blood samples were drawn has not been specified in this report. Hence, the relationship between BALF and plasma concentrations that has been presented may or may not be representing concentrations in both fluids obtained at same time. In spite of this lack of information in this study report, this data does show a relationship between the drug concentrations in plasma and BALF, indicative of availability at the site of action. However, levels are highly variable in patients. It should also be noted that the slopes of the regression line are different for asthmatics vs. healthy volunteers.

22 THE INFLUENCE OF MULTIPLE ORAL DOSES OF ABBOTT-64077 ON THE STEADY-STATE PHARMACOKINETICS OF THEOPHYLLINE

Study No.: M88-265 **Volume:** 1.89 **Pages:** 1 - 64

Investigator and Location:

Objective: To determine the effects of multiple doses of zileuton on the pharmacokinetics of theophylline.

Formulations: **Zileuton:** Capsules (Bulk Lot No. 14-999-AR), containing 200 mg ABBOTT 64077 (Bulk NPRO 4158). **Placebo for zileuton:** Capsules (Bulk No. 27-222-AR, Bulk NPRO 4503R). **Theophylline:** Tablets (Slo-Phyllin[®], Rorer, Bulk Lot No. 81114, List No. L0352), containing 200mg anhydrous theophylline.

Study Design: Randomized, double-blind crossover study with 16 subjects and a 5 day washout period. During each study period, all subjects received theophylline orally as one 200 mg tablet every 6 hours for 5 days. Subjects in treatment group A received zileuton orally as four 200 mg capsules every 12 hours for days 1-5 during period 1 and placebo during period 2. The subjects in treatment group B received the alternate treatment sequence. Breakfast, lunch and dinner were served 2, 5, and 10.5 hours after administration of the morning dose on days 1 through 6 in each period. The consumption of xanthine containing foods and beverages was not permitted from at least three days prior to the start of the study until the final blood collection in each period. Heparinized blood was collected before the 8 a.m. dose on days 1, 3, 4 and 5 as well as 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36 and 48 hours after the 8 a.m. dose on day 5. All samples were analyzed for theophylline and zileuton.

Data Analysis: C_{max} , T_{max} , C_{min} , AUC, $t_{1/2}$ (theophylline), Cl/F , VD_p/f were calculated.

Results: One of eight in treatment group A, and 2 of 8 subjects in treatment group B dropped out of the study. Theophylline results, in Table 67, below. Zileuton results in Table 68, below.

Comments: Theophylline levels in the placebo group (mean trough 7.06 $\mu\text{g/mL}$) are below the therapeutic level of 1 - 20 $\mu\text{g/mL}$ in 12 of 13 cases. Zileuton dosing (800 mg every 12 hours) is different than the proposed dosing regimen (400 - 600 mg every 6 hours). Resulting zileuton plasma levels (mean C_{max} 3.82 $\mu\text{g/mL}$) are also lower than those seen in trials using the proposed dose (M93-034 mean C_{max} 6.00 $\mu\text{g/mL}$). Complete assay validation reports (to include standard curve data, sample chromatograms and individual precision and accuracy data) were not submitted with the study report.

Conclusions: This study has detected a significant decrease (approximately 50%) in the clearance of theophylline when given concomitantly with zileuton. Hence, caution is warranted in the use of zileuton in patients receiving theophylline therapy. In such patients, the theophylline dose should be reduced by approximately 50%, and therapeutic drug monitoring is recommended. No conclusions may be drawn concerning the effect of theophylline on zileuton pharmacokinetics.

Table 68 Pharmacokinetic Parameters of Zileuton (%CV)

	C_{max} ($\mu\text{g/mL}$)	T_{max} (hours)	$C_{min(0)}$ ($\mu\text{g/mL}$)	$C_{min(12)}$ ($\mu\text{g/mL}$)	$AUC_{(0-12)}$ ($\mu\text{g hr/mL}$)	Cl/F (mL/min)
Group A (Period 1)	3.77 (24)	1.3 (38)	0.33 (37)	0.33 (62)	16.93 (28)	855 (35)
Group B (Period 2)	3.87 (28)	1.8 (66)	0.64 (54)	0.31 (64)	21.03 (26)	674 (28)

Table 69 Pharmacokinetic Parameters of Theophylline (%CV) and % Change with Zileuton

	C_{max} ($\mu\text{g/mL}$)	T_{max} (hours)	C_{min} ($\mu\text{g/mL}$)	$AUC_{(0-6)}$ ($\mu\text{g hr/mL}$)	$t_{1/2}$ (hours)	Cl/f (mL/min)	Cl/f (mL/min/kg)	$Vd\beta/f$ (L)	$Vd\beta/f$ (L/kg)
Without Zileuton	12.14 (23)	0.9 (62)	7.06 (29.6)	56.59 (26)	6.74 (19)	62.4 (25)	0.81 (26)	35.1 (14)	0.45 (12)
With Zileuton	20.99 (16)	1.4 (38)	15.92 (23.2)	108.93 (21)	8.36 (21)	31.8 (21)	0.42 (21)	22.3 (15)	0.29 (12)

Change	+73%	+56%	+125%	+92%	+24%	-51%	-64%		

23

ASSESSMENT OF THE PHARMACOKINETIC INTERACTION BETWEEN ZILEUTON (ABBOTT-64077) AND TERFENADINE

Study No.: M94-166 **Volumes:** T3092-T3094
Investigator and Location:

Objective: To determine the effects of zileuton on the pharmacokinetics of terfenadine.

Formulations: **Zileuton:** Tablets (NPRO 5634N, Lot No. 86-149-S2), containing 600 mg zileuton. **Zileuton Placebo:** Tablets (NPRO 5662N, Lot No. 86-150-S2). **Terfenadine:** Tablets (Seldane[®], Marion Merrell Dow, Lot No. P50574, NDC 0068-0723-61, Expiration 04/96), containing 60 mg terfenadine.

Study Design: This randomized, placebo-controlled, two-period, cross-over study enrolled 16 healthy (male, surgically sterile or postmenopausal female) volunteers. During each study period, all 16 subjects received 60 mg of terfenadine every 12 hours on days 1 through 7, and either 600 mg zileuton (Sequence Group A) or placebo (Sequence Group B) every 6 hours on days 1 through 10. A minimum of a fourteen day washout period separated the last dose of study drug in period 1 and the first dose of study drug in period 2. All meals served during the confinement period were standardized with respect to content and volume. Alcohol, tobacco, xanthine derivatives and grapefruit were all prohibited during the course of the study. Blood samples for the determination of terfenadine and its acid metabolite concentrations were obtained immediately prior to the 8:00 am dose (0 hour) on study days 1 through 7 and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after the 8:00 am dose on day 7. Additionally, on days 5, 6 and 7, trough blood samples were collected prior to dosing for zileuton concentration determinations.

*Results given for periods 1 and 2, respectively.

NDA 020471

FIRM: ABBOTT LABS

5 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON



Data Analysis: (Terfenadine and carboxyterfenadine) T_{max} , C_{max} , C_{min} , AUC_{0-12} , C_i/F, terminal phase $t_{1/2}$, metabolite/parent ratios for T_{max} , C_{max} , C_{min} were also calculated. To assess achievement of zileuton steady-state conditions, zileuton and N-dehydrozileuton C_{min} were assayed. To evaluate the pharmacodynamic interaction, maximum observed and average QTc intervals were obtained. Lastly, plasma protein binding of terfenadine was determined when given with zileuton or placebo.

Results: Fifteen of the original sixteen volunteers enrolled completed the study. Results in Tables 71 and 72 below, for terfenadine and carboxyterfenadine, respectively. The mean trough levels of zileuton and N-dehydrozileuton when zileuton was given with terfenadine are listed in Table 73 below. While a significant pharmacokinetic interaction was observed, no significant QTc changes were detected. In an effort to establish a pharmacokinetic/pharmacodynamic link, this reviewer compared individual changes in C_{max} to individual changes in QTc intervals (see figure 5). Pharmacokinetically, change in C_{max} was defined as the difference in C_{max} between the placebo and zileuton arm on study day 7. The pharmacodynamic effect was measured in two ways: (1) difference in QTc-max in the zileuton vs placebo arm on study day 7; (2) difference in QTc-max between baseline (day -1) and post-zileuton treatment (day 7). While a minor trend may be present linking increases in C_{max} to increases in QTc-max, QTc changes appear benign, and relatively random. Zileuton co-administration with terfenadine resulted in a very small decrease in the mean percent bound of terfenadine to plasma protein compared to placebo co-administration with terfenadine ($p=0.0624$).

Table 11 Pharmacokinetic Parameters of Terfenadine (%CV) and % Change with Zileuton

	C_{max} (pg/mL)	T_{max} (hours)	C_{min} (pg/mL)	AUC_{0-12} pg hr/mL	$t_{1/2}$ (hours)	Cl/f (L/hr)
Without Zileuton	3397.9 (49)	2.6 (72)	1518.0 (70)	27271 (59)	21.4 (26)	3055 (59)
With Zileuton	4588.7 (53.5)	2.2 (61.8)	1934.1 (75.9)	36971 (63.6)	22.1 (27.6)	2377 (62)
Change	+35%	-15%	+27%	+36%	+3.3%	-22%

Table 12 Pharmacokinetic Parameters of Carboxyterfenadine (%CV) and % Change with Zileuton

	C_{max} (ng/mL)	T_{max} (hours)	C_{min} (ng/mL)	AUC_{0-12} ng hr/mL	$t_{1/2}$ (hours)	C_{min} M/P ^a	AUC M/P
Without Zileuton	236.2 (36)	2.3 (31)	84.6 (38)	1537 (27)	13.1 (30)	95.7 (76)	81.7 (74)
With Zileuton	274.7 (30)	2.5 (26)	90.1 (32)	1737 (24)	16.6 (54)	82.9 (70)	71.2 (71)
Change	+16%	+8.7%	+6.5%	+13%	+27%	-13%	-13%

^aM/P = Metabolite to Parent ratio

Table 7B Mean Zileuton and N-Dehydrozileuton Trough Plasma Concentrations (%CV)

Zileuton ($\mu\text{g/mL}$)			N-Dehydrozileuton ($\mu\text{g/mL}$)		
	Time (days)			Time (days)	
5	6	7	5	6	7
2.540	2.148	2.318	0.726	0.856	0.724
(45)	(40)	(42)	(56)	(63)	(66)

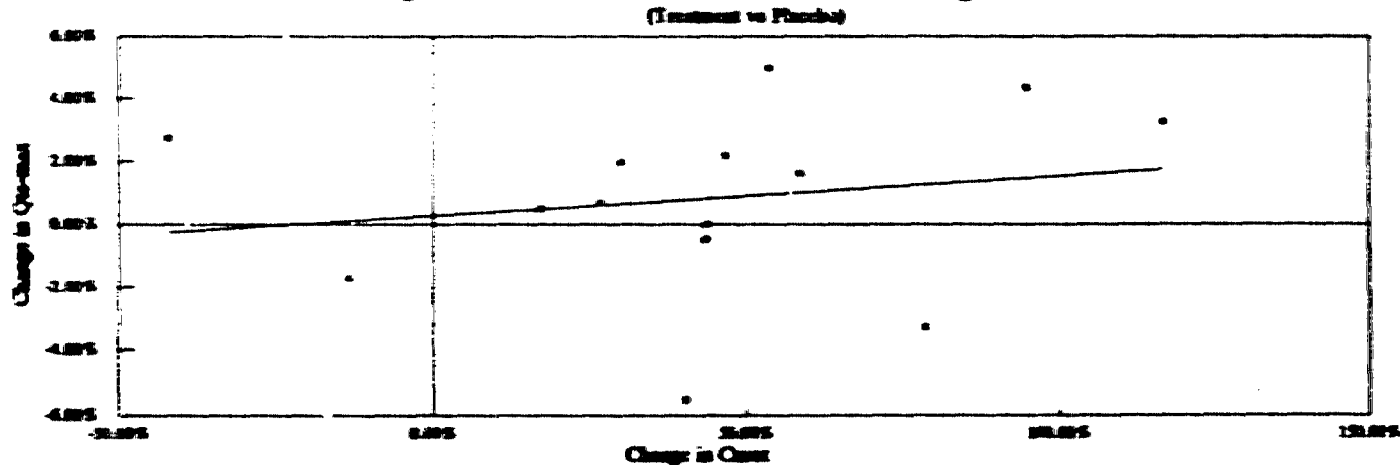
Conclusion: An increase in C_{max} and AUC for terfenadine (+35% and +36%, respectively) was detected when co-administered with zileuton vs placebo. However, no significant QTc changes were observed. Lastly, the plasma protein binding study showed no significant effect of zileuton on the binding of terfenadine.

Subject	Comparison of Changes in QTc-max vs C _{max}				Comparison of Changes in QTc-max vs C _{max}							
	Day 1	Day 7	q change	R	Day 1	Day 7	q change	R				
1	1783	2811	48.44%	0.86%	411	420	2.19%	0.86%				
2	1182	1873	58.48%	1.01%	435	442	1.61%	1.01%				
3	1351	1894	40.19%	0.78%	434	410	-5.53%	0.78%				
5	4793	6215	29.67%	0.65%	409	417	1.96%	0.65%				
6	3989	4664	16.92%	0.49%	414	416	0.48%	0.49%				
7	3002	5844	94.87%	1.46%	413	431	4.36%	1.46%				
9	6711	9627	43.45%	0.82%	398	398	0.00%	0.82%				
10	3942	7038	78.54%	1.26%	425	411	-3.29%	1.26%				
11	1310	1658	26.41%	0.61%	426	429	0.70%	0.61%				
12	3370	2919	-13.38%	0.11%	410	403	-1.71%	0.11%				
13	2329	3340	43.41%	0.82%	407	405	-0.49%	0.82%				
14	5487	8387	51.41%	0.95%	420	441	5.00%	0.95%				
15	2288	4953	116.48%	1.74%	398	411	3.27%	1.74%				
16	4525	2620	-42.15%	-0.25%	398	409	2.76%	-0.25%				
Mean			42.32%				0.81%					1.04%

Regression Output:
 Constant 0.002778
 Std Err of Y Est 0.029772
 R Squared 0.030379
 No. of Observations 14
 Degree of Freedom 12

X Coefficient(s) 0.01271
 Std Err of Coef. 0.020431

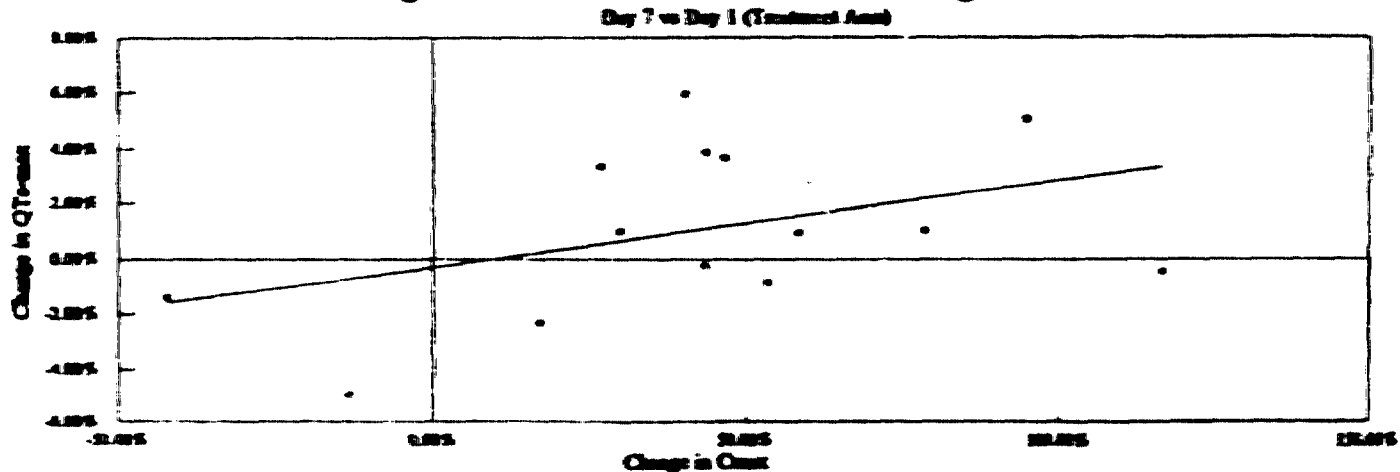
Change in QTc-max vs Change in C_{max}



Regression Output:
 Constant -0.00298
 Std Err of Y Est 0.029107
 R Squared 0.172554
 No. of Observations 14
 Degree of Freedom 12

X Coefficient(s) 0.031582
 Std Err of Coef. 0.019965

Change in QTc-max vs Change in C_{max}



ASSESSMENT OF THE PHARMACOKINETIC INTERACTION BETWEEN ZILEUTON (ABBOTT-64077) AND DIGOXIN IN HUMANS

Study No.: M92-821 **Volume:** 1.92 **Pages:** 1 - 155
Investigator and Location:

Objective: To determine the effects of multiple doses of zileuton on the pharmacokinetics of digoxin.

Formulations: Zileuton: 600 mg tablets (Bulk NPRO 5634R, Lot 62-028-AR), Placebo for zileuton: (Bulk NPRO 5639R, Lot 60-783-AR), Digoxin: 0.25 mg (Lot No. 1Z2102).

Study Design: Twelve healthy male, non-smoking subjects were enrolled in this randomized, double-blind two-period, crossover, multiple-dose Phase I study. During each study period, subjects received either 600mg Zileuton (Treatment A) or placebo (Treatment B) every 6 hours beginning at 8:00 am on Study Day 1 through 2:00 am on Study Day 14. Additionally, subjects received digoxin 0.25 mg during both study periods on Study Days 1 through 11 along with the 8:00 am dose of zileuton/placebo. Subjects abstained from all food and beverages except for the scheduled, standardized meals provided in the study and minimal water to quench thirst from study day -1 until after the final blood collection in each study period. Alcohol, tobacco and xanthine derivatives were prohibited during the course of the study. On study day 11, blood samples were collected for plasma digoxin determinations prior to the 8:00 am dose (0 hour) and at 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, 48, 60 and 72 hours after the digoxin dose in each period.

Data Analysis: Digoxin pharmacokinetic parameters were estimated from plasma concentration-time profiles after the last digoxin dosing on day 11. Parameters estimated included: T_{max} , C_{max} , AUC_{0-24} , $t_{1/2,D}$, CL/F , V_d/F .

Results: Two of the original 12 participants (subjects 5 and 7), were incorrectly dosed and thus were not included in the analysis. Pharmacokinetic results for digoxin are presented in Table 74 below.

Table 74. Mean Digoxin Pharmacokinetic Parameters with Zileuton and Placebo (%CV)

	C_{max} (ng/mL)	T_{max} (hours)	$AUC_{(0-24)}$ ng hr/mL	$t_{1/2,p}$ (hours)	CL/F (L/hr)	V_p/F (Liters)
Without Zileuton	1.356 (17.7)	1.47 (35.4)	14.69 (21.7)	34.7 (14.6)	17.7 (21.4)	878 (21.5)
With Zileuton	1.348 (15.8)	0.89 (30.7)	14.09 (20.8)	37.8 (16.4)	18.5 (22.5)	1010 (28.9)

Change	-1%	-39%	-4%	+9%	+5%	+15%
CI_{95}^c	94-105	44-77	92-100	100-119	100-108	103-128

Comments: Unfortunately, since zileuton levels were not obtained in this study, it can only be assumed that zileuton steady-state levels have been obtained, further, the effect of digoxin on zileuton can not be ascertained. Also, the blood collection schedule did not allow for assessment of digoxin steady-state. Mean trough digoxin concentrations (0.454 ng/mL) may be sub-therapeutic (generally accepted C_{min} s fall between 1-2 ng/mL). These low levels might be explained by the subjects' relatively high creatinine clearance (mean 130±19 mL/min).

Conclusions: No clinically significant digoxin pharmacokinetic changes were detected when given concomitantly with zileuton.

^cBased on two one-sided confidence intervals at $\alpha = 0.05$.

25 ASSESSMENT OF THE PHARMACOKINETIC INTERACTION BETWEEN ZILEUTON (ABBOTT-64077) AND PREDNISONE

Study No.: M91-617 **Volume:** 1.90 **Pages:** 1 - 127
Investigator and Location:

Objective: To determine the effects of concomitant administration of zileuton at steady state on the pharmacokinetics of prednisolone after oral administration of a single 40 mg dose of prednisone.

Formulations: Zileuton: Capsules (Bulk Lot No. 40-393-AR, Bulk NPRO 4924R) containing 200 mg zileuton. Placebo: Capsules (Bulk Lot No. 39-292-AR, Bulk NPRO 4525R).
Prednisone: Tablets, locally acquired.

Study Design: Sixteen healthy, non-smoking adult male subjects were randomly assigned to two treatment groups. Subjects in Treatment Group A received zileuton orally as three 200 mg capsules every 6 hours from day 1 until the 6 a.m. dose on day 7, while the subjects in Treatment Group B received placebo. On day 6, at 6 a.m., prednisone was orally co-administered as two 20 mg tablets to both treatment groups. A one-week washout period separated the last dose of Period 1 and the first dose of Period 2. Each dose of zileuton and prednisone was administered with 180 mL of water. Subjects abstained from all food and beverages except for the scheduled meals provided in the study and minimal water to quench thirst from 8:00 p.m. (Day -1) until after the 30-hour blood collection on study day 7. A blood sample was collected for baseline zileuton and prednisolone determination prior to dosing on study day 1. Blood samples were collected for zileuton assay on Study Days 5 and 6 immediately prior to the 6 a.m. dosing (0 hour) and at 0.5, 1, 2, 3, 4 and 6 hours thereafter. Prednisolone determinations were performed on samples collected prior to prednisone administration (0 hour) and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 and 30 hours after prednisone dosing in both periods.

Data Analysis: C_{max} , T_{max} , AUC_{0-6} (zileuton), C_{max} , T_{max} , AUC_{0-1} , AUC_{0-6} , $t_{1/2}$ (prednisolone).

Results: Three of the 16 subjects were not included in the analysis due to analytical error. Results for zileuton and prednisolone are in Tables 76 and 77, respectively. These data suggest that the interaction of oral prednisone and zileuton pharmacokinetics is either non-existent or slight.

Table 76. Mean Prednisolone Pharmacokinetic Parameters Zileuton and Placebo (%CV)

	C_{max} ng/mL	T_{max} (hours)	AUC_{0-1} ng hr/mL	AUC_{0-6} ng hr/mL	$t_{1/2}$ (hours)
Without Zileuton	400 (11.4)	1.5 (59.9)	2147 (19.4)	2313 (20.7)	2.8 (11.9)
With Zileuton	424 (14.5)	1.2 (48.5)	2190 (16.2)	2361 (16.7)	2.9 (13.0)

Change	+6%	-20%	+2%	+2%	+4%

Table 77. Mean Zileuton Pharmacokinetic Parameters on Day 5 and 6 (%CV)

	C_{max} μ g/mL	T_{max} (hours)	AUC_{0-6} μ g hr/mL
Day 5 (pre-prednisone)	6.28 (29.2)	2.3 (33.7)	22.92 (30.7)
Day 6 (post-prednisone)	5.89 (23.8)	1.7 (42.4)	19.86 (25.0)

Change	-6%	-26%	-13%

Conclusion: According to the results of this study, prednisone and zileuton can be co-administered with little risk of a pharmacokinetic interaction.

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ASSESSMENT OF THE PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTIONS BETWEEN ZILEUTON (ABBOTT-64077) AND WARFARIN

Study No.: M90-541 **Volume:** 1.90 **Pages:** 128 - 434
Investigator and Location

Objective: To determine the potential effects of concomitant administration of zileuton and warfarin on the anticoagulant effect of warfarin, on the steady state pharmacokinetics and safety profile of warfarin.

Formulations: **Zileuton:** Capsules (Bulk Lot No. 40-393-AR, Bulk NPRO 4924R) containing 200 mg zileuton. **Placebo:** Capsules (Bulk Lot No. 27-222-AR, Bulk NPRO 4503R). **Warfarin:** Tablets (Coumadin, DuPont Pharmaceuticals) containing 1, 2, 5 and 10 mg crystalline warfarin sodium of DuPont lots CC080C, CL242A, DC051A and EBH105B, respectively.

Study Design: Thirty normal, healthy males were enrolled in this study. Subjects were titrated (days 1 - 9) then established on a fixed dosage (days 10 - 14) of warfarin to maintain a prothrombin time (PT) of 14 - 18 seconds. Subjects were randomized to receive either Treatment I (fixed dose warfarin and zileuton 600mg every 6 hours) or Treatment II (fixed dose warfarin and placebo every 6 hours) on days 15 through 20. If a subjects PT reached 23 seconds at any time, administration of all drugs was stopped. All meals were standardized with regard to content and were consumed within 30 minutes. Blood was collected on study days 14 and 20 at 0 (pre-dosing) 1.5, 3, 4.5, 6, 9, 12 and 24 hours after warfarin dosing. Additionally, samples were obtained on day 20 at 36, 48, 60 and 72 hours after warfarin dosing. Plasma obtained from these samples were then assayed for R- and S-warfarin. All subjects had PT determinations performed on Day 0, prior to and 12 hours after each warfarin dose on days 1 - 20.

Data Analysis: C_{max} , T_{max} , C_{min} , AUC_{0-24} , CL/F , $t_{1/2, el}$ (R- and S-warfarin), PT.

Results: Five subjects were excluded from the study because their PT did not stabilize. A sixth volunteer was withdrawn due to elevated liver function test values. The mean fixed warfarin dose for subjects randomized to the zileuton group was 6.7 ± 4.2 compared to 6.5 ± 2.5 mg in patients assigned to the placebo group. Analysis of covariance (ANCOVA), utilizing day 14 data as the covariate, detected no difference between treatment groups. However, within group ANOVA observed significant R-warfarin differences (C_{min} , AUC , CL/F) when subjects were given zileuton vs placebo (see Table 77). Significant PT increases were also observed in the zileuton vs placebo treatment groups. However, stratification of patients by baseline PT showed a trend towards more significant changes from baseline correlated with higher baseline PT (see Tables 80 and 81). Since subjects with PT greater than 20 seconds were discontinued, the pharmacodynamic effect observed may be underestimated by this study.

Table 79. Within and Between Treatment Group Comparisons of Warfarin Pharmacokinetic Parameters With and Without Concomitant Zileuton Dosing

Parameters	Zileuton/Warfarin Group (n=9)		Placebo/Warfarin Group (n=11)		ANCOVA Test I vs. II [#]
	Alone	+ Zileuton	Alone	+ Placebo	
R-Warfarin					
T_{max} (hr)	4.2 ± 3.1	5.2 ± 3.9	2.0 ± 0.8	2.3 ± 2.3	0.162
C_{max} (ng/mL)	1059 ± 598	1184 ± 691	1374 ± 529	1394 ± 455	0.758
C_{min} (ng/mL)	650 ± 357	807 ± 483*	722 ± 214	813 ± 356	0.391
AUC_{0-24} (ng·hr/mL)	18994 ± 10359	23109 ± 13812*	22244 ± 6773	24207 ± 8724	0.206
$t_{1/2}$ (hr)		71 ± 17		58 ± 14	0.067 [‡]
CL/F (L/hr)	0.125 ± 0.039	0.106 ± 0.036*	0.145 ± 0.037	0.137 ± 0.042	0.191
S-Warfarin					
T_{max} (hr)	3.3 ± 2.6	2.3 ± 1.3	2.0 ± 0.8	2.2 ± 2.3	0.918
C_{max} (ng/mL)	704 ± 394	642 ± 362	965 ± 372	857 ± 248	0.647
C_{min} (ng/mL)	348 ± 159	340 ± 167	402 ± 116	365 ± 117	0.310
AUC_{0-24} (ng·hr/mL)	11215 ± 5420	11008 ± 5516	13604 ± 3915	12555 ± 3514*	0.136
$t_{1/2}$ (hr)		47 ± 17		35 ± 8	0.059 [‡]
CL/F (L/hr)	0.206 ± 0.075	0.213 ± 0.078	0.235 ± 0.059	0.255 ± 0.065*	0.200

* Significantly different ($p < 0.05$, ANOVA) than the same parameter after administration of warfarin alone for the same treatment group.

[#] Day 20 p-value for comparison of warfarin/zileuton group (I) vs. warfarin/placebo group (II).

[‡] ANOVA instead of ANCOVA since there was no baseline (Day 14) value.

Table 80. Prothrombin Time of Subjects Both With and Without Concomitant Zileuton Dosing

	Zileuton/Warfarin Group			Placebo/Warfarin Group		
	Baseline [*]	Double-Blind ^{**}	Change from Baseline	Baseline [*]	Double-Blind ^{**}	Change from Baseline
Morning PT	17.5 ± 1.7	19.8 ± 3.5	2.3 ± 1.8	18.1 ± 1.4	18.8 ± 1.9	0.7 ± 1.0
Evening PT	17.1 ± 1.9	19.1 ± 3.8	2.0 ± 2.0	17.3 ± 1.5	17.5 ± 1.8	0.3 ± 1.0

^{*} Mean for individuals' average PT on Days 13 and 14.

^{**} Mean for individuals' average PT on Days 19 and 20 for subjects who completed the study and maximum PT for 4 subjects who dropped out.

Table 81. Prothrombin Time of Subjects Both With and Without Concomitant Zileuton Dosing (Stratified by Baseline Prothrombin Time)

Baseline PT (sec)	Estimated Mean for PT Average (sec) on Fifth and Sixth Days of Addition to Warfarin		
	Placebo	Zileuton	ANCOVA p-Value [#]
	Morning PT		
16	16.2	16.8	0.3115
18	18.7	20.8	0.0001
20	21.2	24.8	0.0001
	Evening PT		
16	16.2	17.0	0.1439
18	18.5	20.9	0.0001
20	20.5	24.8	0.0001

[#] For comparison of the two treatment groups

Conclusions: Although the ANCOVA results did not detect significant differences in the pharmacokinetics of warfarin, within group analysis detected a significant effect of zileuton on the pharmacokinetics of R-warfarin. This pharmacokinetic interaction coupled to the increase in subject PT suggests that when used with zileuton, warfarin should be carefully monitored (PT) and adjusted accordingly.

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THE PHARMACOKINETIC INTERACTIONS BETWEEN ZILEUTON (ABBOTT-64077) AND PROPRANOLOL IN HUMANS

Study No.: M92-789 Volume: 1.91 Pages: 1 - 97
Investigator and Location:

Objective: To assess the potential effects of concomitant administration of multiple doses of zileuton on propranolol pharmacokinetics.

Formulations: Zileuton tablets (Bulk Lot No. 60-778-AR, Bulk NPRO 5634N) containing 600 mg. Propranolol tablets (Inderal 80 Tablets, With-Ayerst Laboratories, Philadelphia, PA).

Study Design: 16 healthy, male subjects were enrolled in this open-label, multiple-dose study. Subjects received a single 80 mg dose of propranolol at 8:00 am on days 1 and 7. On days 3 - 7, subjects also received 600 mg zileuton every 6 hours (20 consecutive doses). All subjects fasted for the 12 hours prior to dosing and meals were standardized throughout the course of the study. Serial blood samples were collected prior to (zero hour) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20 and 24 hours after the 8:00 am propranolol dose on study days 1 and 7. All plasma samples were assayed for propranolol and its major metabolite 4-OH propranolol.

Data Analysis: T_{max} , C_{max} , $AUC(t)$, $AUC_{0-\infty}$, β , $t_{1/2, \alpha}$, F , CL/F , V/F .

Results: Day 1 (pre-zileuton) were compared to Day 7 (post-zileuton) data. Results shown in Table 83

Table 83. Mean (%CV) Pharmacokinetic Parameters of Propranolol and 4-OH Propranolol After Single Doses of Propranolol Alone and with Concomitant Zileuton

Parameter	Propranolol			4-OH-Propranolol			CL ₉₀
	Alone	Zileuton	Change	Alone	Zileuton	Change	
C _{max} (ng/mL)	93.2 (44)	142 (45)	+52%	40.2 (68)	44.3 (75)	+10%	60 - 120
T _{max} (hr)	1.4 (50)	1.9 (53)	+36%	0.9 (22)	1.3 (46)	+44%	
AUC _{0-∞} (ng hr/mL)	517 (60)	1056 (72)	+104%	104 (63)	139 (64)	+34%	82 - 156
t _{1/2} (hr)	3.6 (16)	4.5 (28)	+25%	2.4 (28)	3.4 (23)	+42%	
F (%)	23.1 (42)	35.8 (43)	+55%				
CL/F (L/hr)	204 (54)	118 (63)	-42%				
V/F (L)	1013 (52)	707 (58)	-30%				

Comments: No assessment of zileuton pharmacokinetics can be made with this one-armed study.

Conclusion: The results of this study clearly suggest that zileuton decreases the clearance of propranolol and its major metabolite, 4-OH-propranolol and that zileuton may significantly inhibit the clearance of drugs partially utilizing cytochrome P450IID6 for metabolism.

THE PHARMACOKINETIC INTERACTIONS BETWEEN ZILEUTON (ABBOTT-64077) AND PHENYTOIN IN HUMANS

Study No.: M92-820 **Volume:** 1.91 **Pages:** 98 - 401
Investigator and Location:

Objective: To assess the potential effects of concomitant administration of multiple doses of zileuton on phenytoin pharmacokinetics.

Formulations: Zileuton tablets (Bulk Lot No. 62-028-AR, Bulk NPRO 5634N) containing 600 mg. Placebo tablets (Bulk Lot No. 6-783-AR, Bulk NPRO 5639N). Phenytoin Sodium capsules (Dilantin Kapseals[®], Lot No. 01352FA) containing 100 mg.

Study Design: 21 healthy, non-smoking, adult male volunteers were enrolled in this two-period, multiple-dose crossover study. Subjects received either 600 mg zileuton (Treatment Group I) or placebo (Treatment Group II), every 6 hours on study days 1 through 8. On Study Day 5 (in both treatment groups) received a single 300 mg dose of phenytoin along with their 7:00 am zileuton dose. Subjects maintained an overnight fast prior to receiving their first doses of zileuton. After commencement of the study and until after the final blood collection, all meals were standardized with respect to content and schedule. On Study Day 5 in each period, blood samples were collected prior to (zero hour) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60 and 72 hours after the 7:00 am phenytoin dose. All samples were assayed for phenytoin.

Data Analysis: T_{max} , C_{max} , AUC_{0-12} , $AUC_{0-\infty}$, β , $t_{1/2, el}$

Results: 18 of 20 original participants completed the study. Additionally, the results of 3 participants were not included in the analysis due to insufficient sample volume for analysis. Results below, in Table 84.

Comments: Due to phenytoin's non-linear pharmacokinetics, a multiple-dose study design would have provided possibly more meaningful results. Due to study design, the effect of phenytoin on zileuton pharmacokinetics can not be established.

Table 84. Mean (%CV) Pharmacokinetic Parameters of Phenytoin After A Single Dose of Phenytoin Alone and with Concomitant Zileuton

Parameter	Alone	Zileuton	Change	CI ₉₀
C _{max} (μg/mL)	4.32	4.30	-0.4%	89 - 113
T _{max} (hr)	7.13	6.10	-14%	
AUC _{0-t} (mg hr/L)	140.7	151.3	+8%	97 - 115
AUC _{0-∞} (mg hr/L)	164.1	183.5	+12%	97 - 117
t _{1/2} (hr)	16.3	17.51	+7%	
β (hr ⁻¹)	0.051	0.048	-6%	

Conclusion: Zileuton dosed to steady-state did not significantly effect the single-dose pharmacokinetics of phenytoin.

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EFFICACY OF ORAL CONTRACEPTIVES IN VOLUNTEERS RECEIVING CONCOMITANT ZILEUTON

Study No.: M90-543 **Volume:** 1.93 **Pages:** 1 - 78
Investigator and Location:

Objective: Primary pharmacokinetic objectives of this study were to examine the effects of oral zileuton on ethinylestradiol, progestogen and progesterone. Secondly, the study examined the effect on plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) in women receiving oral contraceptives.

Formulations: Zileuton capsules (Lot No. 37-010VH) containing 200 mg. Placebo capsules (Lot No. 41-008VH). **Fixed-Combination Oral Contraceptive** (estrogen content of 30 µg ethinylestradiol and one of three progestogens: levonorgestrel, desogestrel or gestodene) obtained through previous prescription.

Study Design: This placebo-controlled, multiple-dose, parallel group, double-blind study enrolled 18 healthy, female volunteers (all volunteers had been regular users of fixed-combination oral contraceptives having an estrogen content of 30 µg ethinylestradiol). Subjects received either zileuton (600mg every 6 hours) or placebo concomitant with oral contraceptives. The primary endpoints examined in this study were ethinylestradiol and progestogen concentrations during the follicular and luteal phases of the menstrual cycle. Plasma progesterone concentration during the luteal phase was utilized as the marker for ovulation suppression. All subjects were monitored for three complete cycles. Day 1 was defined as the first day of oral contraceptive pill administration. On the first day of the first cycle, subjects received three capsules of placebo four times a day in addition to their oral contraceptives. On day 1 of cycle 2, subjects began the double-blind portion of the study which continued for the duration of cycle 2 and 3 (last dose day 21, cycle 3). One treatment group received 3 zileuton capsules every 6 hours in addition to their usual oral contraceptive regimen, while subjects in the alternative treatment group received placebo. Subjects were permitted to maintain their own diets during the course of the study and alcohol was limited to 2 oz/day. Venous blood was collected for determination of: plasma ethinylestradiol, progestogen, FSH, LH and progesterone on days 5, 6, 7, 19, 20 and 21 at 0800 during cycles 1 and 3. Plasma zileuton concentrations were measured during the second and third cycles (trough, days 5 and 21) as a measure of compliance. Additionally, on day 21 of cycle 3, plasma samples for zileuton pharmacokinetic determination were obtained at 0.75, 1.5, 3, 4.5, 6, 8, 12 and 24 hours post dosing. Subjects were also provided with diaries to record daily menstrual-related questions. All concomitant medications (with the exception of occasional analgesics/NSAID) were discontinued 4 weeks prior to dosing (menstrual cycle 1, day 1).

Table 85

Endocrine Plasma Concentrations. For each of plasma ethinylestradiol, progesterone, FSH, LH and progesterone, the average of the individual's concentrations for days 5-7 (Cycle 1 and 3) was calculated, as was the average for days 19-21. A paired t-test was performed on the change from Cycle 1 (oral contraceptive with placebo) to Cycle 3 (oral contraceptive with zileuton). The same procedure was also performed on the data of subjects who received placebo for zileuton for all three cycles. **Zileuton Plasma Concentrations.** Plasma zileuton concentrations were measured during the second and third cycles (trough, days 5 and 21) as a measure of compliance. Additionally, on day 21 of cycle 3, serial zileuton plasma samples were obtained to determine: C_{max} , T_{max} and AUC.

Results: Of the original 20 subjects, 18 volunteers completed all phases of the study. Results for endocrine plasma results listed, below, in Table 85. Individual and mean zileuton data in Table 86.

Comments: Subjects in this study received one of three different oral contraceptives containing one of three progestogens. Accordingly, the sponsor assayed each subject's specimen for either 3-keto Desogesterol, Gestodene or Levonorgesterol depending on which product the subject was stabilized on prior to the study. However, results were pooled and presented as progestogen concentration (ng/mL). Results might have been more meaningful if the individual hormones were specifically described.

Discussion: No significant changes in ethinylestradiol or progesterone metabolism were detected when given concomitantly with zileuton versus placebo. Pharmacodynamically, no significant changes were detected with respect to either FSH or LH. Significant progesterone increases were observed in the zileuton versus the placebo group. However, this is probably not clinically important since progesterone was still significantly suppressed (Cycle 3 luteal phase 1.61 versus normal levels of 19.1 to 95.4 nmol/L), thus the probability of ovulation would be very low. Examination of zileuton data suggest that study subjects were compliant.

Conclusion: the administration of zileuton (600mg every 6 hours) to women taking oral contraceptives does not cause a significant change in the pharmacokinetics or pharmacodynamics of oral contraceptives.

Table 85

Endocrine Plasma Hormone Levels

		Cycle 1			Cycle 3		
		Days 5, 6 and 7	Days 5, 6 and 7	Δ from Cycle 1	Days 19, 20 and 21	Days 19, 20 and 21	Δ from Cycle 1
Ethinylestradiol (pg/mL) S.D.	Zileuton	29.80	36.67	6.87	37.09	47.81	10.72
		17.99	16.41	20.76	19.26	23.73	18.15
	p-value			0.256			0.055
	Placebo	35.89	33.63	-2.27	50.69	49.53	-1.16
	S.D.	18.92	13.62	25.79	23.56	17.60	17.93
	p-value			0.854			0.892
		Cycle 1			Cycle 3		
		Days 5, 6 and 7	Days 5, 6 and 7	Δ from Cycle 1	Days 19, 20 and 21	Days 19, 20 and 21	Δ from Cycle 1
Progesterone (ng/mL)	Zileuton	2.87	3.05	0.18	4.02	4.16	0.14
		2.06	2.21	0.51	2.46	2.65	0.69
	p-value			0.22			0.479
	Placebo	2.64	2.98	0.34	4.03	4.35	0.32
	S.D.	1.24	1.23	0.71	1.95	2.08	1.81
	p-value			0.347			0.711
		Cycle 1			Cycle 3		
		Days 5, 6 and 7	Days 5, 6 and 7	Δ from Cycle 1	Days 19, 20 and 21	Days 19, 20 and 21	Δ from Cycle 1
FSH (U/L)	Zileuton	2.19	1.60	-0.60	0.45	0.35	-0.09
		0.74	0.76	0.64	0.22	0.12	0.25
	p-value			0.006			0.245
	Placebo	2.17	1.36	-0.81	0.73	0.79	0.06
	S.D.	1.33	1.20	1.10	0.60	0.78	0.62
	p-value			0.173			0.839

Table 85(Continued)

Endocrine Plasma Hormone Levels

		Cycle 1			Cycle 3		
		Days 5, 6 and 7	Days 5, 6 and 7	Δ from Cycle 1	Days 19, 20 and 21	Days 19, 20 and 21	Δ from Cycle 1
L/H (U/L)	Zileuton	2.97	2.75	-0.22	0.45	0.35	-0.09
	S.D.	1.52	1.74	0.97	0.22	0.12	0.25
	p-value			0.433			0.245
	Placebo	3.47	2.71	-0.76	0.73	0.79	0.06
	S.D.	2.17	2.65	1.88	0.60	0.78	0.62
	p-value			0.417			0.839
		Cycle 1			Cycle 3		
		Days 5, 6 and 7	Days 5, 6 and 7	Δ from Cycle 1	Days 19, 20 and 21	Days 19, 20 and 21	Δ from Cycle 1
Progesterone (nmol/L)	Zileuton	0.98	1.63	0.66	1.13	1.61	0.48
	S.D.	0.93	1.14	0.65	0.80	1.00	0.44
	p-value			0.005			0.005
	Placebo	0.68	0.53	-0.15	0.52	0.63	0.12
	S.D.	0.58	0.52	0.45	0.47	0.68	0.21
	p-value			0.491			0.285

Table 86

**Plasma Concentrations of Abbott-64077 in Female volunteers After
Concomitant Treatment with Zileuton (600 mg q.i.d.) and Oral Contraceptives**

Subject	Concentration in $\mu\text{g/mL}$												T_{max} hr	C_{max} $\mu\text{g/mL}$	AUC_{0-6} $\mu\text{g}\cdot\text{hr/mL}$	
	Day 5 Cyc 2	Day 21 Cyc 2	Day 5 Cyc 3	Hours After the Last Dose (Day 21, Cycle 3)												
	0	0	0	0	0.75	1.5	3	4.5	6	8	12	24				
Mean	0.916	0.587	1.058	0.576	2.211	2.506	2.700	2.083	1.097	0.426	0.088	N/A	2.71	3.25	12.69	
SD	1.131	0.632	1.521	0.472	1.345	1.073	0.637	0.916	0.456	0.186	0.045	N/A	1.39	0.85	2.80	
High	4.391	2.355	5.952	1.759	5.005	4.223	3.567	3.705	1.843	0.898	0.180	N/A	4.50	5.01	17.54	
Low	0.031	0.089	0.226	0.060	0.204	1.193	1.370	0.713	0.370	0.243	0.027	N/A	0.75	2.05	3.74	

30 THE INFLUENCE OF MULTIPLE ORAL DOSES OF ABBOTT-64077 ON THE STEADY STATE PHARMACOKINETICS OF SULFASALAZINE

Study No.: M88-218 Volume: 1.88 Page: 127-202
 Investigator and Location:

Objective: To assess the potential effects of concomitant administration of multiple doses of zileuton on the pharmacokinetics of sulfasalazine and its metabolites.

Formulations: Zileuton capsules (Lot No. 22-003VH) containing 200 mg. Placebo capsules. Sulfasalazine tablets (Salazopyrin[®] Pharmacia, LTD U.K.) Containing 500 mg.

Study Design: 14 healthy male volunteers were enrolled in this randomized, double-blind parallel group, placebo-controlled trial. Subjects in both study groups received 1 gram of sulfasalazine orally every 12 hours on days 1 through 7 and one dose on day 8. Volunteers randomized to Treatment Group I received 800 mg zileuton every 12 hours on days 4 through 7 and a single dose on day 8. Subjects in Treatment Group II received placebo according to the same dosing schedule. All subjects fasted 8 hours prior to drug administration and did not eat until 4 hours after the morning dose on days 3 through 8. Breakfast was served 1 hour after the am dose except on days 3 and 8. Heparinized blood was collected prior to (zero hour) and at 1, 2, 4, 8, 12 and 24 hours after the morning dose on days 3 and 8. Additionally, a 16 hour sample was obtained on day 8. All samples were then assayed for zileuton, sulfasalazine (SASP), sulfapyridine (SP) and N-4-acetylsulfapyridine (ASP).

Data Analysis: SASP, SP and ASP (days 3 and 8) C_{max} , T_{max} , C_{min} , $t_{1/2}$ (SASP only), AUC. Zileuton (day 8) T_{max} , C_{max} , C_{min} , AUC. The sponsor classified the genetically controlled acetylation rate as fast or slow as follows: if the acetylated SP AUC expressed as a percentage of the sum of the SP and ASP AUC (%ASP) is less than 40% the subject is a slow acetylator.

$$\%ASP = 100 \times AUC(ASP) / (AUC(SP) + AUC(ASP))$$

Since the subjects of treatment group I received sulfasalazine during study days 1-3, the pharmacokinetic parameters estimated for SASP, SP and ASP were used as baseline values to be compared to the respective parameters measured on day 8 (after zileuton). Analogous placebo data was included for comparison.

Results: All 14 subjects completed the study. Nine subjects were classified as slow acetylators (6 belonged to Treatment Group I and 3 to Treatment Group II; mean ASP 29%), while 5 as fast acetylators (2 belonged to Treatment Group I and 3 to Treatment Group II; mean ASP 53%). Zileuton pharmacokinetic parameters (Group I) are presented in Table 67, below. Individual and mean sulfasalazine parent and metabolite pharmacokinetic parameters are shown in Tables 68, 69 and 70. Changes in mean pharmacokinetic parameters are presented

in Table 9 |.

Table 87. Treatment Group I Mean Zileuton Pharmacokinetic Parameters (%CV)

C_{max} ($\mu\text{g/mL}$)	T_{max} (hour)	C_{min} ($\mu\text{g/mL}$)	AUC ($\mu\text{g hr/mL}$)
3.28 (22)	2.1 (3.9)	0.35 (38)	17.41 (23)

Comments: This study design is fundamentally flawed in: (1) its parallel group design; (2) the number of subjects-- relatively more fast acetylators were randomized to the placebo group (50% fast acetylators) vs the zileuton group (25% fast acetylators); (3) that zileuton was not dosed according to the to-be-marketed regimen (400-600 mg every 6 hours). A more conventional two-way crossover design with appropriate zileuton dosing and ANOVA could have provided more meaningful data. Further, the blood sampling design precludes the evaluation of the effect of sulfasalazine on zileuton. Lastly, no detailed assay validation data were submitted.

Conclusion: Sulfasalazine parent and metabolites appear to trend upwards when sulfasalazine is given concomitantly with zileuton vs baseline (Treatment Group I). However, since sizeable differences were also noted in the placebo arm (Treatment Group II), the results of this study are not conclusive. No evaluation of the interactive potential of sulfasalazine on zileuton can be made from this study.

3 Pages

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Table 91

Sulfasalazine (SASP) Pharmacokinetic Data

		C_{max}	T_{max}	C_{min}	AUC
Group I (Treatment)	SASP Baseline	18.78	7.0	6.16	143.09
	SASP + Zileuton	21.64	5.3	6.28	160.20
	% Change	+15%	-24%	+1.9%	+12%
Group II (Placebo)	SASP Baseline	25.43	7.3	8.58	178.23
	SASP + Placebo	24.22	4.0	4.37	143.62
	% Change	-4.8%	-45%	-49%	-19%

Sulfapyridine Pharmacokinetic Data

		C_{max}	T_{max}	C_{min}	AUC
Group I (Treatment)	SASP Baseline	15.12	7.6	10.57	161.38
	SASP + Zileuton	21.24	6.0	16.56	232.55
	% Change	+40%	-21%	+57%	+71%
Group II (Placebo)	SASP Baseline	12.00	6.8	7.94	118.67
	SASP + Placebo	15.66	7.7	11.81	166.58
	% Change	+31%	+13%	+49%	+40%

N-4 Acetylsulfapyridine Pharmacokinetic Data

		C_{max}	T_{max}	C_{min}	AUC
Group I (Treatment)	SASP Baseline	7.14	9.0	5.60	77.42
	SASP + Zileuton	10.36	5.1	8.45	113.90
	% Change	+45%	-43%	+51%	+47%
Group II (Placebo)	SASP Baseline	7.32	7.5	5.82	80.03
	SASP + Placebo	9.98	8.5	8.10	108.36
	% Change	+36%	+13%	+39%	+35%

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THE INFLUENCE OF CONCOMITANT ORAL DOSES OF ABBOTT-64077 AND NAPROXEN ON THE STEADY STATE PHARMACOKINETICS OF BOTH DRUGS

Study No.: M88-291 **Volume:** 1.88 **Pages:** 203 - 285
Investigator and Location:
Utah.

Objective: To assess the potential pharmacokinetic effects of concomitant administration of zileuton and naproxen (multiple doses) on both drugs.

Formulations: Zileuton capsules (Lot No. 22-003VH) containing 200 mg. Placebo for zileuton not described. Naproxen capsules (Naprosyn[®], Syntex, Lot No. 25-978-AR) containing 500 mg. Placebo for naproxen not described.

Study Design: 24 healthy, male volunteers were enrolled in this randomized, double-blind, placebo-controlled, three-way parallel-group study. Subjects were randomized to receive either 800 mg zileuton (Treatment Groups I and II) or placebo (Treatment Group III) every 12 hours on days 1 through 10. Additionally, subjects also received oral naproxen, 500 mg (Treatment Groups I and III) or placebo (Treatment Group II) every 12 hours on days 5 through 16. All subjects fasted 10 hours prior to and until 2 hours after drug administration. All meals were standardized with respect to time during the course of the study. Heparinized blood was collected prior to dosing (zero hour) on days 1, 3, 4, 9, 10 and 16. Additionally, samples were obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after dosing on days 4, 10 and 16. Samples of the subjects in Treatment Groups I and II were analyzed for zileuton while those of the subjects in Groups I and III were assayed for naproxen.

Data Analysis: C_{max} , T_{max} , C_{min} , AUC, $t_{1/2}$, Cl/F and $Vd\beta/F$. Since subjects of Groups I and II received zileuton alone during days 1 - 4, zileuton parameters estimated for day 4 were used as baseline values to be compared to the respective parameters measured on day 10 (after naproxen). Analogous placebo data was included for comparison. Since subjects in Groups I and II received naproxen alone during days 11 - 16, naproxen parameters estimated on day 16 were used to be compared to the respective parameters measured on day 10 (after zileuton). Analogous placebo data was included for comparison.

Results: Three subjects (2 in Treatment Group I and 1 in Treatment Group III) did not complete the study. Zileuton and naproxen mean and individual pharmacokinetic parameters are presented in Tables 92 and 93 respectively. Changes in mean pharmacokinetic parameters are presented in Table 94.

Comments: This study design is fundamentally flawed in: (1) its parallel group design; (2) that zileuton was not dosed according to the to-be-marketed regimen (400-600 mg every 6 hours). A more conventional two-way crossover design with appropriate zileuton dosing and

ANOVA could have provided more meaningful data. Lastly, no detailed assay validation data were submitted.

Conclusion: No sizeable trends were observed when comparing the pharmacokinetic parameters in the treatment arms versus placebo. However, since some seemingly random differences were observed in all treatment arms, these results can not be considered definitively conclusive.

2 Pages

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Table 94

Zileuton Pharmacokinetic Data

		C _{max}	T _{max}	C _{min-0}	C _{min-12}	AUC	t _{1/2}	Cl/F	Vdβ/F
Group I (Treatment)	Zileuton Baseline 4.04	1.9	0.78	0.28	21.67	2.2	666	130	
	Zileuton + Naproxen	4.77	2.2	1.06	0.26	22.90	2.1	629	111
	% Change	+18%	+16%	+36%	-7.1%	+5.6%	-4.5%	-5.6%	-15%
Group II (Placebo)	Zileuton Baseline 3.97	1.9	0.60	0.22	18.77	2.2	734	135	
	Zileuton + Placebo	4.03	2.2	1.08	0.23	18.45	2.3	762	152
	% Change	+1.5%	+16%	+80%	+4.5%	-1.7%	-4.5%	+3.8%	+13%

Naproxen Pharmacokinetic Data

		C _{max}	T _{max}	C _{min-0}	C _{min-12}	AUC	t _{1/2}	Cl/F	Vdβ/F
Group I (Treatment)	Naproxen Baseline	112	1.3	58	48	869	11.0	9.8	9.0
	Naproxen + Zileuton	113	1.8	57	55	903	13.3	9.3	10.4
	% Change	+1.0%	+38%	-1.7%	+15%	+3.9%	+21%	-5.1%	+16%
Group III (Placebo)	Naproxen Baseline	104	1.6	54	48	829	10.6	10.1	9.2
	Naproxen + Placebo	99	2.0	51	40	780	10.4	10.7	9.6
	% Change	-4.8%	+25%	-5.6%	-20%	-5.9%	-1.9%	+5.9%	+4.3%

ZILEUTON POPULATION ANALYSES REPORTS

Three population analyses (1 pharmacokinetics (PK) and 2 pharmacodynamics (PD) reports were submitted.

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1. META-ANALYSIS:

Data:

PD meta-analysis was carried out on data from 5 single and multiple dose studies (M87-046 - 100 and 200 mg single doses, M88-172 - 400 and 600 mg single doses, M88-173 -200 mg qid, 400 mg tid, and 800 mg bid, M88-346 - 600 mg tid, and M89-366 -600 mg qid). Some studies lasted up to 14 days. Calcium ionophore (A23187 from Sigma) was used to stimulate LTB₄ biosynthesis in blood samples. Percent inhibition of leukotriene B₄ (%I) biosynthesis was used as the PD variable. The LTB₄ inhibition test was carried out *ex vivo*. The Sponsor did not use LTB₄ inhibition data from subjects who received 800 mg doses of zileuton in M87-046 and M88-172 "due to possibly inadequate stimulation with calcium ionophore." Thus 33 subjects were used in the Sponsor's final analysis and these provided 384 observations which were from an all male study population. These subjects on average were 75.02 ± 10.28 kg in weight, 178.86 ± 6.74 cm tall, and 24.15 ± 5.44 years old, and healthy.

Data Analysis:

The percent inhibition of LTB₄ biosynthesis after stimulation of the blood samples with calcium ionophore (I%) was calculated as follows:

$$I\% = 100 * (1 - C_{stim}/CO_{stim} - CO_{unstim})$$

where CO_{stim} is the LTB₄ reference or baseline concentration measured in the reference plasma sample from stimulated blood before drug administration. CO_{unstim} is the LTB₄ background measured in the reference sample from unstimulated blood. In some studies where CO_{unstim} was not measured a value of zero was returned. C_{stim} is the LTB₄ sample concentration measured in plasma from stimulated blood at time t after drug administration.

A population analysis of the inhibition of LTB₄ biosynthesis versus plasma concentration was performed with the NONMEM program. The inhibition of LTB₄ biosynthesis was modeled using a sigmoidal E_{max} model according to the following equation:

$$I = I_{max} * C^\gamma / IC_{50}^\gamma + C^\gamma$$

where I is the percentage inhibition of LTB₄ biosynthesis (effect), I_{max} is the maximum percent LTB₄ inhibition (theoretically 100%), C is the zileuton plasma concentration at the time of inhibition measurement, IC₅₀ is the zileuton plasma concentration at which 50% of the maximum inhibition is observed, and γ is the sigmoidicity factor. Interindividual variability was modeled exponentially for all parameters, except for I_{max} which had a fixed typical value of 100. The residual intraindividual variability in LTB₄ inhibition was modeled as

$$I_{ij}(C) = f(p_i, C_{ij}) + H(\eta) * \epsilon_{ij}$$

where f(p_i, C_{ij}) is the predicted %I as defined by the PD model, p_i is the vector of

pharmacodynamic parameters of the *i*th individual, and C_{ij} is the *j*th plasma concentration of the *i*th individual. The second term on the right hand side of the equation represents the residual departure of the observed %I from the predicted %I. ϵ_{ij} were assumed to have independent normal probability distributions with zero means and a variance of σ^2 . The function $H(f)$ defines how the variance of the residual error depends upon $f(p_i, C_{ij})$. $H(f)$ was expressed as follows:

$$H(f) = (100 - f(p_i, C_{ij})) * f(p_i, C_{ij})$$

The implication of the error structure was to allow maximum variability in %I to occur when the predicted value was 50% with very little variability when %I is near 0 or 100%. None of the covariates were helpful in explaining interindividual variability in IC_{50} . The estimated IC_{50} for zileuton was 0.46 $\mu\text{g/ml}$ and γ was 0.87 (Table 95).

Table 95: Meta Analysis Results: NONMEM Estimates

	Fixed Effects Typical Population Value (%RSE)	Random Effects Interindividual Variability (95% CI)
I_{max} (%)	100 (fixed)	
IC_{50} ($\mu\text{g/ml}$)	0.46 (16.4%)	68.1% (33.5 - 90.0%)
γ	0.87 (8.2%)	27.7% (0 - 41.5%)
Residual CV		17.0%

RSE - percent relative standard error

A subsequent population pharmacokinetics analysis carried out by FDA pharmacokineticist (using NONMEM with first order conditional estimation (FOCE)) on the plasma data from randomly selected 68 subjects from all studies of the meta-analysis studies (weight: 73.65 ± 9.55 kg, age: 25.6 ± 5.2 years, height: 176.84 ± 7.89 cm, 7 black and 61 white) showed that zileuton PK can be described by a one compartment open model with first order input and elimination. The estimated population parameters are summarized in Table 96 below. Weight explained 12% of the variability in CL, and CL was proportional to body weight (i.e. $CL = P * WT$; where P is the typical population value for CL). There was no race effect. Gender effect could not be tested since the data were only from males. None of the covariables were predictors of apparent volume of distribution. Residual variability was modeled proportionally.

Table 9 Zileuton Population PK Parameters: NONMEM Estimates

	Typical Population Parameter Values (%RSE)	Random Effects Interindividual Variability (95% CI)
Ka (h ⁻¹)	0.21 (8.2%)	73.9% (42.0 - 95.7%)
Vd/F (L)	30.20 (15.2%)	81.2% (47.2 - 104.6%)
CL/F (L/h/kg)	0.52 (4.0)	24.2% (19.6 - 28.1%)
Residual error		53.0% (46.5 - 58.8%)

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Phase II Study (M90-460):

Data:

PK samples were collected from a subset of 29 subjects (335 observations) out of a total of 145 patients who participated in a multicenter Phase II safety and efficacy study in which 145 moderately asthmatic patients were enrolled. Two 2 dose levels were used: 600 mg qid (14 patients) and 800 mg bid (15 patients). There 6 females and 23 males in the data set, and subjects weighed between 60.8 and 115 kg (mean 79.2 ± 13.2 kg) with an age and height ranges of 20 - 64 years and 162.6 - 190.5 cm, respectively. Also, there 12 blacks and 26 whites who participated in the PK/PD arm of the study. LTB₄ inhibition assay was also carried out on the blood samples.

Data Analysis:

The data analysis was carried out as per the meta-analysis report for both PK and PD. Weight was a predictor of CL, and the population pharmacokinetic parameters of zileuton in this patient group were similar to those obtained in healthy volunteers from the meta-analysis (Table 97).

Population PD analysis on percent inhibition LTB₄ yielded results similar to those obtained with healthy volunteers (Table 98). %I - zileuton concentrations plot (Fig. 53) shows that there is no hysteresis loop in the relationship between percent inhibition and concentrations of zileuton, suggesting a direct relationship between the two. Concentrations of zileuton beyond 2 µg/ml do not result in any dramatic change in inhibition of LTB₄ biosynthesis since these concentrations produce effect in the maximal inhibitory portion of the curve.

Table 97 Zileuton Population PK Parameters: NONMEM Estimates

	Typical Population Parameter Values (%RSE)	Random Effects Interindividual Variability (95% CI)
Ka (h ⁻¹)	0.33 (19.8)	35.2% (0 - 61.0%)
Vd/F (L)	29.10 (40.5)	35.1% (0 - 86.0%)
CL/F (L/h/kg)	0.43 (6.5)	22.0% (0 - 31.2%)
Residual error 0.5 µg/ml 5.0 µg/ml		146.8% 75.5%

Table 98 PD Analysis Results - NONMEM Estimates

	Fixed Effects Typical Population Value (%RSE)	Random Effects Interindividual Variability (95% CI)
I _{max} (%)	100 (fixed)	12.9 (0 - 18.5%)
IC ₅₀ (µg/ml)	0.43 (16.2%)	57.6% (0 - 107.7%)
γ	0.82 (11.0%)	43.6% (0 - 65.3%)
Residual CV		25.0%

Phase III Study (M91-685):

Data:

Phase III study in moderate asthma 401 patients. 132 and 134 moderately asthmatic patients were administered 400 mg qid and 600 mg qid of zileuton, respectively, while 48 patients were on placebo. A subset of patients (20 patients on 600 mg qid, 24 patients on 400 mg qid, and 21 on placebo) were selected for PK/PD evaluation. The patients were between 18 and 53 years old, weighed between 54.4 and 107.0 kg, and were between 154.9 and 185.4 cm tall. FEV₁, FVC,

and peak expiratory flow rate (PEFR) were measured and expressed as percent change from baseline. 42 patients (16 females and 26 males, 1 black and 41 white) were evaluable for PK/PD. Blood samples were obtained from subjects for the determination of zileuton levels and percent inhibition of LTB₄. Albumin levels were also provided.

Data Analysis: The relationship between %I and zileuton plasma concentration was modeled as described under the Meta-Analysis subsection. Exploratory investigation of covariables predictive of IC₅₀ using generalized additive modeling yielded gender as a predictor, but this did not reach a p < 0.05 level of significance. NONMEM did not select gender or any of the other covariates as a predictor of IC₅₀. The IC₅₀ of 0.47 µg/ml (Table 99) is similar to that obtained in the Phase II study. This is not surprising since patients in the two studies were moderately asthmatic. Again, %I was directly related to zileuton plasma concentration (Fig. 57).

The improvement of FEV₁ occurred within 0.5 h of dosing and continued throughout the dosing interval. The mean time to achieve maximum improvement in FEV₁ was 2 to 3 h later than the time to reach maximum zileuton concentration in plasma after the first dose (Fig. 57), suggesting a time delay in the relationship between zileuton concentration in plasma and improvement in FEV₁. A counterclockwise hysteresis loop was observed between the concentration of zileuton in plasma and the improvement in FEV₁ (Fig. 57), indicating that the site of action of the drug is outside the blood and that the decline in zileuton concentration in tissue lags behind the decline in zileuton concentration in plasma. A similar relationship was observed between improvement in FEV₁ and %I (Fig. 57), suggesting that the mechanism by which zileuton improves lung function is outside the blood.

Table 99 Study M91-685: PD Analysis Results - NONMEM Estimates

	Fixed Effects Typical Population Value (%RSE)	Random Effects Interindividual Variability (95% CI)
I _{max} (%)	100 (fixed)	
IC ₅₀ (µg/ml)	0.47 (12.6)	60.0% (27.7 - 80.3%)
γ	0.99 (8.2)	40.0% (26.0 - 50.2%)
Residual CV		12.8%

SUMMARY:

Given the data set, the IC₅₀ of zileuton was estimated to be 0.46 µg/ml in healthy subjects with an intersubject variability of 68.1%. None of the covariables present in the data set were predictors of this parameter. There was no race differences in PK and weight was found to be a

predictor of CL.

The population pharmacokinetic parameters of zileuton in moderate asthmatics were similar to those obtained in healthy volunteers from the meta-analysis.

Population PD analysis on percent inhibition LTB₄ yielded results similar to those obtained with healthy volunteers. There is a direct relationship between zileuton concentration and inhibition of LTB₄ synthesis. The covariables present in the data set were not predictive of IC₅₀. Concentrations of zileuton beyond 2 µg/ml do not result in any dramatic change in inhibition of LTB₄ biosynthesis. There is an indirect relationship between LTB₄ inhibition / zileuton concentration and improvement in lung function.

COMMENTS:

The total number of subjects for used for the PD population analysis (meta-analysis) was rather small, yielding an imprecise estimate of the variability in IC₅₀. Imprecise estimates of variability in PK parameters could be attributed in part to the sample size.

The age range (15 - 45 years) in the data set was too narrow for any age effect to be picked up in the PK analysis.

In Study M91-685 the subjects studied were either nonsmokers or ex-smokers. Thus the effect of smoking status on IC₅₀ could not be evaluated.

For subsequent applications, the Sponsor should be advised to submit PD data without transformation.

For subsequent population PK/PD submissions, the Sponsor should be advised to test the predictive performance of population models developed.

Figure 53. Observed and Predicted Percent LTB₄ Inhibition as a Function of Zileuton Plasma Concentrations After 600 Mg Q.I.D. and 800 Mg B.I.D. Dosing With Zileuton

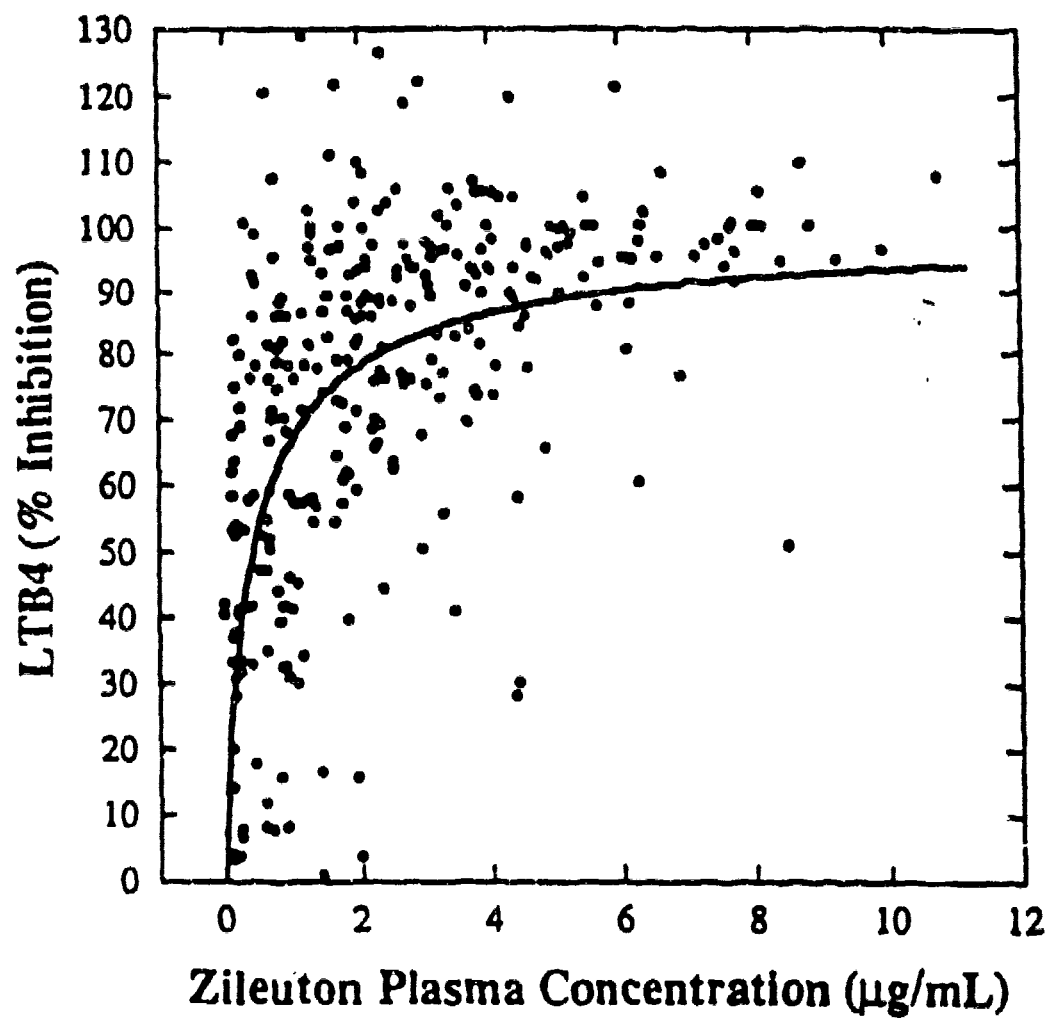


Figure 54 Observed and Predicted Percent LTB₄ Inhibition as a Function of Zileuton Plasma Concentrations After 600 Mg QID and 400 Mg QID Dosing With Zileuton

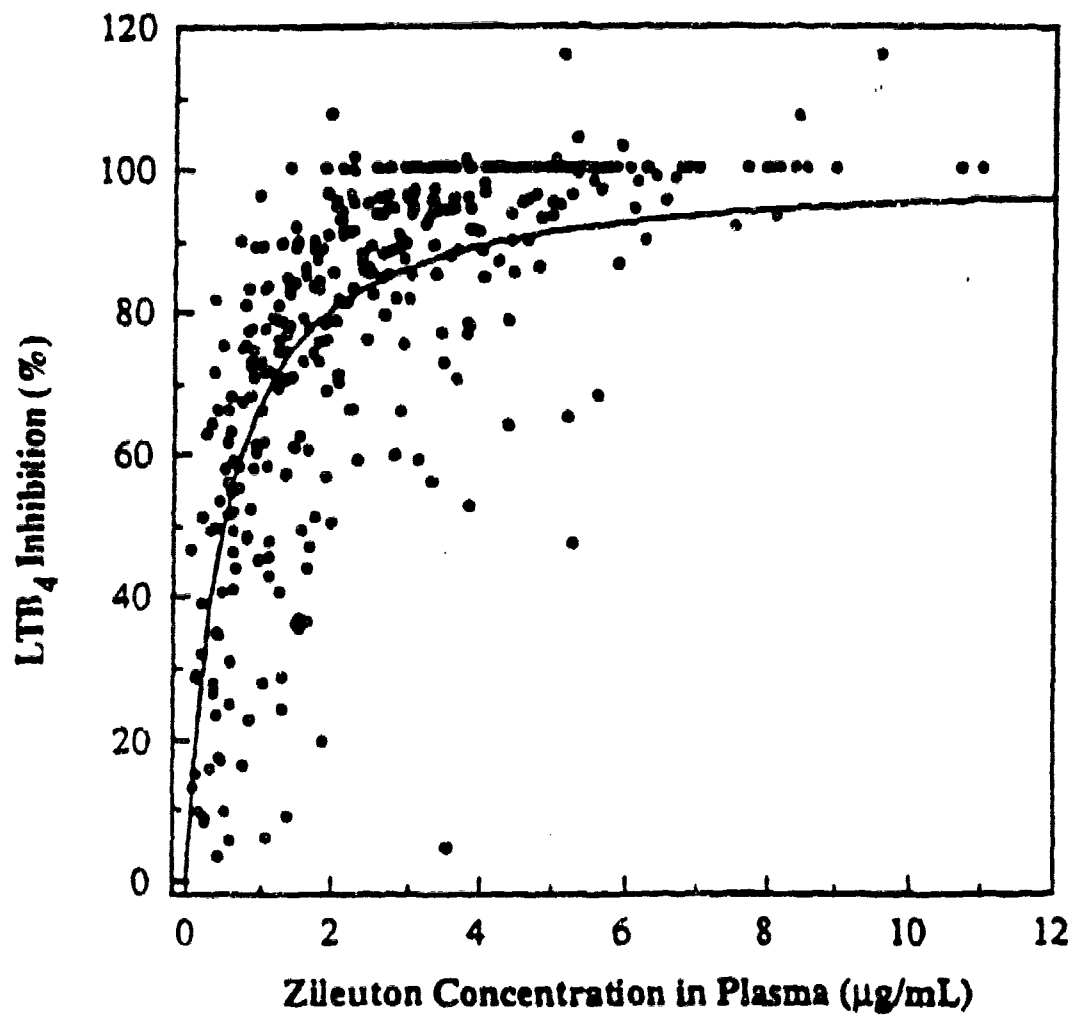


Figure 55 Plot of Mean Zileuton Plasma Concentrations and Percent Change in FEV₁ (From Baseline on Day 1 of Dosing) Versus Time Post-Dosing for Patients Who Received 600 Mg QID Zileuton and Completed the Entire Study

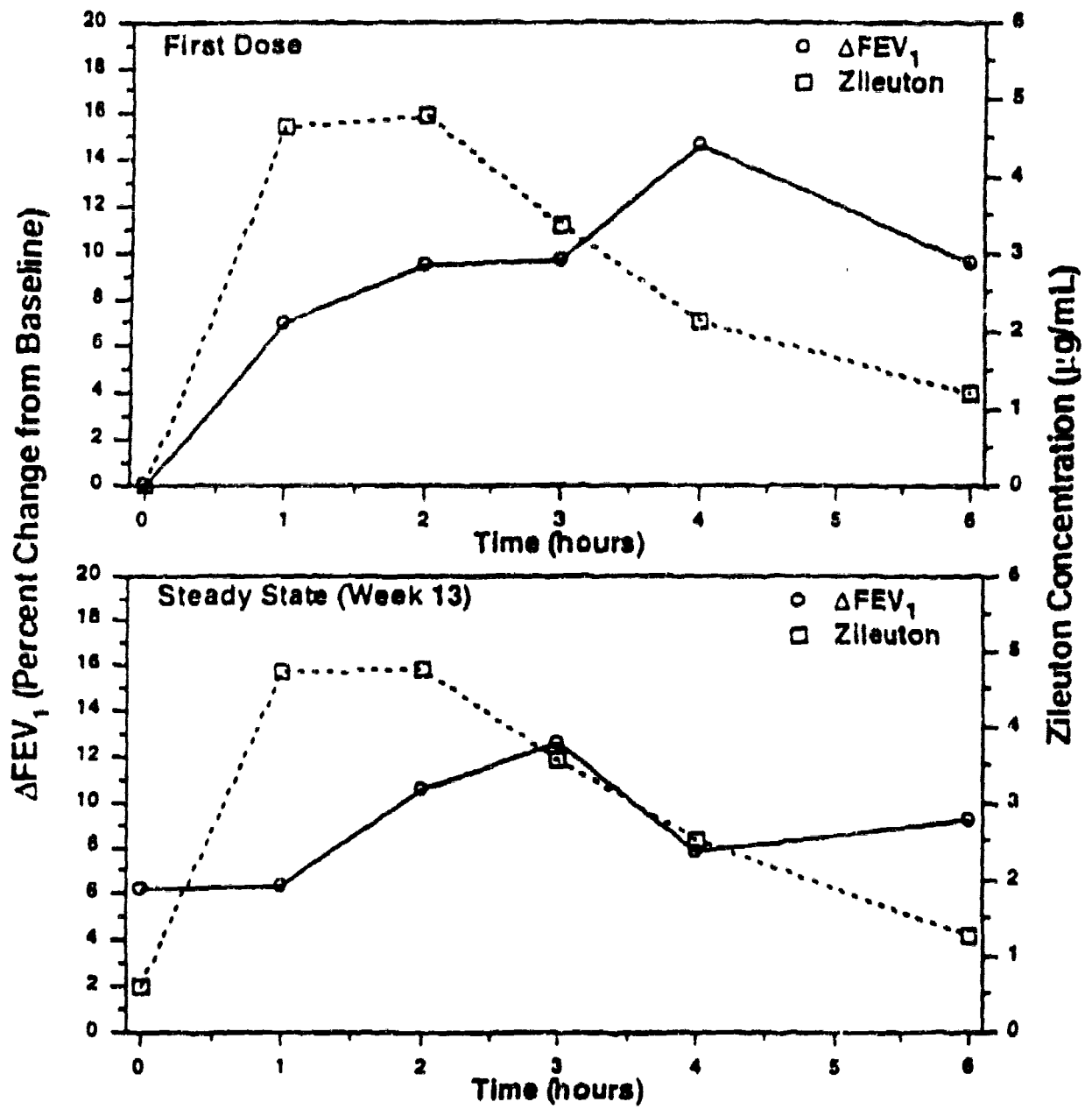
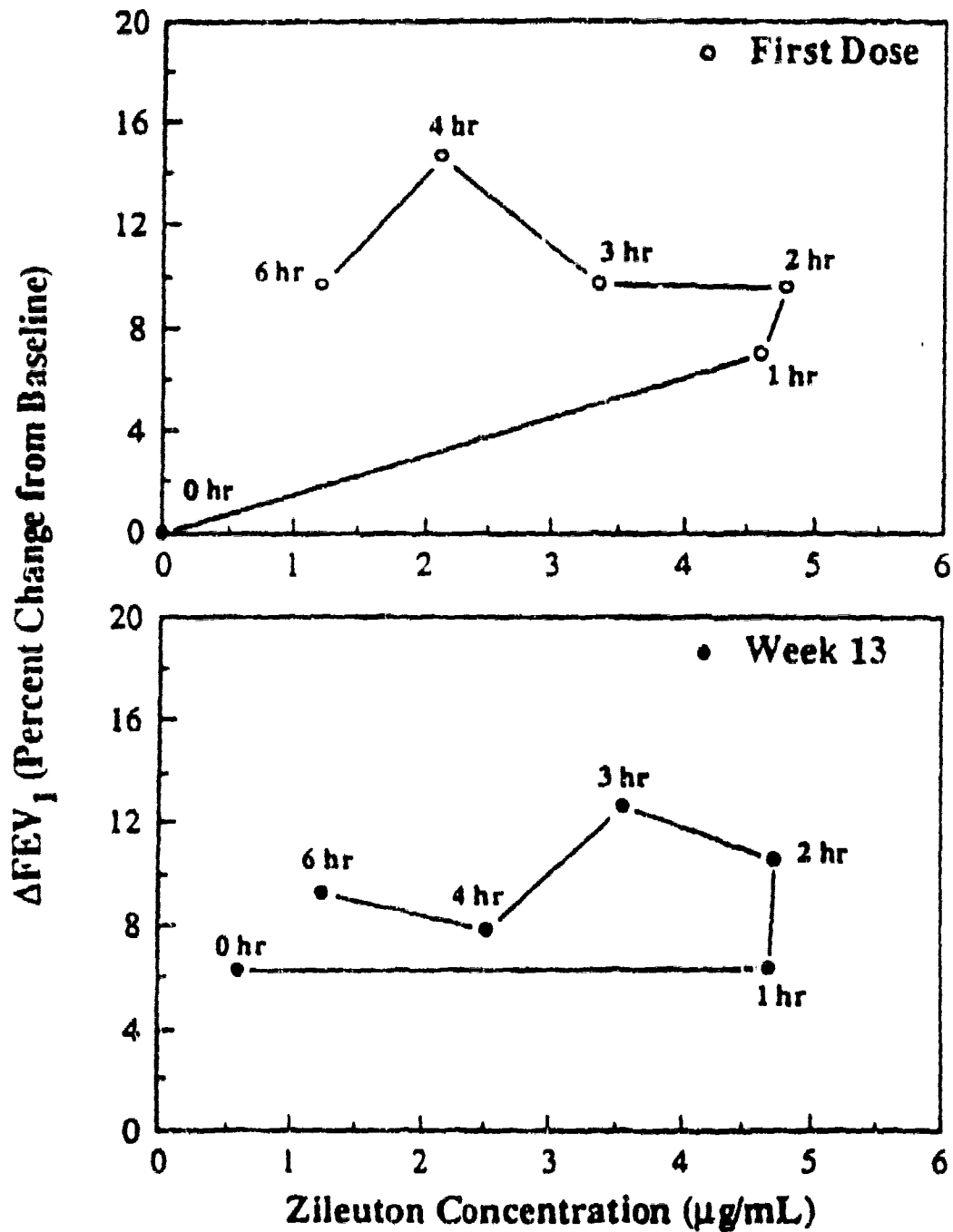
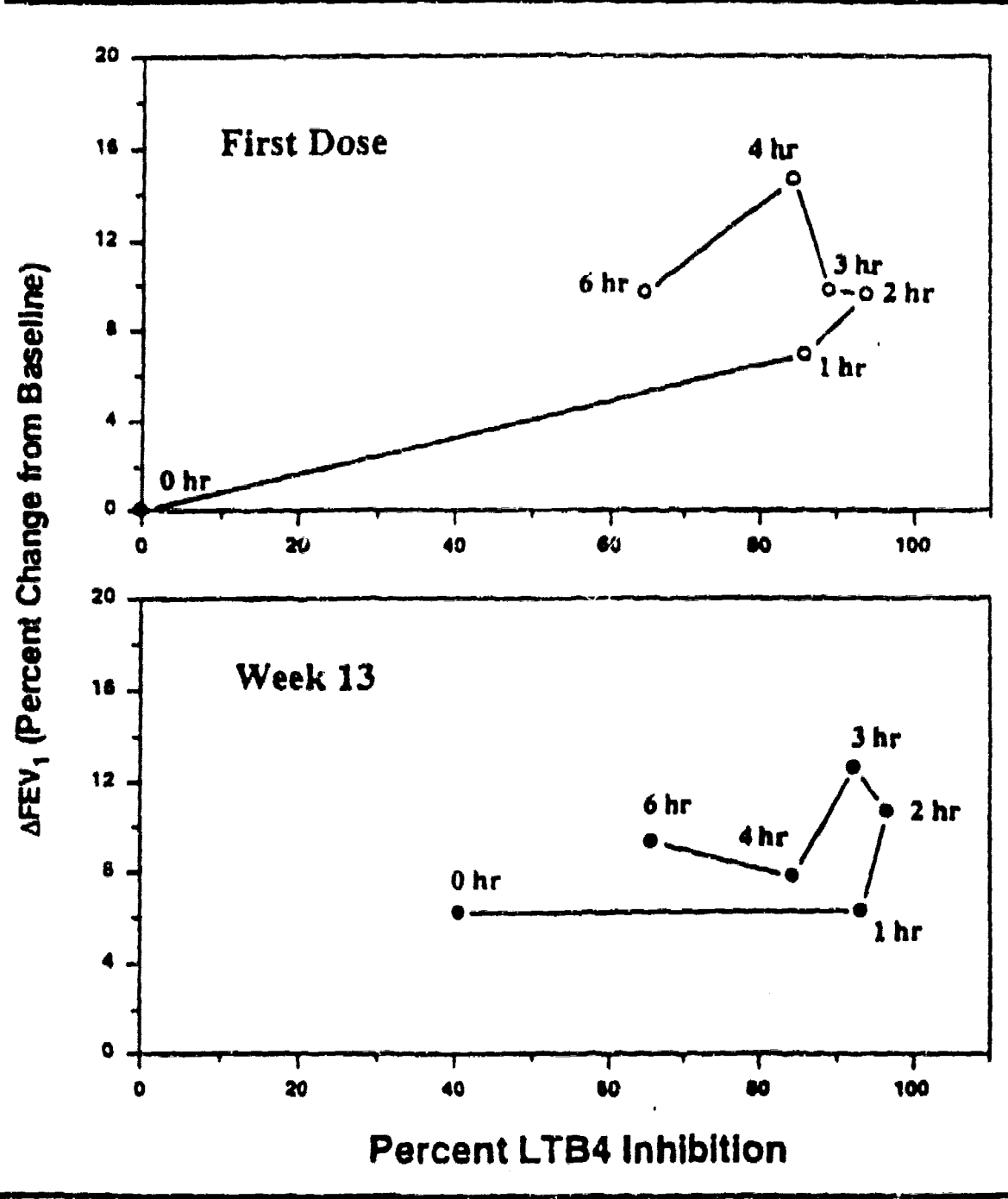


Figure 56. Plot of Mean Zileuton Plasma Concentrations Versus Percent Change in FEV₁ (From Baseline on Day 1 of Dosing) for Patients Who Received 600 Mg QID Zileuton and Completed the Entire Study



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Figure 57. Plot of Mean Percent of LTB₄ Inhibition Versus Percent Change in FEV₁ (From Baseline on Day 1 of Dosing) for Patients Who Received 600 Mg QID Zileuton and Completed the Entire Study



NDA 20,471

Abbott 64077 (Zileuton) Leutrol tablets

Pharmacokinetic section, 161

Submission date: 07/19/94

3A DISSOLUTION METHODOLOGY:

Summary of Dissolution Method and Proposed Specification

Zileuton has a low, pH-independent aqueous solubility. The *in vitro* release procedure used throughout the clinical and dosage form development program employed low agitation intensity. The methodology can be outlined as follows:

Apparatus:

Agitation:

Medium:

Profile Times:

Assay:

Specification proposed by the sponsor: Q = in 45 minutes per USP.

Certain aspects and considerations in selection of this release method are explained below.

Higher agitation intensities were not tested.

Justification of the Dissolution Medium

NDA 20,471

Pharmacokinetic section, ■

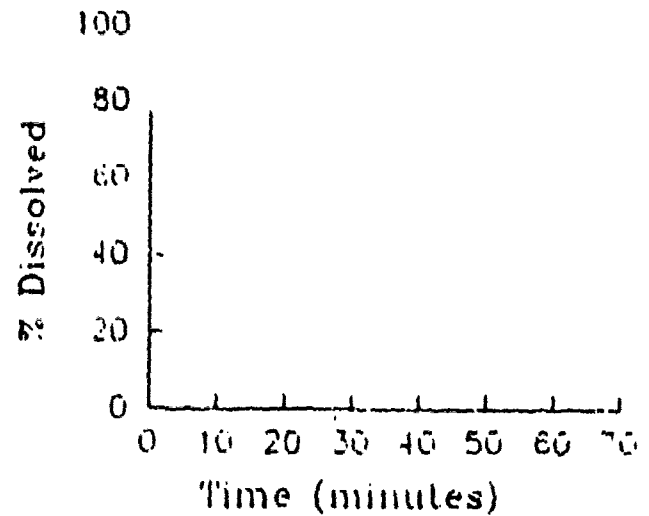
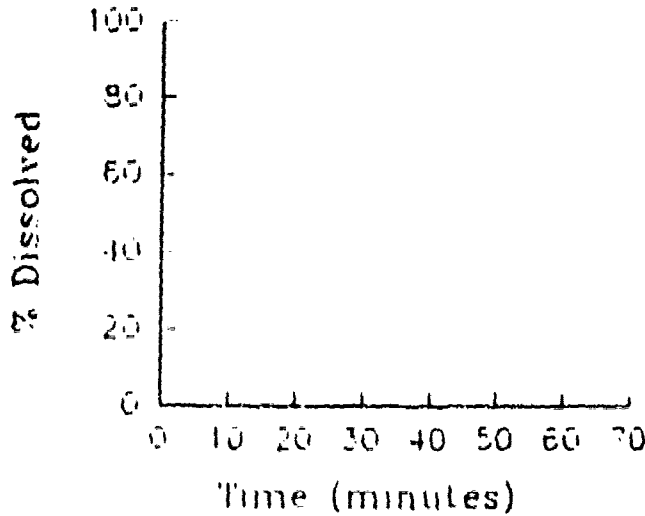
Abbott 64077 (Zileuton) Leutrol tablets

Submission date: 07/19/94

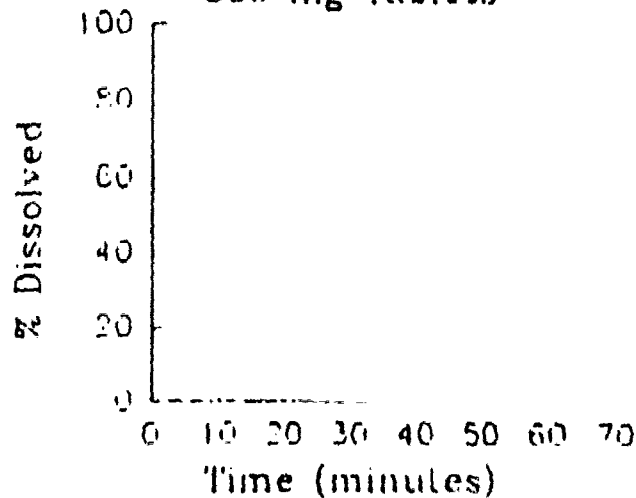
Figure 58

Zileuton Tablets: Mean Dissolution Profiles
in 50 mM SDS, pH 1, Water and pH 7.5

300 mg Tablets



600 mg Tablets



In Vivo-In Vitro Correlation

The relationship between the *in vitro* dissolution profile from the zileuton FF tablet formulations and the extent of absorption (AUC) and the rate of absorption (C_{max}) of the drug was examined by comparing the *in vitro* release data and the C_{max} and AUC values across all bioavailability/bioequivalence studies which used the FF tablets. The data from the following seven bioavailability studies were included in the analysis, F92-216, F92-218, F92-219, F92-226, F92-229, F93-235, and F93-247.

The initial zileuton *in vitro* release (% of label claim released in the first 15 minutes) from the various tablets ranged from [redacted] and in 15 minutes in these studies. The amount of zileuton released in 45 minutes ranged from [redacted]

The average AUC normalized to 100 mg zileuton dose across studies was independent of the initial *in vitro* dissolution at 15 minutes and the extent of dissolution at 45 minutes. AUC did not

correlate to either *in vitro* dissolution parameter.

The average C_{max} value normalized to 100 mg zileuton dose across the FF tablet formulations tested in the bioavailability studies correlated significantly to the initial *in vitro* dissolution of zileuton in 15 minutes in these formulations and the percent zileuton released in 45 minutes. The slope of the line of the plot of the initial *in vitro* dissolution of zileuton and the average C_{max} normalized to 100 mg zileuton dose was significantly different than zero ($C_{max} = 0.492 + 0.006 * \text{Initial dissolution in 15 minutes}$; P-value for the slope = 0.0116) with an R^2 value of 0.398. Similarly, the slope of the line of the plot of the percent zileuton released in 45 minutes and the average C_{max} normalized to 100 mg zileuton dose was significantly different than zero ($C_{max} = -0.508 + 0.015 * \% \text{ released in 45 minutes}$; P-value for the slope = 0.0413) with an R^2 value of 0.283.

The R^2 values of the relationship between the C_{max}/AUC values across bioavailability studies and the initial dissolution in 15 minutes or percent drug released in 45 minutes were 0.553 and 0.588, respectively. Therefore, variability in the initial *in vitro* dissolution in 15 minutes and percent zileuton released in 45 minutes account for _____ respectively, in the variability of the C_{max}/AUC values across bioavailability studies. The slopes of the lines of the plots of the initial *in vitro* dissolution in 15 minutes or percent zileuton released in 45 minutes and the C_{max}/AUC values were also significantly different than zero ($C_{max}/AUC = 0.162 + 0.0018 * \text{Initial dissolution in 15 minutes}$; P-value for the slope = 0.0015; and $C_{max}/AUC = -0.215 + 0.0053 * \% \text{ released in 45 minutes}$, P-value for the slope = 0.0009).

COMMENT: 1. The dissolution method provided is adequate.
2. The sponsor has done an excellent job in trying to show correlation between PK parameters and dissolution data. Although compelling, this is not adequate for setting dissolution specifications.

Dissolution specifications:

The sponsor has provided data on 3 lots for each strength, 2 of which are production lots and one is data from pilot batch (details in table below). The dissolution from the 3 lots for all 3 strengths are shown in figures below. Dissolution data from a reworked tablet that is not bioequivalent to the final formulation is also provided for comparison to show that initial dissolution rate of zileuton might be important.

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Table 10

Zileuton Tablets; Mean (3 lots) Dissolution Profiles,
50 mM SDS Dissolution Medium.

2

Test Method:

Time:	<u>Mean % Dissolved (S.D.)</u>				
Dosage Strength	<u>10</u>	<u>20</u>	<u>30</u>	<u>45</u>	<u>60 Min.</u>

Table 102.

Zileuton Tablets, 300 mg - Dissolution Profiles
of NDA Batches.

(b)

Test Method:

Lot	Time: Trial	% Dissolved				
		10	20	30	45	60 Minutes
60-697-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Mean					
	SD					
60-776-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Mean					
	SD					

Table 102 (Cont.)

©

Lot	Time: Trial	% Dissolved				
		10	20	30	45	60 Minutes

59-594-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Mean					
	SD					

Mean	n=3, lots	
SD		

2 Pages

Purged

Table 104 · Zileuton Tablets, 600 mg - Dissolution Profiles of NDA Batches.

(f)

Test Method:

Lot	Time: Trial	% Dissolved				
		10	20	30	45	60 Minutes
60-699-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
		Mean				
	SD					
60-778-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
		Mean				
	SD					

Table 104(Cont.)

9

Lot	Time: Trial	% Dissolved				
		10	20	30	45	60 Minutes
59-592-AR	1					
	2					
	3					
	4					
	5					
	6					
	7					
	8					
	9					
	10					
	11					
	12					
	Mean					
	SD					
Mean	n=3, lots					
SD						

Pharmacologist Review

7/22

NOV 14 1996

**DIVISION OF PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA**

NDA 20471

Dates of submissions: March 22, June 7, July 23, Oct. 23 and Oct. 24, 1996

Reviewer: C. Joseph Sun, Ph. D.

Date of review: Nov. 14, 1996

Drug: Zileuton Tablets (300 mg and 600 mg)

Drug category: 5-lipogenase inhibitor

Proposed Dose Level: 600 mg QID or 2400 mg daily dose (48 mg/kg based on a 50 kg body weight)

Preclinical studies reviewed: (1) 2-week oral toxicity study of Abbott-68418 in rats submitted on March 22 and July 23, 1996 and (2) response to the FDA's request for information on Oct. 18 and Oct 23 (telephone conversation), 1996 submitted on Oct 23 and Oct 24, 1996, respectively.

Preclinical study previously reviewed: (1) Acute oral toxicity study of Abbott-66193, 65813, 66795 and 68418 in mice submitted on March 22 and July 23, 1996 (See review of the original NDA Submission of July 18, 1994 by Dr. Sun on Sept. 11, 1995) (2) The preliminary results of an initial Unscheduled DNA synthesis (UDS) study in monkey hepatocytes submitted on March 22, 1996 (See consult memorandum of Dr. Casciano of HFT-120 on Feb. 5, 1996 and our letter of March 5, 1996) and (3) The final report of the repeated UDS study in male monkeys primary hepatocytes submitted on June 7, 1996 (see consult memorandum by Dr. Casciano of HFT-120 on Aug. 23, 1996).

Review:

**2-Week Oral Toxicity Study of Abbott-68418 in Rats
(Study no. TA93-260)**

Testing Lab: Abbott

Date of the study: Reporting date July 6, 1994

GLP requirement: A statement of compliance with GLP was not submitted.

Methods: Four groups of Crl:CD^BBR rats (5/sex/group) were administered by gavage of Zileuton dissolved in 0.2 % hydroxypropyl methylcellulose at dose levels of 0 (vehicle), 2.5, 25 and 100 mg/kg/day for two weeks. Following parameters were monitored:

Clinical signs	Daily
Body weight	Twice weekly
Food consumption	Three times during two weeks
Blood chemistry and hematology	Termination
Urinalysis	End of treatment period
Organ weight, gross and histopathology	Termination

Results:

Clinical signs: Decreased activity and increased salivation were observed at 100 mg/kg/day.

Mortality: None.

Body weight: Reduction in body weight gain (14-15%) was observed at 100 mg/kg/day.

Food consumption: Decrease in food consumption (10-12%) was noted at 100 mg/kg/day.

Hematology: Normal.

Blood chemistry: Lower albumin (7%) and higher cholesterol (52%) were observed in the 100 mg/kg/day female group.

Urinalysis: Normal.

Organ weight: An increase (15%) in relative liver weight was seen in the 100 mg/kg female group.

Gross and histological examinations: Normal.

In conclusion, Abbott-68148 was administered by gavage at doses of 2.5, 25 and 100 mg/kg. Clinical signs and slight reduction in body weight gain were observed at 100

mg/kg, an NOAEL. A No effect dose of 25 mg/kg was established.

Summary and Evaluation:

Impurity/degradant specification issue:

Acute oral toxicity studies in animals are not considered appropriate to fully assess the toxicity or safety of the compounds (degradants) to be administered chronically in humans, nor toxicity studies of two weeks in duration. Thus, the submitted acute toxicity studies of four degradants and a 2-week toxicity of one degradant are not acceptable for assessing the safety of degradants at the proposed specifications.

The applicant provided the impurity profile for some lots used in the animal studies as shown below (extracted from sponsor's Table 1 on page 5) :

Table 1.

Study	Lot no. of bulk drug ¹	Duration of Use	Abbot-68418	Abbot-65813	Abbot-66795	Abbot-66193
3-month rat toxicity	05-661-AL	entire study	ND ²	0.08	0.10	0.19
6-month rat toxicity	10-753-AL	entire study	<0.05	ND	0.05	ND
1-year rat toxicity	19-844-AL	entire study	ND	<0.05	ND	0.08
2-year rat carcinogenicity	28-932-AL	ca. 378 days	ND	0.29	ND	0.05
	41-047-AL	ca. 351 days	<0.05	ND	0.06	<0.05
	52-133-AL	ca. 30 days	0.05	ND	<0.05	ND
1-year monkey toxicity	19-844-AL pre-study	entire study	ND	<0.05	ND	0.08
	19-844-AL post-study		0.09 ³	<0.05	ND	0.16

¹ All analyses represent pre-study results with the noted exception.

² ND = none detected

³ This was the result of a re-analysis with the original value being 0.19%.

Abbott-66913 is an inactive metabolite in humans. The AUC of Abbott-66193 was found to be 20% of the zileuton AUC. Furthermore, in the one year oral toxicity in monkeys, no toxicity was observed at 500 mg/kg/day. The exposure to Abbott-66913 at this level in monkeys was 17 times the clinical exposure at the recommended oral dose. Presence of this degradant at the proposed specification of 0.25 % would not have any safety impact. Thus, the specification of 0.25 % is acceptable.

The safety assessment for the degradants (Abbott 68418, 65813 and 66795) at the proposed specifications is summarized below:

Degradant	Specification	Human Intake* (mg/kg/day)	NOAEL in animal species (mg/kg/day)	safety margin (NOAEL of preclinical/ human intake)
Abbott-68418		0.12	0.225 (monkeys**)	2
Abbott-65813		0.09	0.232 (rats***)	2
Abbott-66795		0.12	4.5 (mice****)	40

*: Based on a daily dose of 2400 mg and body weight of 50 kg

** : Based on the NOAEL of zileuton at 500 mg/kg/day from a one year monkey study and an impurity level of 0.045 % (Average of 0% at pre study and 0.09% at post study with detection level of 0.05%).

***: Based on the NOAEL of zileuton at 80 m/kg/day from a 2-year CA study in rats and an impurity level of 0.29% at pre study.

****: Based on the NOAEL of zileuton at 450 mg/kg/day from a 3-month dietary study

in mice and estimated 1% conversion of orally administered dose to Abbot-66795 (14% of the daily dose of radiolabelled 450 mg/kg for four days was excreted in urine. Of the total urine radioactivity, 6.8 % was in the form of conjugated oxime [Abbot-66795 is the oxime metabolite of zileuton]).

For Abbot-68418, pre study impurity level was 0% and post study impurity level was 0.9 % in the one-year monkey study. Thus, an average impurity level of 0.45 % was assumed. Based on the NOAEL of zileuton at 500 mg/kg/day and impurity level of this degradant of 0.45 %, the NOAEL of Abbot-68418 would be 0.225 mg/kg/day. There is only 2-fold of safety margin between the NOAEL of this degradant in monkeys and the estimated human intake of this degradant at the proposed specification of 0.25%. Thus, the proposed specification is not acceptable. If a sensitivity factor of 4 is applied to extrapolating safety in humans from the data from the monkey and if a practical standpoint is considered, 0.15 % would be acceptable.

For Abbot-65813, pre study impurity level was 0.29% in the 2-year carcinogenicity study in rats. No toxicity was observed at 80 mg/kg/day. Thus, an NOAEL of this degradant would be 0.232 mg/kg/day. There is only 2-fold of the safety margin between the NOAEL and the expected human intake of this degradant at the proposed specification of 0.2 %. Thus, the proposed specification is not acceptable. However, if a sensitivity factor of 10 applies to extrapolating safety in humans from rats, the acceptable specification would be 0.05%. However, the specification of a much toxic degradant of Abbott-68418 at 0.15 % based on the LD50s (184 mg/kg for Abbott-68418 vs. 6840 mg/kg for this degradant) would be appropriate for Abbot-65813.

For Abbot-66795, impurity level at the pre study was 0.1% in the 3-month rat toxicity study. No toxicity was observed at 80 mg/kg in the study. Thus, an NOAEL of this degradant would be 0.08 mg/kg. The expected human intake of this degradant at the proposed specification of 0.25 % is 0.12 mg/kg, and safety of this impurity at the proposed specification is not indicated. However, in a PK study where mice were administered by gavage of radiolabelled zileuton at daily dose of 450 mg/kg for 4 days, 14 percent of the oral dose was excreted in urine. Of the total urine radioactivity, 6.8 % was in the form of conjugated oxime (Abbot-66795). Thus, about 1% of an orally administered dose was metabolized to this degradant in mice. No toxicity was seen in mice treated for zileuton at 450 mg/kg/day for three months. An NOAEL of this degradant at 4.5 mg/kg/day in mice was estimated. The expected human intake of this degradant at the proposed specification of 0.25 % would be 0.12 mg/kg/day. Therefore, preclinical data from the 3-month toxicity study and the PK study would provide a

sufficient safety margin of 40-fold for the impurity (degradant) at the acceptable specification of 0.25%.

Genotoxicity issue:

Two unscheduled DNA Synthesis study in male monkey primary hepatocytes were conducted. Preliminary results of an initial study submitted on January 5, 1996 and final results submitted on March 22, 1996 were considered inadequate (see consult review of Feb. 5, 1996 by Dr. Casciano of HFT-120 and our letter of March 5, 1996). A final report for the repeat monkeys UDS study as recommended was submitted on June 7, 1996 and was reviewed by Dr. Casciano of HFT-120 (see Memorandum of Aug 23, 1996). The study was conducted at the Abbott (animal pretreatment, sacrifice, and the hepatocyte isolation and culture) and by SRI International (UDS evaluation) from April 2 to May 29, 1996. A statement of compliance with GLP regulation was provided. Two monkeys were pretreated daily for four days with oral zileuton dissolved in 0.2 % hydroxypropylmethylcellulose at 80 mg/kg/day. An additional three monkeys were pretreated with a single intraperitoneal injection of 50 mg/kg Aroclor 1254 and a fourth monkey was given an additional injection of 50 mg/kg Aroclor 17 hours before sacrifice. All the other test animals were sacrificed 24 hours after the last dose administration. Hepatocytes from each animal were cultured in a plain culture medium, vehicle medium (DMSO) or medium containing zileuton at concentrations of 3, 10, 30, 100, 300 and 600 ug/ml. Four positive controls were used as follow: benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, methylmethanesulfonate, and dimethylnitrosamine. UDS results were negative in all concentrations of zileuton evaluated. At least three of the positive controls yielded typical positive results for UDS in all but one monkey. Thus, zileuton showed no evidence of induction of UDS in an in vitro hepatocyte assay using hepatocytes from male cynomolgus monkeys pretreated with zileuton or Aroclor 1254.

Labeling issue:

Labeling conforms to the format specified under CFR21.Part201.Subpart B dated April 1996. The proposed labeling reflects the preclinical data. The reproductive effects (testicular and prostatic degeneration/atrophy) were reported only in dogs and a sufficient safety margin is demonstrated after factoring in both the receptor-affinity difference of 90-fold between the dogs and humans and the safe drug exposure difference of 6-fold for AUC or Cmax between dogs and humans, pertinence of this findings in dogs to humans is limited. Some revisions are recommended as shown below:

Carcinogenesis, mutagenesis and impairment of fertility:

In 2-year carcinogenicity studies, increases in the incidence of liver, kidney and vascular tumors in female mice and a trend of increase in the incidence of liver tumors in male mice were observed at 450 mg/kg/day (providing 4 times [females] or 7 times [males] the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). No increases in the incidence of tumors were observed at 150 mg/kg/day (providing 2 times the systemic exposure [AUC] achieved at the maximum recommended daily oral dose). In rats, an increase in the incidence of kidney tumors was observed in both sexes at 170 mg/kg/day (providing 6 times [males] or 14 times [females] the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). No increased incidence of kidney tumors was seen at 80 mg/kg/day (providing 4 times [males] or 6 times [females] the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). Although a dose-related increased incidence of benign Leydig cell tumors was observed, Leydig cell tumorigenesis was prevented by supplementing male rats with testosterone. Relevancy of this tumor finding to humans is considered limited.

Zileuton was negative in mutagenicity assays including bacterial reverse mutation (Ames) using *S. Typhirium* and *E. Coli*, HGPRT forward mutation assays in Chinese hamster ovary cells, chromosome aberrations in human lymphocytes, in vitro unscheduled DNA synthesis (UDS) assay in rat hepatocytes with or without zileuton pretreatment or in mouse or rat kidney cells with zileuton pretreatment, mouse lymphoma and micronucleus assays. However, a dose-related increase in DNA adduct formation was reported in kidneys and livers of female mice treated with zileuton. Although some evidence of DNA damage was observed in a UDS assay in hepatocytes isolated from the Aroclor-1254 treated rats, no such finding was noticed in hepatocytes isolated from monkeys where the metabolic profile is more similar to that of humans.

In reproductive performance/fertility studies, zileuton produced no effects on fertility in rats at oral doses up to 300 mg/kg/day (providing 8 times [male rats] and 18 times [female rats] the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). Comparative systemic exposure [AUC] is based on measurements in male rats or nonpregnant female rats at similar dosages. However, a reduction in fetal implants was observed at oral doses of 150 mg/kg/day and higher (providing 9 times the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). Increased in gestation length, prolongation of estrous cycle, and increased in stillbirths were observed at oral doses of 70 mg/kg/day and higher (providing 4 times the systemic

exposure [AUC] achieved at the maximum recommended human daily oral dose). In a perinatal/postnatal study in rats, reduced pup survival and growth were noted at an oral dose of 300 mg/kg/day (providing 18 times the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose).

Pregnancy : Pregnancy category C. Developmental studies indicated adverse effects (reduced body weight and increased skeletal variations) in rats at an oral dose of 300 mg/kg/day (providing 18 times the systemic exposure [AUC] achieved at the maximum recommended human daily oral dose). Three of 118 (2.5%) rabbits fetuses had cleft palates at an oral dose of 150 mg/kg/day (equivalent to the maximum recommended human daily oral dose on a mg/m² basis). Comparative systemic exposure is based on the measurement of nonpregnant female rats at a similar dosage. Zileuton and/or its metabolites cross the placental barrier of rats. There are no adequate and well-controlled studies in humans.....

Nursing Mothers:

..... **Because many drugs are excreted in human milk, and because of the potential for tumorigenicity shown for zileuton in animal studies, a decision should be made whether to discontinue nursing or to discontinue the drug taking into account the importance of the drug to the mother.**

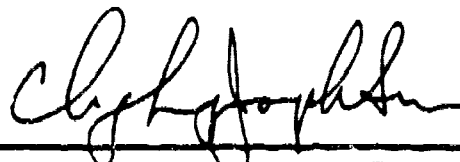
Overdosage:

The oral minimum lethal doses in mice and rats were 500-4000 and 300-1000 mg/kg in various preparations, respectively (approximately 1-7 and 1-3 times the maximum recommended human oral daily dose on a mg/m² basis, respectively). No deaths occurred but nephritis was reported in dogs at an oral dose of 1000 mg/kg (approximately 6 times the maximum recommended oral daily dose on a mg/m² basis).

Recommendation:

Zileuton was carcinogenic in animals and was mutagenic in kidneys and livers of female mice and hepatocytes isolated from the Aroclor-treated rats. However, negative results of a UDS assays from hepatocytes isolated from the Aroclor-treated monkeys where the metabolic profile is more similar to that of humans outweigh the concern for chronic human exposure to zileuton related to its rodent carcinogenic and genotoxic activities. Thus, the NDA is approval from a preclinical standpoint with some revision in the proposed labeling as shown above.

The proposed specification for Abbot-66193 and Abbot-66795 at _____ is acceptable.
The proposed specifications for Abbot-68418 and Abbot-65813 a _____ are
not acceptable. The recommended specification for these two degradants i.



Ching-Long Joseph Sun, Ph.D.
Pharmacology Team Leader

Nov. 14, 1996

Orig. NDA
HFD-570/Division file
HFD-570/Sun
HFD-570/PM
HFD-570/Leak
HFD-570/Honig
HFD-570/Anthrocite
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NDA 20471

Page 1

**DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Review**

NDA 20471

Date of submission: July 18, 1994, March 1, March 29, April 3, 1995 and Aug. 15, 1995

Reviewer: C. Joseph Sun, Ph. D.

Date of review completed: Sept. 8, 1995

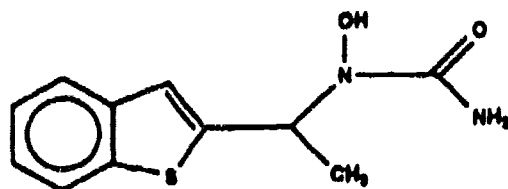
Sponsor: Abbott

Drug name: Leutrol[®] (Zileuton) 300, and 600 mg Tablets

Chemical name: N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea

Other name: Abbott-64077, ABT-077

Structural Formula:



Molecular weight: 236.29

Molecular formula: C₁₁H₁₂N₂O₂S

Related INDs: IND

Class: 5-lipoxygenase inhibitor

Proposed indication: Zileuton is indicated for the prevention and control of symptoms associated with reversible bronchospasm and for maintenance therapy in the management of asthma patients with mild, moderate or severe airway obstruction.

NDA 20471

Page 2

Clinical formulation:

Formulation is listed below (reproduced from sponsor's table 3 on page 121 of vol. 1.1):

Ingredient	Function	Scale (%)

Route of administration: oral

Overall summary:

Pharmacology studies have demonstrated that zileuton is a lipoxygenase inhibitor, inhibiting leukotriene formation. The compound inhibited inflammation in some but not all animal models and should not be considered clearly anti-inflammatory. Zileuton inhibited leukotriene dependent bronchospasm in guinea pigs and late phase bronchospasm and lung reactivity in sheep. The data suggest potential effectiveness for the treatment of asthma based on animal models.

Toxicity studies have revealed hepatocytomegaly associated with microsomal enzyme induction (rats and mice), prolongation of the estrus cycle and increased chronic nephritis/nephropathy (rats), and renal tubular atrophy and inflammation, renal tubular epithelial karyomegaly (dogs), hematologic changes (dogs) and prostatic and testicular atrophy (dogs). Enhancement of toxicity in rats and monkeys was not evident but was observed in dogs with increased duration of administration based on the no effect or tolerated doses. There are some differences in Zileuton metabolism and pharmacokinetics among species. In general, however, the extent of metabolism does not differ so greatly across species as to invalidate the utility of systemic exposure based comparisons using parent drug. Thus, an interspecies comparison of toxicity based on systemic exposure is considered more relevant than dosage-based comparison. Safety margins of the above-mentioned target organs of toxicity based on the relative parent drug exposure at NOAELs to the human exposure at the recommended dosage are given below:

Liver	6 (mice), 18 (rats)
Kidney	3 (rats), 1.5 (dogs)
Hematopoietic system	3.5 (dogs)
Male reproductive organs	6 (dogs)

In immature dogs, a safety margin was not established for the male reproductive effects as the effect was observed at all dose levels tested. Consistent effects of zileuton on adult male dog reproductive organs were observed at doses > 6 times the human exposure. Factoring in both the receptor-affinity difference of 90-fold between adult dogs and humans and the safe drug exposure difference for AUC or C_{max} between dogs and humans, there appears to be a sufficient safety margin. The male reproductive effects caused by zileuton in adult and immature dogs should be described in the labeling.

Zileuton increased the incidence of Leydig cell tumors in rats, presumably due to a disruption of the hypothalamic-pituitary-gonadal axis as demonstrated by lower testosterone response following HCG challenge in Zileuton-treated rats. The applicant's explanation of a hormonal negative feedback mechanism is further supported by a preliminary study report that Leydig cell tumorigenesis was prevented by replacement therapy with testosterone in male rats. An absence of any hormonal change in humans supports the absence of relevant risk for Leydig cell tumors in humans. Hepatic tumors observed following Zileuton treatment of rats were associated with peroxisome proliferation. Abbott suggested that this tumor effect is similar to that caused by

phenobarbital, and has demonstrated that like phenobarbital Zileuton produced enzyme induction in rodents. Since other rodent peroxisome proliferators have been shown to produce neither peroxisome proliferation nor tumors in humans, the relevance of this rodent tumor finding to humans is questioned. However, the sponsor has not ruled out other factors (e.g. DNA modification) from contributory to Zileuton's effect on liver tumors. An increase in the number of kidney tumors in female mice and rats of both sexes was suggested to result from increased renal cell proliferation and renal toxicity. However, renal toxicity was not observed in the carcinogenicity study, although at similar dose renal toxicity was seen in a chronic toxicity study in rats. Finally, Zileuton treatment was associated with an increase in hemangiosarcomas, mostly ovarian, in female mice administered 450 mg/kg/day. The applicant argued (amendment of 3/29/94) that this tumor type was partly due to Zileuton's immunosuppressive activity. The finding of hemangiosarcoma, considered significant, is a concern, and should be stated in the labeling based on available data if the drug is approved.

Although in battery of genotoxicity studies Zileuton was generally not found to be genotoxic, DNA adduct formation was reported in liver and kidneys of female mice administered Zileuton using the P³² post-labeling method. This finding suggests genotoxic potential and should be indicated in the labeling as such. Furthermore, the chinese hamster ovary/HGPRT assay originally submitted was not considered adequate because of the low cytotoxicity (See Memo of May 11, 1995 by Dr. Casciano of HFT-120). The resolution of the biological significance of this positive finding of DNA adduct formation regarding the genetic mechanism of tumorigenesis and the inadequacy of the CHO/HGPRT assay could be determined by conducting a series of further tests. The two recommended follow-up genotoxicity studies agreed to by the applicant (mouse lymphoma assay and in vivo/in vitro UDS of rat hepatocytes) were submitted and reviewed (see memo of Sept. 1, 1995 by Dr. Casciano of HFT-120). For the lymphoma assay, although negative findings in both activation and non-activation phases of the study were reported, it was considered inadequate for the activation phase because of the low cytotoxicity. For the in vivo/in vitro UDS assay, hepatocytes isolated from the zileuton pretreatment group did not show any DNA damage. However, hepatocytes isolated from the Aroclor 1254-treated rats with in vitro zileuton treatment showed a positive dose response of DNA damage. This finding indicated that with this pretreatment regimen enzymes were induced that are capable of activating zileuton to cause DNA damage. Thus, the data show that zileuton induced UDS and should be considered genotoxic (see Memo of Dr. Casciano of HFT-120 on 9/1/95).

The applicant's explanations for tumor findings are theoretically reasonable, although only in some instances supported by mechanistic studies. However, taken together the findings of multiple tumor types, some of which cross species and sexes (but not in equal strength in each) and the evidence for genotoxicity, suggests that a careful appraisal of risk-benefit of zileuton for treatment of asthma is essential. With the exception of Leydig cell tumors in male rats, all other tumors were only observed at the highest dosages tested. However, based on drug exposure for the parent compound at doses where no tumors (except Leydig cell tumor) were found, the safety margin for humans treated with Zileuton for tumor findings is only of 2-fold to 6-fold based on

mouse and rat results.

Based on the preclinical data zileuton is considered a genotoxic carcinogen. There is, however, some indication that the metabolic profile of zileuton in rodents differs from that in humans and therefore genotoxic and carcinogenic findings in rodents may not be relevant to humans. A series of studies to demonstrate the irrelevance of rodent genotoxicity findings for humans could be conducted. These new studies should focus on the particular studies suggesting the genotoxic potential of zileuton but should use animals (or human tissue) with a more appropriate metabolic profile (for assessing human risk). Such studies, if negative, may alleviate the concern for human use of the product that is based on its rodent carcinogenic and genotoxic findings.

There were no outstanding IND issues, all issues currently outstanding related to the NDA review.

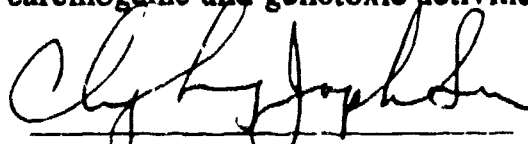
Labeling conforms to the format specified under CFR21.Part201.Subpart B dated April 1, 1995. The proposed labeling generally reflects the preclinical data. Hemangiosarcoma in mice, positive DNA adduct formation and dose-related increase in DNA damage of the in vivo/in vitro UDS assay, prostatic and testicular atrophy in immature dogs and target organs of toxicity should be mentioned in the appropriate sections. A final review of the labeling is deferred at this time.

Recommendation:

Based on currently available pharmacology and toxicology findings of tumorigenicity and genotoxicity, it is recommended that Zileuton not be approved for treatment of mild or moderate asthmatics.

Letter to the applicant:

We have reviewed your new drug application for Zileuton. Based on currently available pharmacology and toxicology findings of tumorigenicity and genotoxicity, we consider that zileuton is a rodent genotoxic carcinogen and therefore it is not recommended for approval to treat mild, moderate and severe asthmatics. However, there is some indication that metabolic profile for zileuton in rodents differs from that in humans. The genotoxicity and carcinogenicity findings in rodents may not be relevant to humans. A series of studies could be conducted to demonstrate the irrelevancy of rodent genotoxicity for humans. These studies should address the genotoxicity of zileuton using animals (or human tissue) with an appropriate metabolic profile for zileuton. Negative results of such studies appropriately conducted could outweigh the concern for human use of the product related to its rodent carcinogenic and genotoxic activities.


C. Joseph Sun, Ph. D.

Sept 8, 1995

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Attachments (Review, Appendix, Memos and Previous IND Reviews)
Orig. NDA
HFD-155/Division file
HFD-150/Dr. DeGeorge
HFD-155/Dr. Sun

WJY 9/11/95

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Previous reviews, dates of reviews:

IND

IND

IND

6/4/95

Studies reviewed in the NDA:

Acute oral toxicity of two enantiomers

Acute oral toxicity of Abbot-66193, 65813, 66795 and 68418

Ames test of Abbot-66193, 65813, 66795, and 68418

3 month combination study of zileuton with prednisone or naproxen in rats

Inhibition of testosterone production in rat Leydig cell bioassay

14 day immunological evaluation in female mice

14 day immunological evaluation in female rats

Preliminary DNA adduct formation in kidneys and liver of female mice

Preliminary supplementary testosterone on zileuton-induced Leydig cell tumor in rats

Studies not reviewed in this NDA:

Hydrocortisone challenge of dogs made neutropenic with zileuton

Dermal formulation studies

Acute oral toxicity of zileuton in combination with several bronchodilator and antiinflammatory agents in male mice

Acute oral toxicity of zileuton in combination with acetaminophen in male mice

Acute oral toxicity of zileuton in combination with indomethacin in rats

Studies previously reviewed:

Pharmacology (primary and secondary pharmacological effects)

Pharmacokinetics (Absorption, distribution, metabolism and excretion)

Toxicology

Acute oral toxicity studies in mice, rats and dogs

Acute s.c. and i.p. toxicity studies in mice and rats

3 month dietary MTD study in mice

2 year carcinogenicity study in mice

3 month oral toxicity study in rats

3 month dietary MTD study in rats

6 month oral toxicity in rats

1 year oral toxicity study in rats

2 year carcinogenicity study in rats

6 week oral toxicity study in immature rats

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3 month oral toxicity study in dogs

1 month oral toxicity study in male dogs with 1, 3 and 6 month recovery periods

6 month oral toxicity study in dogs

1 year oral toxicity study in dogs

1 month oral toxicity study in monkeys

6 month oral toxicity study in monkeys

1 year oral toxicity study in monkeys

Special acute toxicity studies

Passive cutaneous sensitization in guinea pigs

Active systemic anaphylaxis in guinea pigs

Antigenicity study in guinea pigs

Liver peroxisome proliferation and enzyme induction studies

Kidney cell proliferation studies

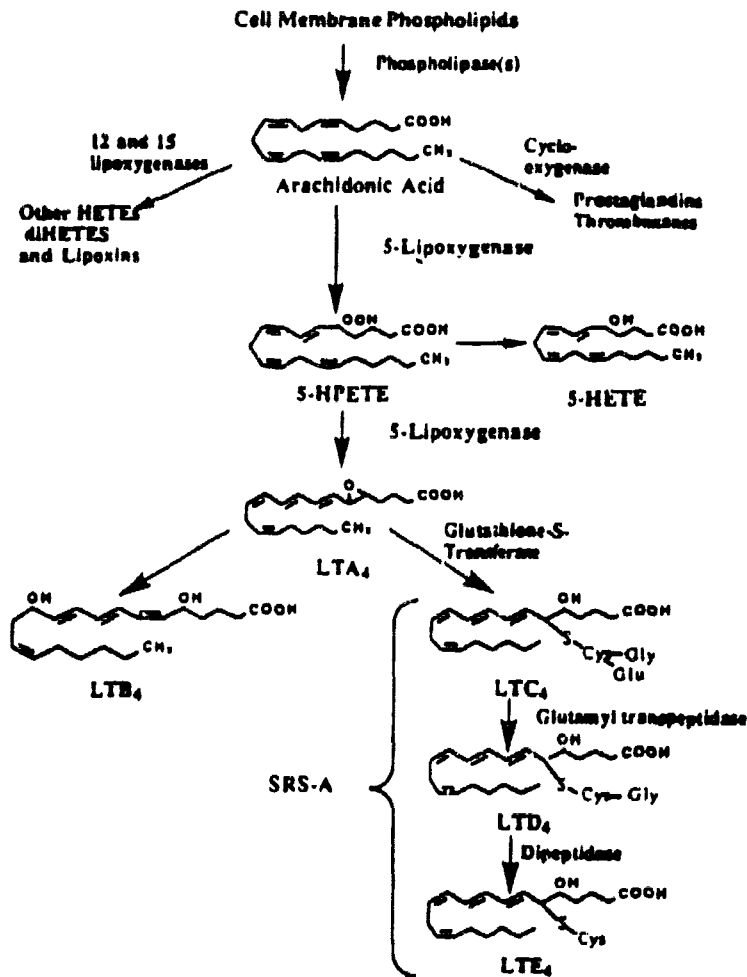
Reproductive toxicity studies (Segment I in rats, Segment II in rats and rabbits and Segment III in rats)

Mutagenicity studies

Review

Pharmacology

Primary pharmacology studies: 5-Lipoxygenase is the enzyme in the arachidonic acid cascade (as shown below) leading to the leukotrienes. Zileuton (Abbott-64077) is a direct 5-lipoxygenase inhibitor.



The inhibition of the formation of 5-HPETE by zileuton leads to the inhibition of all possible products in the cascade including 5-HPETE and leukotriene A₄ (LTA₄), LTB₄, LTC₄, LTD₄ and LTE₄.

Zileuton has been investigated for the following pharmacological effects:

Inhibition of rat basophil leukemia (RBL) lysate and human whole blood 5-Lipoxygenase: In RBL assay, IC₅₀ was 92-670 nM at arachidonic acid concentrations of 6-164 μM. Inhibition was reversible and competitive. The potencies (IC₅₀s) of two enantiomers (Abbott-68967 or R-enantiomer, 0.51 μM; Abbott-68968 or S-enantiomer, 0.91 μM) were found to differ only slightly from racemic zileuton (0.61 μM). In the human whole blood assay, R-enantiomer was more potent

(IC₅₀ = 0.79 μM) than S-enantiomer (1.6 μM). Zileuton had an intermediate IC₅₀ value of 0.92 μM.

Inhibition of ionophore-induced LTB₄ formation in polymorphonuclear leukocyte (PMNL) from rat and human: IC₅₀s of 0.2 μM (human) and 0.3-0.7 μM (rat) were obtained.

Inhibition of LTB₄ in ionophore and antigen-stimulated leukocytes in mast cells of guinea pig, mouse and rat: IC₅₀s were 0.8-1.6 μM.

Inhibition of ionophore and zymosan-stimulated LTB₄ production in whole blood of human, rat, dog and guinea pig: IC₅₀s were 0.2-0.9 μM using ionophore as stimulant. In human whole blood, IC₅₀ was 1.9 μM using zymosan as stimulant.

Inhibition of LTB₄ in chopped lung fragment of human, monkey, rabbit, sheep and guinea pig: IC₅₀s were 0.4 μM (human), 0.6-1.4 μM (monkey), 1.0 μM (rabbit), 1.5 μM (sheep), and 3.2 μM (guinea pig).

Inhibition of cyclooxygenase of sheep seminal vesicle, human platelet 12-lipoxygenase, soybean and reticulocyte 15-lipoxygenase enzyme: Very weak inhibition was observed only at concentrations > 100 μM. Using rat PMNL, zileuton was 28-fold more potent in inhibiting 5-lipoxygenase than cyclo-oxygenase, indicating the specificity of inhibition on 5-lipoxygenase.

Inhibition of eicosanoid production in ionophore-stimulated whole human blood: IC₅₀s for LTB₄, TXB₂, 12-HETE and 15-HETE were 0.74, 84, 32 and 30 μM, respectively. A ratio of 100 was found by comparing inhibition of LTB₄ and TXB₂ in ionophore-stimulated human whole blood.

Effects on LTB₄ and C5a receptor bindings in human neutrophil: At 100 μM, it exhibited less than 50 % of inhibition of LTB₄ receptor of human neutrophil membrane. No inhibition of C5a binding of human neutrophil membranes reported at same concentration.

Inhibition of human PMNL myeloperoxidase: No inhibition was observed at 100 μM.

Inhibition of airway smooth muscle (trachea) contraction in guinea pig and human: In guinea pig trachea, inhibition of agonist (acetylcholine, prostaglandin, U-44069, histamine) induced contraction was not seen at concentrations up to 100 μM. At 100 μM, zileuton failed to exhibit any effect on isoproterenol-induced relaxation of guinea pig trachea. However, zileuton reduced the LTD₄-induced contraction. The IC₅₀ for inhibition of antigen-induced contraction was 7 μM. Inhibition of IgE-induced contraction in human lung tissue was also observed at 10 μM.

Ex vivo LTB₄ inhibition in monkey, dog, sheep and rabbit: Inhibition was seen for 2 hours after oral administration in monkey at doses of 5 and 20 mg/kg. In dogs, complete inhibition was seen for 24 hours at 5 mg/kg. At 0.5 mg/kg, it produced > 50 % inhibition for 3 hours. In sheep, oral

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doses of 5 and 20 mg/kg produced complete inhibition for 24 hours. Oral dose of 20 mg/kg produced complete inhibition in rabbits at 9 hours.

Inhibition of leukotriene formation in rat peritoneal anaphylaxis model: Oral ED50s for inhibition of LTB₄ and LTE₄ were 3-7 mg/kg. I.V. dose of 5 mg/kg produced inhibition (64%) of immunoreactive-sulfidopeptide leukotriene levels in peritoneal fluid.

Effect on allergic sheep model of bronchospasm and airway hyperactivity: Oral dose of 10 mg/kg inhibited the second phase of bronchoconstriction while having little inhibitory activity against the early phase.

Secondary Pharmacology studies:

Neuropharmacology:

a. Neuropharmacological profile in mice: It had minimal activity at ip doses of 10 and 30 mg/kg. At 100 and 300 mg/kg, it produced hypothermia, decreased grip strength, piloerection, ptosis, dyspnea and changes in motor activity. Similar signs were seen with oral doses of 100 and 300 mg/kg.

b. Spontaneous motor activity in mice and rats: It had no effects in spontaneous motor activity at oral doses up to 100 mg/kg.

c. Rotarod performance in mice and rats: No effects were reported at oral doses up to 100 mg/kg.

Cardiovascular effects in dogs: Zileuton given at a oral dose of 5 mg/kg caused an increase (123%) in pulmonary arterial pressure and a reduction (37%) in cardiac output and increase in pulmonary vascular resistance (358%) in anesthetized dogs. However, in conscious dogs, intragastric doses up to 50 mg/kg did not have effects on heart rate, blood pressure or pulmonary arterial pressure. Thus, the previous-observed cardiovascular effects in anesthetized dogs could be attributable to interaction between the pentobarbital and arachidonic acid metabolism. In anesthetized dogs in which the left coronary artery was occluded, zileuton at iv dose of 20 mg/kg had no effects on myocardial infarct size, blood pressure, heart rate or coronary blood flow. In the endotoxin-induced cardiovascular change, 30 minutes pretreatment with Zileuton (iv dose of 20 mg/kg) demonstrated more rapid recovery in the decreased arterial pressure than was seen in controls. Intraduodenal dose of 50 mg/kg had no effect on this model.

Binding affinity to prostatic androgen receptors in prostates of dogs, monkeys, rats and human: The highest affinity was observed in dog prostate (pK₁ of 4.94). Monkey and rat had affinity of pK₁ of 4.32 and 4.17, respectively. Affinity in human prostate was pK₁ of 2.98, which is 90 fold less than in dog prostate. In all species studied, S-enantiomer demonstrated higher affinity (1.5-5 fold) than the R-enantiomer.

Antipyretic effects in rats and rabbits: Pretreatment of zileuton at oral doses of 10-100 mg/kg reduced yeast-induced fever in rats. However, at same dose levels, it had no effect on lipopolysaccharide-induced fever in rabbits. Aspirin (80mg/kg) and indomethacin (20 mg/kg) produced antipyretic effect in rats and rabbits, respectively.

Intraocular pressure in rabbits: Zileuton had minimal effect on intraocular pressure at 0.03, 0.1 and 0.3 %. However, pilocarpine (0.3%) produced a significant decrease in IOP.

Summary of pharmacology:

Zileuton was found to inhibit the formation of 5-lipoxygenase products. In cell lysate the compound inhibited the formation of 5-hydroxyeicosatetraenoic acid (5-HETE). Zileuton also inhibited the formation of leukotrienes in a variety of cell types using different stimuli.

Zileuton was tested against other related enzymes such as cyclooxygenase, 15-lipoxygenase from soybean and rabbit reticulocyte as well as the 12-lipoxygenase from human platelet. Zileuton was a weak inhibitor against these enzymes being at least two orders of magnitude less potent than against 5-lipoxygenase. In addition, in ionophore A 23187 stimulated whole blood, zileuton showed selectivity being 40 - 100 fold more potent in inhibiting leukotriene B₄ (LTB₄) formation than in inhibiting 12-hydroxyeicosatetraenoic acid (12-HETE) or thromboxane B₂ (TXB₂).

Against 5-HETE biosynthesis in rat basophil leukemia (RBL) cell lysate, the R (+) enantiomer was slightly more potent (approximately two fold) than the S (-) enantiomer. In the human whole blood assay this difference was not significant. Zileuton was found to be an orally active inhibitor of leukotriene formation in several species. Inhibition of *ex vivo* LTB₄ formation was demonstrated in dog, monkey, rat, sheep and rabbit after oral doses ranging from 0.5 mg/kg in the dog to 20 mg/kg in the monkey.

Zileuton also inhibited both LTB₄ and leukotriene E₄ (LTE₄) biosynthesis induced by an anaphylactic reaction in the rat peritoneal cavity. Inhibition was seen with both enantiomers with no significant difference in potency between them or zileuton.

compound.

Zileuton showed activity against arachidonic acid and antigen-induced bronchospasm. Zileuton did not inhibit smooth muscle contraction caused by several agonists. In addition, zileuton was effective at inhibiting antigen induced late phase bronchoconstriction and hyperactivity in sheep.

Zileuton had no important CNS activity. Zileuton has little cardiovascular effects.

Zileuton bound with *in vitro* to androgen receptors from prostate glands of dogs, rats, monkeys and man. The highest affinity was found in dog prostate as compare to androgen receptor affinity in human prostate, which is 91-fold weaker. The relative high species-specific affinity of zileuton

to androgen receptor in dogs may explain the occurrence of prostatic and testicular atrophy in this species.

It had a minimal antipyretic effect in rats that was not as great as that of aspirin. Zileuton did not produce an antipyretic effect in lipopolysaccharide-treated rabbits. Zileuton had minimal effects on intraocular pressure in rabbits.

Absorption, Distribution, Metabolism and Excretion

The absorption, distribution, metabolism and excretion of zileuton were investigated in mice, rats, dogs and monkeys. The disposition of [¹⁴C]-R(+)/unlabeled S(-) and [¹⁴C]-S(-)/unlabeled R(+) zileuton pseudoracemates was studied in dogs to determine the fate of each enantiomer in the presence of the other. The doses used in these studies range from those which approximate therapeutic doses in humans to those used in subchronic or chronic toxicity studies in animals. Most of the metabolism studies were done using single oral doses, but some studies in rats and monkeys involved multiple doses for 4 to 9 days. Intravenous doses were included in rat and dog studies to provide bioavailabilities. Data obtained from many of these studies were evaluated using a modified chromatographic procedure capable of resolving zileuton and Abbott-66193. Zileuton labeled with carbon-14 was used in all of the metabolism studies. Metabolic patterns in biological samples were determined using high pressure liquid chromatography in conjunction with a radioactivity flow monitor.

Absorption

Oral doses of zileuton are well absorbed in mice, rats, dogs and monkeys. Peak times of parent drug occurred between 1 and 6 hours. Peak plasma concentrations and AUC values increased nearly proportionally with increasing dose size in mice and rats. The plasma levels of zileuton were 1-2 fold greater in female rats than in males. Increases in zileuton C_{max} and AUC values were less than proportional to the dose in monkeys due to diminished absorption at higher doses, and did not increase with treatment duration. Following an oral dose of zileuton, AUC values ranged from 4-27 $\mu\text{g}\cdot\text{h}/\text{mL}$ in mice (5-20 mg/kg), 6-1294 $\mu\text{g}\cdot\text{h}/\text{mL}$ in rats (5-300 mg/kg), 40-1398 $\mu\text{g}\cdot\text{h}/\text{mL}$ in dogs (5-400 mg/kg) and 0.3-107 $\mu\text{g}\cdot\text{h}/\text{mL}$ in monkeys (5-400 mg/kg). The normalized AUC based on mg/kg among all species were in the following order: Dog > rat > human > mouse > monkey. However, the normalized AUC based on mg/m^2 were in the following order: rat > mouse > dog > human > monkey. The elimination half-life of zileuton was species dependent, ranging from 0.3 hours in monkeys, 0.9 hours in mice, 2-3 hours in rats, and 7-8 hours in dogs. Comparison of the AUC of zileuton after oral and intravenous doses (5-20 mg/kg) showed that bioavailability of an oral dose was greater in dogs (87-92%) and mice (64%) than in rats (26%) and monkeys (23%). Differences in the bioavailabilities most likely indicate the extent of first pass metabolism.

The high bioavailability estimate of zileuton in dogs is probably related to the slower rate of

metabolism and longer half-life of the R(+) enantiomer. The half-life of the R(+) enantiomer (7.2-7.5 hours) was 1.5 -1.8 fold greater than that of the S(-) enantiomer (4.3-4.8 hours) and the corresponding intravenous AUC values for the R(+) isomer (25-36 $\mu\text{g}\cdot\text{h}/\text{mL}$) were 3-4 fold greater than that of the S(-) isomer (8-10 $\mu\text{g}\cdot\text{h}/\text{mL}$). No evidence for chiral inversion of the R(+) to the S(-) enantiomer was found. A similar difference in half-lives between the R(+) enantiomer (0.44 hours) and the S(-) enantiomer (0.22 hours) was observed in monkeys.

Protein Binding

Protein binding of zileuton at concentrations ranging from 0.1 to 100 $\mu\text{g}/\text{mL}$ ranges from 87-94% in mouse, rat, dog and monkey plasma.

Distribution

Rat: Radioactivity (^{14}C) was rapidly distributed throughout the body of rats 1 hour after a 5 mg/kg oral dose. The highest concentrations were found in the stomach (7 times the plasma concentration of 5.5 $\mu\text{g eq}/\text{ml}$), small intestine (3.6X), bladder (3.5X), thyroid (3X), liver (2X), adrenal (! 7X) and kidney (1.5X). However, peak concentrations in the testes (2.2 $\mu\text{g eq}/\text{ml}$) and prostate (5.7 $\mu\text{g eq}/\text{ml}$) were not reached until 3 hours after dosing.

Pregnant and lactating rats: Whole body autoradiography of an 18 day gravid rat after an oral 5 mg/kg dose of [^{14}C]zileuton demonstrated that radioactivity crossed the placental barrier. Following an oral 70 mg/kg dose of radiolabeled zileuton to lactating rats, total radioactivity was distributed into the milk of dams, but the mean carbon-14 concentrations did not exceed those in plasma (0.5-0.8 the plasma level).

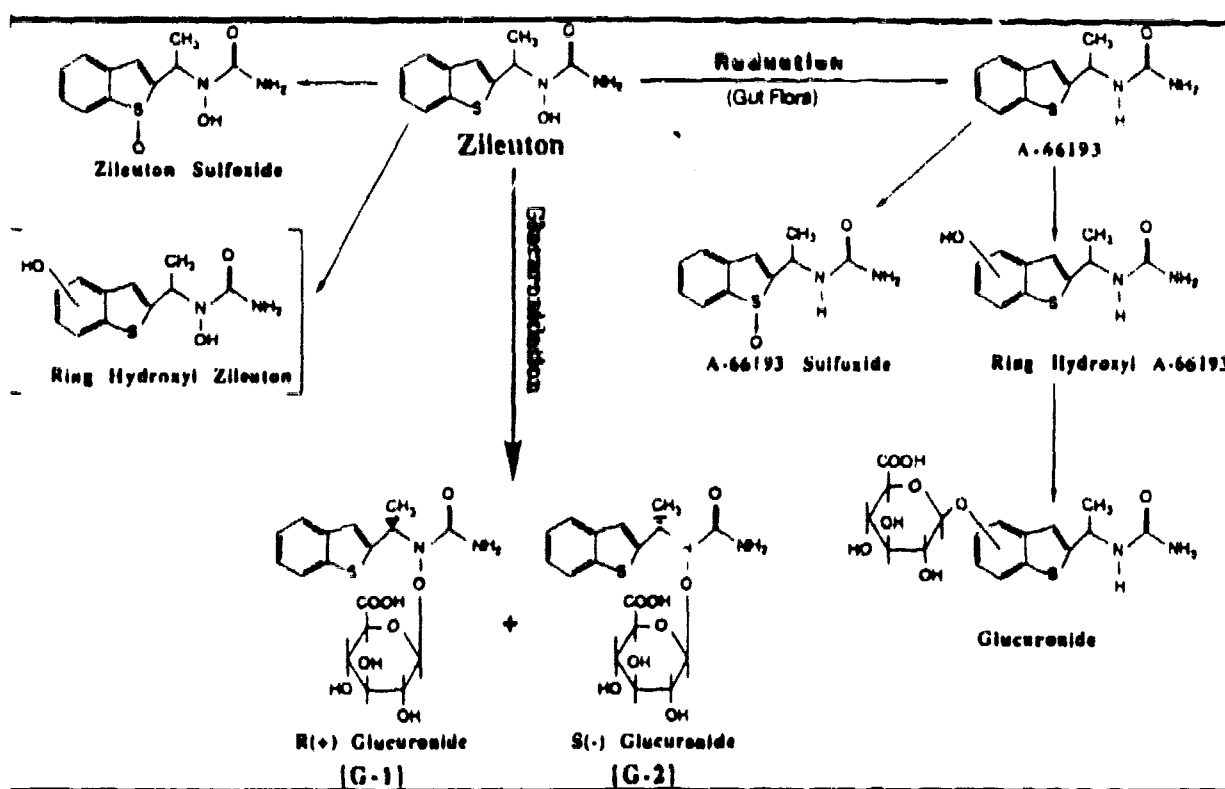
Dog: At 1 hour following a 5 mg/kg oral dose of [^{14}C]zileuton to dogs, the highest concentrations of radioactivity were found in the liver (4X the plasma concentration of 8.1 $\mu\text{g eq}/\text{ml}$) and kidney (1.3X). Carbon-14 concentrations in the lung, testis, prostate, brain, muscle and fat were all less than plasma levels. For the prostate, Tissue/Plasma ratios increased from 0.67 at 1 hour to 1.3 at 8 hours. Very low levels of radioactivity were found in cerebrospinal fluid at 1 or 8 hours. Levels of radioactivity in the retina and uvea were considerably higher and increased from 4.3 $\mu\text{g eq}/\text{g}$ at 1 hour to 16.3 $\mu\text{g eq}/\text{g}$ at 8 hours, indicating a greater affinity of the drug and/or metabolites for pigmented ocular tissue.

Enzyme induction and Inhibition: Daily oral administration of zileuton (20 to 450 mg/kg/day) to rats and CD-1 mice resulted in a moderate induction of hepatic microsomal mixed function oxidase enzymes. Increases in liver weight and microsomal protein in all treatment groups suggested proliferation of the smooth endoplasmic reticulum. In both species, approximately dose related elevations in total cytochrome P-450, benzphetamine N-demethylase, 7-ethoxycoumarin O-dealkylase or 7-pentoxiresorufin O-dealkylase (P-450IIB) activities were similar to phenobarbital treated animals. However, 4-9 fold increases in the cytochrome P-450IVA

mediated 12-hydroxylation of lauric acid were observed only in female mice treated with zileuton and clofibrate. Thus, zileuton is a phenobarbital-type inducing agent in rats and a phenobarbital- or clofibrate-type inducer in mice.

Metabolism:

Metabolic Pathways: Zileuton metabolism is species dependent. The proposed metabolic pathway is given below (reproduced from sponsor's Figure 1 on page 91 on Vol. 68):



In monkeys, the absorbed zileuton is glucuronidated in the liver to form approximately equal amounts of the R(+) and S(-) zileuton glucuronides (G-1 and G-2, respectively). Metabolism of zileuton in the dog is stereoselective. The S(-) enantiomer appears to be exclusively glucuronidated to G-2, while the R(+) enantiomer is metabolized to a variety of uncharacterized metabolites. Formation of G-1 in the dog is a minor pathway. The route of biotransformation in rats is characterized by the formation of a number of polar metabolites (hydroxylated zileuton, its corresponding glucuronide, and zileuton sulfoxide). Formation of G-1 and G-2 is not significant in this species. The pathway in mice is comparable to that found in rats, except that G-2 is also a major metabolite, indicating stereoselectivity in glucuronidation. In all species, the zileuton was reduced to the N-dehydroxylated metabolite (Abbott-66193). This metabolite is not pharmacologically active, but can be further metabolized in the liver to products in rats as ring

hydroxylated Abbott-66193 (M1), its corresponding glucuronide, and Abbott-66193 sulfoxide. Major plasma metabolites of zileuton were variable among species. Circulating metabolites in mice and rats included large amounts of polar material. Significant levels of S(-) zileuton glucuronide (G-2) were found in mouse plasma; R(+) zileuton glucuronide (G-1) and G-2 were minor components of rat plasma. The main metabolites in monkey plasma were approximately equal amounts of G-1 and G-2, while only G-2 was the predominant metabolite in dog plasma. Lesser amounts of circulating polar material in dogs probably represent metabolites of the R(+) enantiomer. Circulating levels of Abbott-66193 were also found in all species and occurred from 3 to 21 hours post-dosing. After single doses (5-400 mg/kg), concentrations expressed as the percentage of parent drug exposure were about 2-6% in mice, 1-3% in dogs, 10-16% in rats and 134% in monkeys and 19% in human. The Abbott-66193 levels increased with the zileuton dose and were as high as 1400% in monkeys after 800 mg/kg/day for 7 days. Interspecies comparison of AUCs of zileuton and Abbott-66193 after multiple oral dosing is given on next page (reproduced from sponsor's table on page 18 of Vol. 22):

Species	24hr Dose (mg/kg/day)	Doseage Regimen	Sampling Period	Sex	AUC 0-24hr ($\mu\text{g}\cdot\text{hr/mL}$)	
					ZILEUTON	Abbott-66193 ^b
MAN	35 ^a	q6hr	-	M+F	77.0	15.3
MONKEY	50	q24hr	day 153	M+F	4.1	12.2
	150	q24hr	day 153	M+F	16.4	94.8
	500	q24hr	day 153	M+F	32.9	262.8
DOG	5	q24hr	day 6	M+F	42.0	1.3
	20	q24hr	day 6	M+F	155.1	4.3
	100	q24hr	day 6	M+F	367.7	3.3
	400	q24hr	day 6	M+F	1239.7	10.8
	20	q24hr	week 22	M+F	288.5	4.9
	50	q24hr	week 22	M+F	434.7	10.2
	100	q24hr	week 22	M+F	624.8	5.3
	200	q24hr	week 22	M+F	890.5	13.9
	10	q24hr	week 48	M+F	115.0	ND ^c
	25	q24hr	week 48	M+F	283.6	ND
	50	q24hr	week 48	M+F	436.5	ND
RAT	300	q24hr	day 4	M	646.8	151.9
			day 4	F	1366.6	257.1
	80	SD ^a	day 1	M	333.2	30.7
	300	SD	day 1	M	1294.3	133.6
	20	q24hr	day 345	M	102.3	20.1
				F	201.9	29.0
	50	q24hr	day 345	M	263.4	59.7
				F	596.8	100.9
	150	q24hr	day 345	M	700.6	188.0
				F	1262.7	249.9
	45	via diet	week 75-76	M	187	32
				F	250	49
				M	285	74
F				456	90	
M				498	131	
F				1043	237	
170	diet	week 75-76	M	45	0.13	
			F	69	0.26	
			M	139	4.01	
			F	160	5.13	
			M	529	22.60	
			F	340	17.22	
MOUSE	50	via diet	Week 93-94	M	45	0.13
				F	69	0.26
	150	via diet	Week 93-94	M	139	4.01
				F	160	5.13
	450	via diet	Week 93-94	M	529	22.60
				F	340	17.22

Excretion

Urinary was a major route of excretion (53-96%) and fecal excretion was a minor route of excretion for eliminating zileuton and its metabolites from the body. Biliary excretion does not appear to be a major route of elimination for zileuton. Only 0.1-2% of free parent drug was recovered in the urine after an oral dose. The pattern of urinary metabolites is species dependent and similar to that seen in plasma. Major metabolites in monkey urine are G-1 (22-38% of the dose) and G-2 (26-49% of the dose), whereas G-2 predominates in dog urine, accounting for about

60% of the dose. In rat and mouse, polar metabolites represent 30-54% of the radioactive dose. The polar material in rat urine were ring hydroxylated and sulfoxide metabolites of zileuton and/or Abbott-66193. Glucuronide conjugates of ring hydroxylated zileuton/Abbott-66193 have also been detected. Urine levels of G-1 and G-2 were 0.6-1 and 2-5% of the dose in rats and 0.0 and 16% of the dose in mice, respectively. Interspecies comparison of urinary excretion of zileuton and its metabolites is given below (reproduced from sponsor's table 3 on page 212 of vol. 1):

Dose (mg/kg)	Route	Zil	Polar	Percent of the Carbon-14 Dose				
				RPM	G-1	G-2	M-1	Others
				Urine				
5	po	nd	15.15	nd	0.6	15.88	2.04	4.36
5	po	1.77	53.9	nd	1.06	3.2	1.4	6.25
	iv	8.23	45.9	nd	0.89	2.01	1.3	6
170	po	0.09	30.27	0.24	0.59	1.91	0.34	4.46
170 (2 yr)	po	0.19	37.24	0.16	1.14	5.05	0.19	2.83
170-D*	po	0.19	46.11	0.24	1.89	3.9	0.44	8.01
170-D (2 yr)*	po	nd	41.51	nd	4.69	10.38	0.04	3.6
5	po	nd	15.3	nd	3.1	60.4	nd	2.2
	iv	nd	14.5	nd	2.8	56.7		3.8
5, [¹⁴ C] R	po	nd	32.29	nd	7.62	0.65	0.23	4.08
	iv	nd	32.31	nd	6.76	0.55	0.51	4.93
5, [¹⁴ C]-S	po	nd	0.14	nd	nd	77.5	nd	0.73
	iv	nd	0.11	nd	nd	75.56	nd	1.36
5	po	nd	4.3	nd	38.2	48.8	nd	0.9
400	po	0.16	14.14	nd	22.26	26.12	0.46	0.96
		0.29	20.51	nd	18.87	22.66	0.04	1.57
ca. 5.7, po	po	nd	8.2	nd	35.3	43.2	nd	0.4

Summary of ADME:

Oral doses zileuton were well absorbed, extensively metabolized and excreted primarily into the urine of mice, rats, dogs, monkeys and humans. Interspecies comparison of ADME is shown below (reproduced from sponsor's table 5 on page 214 of Vol. 1) :

Parameter	Mouse (5-20)	Rat (5-300)	Dog (5-400)	Monkey (5-400)	Human (ca. 5.7)
Plasma					
<u>Zileuton</u>					
C _{max} (µg/mL)	1.8-16.1	2.3-104	4.6-89.7	0.3-9.0	2.26
T _{max} (h)	1	1 to 6	1-11.3	0.5-2	3
t _{1/2} (h)	1	1.4-3.3	7.3-11.9	0.4	3.8
AUC (µg·h/mL)	4.0-26.5	6.4-1294	39.7-1398	0.3-107	13.78
<u>Metabolites</u>					
AUC (µg·h/mL)					
Abbott-66193	0.08	0-54.1	nd	0-143	2.87
Polar	6.64	9.6-330	1.64	nd	nd
G-1	nd	nd	0.28	4.4-523	9.82
G-2	1.42	nd	11.14	3.9-399	9.30
M-1	nd	nd	nd	nd	nd
RPM	4.74	9-44.1	nd	nd	nd
<u>Excretion</u>					
Urine (% Dose)	74.3*	52.7-72.8*	88.1*	71.6-96*	94.48
Feces (% Dose)	15.6	20.7-24.8	14.2	0.33-18.5	2.21
Bile (% Dose)	na	na	6.01	na	na
<u>Metabolism</u>					
<u>Urine (% Dose)</u>					
Zileuton	nd	0.09-1.8	nd	0-0.2	nd
Abbott-66193	nd	nd	nd	nd	nd
Polar	45.45	30.3-53.9	15.3	4.3-14.1	8.20
G-1	0.6	0.59-1.14	3.1	22.3-38.2	35.30
G-2	15.88	1.9-5.1	60.4	26.1-48.8	43.20
M-1	4.36	0.34-1.4	nd	0-0.5	nd
RPM	nd	0-0.24	2.2	nd	nd
<u>Feces (% Dose)</u>					
Zileuton	0.1	nd	na	1.7	na
Abbott-66193	4.8	3.1-4.2	na	16.22	na
Polar	0.64	1.4-2.0	na	nd	na
G-1	nd	0.5-0.8	na	nd	na
G-2	nd	0-0.06	na	nd	na
M-1	9.55	1.6-6.6	na	nd	na
Unknown (14 min.)	nd	6.3-9.4	na	nd	na
<u>Bile (% Dose)</u>					

Major organs of distribution in rats were GI tract, bladder, thyroid, liver, adrenal and kidney. Presence of drug in testes and prostate was indicated at slower rate. In dogs, liver and kidney were major organs of distribution. Accumulation of drug in prostate was indicated.

It was highly protein-bound in all species. It was an enzyme inducer in rodents.

In pregnant or lactating rats, zileuton crossed the placental barrier and excreted into milk.

No single animal species represents an ideal model for both the pharmacokinetics and metabolism of zileuton in humans. Comparison of urinary and plasma metabolites indicates that the metabolic pathways for the absorbed zileuton, as well as the extent of its reduction to Abbott-66193, are species-specific. In the rodent species, the metabolic profile in urine are unlike those observed in humans. However, plasma profile of zileuton and Abbott-66193 were similar between human and rats. In man and monkey, zileuton was glucuronized in the liver. However, the very short half-life of zileuton in monkeys (0.3 hours) as compared to humans (2-4 hours) suggests that the rate of glucuronidation and excretion were rapid in monkeys, resulting in less systemic exposure. Thus, the monkey may not be a suitable pharmacokinetic model for human. Metabolism studies in the dog have shown that the R(+) enantiomer persists in the plasma and is responsible for the long half-life (7.5 hours) of zileuton in dogs. Furthermore, different plasma profile of zileuton and Abbott-66913 in dogs suggests that the dog may not be a relevant pharmacokinetic model for human.

Toxicology

Acute Oral Toxicity Evaluation of Two Enantiomers (Abbott-68967 and 68968) in Mice (Study no. TD-92051, Vol 22)

Testing laboratory: Abbot Lab

GLP requirement; A statement of compliance with GLP regulation was included

Methods: Five/sex/group were administered with zileuton, R-enantiomer (Abbott-68967) and S-enantiomer (Abbott-68968) suspended in 0.2 % HPMC at dosing volume of 30 ml/kg and at dose ranges of 2.38-3.36, 0.66-1.97 and 1-4.4 mg/kg, respectively. Observation period was 2 weeks.

Results: Clinical signs of decreased activity, ataxia, dyspnea, squinting, prostration, unconsciousness, coma or death were observed in all drug-dosed groups. All signs were noted on or before day 5. Decreased body weight gains and /or body weight losses were noted. No gross pathology were reported. LD50s for zileuton, Abbott-68967 and Abbott-68968 were 2.72, 1.21 and 2.1 mg/kg, respectively. Thus, the oral LD50s for both R and S enantiomers were lower than the oral LD50 for zileuton, indicating higher toxic potential of either enantiomer. The data suggested that further development of either enantiomer was not warranted.

**Acute Oral Toxicity of Abbott-66913 in Mice
(Study no. T87-440, Vol. 22)**

GLP requirement: The study was not conducted with the intention of being used in critical support of an application.

Methods: Ten male mice were given Abbott-66913 in suspension of 0.2 % methylcellulose by gavage at dose level of 4 g/kg. Observation was 14 days

Results: Decreased activity and squinting was observed.

**Acute Oral Toxicity of Abbott-66193, 65813, 66795 and 68418 in Male Mice
(Study nos. TX-92-276, 275, 274 and 280, Vol. 22)**

GLP requirement: No statements of compliance with GLP regulations were included.

Methods: Five mice/sex/group were administered a single oral dose of Abbott-66193 (metabolite), 65813, 68418 and 66795 (degradants of zileuton) at doses of 1000-6000, 800-4000, 20-100 and 1000-6000 mg/kg, respectively. Clinical signs of decreased activity, ataxia, squinting and dyspnea were reported in the 65813 and 66913 groups. Increased activity, ataxia, jerks, dyspnea and clonic convulsions were observed in the 68418 group whereas decreased activity, ataxia, tremors, squinting, dyspnea and prostration were seen in the 66795 group. The lethal doses were 6, 4, 4 and >0.1 g/kg for 66913, 65813, 66795 and 68418, respectively. No effect doses were 2, 0.8, 1 and 0.04 g/kg, respectively.

**Ames Test of Abbott-66193, 65813, 66795 and 68418
(Study no. TX 92-382, Vol. 65)**

Testing laboratory: Sponsor

Methods: Detail description of methodology was not provided. Abbott-66193, 65813, 66795, and 68418 at concentrations of 1-10000 ug/plate were tested on TA 1535, 1537, 98 and 100 strains of *Salmonella typhimurium* for mutagenicity in the presence or absence of metabolic activation. Dimethyl sulfoxide was used as a negative control. N-methyl-N'-nitro-N-nitrosoguanidine, quacrine mustard, 2-nitrofluorene were used as the positive controls in the presence of metabolic activation and 2-Aminoanthracene was used as the positive control in the absence of metabolic activation. A dose-related 2-3 fold increase over vehicle control colony was the criteria of a positive response.

Results: Lack of 2-3 fold nor dose-related increase over vehicle control colony counts in all four strains by all four compounds, indicating all of the degradants were non-mutagenic to *Salmonella typhimurium*.

3 Month Oral Toxicity of Combination with Prednisone or Naproxen in Rats
(Study no. TA90-324, Vol. 54)

Testing laboratory: Sponsor

GLP requirement: A statement of GLP compliance was included.

Methods: Six groups of rats (6/sex/groups) were given vehicle, zileuton, prednisone, naproxen, zileuton/prednisone and zileuton/naproxen at oral doses of 80, 0.9, 21, 80/0.9 and 80/21 mg/kg/day, respectively, for 3 months. Following parameters were monitored.

Clinical signs	daily
Body weight	weekly
Food consumption	weekly
Ophthalmoscopy,	end of 3 month
Urinalysis	termination
Hematology and blood chemistry	termination
Pharmacokinetics	3 hours after treatment on days 2 and 33
Organ weight	termination
Gross and histopathology	termination

Results:

Mortality: One female rat in the pharmacokinetic group that received 80/21 was found dead on day 27 but without any clinical signs of toxicity. One female given 21 mg/kg/day of naproxen was found dead on day 89. Weakness, dehydration and emaciation were observed prior to death. The death was drug-related.

Clinical signs: Except those mentioned above, one additional male rats received 80/21 exhibited emaciation, dehydration, decreased activity and red dry discharge from the nose were observed.

Body weight: Normal.

Food consumption: Normal.

Ophthalmoscopy: Normal.

Plasma drug level: Plasma levels (ug/ml, 3 hours after dosing) are summarized below:

test material	zileuton	prednisone	naproxen	zileuton/ prednisone	zileuton/ naproxen
dose (mg/kg)	80	0.9	21	80/0.9	80/21
day 2 M zileuton F	26 47			32 49	20 26
day 2 M naproxen F			30 45		22 30
day 33 M zileuton F	47 58			42 61	28 24
day 33 M naproxen F			53 51		48 43

Slight accumulations of zileuton and naproxen were observed in male rats on day 33. Plasma level of zileuton treated with zileuton/naproxen appeared to be lower than those receiving zileuton alone.

Urinalysis: Normal

Hematology: Similar lower hematocrit (10%), hemoglobin (12%) and red blood cell (15%) were observed in naproxen and zileuton/naproxen groups. Again, same degree of elevation (30%) of platelet count was reported in naproxen and zileuton/naproxen group. Thus, data indicate the changes were related to naproxen with no drug interaction due to zileuton.

Blood chemistry: Lower serum protein with same degree of depression (17%) were reported in both naproxen and zileuton/naproxen groups, again indicating no drug interaction.

Organ weight: Female rats given zileuton/naproxen combination had higher absolute and relative kidney weight (22%) than the control, zileuton or naproxen groups. Zileuton alone (18%) or zileuton/naproxen (25%) combination produced higher liver weight in females.

Gross pathology: Findings in small intestine were observed in two zileuton/naproxen group and changes in the abdominal cavity of one rat given naproxen were reported.

Histopathology: Treatment related changes were observed in the liver (hypertrophy) of the zileuton, zileuton/prednisone and zileuton/naproxen groups as well as the kidney (papillary degeneration and/or necrosis) and nephritis) and GI tract (gastritis and ulceration) of naproxen and zileuton/naproxen groups. Incidence of liver changes was observed similar between two group. Slight higher incidence

of papillary necrosis and gastric ulcers (20 vs 10%) were observed in zileuton/naproxen group.

Inhibition by Zileuton of Testosterone Production in Rat Leydig Cell Bioassay
(Study no. TX92-512, Vol. 58)

Methods: The Leydig cells were incubated with various concentrations of test compound for two hours, LH was added, and the cultures incubated for an additional one hours. Ketoconazole was included as a comparative control.

Results: EC 50's for zileuton and Abbott-66193 were 80 and 56 ug/ml, respectively. No inhibition was seen with two urinary metabolites at concentrations up to 200 ug/ml.

Plasma Clearance of Testosterone in Male Rats after Chronic Administration of Zileuton
(Study no. 93-097, Vol. 52)

Methods: Dietary administration of zileuton at dose of 170 mg/kg/day or control for 21 days. On day 22 ³H-testosterone (i.v.) was given, blood samples were taken at 0.12, 0.25, 0.5, 0.75, 1 and 1.25 hr.

Results: Plasma concentrations, AUC and plasma clearance were similar between the zileuton-treated and control groups. The data indicated that the administration of zileuton for 21 days had no effect on the plasma clearance of testosterone.

Preliminary DNA Adduct Study
(Amendment of 3/1/95)

Methods: Female CD-1 mice were treated with either zileuton at 0, 50, 150 and 450 mg/kg (po) or the positive controls (2-naphthylamine and benzo[a]pyrene, 50 mg/kg, ip) for 4 days. The mice were euthanized 2-4 hours after the last dose. DNA was extracted from the liver and kidneys and examined for DNA adducts using the P32-postlabeling technique.

Results: Mean relative adduct levels (RAL) per one billion nucleotides in liver and kidneys following 4 daily treatment of zileuton are summarized below:

Dose (mg/kg/day)	Liver RAL	Kidney RAL
0	8	8
50	13	24
150	31	52
450	37	115
Positive controls	1146	883

Dose-related increases in DNA adducts of liver and kidneys were observed. Positive control yielded a typical result. Thus, oral administration of zileuton produced DNA adduct formation in liver and kidneys of female mice.

14 Day Immunological Evaluation in Female Mice
(Study no. TD-91-387, Vol 56)

Methods: Five groups of female mice each consisting of 8 animals were administered Zileuton orally at dose levels of 0 (0.2 % hydroxypropylmethylcellulose), 0 (saline), 150, 300 or 450 mg/kg for 15 days. A positive control group received cyclophosphamide (ip, 25 mg/kg/day) during days 11 through 15. On day 11, all mice were immunized with sheep erythrocytes and on day 15, 2.5 hrs after the last drug administration, the IgM spleen AFC response was measured. The data were expressed as AFC per one million spleen cells or per spleen.

Results: Dose-related decreases in spleen IgM antibody-forming cell (AFC) were seen in the zileuton-treated groups (21-44%). There were no zileuton-related changes in spleen weight, spleen cellularity or thymus weight. Positive control yielded typical decrease (99.6%) in AFC and reduced thymus weight, spleen weight and spleen cellularity.

14 Day Immunological Evaluation in Female Rats
(Study no. TA 90-256, Vol 56)

Methods: Similar procedure as in the above-mentioned female mice study, zileuton was administered to female rats at dose levels of 80, 150 and 300 mg/kg/day for 15 days.

Results: No immunotoxicity was observed following oral administration of zileuton to rats at doses up to 300 mg/kg/day.

Effect of Testosterone Supplement on the Development of Leydig Cell Tumor by Zileuton

This is an preliminary summary report submitted in the Amendment of 3/29/95.

Methods: Zileuton at dietary dose of 170 mg/kg/day was administered to male Fisher 344 rats (10/group) of 9 months of age with or without subcutaneous testosterone implants for 24 weeks.

Results: The incidence of Leydig cell tumor is summarized below:

Dose	0	170 mg/kg/day	170 mg/kg/day
Testosterone Supplement	-	-	+
Leydig cell tumor	0/10	4/10	0/10

The data indicates that supplementation with exogenous testosterone prevents the development of Leydig cell tumors induced by zileuton.

Toxicology Summary

The toxicology of zileuton has been studied in mice, rats, dogs, and monkeys in studies ranging in duration from a single dose to two years of oral administration. Drug-induced effects in animals in kidney, and on hematopoietic and reproductive systems.

Single Dose Toxicity

When given orally in 0.2% HPMC, the median lethal dose (LD_{50}) generally exceeds the limit dose of 4.0 g/kg for both mice and rats. Toxic signs for both species consisted of decreased activity, ataxia, prostration and coma. Dark brown material in the small intestine and mucosal reddening and hemorrhages in the stomachs of some animals at necropsy indicated gastrointestinal irritation. In rats, metabolite, Abbott 66913 or degradant, Abbott-65813 was less toxic than the parent compound. Degradants, abbott-66795 and 68418 were more toxic than the parent compound.

When administered subcutaneously, the LD_{50} for both mice and rats was greater than the limit dosage of 5.0 g/kg. Toxic signs in mice included decreased activity, ataxia, squinting and dyspnea; no signs of toxicity were observed in rats. When administered intraperitoneally, LD_{50} values ranged from 0.72 to 1.03 g/kg for both species. Signs of toxicity included decreased activity, ataxia, prostration and coma.

Ataxia, emesis, loose or mucoid stools, lacrimation, hyperactivity and excessive thirst were observed at doses of 500 mg/kg or higher in dogs orally administered zileuton. Nephritis was seen at doses of 500 and 1000 mg/kg.

The LD_{50} s for the individual enantiomers administered orally to mice in 0.2% HPMC were 1.21 g/kg for the R-enantiomer (Abbott-68967) and 2.10 g/kg for the S-enantiomer (Abbott 68968). These values were similar to or lower than that for the racemic mixture, 2.72 g/kg. There were no differences in toxic signs among the three treatments. In addition to pharmacodynamic data of no apparent difference being observed among these three compounds, the acute toxic data may further suggest that development of either enantiomer rather than racemic mixture is not warranted.

Subchronic toxicity

Mouse: A three month dietary study was conducted in mice at dosages of 0, 80, 200, 450 and 1000 mg/kg/day to select dosages for the two-year carcinogenicity study. Increased liver weights and centriobular hepatic cytomegaly observed at 450 mg/kg/day or higher were considered secondary to hepatic enzyme induction. Toxicity consisted of hepatocellular vacuolation at 1000

mg/kg/day. Judging from no effect on body weight gain but some liver effects, we concluded that MTD could be 1000 mg/kg. However, lower dose could be considered the MTD if additional effects are seen at end of the two year carcinogenicity or sufficient exposure of compound is demonstrated.

Rat: A three-month rat study was conducted by gavage at 0, 5, 20, 80, and 300 mg/kg/day. No drug-related deaths were observed, clinical signs of alopecia and salivation was reported. Increases in liver weight and centrilobular hepatic cytomegaly were considered secondary to hepatic enzyme induction at 80 mg/kg and above. Elevated serum cholesterol, calcium, phosphorus and protein values were also seen at 300 mg/kg/day. Prolonged estrus was observed for female rats administered 300 mg/kg/day. Use of a specific histochemical technique for proliferating cell nuclear antigen (PCNA) indicated an increase in kidney proximal tubular epithelial cell proliferation at one and three months in male rats and three months in females. A dosage of 20 mg/kg/day was considered to be the no-toxic-effect dosage for both treatment durations. Tolerated dose of 80 mg/kg was established.

A three-month dietary study was conducted in rats at dosages of 0, 80, 170, 350 and 750 mg/kg/day to select dosages for the two-year carcinogenicity study. Decreased body weight gain and food consumption were observed at 350 mg/kg/day and above. Increased urinary protein loss and serum proteins were observed at these same dosages. Liver and kidney weights were increased at 170 mg/kg/day and uterine and ovarian weights were reduced at 750 mg/kg/day. Hepatocellular hypertrophy was observed at all dosage levels and chronic nephritis was observed at 350 mg/kg/day and higher. Renal proximal tubular epithelial cell proliferation, as determined by PCNA staining, was increased in females at 80 mg/kg/day and above and in males at 170 mg/kg/day. A tolerated dose of 80 mg/kg was established.

A six-week oral toxicity study was conducted in immature (14 days old) rats at dosages of 0, 20, 80 and 300 mg/kg/day. Salivation and alopecia; reduced body weight gain; decreased food consumption; increased RBC, serum cholesterol and serum protein values; increased liver weight and hepatocellular hypertrophy were all observed at 80 mg/kg/day and higher. Adverse effects, were reversible within a two-week recovery period. It prolonged estrus at 300 mg/kg. Thus, a tolerated dose of 80 mg/kg/day was established. It appears that immature rats was not more sensitive than adult rats in term of ziletuon's toxicity.

Dog: A three-month oral dog toxicity study was conducted at dosages of 0, 5, 20, 100 and 400 mg/kg/day. Plasma level exposures were approximately 1, 3, 9 and 18 times the expected clinical exposure to ziletuon. Lethal dose was 400 mg/kg. Clinical signs of decreased activity, dehydration, emesis, emaciation, ataxia, mucoid stools and decreased body weights were limited to the highest dosage group. Slightly decreased RBC parameters and evidence of bone marrow hyperplasia (males only) were observed for dogs at 400 mg/kg/day. Testicular and prostate weights were decreased for males at 100 and 400 mg/kg/day after one month of treatment, but only at 400 mg/kg/day after three months. Prostatic and testicular atrophy were observed in males

at 20 (one dog), 100 and 400 mg/kg/day after one month of treatment, but testicular atrophy/degeneration was only observed at 100 (one dog) and 400 mg/kg/day and no prostatic atrophy was observed after three months. No other consistent changes were observed in this study. The no effect dose was considered 5 mg/kg/day after one month of treatment and 20 mg/kg/day after three months.

In a separate study, dogs were administered zileuton orally at a dosage of either 0 or 100 mg/kg/day for one month and groups were then allowed to remain untreated for one, three or six months to determine the reversibility of any testicular or prostatic changes induced. The plasma level exposure in the zileuton-treated group was 9 times the anticipated clinical plasma exposure. At the end of the one-month treatment and one-month recovery periods, decreased spermatogenesis and testicular cellularity were observed. No drug-related testicular changes were observed after the three- or six-month recovery periods. Prostatic atrophy was still observed after the three- and six-month recovery periods.

Monkey: A one-month oral toxicity study was conducted in monkeys at doses of 0, 20, 50, 150 and 360 mg/kg/day. No drug-induced toxicity was observed at any of these dosage levels, no effect dosage was considered to be at least 360 mg/kg/day.

Chronic Toxicity

Rat: Zileuton was administered to rats by oral gavage at dose levels of 0, 20, 80, 150 or 300 mg/kg/day for six months. Salivation was observed at 150 and 300 mg/kg. Increased liver weights and hepatocellular hypertrophy were seen at all dosage levels. Proteinuria and nephropathy were also observed at 150 and 300 mg/kg/day. Increased thyroid weight in males and luminal dilatation and subacute endometritis of uterus in females were observed at 300 mg/kg/day. These changes were reversible following a one-month recovery period. The NOAEL dose of 80 mg/kg/day was established.

Zileuton was also administered to rats by gavage at dosages of 0, 20, 50, and 150 mg/kg/day for one year. Plasma level exposures near the end of the treatment period for males were approximately 1, 3 and 9 times (males) and 3, 8 and 16 times (females) expected clinical exposure. No drug-related deaths occurred and clinical signs were limited to salivation and red discharge from the eyes, nose and mouth at 150 mg/kg/day. Increased liver weights and hepatocellular cytomegaly were seen at 50 and 150 mg/kg/day. Proteinuria, increased kidney weights and increased nephropathy were observed at 150 mg/kg/day. The tolerated dose of 50 mg/kg/day was established.

Dog: Dogs were administered zileuton at dose level of 0, 20, 50, 100 and 200 mg/kg/day for six months. Plasma level exposures were approximately 3, 5, 11 and 16 times, respectively, the proposed clinical exposure. Drug accumulation was not observed. Mortality was observed at 100 mg/kg/day and higher. Body weight loss, glucosuria and ketonuria were observed at 200

mg/kg/day. Hematological changes were reported at 50 mg/kg and higher. Prostatic atrophy and testicular degeneration seen at 200 mg/kg/day were not reversible at end of 4 week recovery period. Prostatic atrophy was observed at 100 mg/kg. Renal anisokaryosis and karyomegaly were observed at 50 mg/kg/day and above with minimum being observed at 20 mg/kg. Thus, 20 mg/kg/day was considered to be the NOAEL. Lethal dose was 100 mg/kg.

A one-year study was also conducted in dogs with oral administration of zileuton at dosages of 0, 10, 25 and 75/50 mg/kg/day. The dose of 75 mg/kg was reduced on day 74 to 50 mg/kg because of excessive toxicity. Plasma exposures were approximately 1.5, 4 and 6 times, respectively, the proposed clinical exposure. Inappetence, emesis and decreased body weight gain were observed at the highest dosage. Marked hematological changes (neutropenia, thrombocytopenia and/or anemia) were observed in high-dosage dogs. Glucosuria (high dosage only). Renal tubular epithelial karyomegaly, cortical tubular atrophy and subacute inflammation were observed in the 50 mg/kg/day group and occasionally in the 10 and 25 mg/kg/day group dogs. An increased severity of renal cortical tubular vacuolation was also observed in dogs administered 25 or 50 mg/kg/day. Most of toxic effects were nonreversible or incomplete reversible. Testicular degeneration was observed in the highest dose group. Drug-related effects observed in the 10 mg/kg were limited to minimal renal histologic changes in kidney and urinary bladder without measurable renal function deficit. Thus, a NOAEL of 10 mg/kg was estimated. A narrow safety margin was provided by this study based on the drug exposure (AUC) at this dose since it represents only 1.5 times the human exposure. Lethal dose was considered to be 75/50 mg/kg. Based on the AUCs of the no male-reprotoxic doses of 20, 50 and 25 mg/kg in the 3, 6 and 12 month toxicity studies, respectively, as compared to that of the human dose of 600 mg qid, there was a safety margin of 2-6 fold between the dog and human.

Monkey: Six-month and one-year oral toxicity studies were conducted in monkeys at dosages of 0, 50, 150 and 500 mg/kg/day. Systemic exposures to zileuton at the highest dosage were only about 50% of the expected clinical exposure. However, exposures for Abbott-66193, the N-dehydroxylated metabolite, were up to 10 times the expected clinical exposure for this metabolite. No treatment-related toxicity was seen in either of these studies, so the no-toxic-effect dosage was at least 500 mg/kg/day.

Interaction Studies: A special three-month toxicity study was conducted in rats to investigate the potential for toxic interactions between zileuton and either prednisone or naproxen. Rats received oral dosages of zileuton (80 mg/kg/day), prednisone (0.9 mg/kg/day) or naproxen (21 mg/kg/day) either alone or in combination. No significant drug interaction was observed between zileuton and either naproxen or prednisone.

Reproduction Studies

Fertility and General Reproductive Performance: Zileuton was administered to female rats at dosages of 0, 15, 70 and 300 mg/kg/day for two weeks prior to mating with untreated males to

determine any effects on fertility and general reproductive performance. No effects were observed on the estrus cycle, or fertility. Prolongation of gestation periods, and decreased litter size were observed at 70 mg/kg and above. Decreased pup weight was observed at 300 mg/kg.

A second study was conducted in which both male and female rats were administered zileuton orally at 0, 15, 75, 150 and 300 mg/kg/day. Males were treated for at least 80 days prior to mating with either untreated or treated females. It had no antifertility effects on Males. It induced occurrence of protracted estrus period, decreased the number of implantation sites and viable fetuses, and decreased pup weight at 150 mg/kg. At 300 mg/kg, it produced lower fetal weight, decreased pup viability, prolonged gestation period and increased still birth. However, 300 mg/kg appear to be maternally toxic. Thus, it had no antifertility effects on females but produced embryo and fetal toxicity and should be indicated in the labeling.

Teratogenicity: Zileuton was administered orally to mated female rats at dosages of 0, 15, 70, 150 and 300 mg/kg/day on gestation days 6-15. No treatment-related abnormalities were observed at any dosage, but an increase in skeletal variations and ossification delays were observed at 300 mg/kg/day.

Zileuton was administered to mated female rabbits by gavage at dosages of 0, 15, 50 and 150 mg/kg/day on gestation days 6-18. Three instances of cleft palate in three fetuses (3/118 or 2.5 %) and in two litters (2/17 or 11 %) from the highest dosage group were slightly above historical ranges (0-2 and 0-7%, respectively) for the laboratory and may be considered treatment related. Moreover, two fetuses of the 150 mg/kg group had domed head and hydrocephalus. Therefore, it may poses a teratogenic potential. Pregnancy category C has been appropriately indicated in labeling.

Peri-/postnatal development study: Mated female rats were administered zileuton orally at dosages of 0, 15, 70 and 300 mg/kg/day from gestational day 15 through postpartum day 20. Ataxia, decreased activity, decreased weight gain and food consumption and increased gestation length were observed at 300 mg/kg/day. A tendency for increased gestation length was also observed at 70 mg/kg/day. Decreased pup weight and pup survival rate were noted for dams administered 300 mg/kg/day.

Mutagenic Potential: It did not induce any increase in revertant colonies in *E. coli* WP2A *trp*^{uvrA} at concentrations up to 1 mg/plate. A test for chromosomal aberrations in mammalian cells *in vitro* consisted of a chromosome aberration assay using human lymphocytes at concentrations up 185-370 ug/ml. Tests for gene mutations *in vitro* in eukaryotic systems included a forward mutation assay at the HGPRT locus in Chinese hamster ovary (CHO) cells and an unscheduled DNA synthesis (UDS) assay using rat primary hepatocyte at concentration up to 30 ug/ml. *In vivo* tests for genetic damage included a mouse micronucleus test at ip doses up to 940-1700 mg/kg. All of the mutagenicity assays except the CHO/HGPRT assay were negative. The CHO/HGPRT was

considered inadequate in the activation phase because of the low cytotoxicity (see Memo of May 11, 1995 by Dr. Casciano). However, a dose-related increase in DNA adduct formation in kidneys and liver of female mice treated with zileuton was observed at doses of 50-450 mg/kg. Two additional follow-up genotoxicity studies (mouse lymphoma assay and in vivo/in vitro UDS assay) were recommended following consultation on the interpretation of these findings from FDA experts. These studies were reviewed by Dr. Casciano of HFT-120 (see Memo of Sept. 1, 1995). For the lymphoma assay negative results were reported at concentrations up to 250 ug/ml in both activation and non-activation phases, however, it was considered inadequate in the activation phase because of the low cytotoxicity. For in vivo/in vitro UDS assay, zileuton at concentrations of 2.5-101 ug/ml did not induce any DNA damage in hepatocytes isolated from the zileuton pretreatment group (150 mg/kg/day, p.o., x4). However, zileuton at concentrations of 10-505 ug/ml produced a dose-related increase in mean net nuclear grains in hepatocytes isolated from the Aroclor 1254 (500 mg/kg, i.p., x1) treated rats. Thus, the data show that zileuton should be considered genotoxic.

Several degradants/metabolites of zileuton, Abbott-56193, Abbott-65813, Abbott 66795 and Abbott 68418 were also tested in the Ames test. All were found to be negative in this assay.

Carcinogenic Potential: A two-year dietary carcinogenicity study was conducted in mice at dosages of 0, 50, 150 and 450 mg/kg/day. A statistically significant increase in hepatic tumors was observed among female mice administered 450 mg/kg/day. Increased incidence of kidney tubular tumors was also observed for mice administered 450 mg/kg/day. A significantly high incidence of hemangiosarcomas in several tissues or organs, ovarian in particular, was also observed among female mice administered 450 mg/kg/day. Although a trend of increased incidence of hemangiosarcoma was suggested in male rats but was within the range of the historical control.

A two-year carcinogenicity study with zileuton was conducted in rats administered 0, 40, 80 and 170 mg/kg/day. Plasma level exposures were approximately 2, 4 and 6 times clinical exposure for male rats and 3, 6 and 14 times clinical exposure for female rats. A statistically significant, dosage-related increase in benign Leydig cell tumors was observed in the testes of male rats. An significant increase in the numbers of renal tubular tumor was observed among both male and female rats administered 170 mg/kg/day.

JAN 18 1994

SUN C-67

**Pharmacologist Review of IND
(Amendments of Feb. 10 and April 26, 1993)**

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)

SPONSOR: Abbott

DRUG: Zileuton (Abbott-64077) Tablets

DRUG CATEGORY: Lipoxygenase inhibitor

Date of receipt by HFD-150: Feb. 12 and April 27, 1993

Date of review: January 14, 1994

SUBMISSION CONTENTS:

- (1) Carcinogenicity studies in mice and rats,
- (2) Ames test,
- (3) in vivo unscheduled DNA synthesis in rat and mouse kidney cells,
- (4) hypothalamic-pituitary-gonads challenge in rats,
- (5) peroxisome proliferation in liver and kidney in rats,
- (6) hepatic and renal proximal and hepatic drug metabolizing activity in mice,
- (7) cell proliferation in mice,
- (8) cell proliferation in rats, and
- (9) PCNA (Proliferating cell nuclear antigen) studies in rat kidneys of 3 month oral and 3 month dietary MTD studies and in mouse kidney of 3 month MTD dietary study.
- (10) Historical control incidence rates of four tumor types

BRIEF COVERSHEET FOR THE REVIEW OF CARCINOGENICITY STUDIES

	Mouse		Rat	
1. No. of studies	1		1	
2. Name of laboratory		Sponsor		
3. Strain	CD-1		CD	
4. No/sex/group	60		60	
5. Dose level (mg/kg)	0 0 50 150 450		0 0 40 80 170	
		by dietary		
6. Basis for dose selection stated	yes (3 month dietary MTD studies)		yes	
7. Interim sacrifice	no		no	
8. Total duration	2 years		2 years	
9. Survival rates (%) at termination				
	Male	female	male	female
0	42	33	38	32
0	43	48	50	48
L	45	37	38	53
M	42	43	48	50
H	30	28	60	63

11. Statistical methods used

**Peto method
Westfall and Young method
Cochran-Armitage test**

**2 Year Carcinogenicity Study in Rats
(Study no. TA 89-279)**

Testing laboratory: Sponsor.

Date of the study: Reporting date is January 15, 1993.

GLP requirement: A statement of compliance with GLP regulations was included.

Methods: Five groups of CD-1 rats each consisting of 60 males and females were given zileuton (Lot nos. 28-932-AL, 41-047-AL and 52-133-AL) at dietary dose levels of 0, 0, 40, 80 and 170 mg/kg/day for two years. Additional 10 males and 10 females per dose group were assigned for serology tests. Dose selection was based on a 3 month dietary MTD study at dose range of 80-750 mg/kg. Following parameters were monitored:

Clinical sign	daily
Body weight and food consumption	weekly before week 13 biweekly thereafter
Palpation	weekly for 6 months
Hematology	months 12, 18 and 24. days 345-346 in control and 170 mg/kg groups
Plasma drug level	weeks 1 and 52 at 7-9 am, weeks 57-58, 75-76 and 99-100 at 12:30-1:30 pm, 5-6 pm, 12:30-1:30 am and 6-7 am
Urinalysis	days 345-346 on control and high-dosage groups
Gross and histopathology	termination

Results:

Stability of drug in mixture: At end of two year, lots remained 100.-100.9 % of their initial potencies.

Drug intake: The actual drug intake was approximately 70-88 % of the intended daily dosages.

Survival data: No differences in the survival rates were found. A positive dose-related trend in the survival rate was detected for the female rats. Survival rates in 0, 0, 40, 80 and 170 mg/kg groups were 38, 50, 38, 48 and 60 for males and 32, 48, 53, 50 and 63

% for females, respectively.

Clinical signs: Normal.

Body weight: At end of two-year treatment, mean body weights compared to the controls were significantly lower by 28 % for males at 170 mg/kg and 11, 22, 36 % for females at dose levels of 40, 80 and 170 mg/kg/day, respectively. Body weight changes are given below (extracted from sponsor's figures on pages 73 and 74):

Figure 1. Mean Body Weight (g) - Males

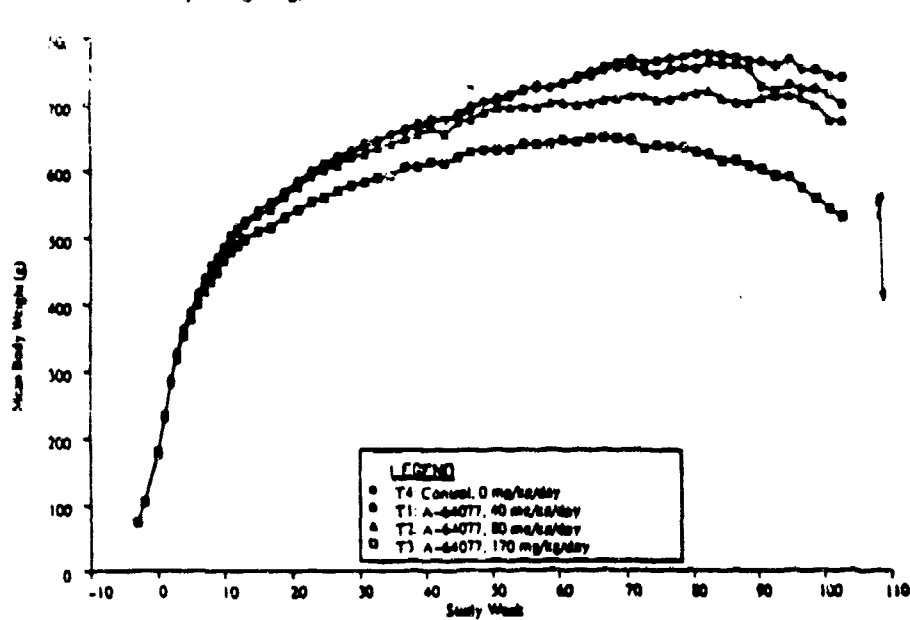
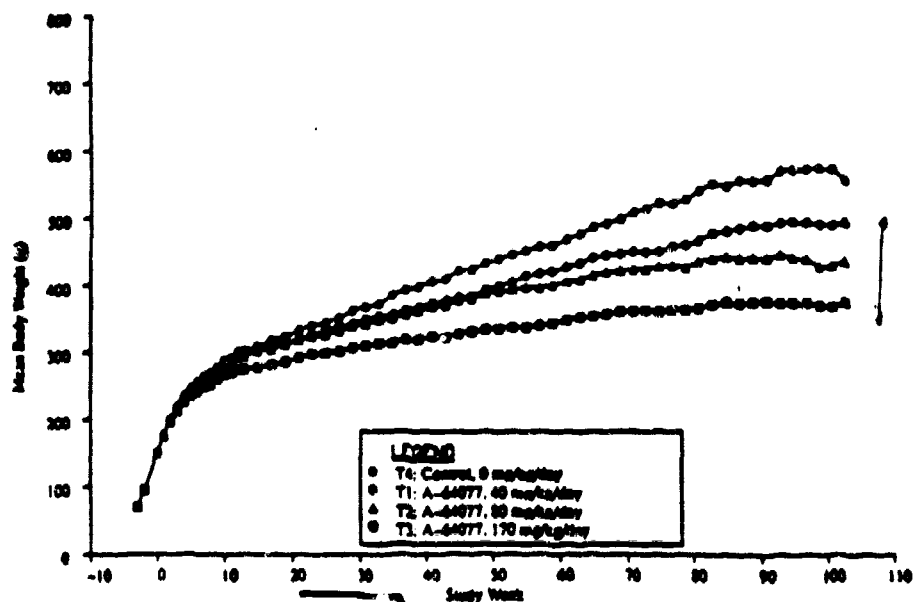


Figure 2. Mean Body Weight (g) - Females



Food consumption: At dose level of 170 mg/kg, it produced significant decrease (17-23%) in food consumption. At dose of 80 mg/kg, it produced decreases sporadically during the study (4-13%).

Plasma drug levels: The following table summarizes the mean $AUC_{0-24\text{ hr}}$ of the parent compound and metabolite, Abbott-66913 (extracted from sponsor's table on page 4-164):

Duration of Dosing	Mean Plasma Concentration (µg/mL)†		Mean 24-hr AUC (µg·hr/mL)††	
	Zileuton	Metabolite	Zileuton	Metabolite
40 mg/kg/day				
Week 4	3.961±1.845	1.018±0.321		
Week 52	8.963±2.413	1.956±0.457		
Week 58			128.48±43.29	22.65±10.81
Week 76			218.60±61.21	40.50±11.77
Week 100			308.30±79.20	56.54±15.57
80 mg/kg/day				
Week 4	9.772±2.955	2.964±0.601		
Week 52	18.256±7.350	5.198±2.045#		
Week 58			31.22±144.32	80.95±34.17#
Week 76			370.38±124.60	82.05±30.00
Week 100			522.29±145.83	127.34±37.69
170 mg/kg/day				
Week 4	20.645± 8.930	6.907±1.664		
Week 52	33.669±15.201	9.230±3.497		
Week 58			683.58±249.43	170.62±56.92
Week 76			770.55±322.15	184.02±61.00
Week 100			792.24±189.78	260.41±96.88

† Single plasma sample obtained in the morning (n≈approximately 20 rats per dose group)

†† Estimated from plasma samples collected at four different times on four separate days in the same rat (n≈approximately 10 rats per dose group)

Calculated from estimated values

The concentrations of parent compound increased with increasing dosage and duration of treatment. Female rats had higher AUCs than those recorded for male rats. The AUC for parent compound in humans after the intended clinical dosage of 600 mg qid was about 70 µg·hr/ml. Thus, the ratios of the AUCs that received 40, 80 and 170 mg/kg/day to that expected in humans were 4, 6.2 and 9.2 for males and 4.9, 8.7 and 13.1 for females, respectively.

Serology: Common viruses in rats were detected with no effect on the study being observed.

Hematology: Normal.

Gross pathology: Normal.

Histopathology:

Non-neoplastic findings: Complete listing of non-neoplastic findings are given in the Appendix I (extracted from sponsor's tables of 15-16). No drug-related non-neoplastic findings were reported except dose-related increases of cytomegaly in liver were found. The incidence of this change are list below:

dose level (mg/kg)	0	40	80	170	0
males	0/60	14/60	39/60	48/60	0/60
females	2/61	2/60	27/60	49/60	0/60

Neoplastic findings: Complete listing of neoplastic findings are given in Appendix II (extracted from sponsor's tables 17-18). There were statistically significant increases in the incidence of leydig cell adenoma in testes. There were increases in incidence of renal tubular adenoma or combined renal adenoma/carcinoma. The above-mentioned finding are summarized below:

Tumor	0	40	80	170	Peto	Pairwise
Males						
Leydig cell adenoma	3/60	12/60	12/60	24/60	0.0000	0.0000
Renal tubular adenoma	3/60	1/60	1/60	7/60	0.026	0.0414
Combined renal tubular adenoma and carcinoma	3/60	2/60	1/60	9/60	0.0062	0.0494
Females						
Renal tubular adenoma	0/60	0/60	2/60	5/60	0.0039	0.045
Combined Renal tubular adenoma and carcinoma	0/60	1/60	2/60	5/60	0.0113	0.045

Statistical analysis was performed by HFD-715 (see statistical reviews of 4/21, 5/5 and 5/14/93 by Dr. Chen). Renal tubular tumor is considered a rare type in rats based on the average historical control incidence of 0.4% as submitted by the sponsor. Thus, P values of less than 0.05 would be considered significant.

In conclusion, zileuton at dietary doses up to 170 mg/kg was tested for its carcinogenic

potential. The dose selection was based on a 3 month dietary study that retardation of body weight gain, nephritis and increased liver weight were found at 350 mg/kg. No effects were seen at 170 mg/kg. In the carcinogenicity study, dose-related decreases in food consumption and body weight and hepatocytomegaly were observed at 170 mg/kg. The hepatic effects may be considered an adaptive change related to enzyme induction. Drug exposure at the high dosage group represents 9.2-13 fold the human's at intended dosage of 600 mg q.i.d.. Thus, the high dose appears to be approximate the MTD. The study is acceptable. Zileuton administered for 2 years in rats was associated with significant increases in the incidence of leydig cell tumors in testes and renal tubular adenoma/carcinoma.

24 Month Carcinogenicity Study in Mice (Study no. TD89-279)

Testing laboratory: Sponsor.

Date of the study: Reporting date is Jan. 20, 1993

Methods: Five groups of CD mice each consisting of 60 males and 60 females were given zileuton (Lot nos. 28-932-AL and 41-047-AL) at dietary dose levels of 0, 0, 50, 150 and 450 mg/kg/day. Dose selection was based on a 3 month dietary study in which increased liver weight and hepatic cytomegaly were seen in the 450 and 1000 mg/kg/day groups and degenerative changes in liver at 1000 mg/kg/day. Additional 20 males and 20 females were assigned to control and drug-treated group for pharmacokinetics study. Following parameters were monitored:

Clinical signs	daily
Body weight and food consumption	weekly until week 13 and biweekly thereafter
Palpations	weekly during the 6th month and at necropsy.
Hematology	months 12 and 18
Plasma drug level	weeks 4, 52, 93 and 94 between 6 to 7 pm, 12
to	1 pm, 5-6 pm and 12-1 am. Detection limits
	were 0.01 ug/ml for zileuton and Abbott-66913
Gross and histopathology	Termination at 101-102 week for females and
	week 103-104 for males.

Results:

Stability of diet mixture: The concentrations of the formulations in weeks 1,4,12,26,52,

NDA 020471

FIRM: ABBOTT LABS

6 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

70,78 and 101 were between 89-112 % of the nominal content.

Mortality: Mortality rate was greater than 50 % in all groups. At terminal necropsy numbers of survival were 25,27,25,18 and 26 for males and 20,22,26,17 and 29 for females at 0, 50, 150, 450 and 0 mg/kg/day, respectively. However, mortality was 50 % at day 650 (450 mg/kg male), day 628 (150 mg/kg male), day 668 (50 mg/kg male), day 663 (control male), day 622 (450 mg/kg female), day 629 (150 mg/kg female), day 650 (50 mg/kg female), day 613 (control female). Thus, survival rates were about 50 % at weeks 87-95 in all groups.

Clinical signs: No drug-related signs were observed.

Body weight: Transient body weight loss was observed in males (19%) and females (15%) during weeks 0-77 at dose level of 450 mg/kg. The body weight data are shown below (extracted from sponsor's figures on pages 8-113 and 8-114):

Figure 1. Mean Body Weight (g) - Males

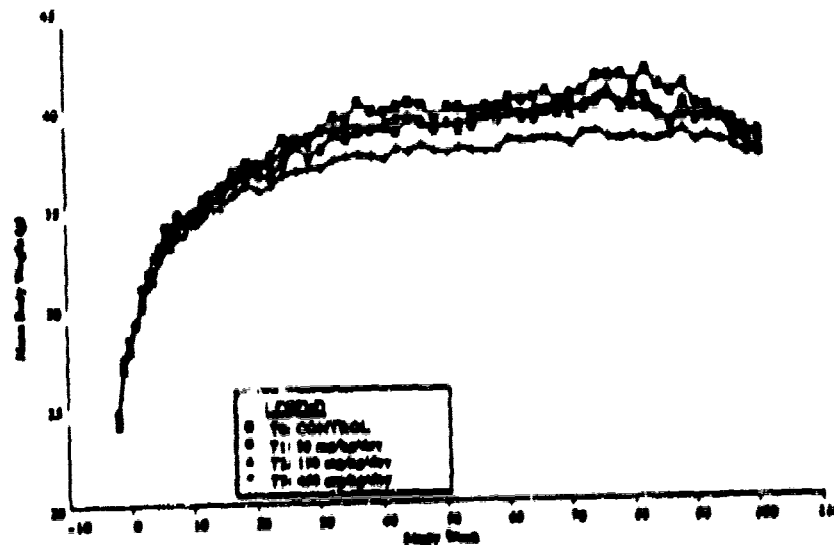
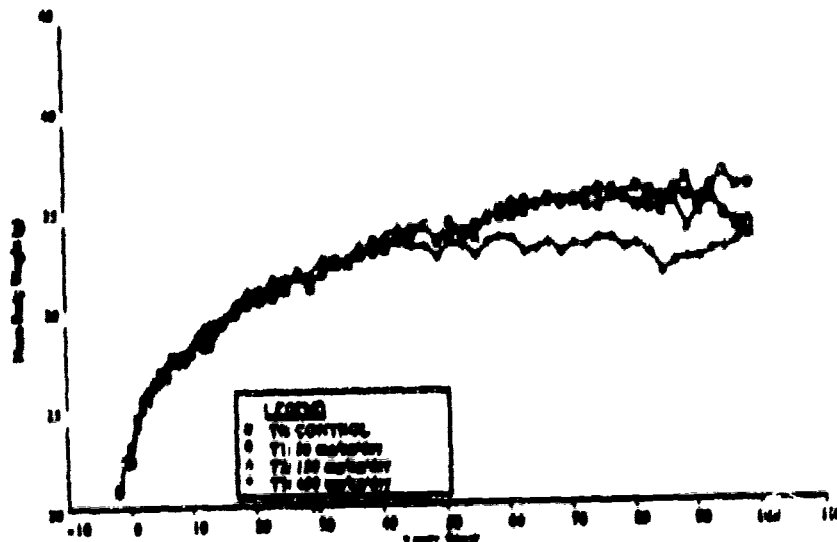


Figure 2. Mean Body Weight (g) - Females



Food consumption: Decreased food consumption (5-12%) was seen in males during first 6 weeks and in females during weeks 41-95 at dosage levels of 150 and 450 mg/kg.

Drug intake: The daily intake was generally within 10% of the intended dosage.

Plasma drug levels: The PK data for zileuton and metabolite (Abbott-66913) are summarized below (extracted from sponsor's table on page 8-149):

Duration of Dosing	Mean Plasma Concentration (µg/mL) [†]		Mean 24-hr AUC (µg·hr/mL) ^{††}	
	Zileuton	Metabolite	Zileuton	Metabolite
50 mg/kg/day				
Week 4	1.17±0.71	0.05±0.11		
Week 52	1.82±0.78	0.14±0.16		
Week 93/94			50.46±13.07	0.18±0.33
150 mg/kg/day				
Week 4	2.96±1.22	0.21±0.16		
Week 52	4.06±3.34	0.24±0.17		
Week 93/94			153.69±26.88	4.80±2.24
450 mg/kg/day				
Week 4	8.52±5.39	0.59±0.34		
Week 52	11.43±4.49	0.63±0.27		
Week 93/94			396.44±172.61	18.84±8.10

[†] Single plasma sample obtained in the morning.

^{††} Calculated from plasma samples collected at four different times on four separate days in the same mice.

Dose proportionalities to AUC and average plasma concentration were reported. Metabolite concentrations account 4.3-7.7% and 0.4-4.8 % of parent compound concentrations based on concentration and AUC, respectively. At dose of 50, 150 and 450 mg/kg/day, the ratios of the AUCs for zileuton to that of humans at 600 mg qid were 0.7, 2.2, and 5.7, respectively.

Serological examination: Some common murine viruses were detected with no detrimental effect on the study being observed.

Hematology: Normal.

Gross pathology: Normal.

Histopathology:

Non-neoplastic findings: Summarized histopathological findings are given in the Appendix III (extracted from sponsor's tables 12-13 on pages 7-296 to 7-359). Dose-related hepatomegaly, mostly centrilobular, was observed in the 150 and 450 mg/kg/day groups. Electron microscopic examinations revealed a marked gender-dependent proliferation in females. Electron microscopic examination did not indicate any cell proliferation in kidney. However, increased cellular proliferation in kidney was suggested at the high dosage by an increase in proliferating cell nuclear antigen (PCNA) staining which will be elaborated later in the review. Therefore, hepatic change was compatible with proliferation of the smooth endoplasmic reticulum and was a reflection of enzyme induction.

Neoplastic findings: A summarized findings are given in the Appendix IV (extracted from sponsor's tables 8-9 on pages 9-94 to 8-112). Significant increases in the incidence of hepatocellular adenomas and combined hepatocellular adenoma and carcinoma was observed in the females. Significant increases in hemangiosarcoma in ovary was reported. Similarly, a statistically significant increase in the incidence of whole-body hemangiosarcoma and combined hemangioma/hemangiosarcomas was seen in females. Trend of increasing incidence of combined kidney tubular adenoma and carcinoma was seen in females. The findings of the above-mentioned tumors are listed below:

Tumor types	0	50	150	450 mg/kg	p value Trend (Peto)	p value Pairwise (H) vs (C)
Males						
Liver adenoma and carcinoma	14/60	10/60	9/60	21/60	0.0021	0.03
Kidney tubular adenoma	0/60	0/60	1/60	2/60	0.0335	NS
Females						
Liver adenoma	0/60	2/60	0/60	8/58	0.0000	0.0013

carcinoma	0/60	1/60	0/60	3/58	0.0224	NS
combined adenoma carcinoma	0/60	3/60	0/60	11/58	0.0000	0.0005
Whole body						
hemangiomas	1/60	3/60	3/60	3/58	NS	NS
hemangiosarcoma	5/60	1/60	2/60	16/58	0.0000	0.0034
combined	6/60	4/60	5/60	19/58	0.0000	0.0015
Ovary						
hemangiosarcoma	0/60	0/60	0/60	5/57	0.0003	0.0214
Skin						
hemangiosarcoma	0/60	0/60	1/60	3/58	0.0150	NS
Vagina						
combined hemangioma/hemangiosarcoma	0/60	0/60	0/60	2/57	0.0485	NS
Kidney						
adenoma	0/60	0/60	0/60	3/58	0.0143	NS
combined adenoma /adenocarcinoma	0/60	0/60	0/60	4/58	0.0029	0.048

Statistical analysis was performed by HFD-715 (see statistical reviews of 4/21, 5/5 and 5/14/93 by Dr. Chen). Combined liver adenoma and carcinoma is considered a common tumor. Thus, P value of 0.03 for pairwise analysis for male mice is not considered statistically significant.

In conclusion, zileuton at dietary doses up to 450 mg/kg was tested for its carcinogenic potential. Dose selection was based on a 3 month dietary dose-range finding study in which increased liver weight and hepatomegaly were seen at 450 and 1000 mg/kg and degenerative changes in liver at 1000 mg/kg. We concluded that the maximal tolerated dose should be 1000 mg/kg (see review of 6/20/89 amendment by Dr. Sun on 2/22/93). In the carcinogenicity study, transient decreases in body weight and food consumption and hepatic cytomegaly were observed at 450 mg/kg, indicating no employment of maximal tolerated dose for the study. The highest dose used in the study represents approximately one half of the MTD. Survival rates were about 50 % at weeks 87-95. Drug exposure in the high dosage group was 5.7 times the human exposure at the

proposed human dose. However, the data indicates that zileuton administered at 1/2 of MTD for 2 years in mice was associated with significant increases in the incidence of tumors in liver, vascular tumor in ovary and whole body as well as renal tubular tumor in female mice.

**Ames Test
(Study no. TX92-224)**

Testing laboratory: Sponsor.

Methods: Zileuton was evaluated for mutagenicity activity in the bacterial reverse mutation assay using *Salmonella typhimurium* strains TA-1535, 1537, 1538, 98 and 100. The assay was conducted at concentrations over a range of 100-5000 ug/plate in the absence of metabolic activation or 1-5000 ug/plate in the presence of metabolic activation. Following positive controls were used: N-methyl-N'-nitrosoquinidine, quinacrine mustard, 2-nitrofluorene and 2-aminoanthracene. Incubation period was 48 hours at 37 degrees C.

Results: Positive controls yielded typical positive responses. Cytotoxicity was seen at 2000 ug/plate for most strains and 5000 ug/plate for all strains. No dose-related increases in the number of colonies on plates treated with zileuton were seen when compared to that of vehicle control. Thus, Zileuton was non-mutagenic in this assay.

**In Vivo Unscheduled DNA Synthesis (UDS) in Rat and Mouse Kidney Cells
(Study no. TX92-341)**

Testing laboratory:

Methods: Sprague Dawley rats and CD-1 mice were given single doses of zileuton at dose levels of 0, 400, 800 and 1600 mg/kg for male rats, 0, 225, 450 and 900 mg/kg for female rats, and 0, 450, 900 and 1800 mg/kg for mice. Kidneys cells were isolated by a collagenase digestion technique at 2 and 16 hours after treatment. The cells were incubated in culture with radiolabelled thymidine and processed for autoradiography. Measurement of UDS and DNA replication were performed by counting autoradiography grains over the nuclei of kidney cells incubated for 20-22 hours with radiolabelled thymidine. Fifty cells per slide were evaluated for UDS (3 slide/animal and 3 animals/sex/dose group) and net grains(NG)/nucleus and percentage of cells in repair (% IR) were determined. It was considered positive if the NG was greater than zero and the % IR was greater than 20 %. S-phase cells are those with at least 30 nuclear grains.

Streptozocin (400 mg, i.p.) was used as the positive control.

Results: NG and % IR were never reached the positive criteria of greater than zero and greater than 20%, respectively, except in one male mice in the vehicle group had 0.05 NG and 24 % IR. Positive control yielded a typical response. Thus, zileuton had no UDS induction or DNA repair in kidney cells.

**Two week Oral hypothalamic-pituitary-gonad challenge in Rats
(Study no. TA 92-313)**

Testing laboratory: Sponsor.

Methods: Six groups of animal each consisting of 15 CD male rats were given 0 and 170 mg/kg of zileuton for 14 days. Same dose was used in the 2 year rat carcinogenicity study in which leydig cell tumor was found. Rats received either human chorionic gonadotropin (hCG, 100 I.U./rat, s.c.), gonadotropin releasing-hormone (GnRH, 3 ug/kg, i.p.) or naloxone (2 mg/kg, s.c.) on day 14 one hour after administration of zileuton. Blood samples were collected at 0, 0.5, 1,3 and 6 hours after the administration of either hCG, GnRH or naloxone. The samples were analyzed for LH and testosterone by using radioimmunoassay. Rats were sacrificed 6.5 hour after receiving the administration of hCG, GnRH or naloxone and testicular .

Results:

Mortality: No zileuton-related mortality was observed. Several rats died on day 14 after receiving the appropriate challenge. They were not considered to be treatment related.

Clinical signs and body weight: Normal.

Serum hormones: Zileuton had no effect on the levels of serum^{LH} or testosterone in male rats following 14 day oral administration. Zileuton-treated rats had a lower (43%) serum testosterone response three hours following the hCG challenge. No difference was seen in serum LH between the control or zileuton-treated rats after the hCG challenge. Six hours time period might not be sufficient to observe a negative feedback-induced change in serum LH in response to the change in serum testosterone. Zileuton treatment did not alter the response of either serum LH or testosterone to the GnRH or naloxone challenge, indicating its direct effect on LH receptors on Leydig cells.

Testicular testosterone and organ weights: Normal.

The data demonstrated zileuton-treated rats had a lower serum testosterone response but had no effect on serum LH following and hCG challenge. The data may imply that this effect would be expected to alter the balance of the hypothalamic-pituitary-gonadal axis and may provide a mechanism by which zileuton increases the incidence of leydig cell tumors. However, no effects on serum testosterone and LH were observed following 14 daily oral administrations of zileuton. It would be more convincing by demonstrating lower testosterone level and elevated LH level after long term administration of zileuton. Moreover, the proposed mechanism of tumorigenesis could be further supported by reversal of tumorigenesis with testosterone replacement therapy.

Peroxisome Proliferation in liver and kidney in Rats (CMR no. 92102)

Testing laboratory: Sponsor.

Methods: Seven groups of animals each consisting of 10 male and 10 female CD rats were given oral dose of zileuton at doses of 0 (HPMC), 40, 80, 170 and 300 mg/kg/day, 0 (corn oil) and 308 mg/kg of clofibrate (positive control) for 14 days. Electron microscopic examination were performed. Peroxisome palmitoyl-CoA beta oxidation assay and lauric acid hydroxylase assay were conducted.

Results:

Body weight: Retardation of body weight gain was observed in male 300 mg/kg zileuton group only.

Organ weight: Increased relative liver weight was seen in the zileuton-treated male groups and clofibrate group. Increases kidney weight was seen in the male clofibrate group. In females, increased relative liver and kidney weight were seen in the zileuton 300 mg/kg and clofibrate groups.

Electron microscopic observation in liver and kidney: There was no evidence of peroxisome proliferation by electron microscopic examination in the zileuton-treated rats. In contrast, clofibrate induced increase in the number of peroxisome in liver but not in kidney. In a previous report (see review of amendment of 2/3/90 by Dr. Sun on 2/23/93), induction of microsomal drug-metabolizing enzymes have been observed in livers of rats following zileuton treatment. In the 6 month oral toxicity study in rats in liver, no evidence of peroxisomal proliferation was observed by electron microscopic examination.

Peroxisome palmitoyl-CoA beta-oxidation activity in liver and kidney: In males, increased activity in liver was observed in zileuton treated rats (34%) and clofibrate-treated rats (443%). In kidney, decreased activity (20%) was seen at 80 mg/kg zileuton group whereas increased activity was noted in clofibrate group (82%). In females, activity in liver was increased in clofibrate-treated rats (78%). In kidney, increased activity was reported for zileuton group (58%) and clofibrate group (77%).

Lauric acid hydroxylase in liver: In male zileuton-treated rats, dose-related increase (54-94%) in 11-hydroxylation activity was found. In female zileuton-treated rats, decrease in 12-hydroxylation activity was observed. LAH (11 and 12 hydroxylation) activities were increased significantly (145-384%) in the clofibrate-treated rat livers.

In conclusion, an increase in the relative liver and kidney weights was reported following 14 days oral dosing with zileuton. Increase in 11-hydroxy lauric acid formation in male livers indicting zileuton to be a cytochrome P-450 inducer. A dose dependent increase in peroxisome, peroxisomal beta-oxidation activity and 12-hydroxy lauric acid formation was not observed whereas clofibrate produced a marked effect in liver or kidney. Thus, clifibrate, a known peroxisome proliferator, had a marked effect on hepatic and kidney peroxisomal proliferation whereas zileuton had only a marginal effect.

Peroxisomal and drug metabolizing activity in Mice (Study no. 92-575)

Testing laboratory: Sponsor.

Methods: Eleven groups of CD-1 mice each consisting of 10-13 males and 10-13 females were administered orally with zileuton at dose levels of 0 (HPMC, 2 groups), 50, 150, 450 mg/kg (A and B groups), phenobarbital (50 mg/kg), 0 (corn oil, 2 groups), and clofibrate (250 mg/kg and 462 mg/kg) for 14 days. Immunoassay analysis of cytochrome P-450 was performed with immunoblot method using rabbit polyclonal IgG antibodies against rat cytochrome P-450 (IIB1 or IVA). Catalase and palmitoyl CoA oxidase activities in liver and kidney were measured. Liver microsomal total cytochrome P-450 was determined spectrophotometrically and monooxygenase activities were determined. Hydroxylation of lauric acid and ERNase, DMNASE, 7-PRODase, BZNase and 7-ERODase activities in liver were measured.

Results:

Body weight and relative liver and kidney weight: At dose of 450 mg/kg, zileuton

Body weight and relative liver and kidney weight: At dose of 450 mg/kg, zileuton caused increased relative liver weight (25-30%). Phenobarbital and clofibrate produced lesser degree of increased liver weight by 6-10 and 26 %, respectively.

Peroxisomal enzyme marker activities and microsomal drug metabolizing activity in livers: Summarized data are given below (extracted from sponsor's tables 10 and 11 on pages 9-224 and 225):

Parameter	% Change in Activity Relative to Vehicle Control						
	Zileuton (mg/kg)				Clofibrate (mg/kg)		Phenobarbital (mg/kg)
	50 (T ₁)	150 (T ₂)	450A (T ₃)	450B (T ₈)	250 (T ₆)	462 (T ₁₀)	50 (T ₄)
Peroxisomes:							
PCoA	26.6*	42.9*	76.5*	16.2*	111*	385*	-6.0
Catalase	0.70	19.3	53.7*	-10.8	29.9*	58.0*	-8.0
Microsomes:							
Total protein	32.3*	56.6*	82.2*	4.10	1.31	17.0*	57.0*
Total cytochrome P-450	2.20	21.9*	55.9*	77.8*	1.10	23.0*	25.0*
7-ERODase (P-450IA)	15.6*	34.4*	126*	122*	-2.10	13.0	62.0*
BZNase (P-450IB + others)	22.7	46.0*	81.3*	ND	-1.03	ND	54.0*
7-PRODase (P-450IIB)	131*	354*	1214*	1324*	-37.0*	-34.0*	902*
DMNase (P-450IIE)	-6.60	3.80	34.6*	ND	33.3*	ND	6.0
ERNase (P-450IIA)	14.1	6.50	60.6*	ND	-16.9	ND	53.0*
LAHase (P-450IVA)	4.0	32.0	111*	109*	456*	963*	-4.0

Table 11. Summary of Effects of 14 Day Oral Zileuton Pretreatment on Female CD-1 Mouse Liver Peroxisome and Microsomal Drug Metabolizing Activity

Parameter	% Change in Activity Relative to Vehicle Control						
	Zileuton (mg/kg)				Clofibrate (mg/kg)		Phenobarbital (mg/kg)
	50 (T ₁)	150 (T ₂)	450A (T ₃)	450B (T ₈)	250 (T ₆)	462 (T ₁₀)	50 (T ₄)
Peroxisomes:							
PCoA	-7.60	44.5*	121*	97.1*	170*	358*	-45.0*
Catalase	18.0*	25.1*	50.6*	14.9*	21.0*	44.0*	7.10
Microsomes:							
Total protein	18.8	32.6*	48.6*	69.4*	6.30	67.0*	51.0*
Total cytochrome P-450	25.6*	82.1*	147*	94.3*	-4.10	20.0	85.0*
7-ERODase (P-450IA)	44.7*	184*	184*	208*	-32.4*	-24.0*	145*
BZNase (P-450IB + others)	46.4*	77.1*	104*	ND	-10.6	ND	99.0*
7-PRODase (P-450IIB)	96.3*	586*	1264*	1399*	-45.3*	-50.0*	1354*
DMNase (P-450IIE)	-5.90	18.5	40.1*	ND	-6.80	ND	-14.0
ERNase (P-450IIA)	-35.1	-11.3	11.3*	ND	4.50	ND	-7.10
LAHase (P-450IVA)	51.2	279*	851*	396*	499*	1240*	-44.0

Activities are abbreviated as follows: PCoA, KCN-insensitive palmitoyl CoA oxidase; 7-ERODase, 7-ethoxycoumarin O-deethylase; BZNase, benzphetamine N-demethylase; 7-PRODase, 7-pentoxycoumarin O-deethylase; DMNase, N,N-dimethyl-nicotinamide N-demethylase; ERNase, erythromycin N-demethylase; LAHase, lauric acid 12-hydroxylase.

* Statistically significant change ($p \leq 0.05$). ND, not determined.

Increases in PCoA activity (77% in males and 121% in females at 450 mg/kg/day) were reported. Clofibrate brought higher degree of PCoA activity (358-385%). Zileuton had no dose-related increases in catalase activity. Phenobarbital did not induce liver PCoA or catalase activity, indicating no cell proliferating activity. Thus, data suggests that zileuton had weak proliferating activity as compared to the known peroxisome proliferator, clofibrate.

Zileuton exhibited a moderate cytochrome P-450 induction profile which is similar to phenobarbital (P-450IA, P-450IIB, P-450IIC, P-450IIE, P-450IIA) and clofibrate (P-450IVA or LAHase).

PCoA activity in kidney: Effect on PCoA activity is summarized below (extracted from sponsor's tables 1 and 2 from pages 9-215 and 9-216):

Treatment Group	Dose (mg/kg)	Kidney PCoA ^a	Kidney PCoA ^b
HPMC (T ₀)	-	3.46 ± 0.12	1.09 ± 0.08
Zileuton (T ₁)	50	3.11 ± 0.10	1.15 ± 0.04
Zileuton (T ₂)	150	3.16 ± 0.08	1.37 ± 0.03**
Zileuton (T ₃)	450	3.40 ± 0.20	1.33 ± 0.17
Phenobarbital (T ₄)	50	2.38 ± 0.25	0.93 ± 0.20
Corn Oil (T ₅)	-	3.98 ± 0.10	1.18 ± 0.15
Clofibrate (T ₆)	250	3.63 ± 0.23	2.04 ± 0.18**
HPMC (T ₇)	-	3.68 ± 0.19	0.95 ± 0.03
Zileuton (T ₈)	450	2.02 ± 0.28***	1.67 ± 0.17**
Corn Oil (T ₉)	-	2.73 ± 0.11	0.98 ± 0.12
Clofibrate (T ₁₀)	462	4.33 ± 0.03***	3.48 ± 0.24***

** : P < 0.01; *** : P < 0.001

No obvious increase in activity was found in both zileuton (-46% to 75%) and phenobarbital-treated groups whereas clofibrate caused increase in activity (-9 to 72% at 250 mg/kg and 58 to 255% at 462 mg/kg) in kidney.

In conclusion, zileuton is a mixed phenobarbital and clofibrate-type cytochrome p-450 inducer and a relatively weak peroxisome proliferator in liver of mice.

**Kidney Cell Proliferation Study in Mice
(Study no. TD92-303)**

Testing laboratory:

Methods: Zileuton was given to CD-1 mice (5 mice/sex/dose) at dietary dosages of 0, 50, 150 and 450 mg/kg/day for 5 or 26 days. Alzet minipumps containing 20 mg/ml BrdU were implanted s.c. 4 days prior to euthanasia. The kidney tissue section were evaluated for BrdU uptake as a measure of DNA synthesis using immunohistochemical techniques.

Results: No drug-related mortality was reported. No differences in body weights and food consumption were seen. No gross lesion were observed. BrdU labeling was increased (240%) in proximal tubules in males at 450 mg/kg on day 5 but was decreased (78%) on day 26. In female, increases (520-600%) in the proximal tubules of the corticomedullary junction in high-dosage female mice were observed on both day 5 and 26.

**Kidney Cell Proliferation Study in Rats
(Study no TA 92-302)**

Testing laboratory:

Methods: Similar procedure as previously described was performed. Zileuton at dietary dose levels of 0, 40, 80 and 170 mg/kg/day was administered for 5 or 26 days.

Results: No effects on body weight, mortality and gross pathology were reported. Increase (220 %) in BrdU labeling was observed in the proximal renal tubules of males treated for 26 days.

Proliferation Cell Nuclear Antigen Study in Rodent Kidney

Testing laboratory:

Methods: Cell proliferation was quantified in rat kidneys from the high dosage group one-month time point and at end of the three month oral toxicity study in rats (study no

TA87-140), and control low and high-dosage groups in the 3 month dietary MTD study in rats (Study no. TA88-172) and the three month MTD dietary study in mice (study no. TD 88-173). A labeling index was quantified as the percentage of cells in S-phase. 5/sex/drug-treated or control groups was used.

Results:

One month interim point of the 3 month oral toxicity study in rats: In the high-dosage males, a 5-fold increase in renal cell proliferation over controls was observed. According to the sponsor, the cell proliferative response in treated male rats was greater than the proliferative response seen in 1,4-dichlorobenzene (a nongenotoxic kidney carcinogen) treated male rats. However, no data was provided.

Three month oral toxicity study in rats: In the male and female rats, a 6- and 4- fold increases in renal cell proliferation were observed, respectively. Mild nephropathy was observed.

Three month dietary MTD study in rats: In the male rats, a dose-related increase in renal cell proliferation but was significantly greater than control at the high dose only (2.2 fold). In the female rats, renal cell proliferation was significant only at the lower dose (2.6 fold). Histopathology findings in rats were not significant. There were no correlation of nephropathy with cell proliferation.

Three month dietary MTD study in mice: A treatment-related cell proliferation were not observed. Histopathology findings in males and females were not significant.

In conclusion, kidney cell proliferation were seen in rats following oral dosing of dietary administration. The cell proliferative response (2.2-5 fold) in rats was comparable or greater than the proliferative response (2 fold) seen in 1,4-dichlorobenzene treated male rats (Carcinogenesis, 11,2245-2251, 1990). 1,4-dichlorobenzene is a male rat kidney carcinogen. These data may support a chemically induced increase in renal proliferation in the kidney of rats, which may play role in renal carcinogenicity. In mice, although such proliferation was not observed, similar finding of tubular adenoma or carcinoma in females were reported in the 2 year carcinogenicity study.

SUMMARY AND EVALUATION:

In the 2 year carcinogenicity study in mice, zileuton at dose levels of 0, 70, 150 and 450 mg/kg/day was tested. Dose selection was based on a 3 month dietary MTD study that

increased liver weight and hepatic cytomegaly at 450 and 1000 mg/kg and retardation of food consumption and hepatic degenerative change at 1000 mg/kg were observed. In the current study, no toxicities were observed at the high dosage group except transient decreases in body weight and food consumption and hepatic cytomegaly, indicating MTD was not inadequately employed. At this dose level, drug exposure was 5.7 times that of human dosage at 600 mg qid. Survival was acceptable at week 87-92. The data indicate that zileuton administered at approximately 1/2 MTD produced hepatic tumors, vascular tumor in whole body with ovary in particular and renal tubular tumor in female mice.

In the 2 year carcinogenicity study in rats, zileuton at dietary doses of 0, 40, 80 and 170 mg/kg was tested. Dose selection was based on a 3 month MTD study. Dose-related decreases in body weight and food consumption as well as cytomegaly in liver were reported, indicating employment of MTD. Most of survival rates were better than 50 % at end of study. Therefore, the study is valid. Zileuton produced tumors in testis (leydig cell tumor) and kidney (renal tubular tumor).

The incidence of the above-mentioned tumors were above the upper range of the historical control incidence provided by the sponsor.

Zileuton was not mutagenic as indicated by the negative results in the currently submitted two studies (Ames test and in vivo unscheduled DNA synthesis in rat and mouse kidney cells) as well as those mutagenicity studies (Ames test, unscheduled DNA synthesis in rat hepatocyte, CHO/HGPRT mutation assay, micronucleus test in mice and chromosome aberration in human lymphocytes) previously submitted (see original IND 34958 review of 10/17/90 by Dr. Sun). Thus, tumorigenesis induced by zileuton could be by way of epigenetic mechanism.

Leydig cell tumor is the most common testicular neoplasm in the rats. The majority of leydig cell tumors are benign, only about 10 % being malignant. It is rare (2-3% of human testicular tumors) and usually benign in humans. Zileuton inhibited the hCG-induced testosterone release but not GnRH- or naloxone-induced testosterone release, indicating its effect on hypothalamic-pituitary-gonad axis at testicular level. Therefore, the sponsor attribute hormonal influence rather than genetic alteration to its tumorigenesis. Although the mechanism is possible, it would be more compelling if elevation of LH level and reduction of serum testosterone could be demonstrated after long term administration of zileuton or reversal of tumorigenesis with replacement therapy of testosterone could be demonstrated. Similar tumor findings were reported by an approved product, Cimetidine[®].

Zileuton produced hepatic tumor in female mice. Zileuton exhibited phenobarbital-like enzyme induction in rodents with some clofibrate-like activity in mice. The relevance of this tumor findings to humans because of their enzyme induction property is still debatable.

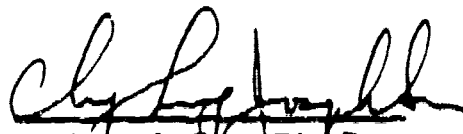
Zileuton caused renal tubular tumor in rats and female mice. Since renal neoplasms found in rats have comparable counterpart in humans, neoplastic finding in this organ found in rats may have considerable relevance to the process in humans. The tumorigenesis of this tumor type in rats could not be completely explained by its epigenetic mechanism of cell proliferating activity because of marginal or no increase in peroxisomal proliferation markers but increased kidney cell proliferation and PCNA activity were reported in kidney cells.

In female mice, zileuton caused hemangiosarcoma in whole body (skin, vagina, liver, ovary, spleen and uterus) and ovary in particular. This tumor type is common in mice but rare in humans. Comparative aspect of the findings in mice to humans is difficult to draw because limited studied in this neoplasm of humans and laboratory animals. The sponsor should be informed to explain the significance of this tumor finding to humans. An approved product, etretinate (Tegison[®]), produced similar blood vessel tumors (hemangioma and hemangiosarcoma in several different tissue sites) in male mice.

RECOMMENDATION:

Given the significant findings in the carcinogenicity studies, risk and benefit assessment of long term administration of zileuton to asthmatic patients should be carefully analyzed. Although the relevance of neoplastic findings in the carcinogenicity studies to humans is unknown, such neoplastic findings should be described in patient consent form if the proposed clinical trial of longer than 6 month is allowed. The sponsor should be asked to explain the significance of neoplastic findings of hemangiosarcoma and renal tubular tumor in particular.

Furthermore, renal function test, male reproductive activities and hormonal levels should be monitored for long term administration of more than 6 month as recommended in the review of 2/23/93.


C. Joseph Sun, Ph. D.

Jan. 14, 1994

SUN

JUN 7 1993

communication to sponsor required.

Pharmacologist Review of IN.
(amendment of May 10, 1993)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-155)

SPONSOR: Abbott Lab.

DRUG: Zileuton

DRUG CATEGORY: lipxygenase inhibitor

Date of receipt by HFD-155: May 21, 1993

Date of review: June 4, 1993

Submission content: Investigator brochure and Patient information and consent form

Evaluation and comments:

The informed consent and omvestigator brochure do not properly address the significance of preclinical findings of the carcinogenicity studies in rodents and the toxicity studies in dogs.

RECOMMENDATION:

The sponsor should be conveyed as shown in the letter to sponsor below:

Letter to sponsor:

We have completed the review of your submission of May 10, 1993. At this time, we have the following recommendations:

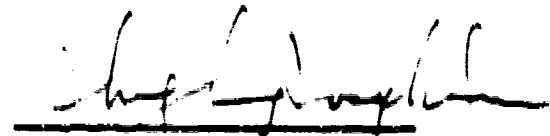
1. The carcinogenicity results indicate tumors in multiple organs. However, those of most concern appear to be the vascular tumors (hemangiosarcomas) and the renal tumors. While there is mechanistic information which suggests that the liver and testicular tumors may be of questionable relevance for humans, there is no such data for the vascular and renal tumors. The carcinogenicity section of the informed consent should be modified to read:

"Administration of zileuton in two year carcinogenicity studies produced vascular and renal (kidney) tumors in female mice at doses resulting in plasma exposures (AUC) which were 5 times that in humans and renal tumors in male and female rats at 9-13 times the human plasma AUC."

Furthermore, renal tubular adenoma or combined renal tubular adenoma and adenocarcinoma observed in rats and combined renal tubular adenoma and adenocarcinoma reported in female mice should be incorporated in animal toxicology section of the investigator brochure.

2. The section in the informed consent dealing with toxicity in dogs should be modified to read:

"After Zileuton administration to dogs for one year at doses producing plasma exposures 7 times that in humans, evidence of toxicity to the testis and kidneys was found. In human studies performed to date, ..."


C. Joseph Sun, Ph.D.

Orig. IND
HFD-155/Division file
HFD-155/Dr. Jenkins
HFD-155/Dr. Sun
HFD-151/CSO

June 4, 1993



MAR 19 1993

Pharmacologist Review of IND
(Amendments of Feb. 24, 1993 and March 8, 1993)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)
DRUG: Zileuton Tablets
DRUG CATEGORY: Leukotriene synthesis inhibitor (lipoxygenase inhibitor)

Date of receipt by HFD-150: Feb. 25 and March 10, 1993

Date of review: March 19, 1993

SUBMISSION CONTENTS: (1) Overview of testicular and prostatic changes in the dogs
(2) 12 month oral toxicity study in dogs

**Twelve Month Oral Toxicity Study in Dogs
(Study no. TB91-219)**

Testing laboratory: Sponsor.

Date of the study: Aug. 8, 1991 to Jan. 31, 1993

GLP requirement: A statement of compliance with GLP regulations was included.

Methods: Four groups of beagle dogs (7.9 months of age) each consisting of 6 males and 6 females were given zileuton (Lot no.51-125-AL) in gelatin capsules at dose levels of 0, 10, 25 and 75 mg/kg/day for 12 months. The dosage for the 75 mg/kg/day group were reduced to 50 mg/kg on day 74 because severe effects were observed in several dogs. Two control and two high-dosage dogs were held for an eight-week recovery period. Following parameters were monitored:

Clinical signs	daily
Body weight	twice weekly during first month and weekly thereafter
Food consumption	weekly
Ophthalmologic examinations and EKG	months 0, 6 and 12
Plasma drug level	0, 1,3,6,10,16 and 24 hours during weeks 2, 17, 32, and 48. Detection limit was 0.001 ug/ml.
Metabolism and disposition	days 277 to 284, 50 mg/kg of R(+) enantiomer (88.6uCi/mg) were given to two severely affected (neutropenia) dogs

Urinalysis
Blood chemistry and hematology
Organ weight, gross and histopathology

and other two dogs in the high-dosage group. Blood samples were collected at 0-120 hrs and urine and feces were collected daily for 5 days.

weeks 26, 36, 44, 52 and 60.

months 3, 6, 8 and 12.

termination and recovery period. Two severely affected dogs in the high-dosage group were not treated after day 278 and were not necropsied in the study. These two animals were transferred to the metabolism and disposition study (TB92-343).

Results:

Stability of test materials: Potency after the study was 98.4 % as compared to the reference.

Clinical signs: Decreased activity, hyperthermia, inappetence, dehydration, infection and emesis were observed in the 50-75 mg/kg group. One male and one female high-dosage dogs were removed from study on day 329 because of severe infection probably due to marked neutropenia. These two dogs were not necropsied in the study but transferred to the metabolism and disposition study (TB92-343).

Body weight: Body weight loss (-0.07 kg) in females and retardation of body weight gain (+0.4 kg) in males were observed in the high-dosage groups vs body weight gains in control female and male groups of 1.75 kg and 2.31 kg, respectively.

Food consumption: Marked inappetence to basal diet was observed in the high-dosage group. The dogs continued on the special diet or canned food for most of the treatment period.

Ophthalmology: Normal.

EKG: Ventricular extrasystole were observed at the mid-treatment interval in one high dosage dog, and in a mid-dosage dog at the end of treatment period. However, significance of this is unknown.

Plasma drug level: C_{max} and AUC values are summarized below (extracted from sponsor's table on page 22):

Dosage (mg/kg/day)		C _{max} (µg/ml) and AUC (µg·hr/ml) Values (Mean ±SD)			
		Week 2	Week 17	Week 32	Week 48
10	C _{max}	11.3±4.3	11.1±2.4	13.0±3.5	12.5±3.8
	AUC	105.1±37.5	95.1±14.1	110.9±20.2	115.0±26.5
25	C _{max}	25.4±5.8	27.4±11.7	26.2±6.2	28.2±5.4
	AUC	246.5±36.2	236.7±86.0	248.5±60.9	283.6±79.4
75 ^a	C _{max}	42.9±16.8	—	—	—
	AUC	540.8±172.0	—	—	—
50 ^a	C _{max}	—	43.3±14.2	40.3±11.5	38.9±7.0
	AUC	—	425.7±157.6	466.1±135.3	456.5±76.8

^aThe dosage for the high-dosage group was reduced from 75 to 50 mg/kg/day on Day 73.

Proportionality to increasing dose levels was demonstrated. Drug accumulation was not indicated over the one-year dosing period. T_{max} values were 1-4 hrs and terminal T_{1/2} was 5.28-6.66 hrs. Thus, systemic exposure to zileuton in dogs administered the 10, 25, 50 and 75 mg/kg/day dosages were 1.5, 3.6, 6.4 and 7.7 times the human exposure of 70 ug·hr/ml (600 mg qid).

Metabolism and disposition: There were no difference in metabolism and excretion between two high-dosage dogs which developed neutropenia and two did not following administration of radiolabeled ¹⁴C-R(+)-zileuton, indicating no relationship between the development of neutropenia and metabolism of zileuton. There was gender difference in metabolism and excretion in these four dogs. Plasma AUC₀₋₉₆ values of the N-dehydroxylated metabolite (Abbott-66913) were higher in males (75-89 ug·hr/ml) than in females (9.5-13.5 ug·hr/ml). In contrast, plasma AUC of zileuton were higher in females (558-605 ug·hr/ml) than in males (206-268 ug·hr/ml). The Pk data may suggest higher oral absorption in female dogs. In males, urinary excretion accounted for 46-73% of dose while 27-56% was recovered in feces. In contrast, in females urinary excretion

represented 16-34%, while fecal excretion accounted for 73.5-84% of the dose.

Urinalysis: Glycosuria was observed in the high-dosage dogs and was still present at end of recovery period in males, indicating nonreversibility in males or incomplete reversibility in one female dog. Higher potassium (225%) was seen in the high dosage females.

Hematology: Persistent neutropenia, thrombocytopenia anemia were observed in two high-dosage dogs. Sporadic findings were seen in one high-dosage dog. The M:E ratio of bone marrow smear was 5-50:1.

Blood chemistry: Elevation of chloride (2-5%) and reduction of CO₂ (16-19%) were seen in the 25 and 50-75 mg/kg/day groups. They were not reversible in the high-dosage group. Elevations of creatinine were reported in the 25 (24-44%) and 50-75 mg/kg/day (29-73%) groups. It was not reversible in the high-dosage group. Thus, clinicalopathological findings of decreased serum bicarbonate, hyperchloridemia and elevation of creatinine, couples with glucosuria are indicative of a renal tubular transport defect at 25 mg/kg/day dose level. it was not completely reversible within the recovery period.

Organ weight: Increased relative liver weight (19%) was observed in the male 50-75 mg/kg group.

Gross pathology: Pale kidney was observed in three dogs of the 50-75 mg/kg group. Reversible discoloration of urinary bladder was observed in all drug-treated groups (1/12, 2/12 and 4/8 dogs at 10, 25 and 50-75 mg/kg/day, respectively)

Histopathology: Submucosal hemorrhage in the urinary bladder were noted in 1/12, 5/12 and 6/10 dogs which received 10, 25 and 50-75 mg/kg/day, respectively. It was not observed in the recovery group, indicating reversibility. Renal cortical tubular atrophy was noted in 3/12 (minimal degree), 4/12 (minimal to mild degree) and 7/8 (mild to moderate degree) dogs which received 10, 25 and 50-75 mg/kg, respectively. It was not reversible. Irreversible inflammation was accompanied in the 50-75 mg/kg group. Cortical tubular vacuolation was present in the 25 and 50-75 mg/kg/day groups. In addition, karyomegaly of renal tubular epithelial cells was present in 6/12, 5/12 and 6/8 dogs which received 10, 25 and 50-75 mg/kg/day, respectively. It was reversible but not completely. Degeneration in testis was observed in the high dosage group (2/4 vs 1/5 in the control group). One female and one male from the high-dosage group were removed from the study on day 329 because of severe infection and were not necropsied in the

study.

In conclusion, zileuton at oral doses up to 50 mg/kg produced clinical signs, target organs of toxicity in kidney and urinary bladder and hematologic effects (anemia, neutropenia and thrombocytopenia). Most of toxic effect were nonreversible or incomplete if reversible. Degeneration in testis was observed in the high-dosage group. The drug-related effect observed in the 10 mg/kg were limited to minimal renal histologic changes in kidney and urinary bladder without measurable renal function deficit. Narrow of safety margin was provided by the study based on the drug exposure (AUC) at this dose since it represents only 1.5 times the human exposure.

SUMMARY AND EVALUATION:

Reports of the 3 month and 6 month oral toxicity studies were submitted on Sept. 29, 1987 and May 14, 1992 were reviewed by Dr. Tai on Nov. 29, 1987 and Dr. Sun on Feb. 12, 1993, respectively.

In the 3 month oral toxicity study, zileuton at dose levels of 0, 5, 20, 100 and 400 mg/kg was tested. It produced mortality, body weight loss, clinical signs of toxicity, hematological changes, increases in liver and adrenal weights, decrease in heart and prostate weights and histopathological changes in prostate, testis and uterus at 400 mg/kg. At 100 mg/kg, it exhibited clinical signs, retardation of body weight gain and histological changes in testis. However, prostate atrophy and testicular atrophy were found also at 20 mg/kg/day in the one month interim study.

In the 6 month oral toxicity study with a recover period of 4 weeks, at 200 mg/kg it produced hematopoietic toxicity, mortality, clinical signs, elevation of AP and GGT, renal toxicity, nonreversible prostatic atrophy but reversible testicular degeneration. At 100 mg/kg, it exhibited body weight loss, mortality, clinical signs, renal toxicity and prostatic atrophy. At 50 mg/kg, body weight loss, emesis and morphological changes in kidney were seen. A no effect dose of 20 mg/kg and a no male-reprotoxic dose of 50 mg/kg were established.

In the current 12 month study with a recovery period of 8 weeks, at 50 mg/kg it produced retardation of body weight gain, severe hematotoxic effects, clinical signs, elevations of creatinine and chloride, glycosuria, reduction of bicarbonate, degeneration in testis and toxicities in kidney and urinary bladder. Most of the toxicities were nonreversible or incomplete if they were reversible except the effect in testis. At 25 mg/kg, it still caused renal toxicity (pale kidney, renal cortical atrophy) and urinary

bladder (discoloration and submucosa hemorrhage). At lowest dose of 10 mg/kg, minimal to mild effects on kidney and urinary bladder were present but without any renal function deficit. Thus, an acceptable tolerated dose of 10 mg/kg was established.

Histopathological changes in testis and prostate observed in the one-month interim report of the 3 month study at 20 mg/kg maybe artifact since it was not reproducible at end of 3 month as well as in the 6 and 12 month studies at higher dose levels 50 and 25 mg/kg, respectively. Furthermore, according to the submitted summarized report of the reversibility study in dogs with various recovery periods of 1, 3 and 6 months, dogs received 100 mg/kg/day for one month developed some changes in testis (lower organ weight, decreased spermatogenesis and diminished cellularity). However, after one or three month recovery period, testicular weight returned to normal and no changes were found in testis. Although unilateral changes in testis (tubular hypocellularity and multinucleated giant cells) were seen in three dogs at end of 6 month recovery period, they were not considered to be treatment related due to their similarity to changed in one control dog and they were not seen at ends of one and three month recovery periods. Thus, the summarized report suggests reversibility of testicular changes after one month oral administration of zileuton. In summary, based on the AUCs of the no male-reprotoxic doses of 20, 50 and 25 mg/kg in the 3, 6 and 12 month toxicity studies, respectively, as compared that of the humans dosage of 600 mg qid, there are safety margin of 2.2-6.2 folds between the ~~rats~~ and human dosage.

Although drug accumulation was not demonstrated, ^{dog}enhancement of toxicity was indicated by lower lethal dose established in the 6 month study (100 mg/kg) as compared to that in the 3 month study (400 mg/kg) and severe toxicity exhibited in the 12 month study at 50 mg/kg. Additionally, renal toxicity was prevalent at lower dose in the 12 month study (20 mg/kg) as compared to that in the 6 month study (100 mg/kg) and no renal toxicity was seen in the 3 month study at doses up to 400 mg/kg. Therefore, with mild degree of renal effect observed at low dose of 10 mg/kg, it represent only 1.5 fold of safety margin based on AUC of 105 ug.hr/ml in dogs and 70 ug.hr/ml of the recommended human dosage (600 mg qid).

In the one year oral toxicity study in monkeys submitted in the amendment of Aug. 10, 1990, zileuton at doses up to 500 mg/kg/day did not produce any toxicity. However, as indicated in the original IND review by Dr. Sun on Oct. 17, 1990 that monkeys was not the appropriate animal model for predicting safety margin of zileuton for clinical trial due to its marked differences in extends of metabolism and elimination as compared with those in man. Moreover, AUC values at 150 mg/kg/day and 500 mg/kg/day in the 12 month monkey study were 29 and 27 ug.hr/ml, respectively, which represent less than

half of that in humans at the intended dosage of 600 mg qid.

Since narrower safety margin was indicated after one-year administration in dogs, titration of human dosage for duration of administration longer than 6 months and careful monitoring of renal function and male reproductive system should be exercised.

RECOMMENDATION:

Titration of human dosage may be necessary and careful monitoring of renal function and male reproductive performance should be exercised for intended duration in humans of longer than 6 months.


C. Joseph Sun, Ph. D.

March 19, 1993

Orig. IND
HFD-150/Division File
HFD-150/Dr. Sun
HFD-150/Dr. Jenkins

WJL 3-19-93

Pharmacologist Review of IND
(Amendment of June 23, 1992)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)
SPONSOR: Abbott Laboratories
Abbott Park, Illinois
DRUG: Zileuton (Abbott-64077)
DRUG CATEGORY: Leukotriene synthesis inhibitor (lipoxygenase inhibitor)

Date of receipt by HFD-150: June 25, 1992

Date of review: July 21, 1992

SUBMISSION CONTENT: A description of some preliminary findings in mouse and rat carcinogenicity studies.

A review has been completed by Dr. Choudary of HFD-180 (see attached memorandum to IND on July 9, 1992).

RECOMMENDATION:

Recommendations and comments listed in the Memorandum of July 9, 1992 should be conveyed to the sponsor.


C. Joseph Sun, Ph.D.

July 21, 1992

Orig. IND
HFD-150/Division file
HFD-151/CSO
HFD-150/Dr. Sun
HFD-150/Dr. Jenkins a/s 7/21/92

MEMORANDUM

DATE: July 9, 1992

FROM: Supervisory Pharmacologist
HFD-180

SUBJECT: INL (Zileuton/Abbott-64077) - Amendment (# 039)
Dated June 23, 1992 - Some Preliminary Findings in Mouse & Rat
Carcinogenicity Studies.

TO: IND

In amendment dated June 23, 1992, sponsor submitted a letter describing the status of the carcinogenicity studies of Zileuton in mice and rats. The following listed findings were reported as preliminary data:

1. Occurrence of hepatocellular adenomas in female mice (3.3, 0 and 13.8% incidence in 50, 150 and 450 mg/kg/day groups Vs 0% in concurrent controls). The incidence in the highest dose group was significantly ($P=0.0055$) higher.
2. Occurrence of hepatocellular adenocarcinomas in female mice of 450 mg/kg/day group (5% incidence). Sponsor claimed that this was not statistically significant when compared to concurrent controls, but did not provide the incidence in the concurrent control group.
3. Occurrence of dose related increased incidence of benign testicular interstitial cell tumors in the rats (18.3, 20.0 and 40.0% incidence in 40, 80 and 170 mg/kg/day groups Vs 5.0% in concurrent controls).

Sponsor tried to convey an impression of apparent safety of Zileuton at the maximum recommended human dose of 600 mg q.i.d. (48 mg/kg/day) in spite of the above listed findings in the carcinogenicity studies. However, the supporting logic for sponsor's contention is flawed. The data from the two carcinogenicity studies are apparently not completely assessed. There are "other tumor data" which need to be reviewed and analyzed. Even though Zileuton was negative in the completed mutagenicity tests, occurrence of tumors in different organs in different species is in itself unsettling. Sponsor claimed that the highest doses employed in the carcinogenicity studies are markedly "supratherapeutic". This claim is neither correct nor well founded if the dose comparisons are based on surface area. Thus the maximum recommended human dose (600 mg q.i.d., 48 mg/kg/day) of 1776 mg/m² is much higher than the highest doses employed in the mouse (450 mg/kg/day = 1350 mg/m²) and rat (170 mg/kg/day = 1003 mg/m²) carcinogenicity studies. The other doses from these studies would amount to small fractions of the human dose.

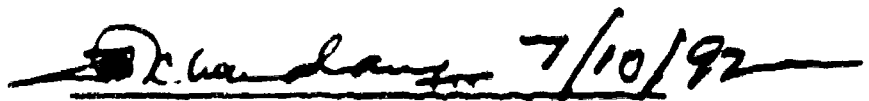
Even though sponsor stated that the incidence of hepatocellular adenocarcinomas in female mice failed to reach statistical significance, it probably will attain significance if the comparisons are correctly based, i.e. compare the combined incidences of hepatocellular adenomas and adenocarcinomas. As per sponsor's account, studies on hepatocellular enzyme induction in mice (specific P450 families) are still pending.

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Sponsor implied that the dose related increased incidence of benign testicular interstitial cell tumors in rats is somehow related to some presumed influence of Zileuton on hypothalamo-hypophysio-gonadal axis interfering with GnRH, LH or testosterone and on the hepatic 2B and 3A families of P450 affecting testosterone catabolism. No data in rats exist to support the aforementioned presumptions. Sponsor's claim that Zileuton does not exert such effects in humans is irrelevant, since such effects are not demonstrated in rats.

The incidences of hepatocellular adenoma (13.8%) and hepatocellular adenocarcinoma (5%) in mice at the highest dose of Zileuton and the dose related increased incidences of benign testicular interstitial cell tumors in rats (18.3 to 40.0%) are higher than the incidences in their respective concurrent control animals and the historical strain control incidences (For female Charles River CD₁ mice: hepatocellular adenoma-0.8% and hepatocellular carcinoma-1.0%; For male Charles River Sprague-Dawley rats: testicular interstitial cell tumors-3.5%). The doses which produced these varied tumorigenic effects in these species of animals represent only fractions of maximum recommended human dose on surface area basis. Sponsor's reasoning for considering these findings as not relevant to human safety is not convincing or well founded. Sponsor should therefore be asked to provide the following expeditiously for an accurate and full safety assessment of the findings of mouse and rat carcinogenicity studies of Zileuton:

1. Full reports of the mouse and rat carcinogenicity studies.
2. A comprehensive report on the effects of Zileuton on hepatocellular enzyme induction, hepatic P450 families and hepatic peroxisomal proliferation in CD₁ mice and Sprague-Dawley rats.
3. A thorough investigation in to short term and long term effects of modest doses of Zileuton on male reproductive hormones and the hypothalamo-hypophysio-gonadal axis and their relation to the induction of testicular interstitial cell tumors.
4. Include in the Investigator Brochure the information on the occurrence of tumors in mouse and rat carcinogenicity studies.


Jasti B. Choudary, Ph.D., B.V.Sc.

cc:
IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-180/Dr. Mukherjee (IND)
HFD-150/Dr. Taylor (IND)
HFD-180/Dr. Retrie - Suh
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**Pharmacologist Review of IND
(Amendment of May 14, 1992)**

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)

SPONSOR: Abbott Laboratories
Abbott Park, Illinois

DRUG: Zileuton (Abbott-64077)

DRUG CATEGORY: Leukotriene synthesis inhibitor (lipoxygenase inhibitor)

Date of receipt by HFD-150: May 19, 1992

Date of Review: Feb. 12, 1993

SUBMISSION CONTENTS: 6 month oral toxicity study in dogs

**6 Month Oral Toxicity Study in Dogs
(Report no. 91/296)**

Testing laboratory: Sponsor

Date of the study: Nov. 21, 1990 to March 16, 1992

GLP requirement: A statement of compliance with GLP regulations was included.

Methods: Five groups of Beagle dogs (7-14 kg and 13-15 months of age) each consisting of four males and 4 females were given Zileuton (lot no. 46-097.AL) in capsules at oral doses of 0, 20, 50, 100 and 200 mg/kg/day for 26 weeks. Additional two males and two females were assigned to each of the control and 100 and 200 mg/kg/day group for a recovery period of 4 weeks. Following parameters were monitored:

Clinical signs	daily
Body weight and food consumption	weekly
Ophthalmologic examination	weeks 0, 13 and 26
Prostate palpation	months 0, 1, 3 and 6
EKG	weeks 0 and 26
Plasma drug level	0, 1,3,6 and 24 hours after dosing during weeks 1,6,11 and 22 of treatment
serum hormone (luteinizing hormone and testosterone)	weeks 7, 15 and 25

Hematology and blood chemistry	weeks 0, 13, 26 and 30
Urinalysis	week 26
Organ weight, gross and histopathology	weeks 26 and 30

Plasma levels of the parent compound and its metabolite were measured by HPLC method. The lower limit of detection were 0.01 ug/ml and 0.1 ug/ml for the parent and metabolite, respectively.

Results:

Clinical signs: Decreased activity, prostration, weakness, increased salivation, emesis, lacrimation and changes in the stool were observed in the 100 and 200 mg/kg/day groups. Dehydration was also seen at the 200 mg/kg/day dose level. Slight increase in the frequency of emesis and changes in stool pattern were seen at the 20 and 50 mg/kg/day dosage levels. The above-mentioned clinical signs were not observed at end of recovery period.

Mortality: Drug-related deaths were seen in the 100 (4/8) and 200 mg/kg/day (5/8) groups.

Body weight: Significant body weight loss (4 kg) was observed in the male 200 mg/kg group. Some individual 50 and 100 mg/kg treated dogs showed marked body weight loss (0.7-1 kg) during drug treatment.

Food consumption: No statistical changes were reported.

Ophthalmologic examination and EKG: Normal.

Plasma drug level: The Pk data of parent compound are summarized on the next page:

	week 1				week 6			
	Tmax	Cmax	AUC	t1/2	Tmax	Cmax	AUC	t1/2
20 mg/kg	1.5	15.39	185.56	8.34	2.6	20.06	229.04	7.22
50 mg/kg	4.1	17.51	272.74	10.28	2.6	32.98	409.80	7.68
100 mg/kg	3.3	40.38	697.43	9.35	4.8	66.51	950.88	8.25
200 mg/kg	9.3	68.43	1294.10	25.07	3.5	66.37	1030.22	9.65
	week 11				week 22			
20 mg/kg	4.9	19.63	272.51	16.43	3.4	22.19	288.54	7.69
50 mg/kg	2.8	25.47	352.68	8.32	3.6	31.64	434.71	8.01
100 mg/kg	2.9	41.15	593.62	8.29	3.5	44	624.82	8.40
200 mg/kg	5.1	80.82	1092.08	7.68	3.4	63.21	890.46	9.70

Unit for the parameters are: Tmax,hr; Cmax,ug/ml; AUC, ug.hr/ml and t 1/2, hr.

Oral absorption was moderate to slow as indicated by the Tmax being 1.5-4.9 hrs for the 20 and 50 mg/kg/dy groups and 2.9-9.3 hrs for the 100 and 200 mg/kg/day groups. Elimination was slow with T 1/2 being 7-10 hrs. Plasma levels of Zileuton did not increase in a dose-proportional manner.

No accumulation of parent drug was observed and steady state was achieved by week 6 as shown in the figure below (extracted from sponsor's figure 5 on page 405):

The ratios of AUCs of parent compound for dogs to that anticipated in man after 2400 mg/day dosing (70 ug.hr/ml) were 2.7-4.1, 3.9-6.2, 8.5-13.6 and 12.7-18.5 for the 20, 50, 100 and 200 mg/kg/day dose groups, respectively.

Plasma level of Abbott-66193 were quite low representing 0.5-4.5 % of the plasma levels of parent compound indicating minimal production or metabolism.

Hormone level: Transient decreases in LH and testosterone were observed in two dogs each of the 100 and 200 mg/kg/day groups at weeks 7 and 15. All four animals also exhibited body weight loss.

Urinalysis: Ketonuria and glycosuria were found in the 200 mg/kg/day group. Glycosuria was still observed in one of the 200 mg/kg recovery group.

Hematology: Decreases in RBC (15-40%), hematocrit (14-57%), hemoglobin (40%) and platelet (56%), increased APTT (2.16 sec) in males and granulocytic hyperplasia were found in the 200 mg/kg/day group. Decrease in RBC but not statistically significant was reported at 50 and 100 mg/kg.

Blood chemistry: Increase in chloride (5-9%), decrease in aspartate aminotransferase (35-41%) were observed in the 200 mg/kg/day group. In addition, decrease in albumin (48%), elevations of AP (900%) and GGT (260%) in males and increase in creatinine (57%) and decrease in protein (9%) in females were seen in the 200 mg/kg/day group.

Organ weight: Increase in relative liver weight (5% vs 2.8%) and decrease in absolute heart weight (50%) were seen in one surviving male of the 200 mg/kg/day group. Increased liver weight was also seen in the 100 mg/kg/day group but not statistically significant.

Gross pathology: Duodenal ulcer was seen in 100 and 200 mg/kg/day groups. Greenish discoloration in kidney and small prostate were found in the 200 mg/kg/day group.

Histopathology: Karyomegaly in tubular cells were found in all dose-treated groups (1/8, 4/8, 4/12 and 4/12 for 20, 50, 100 and 200 mg/kg, respectively). Anisokaryosis (enlarged nuclei in renal cells) was seen in the 50 and 200 mg/kg/day groups. Prostatic atrophy (3/4) and testicular degeneration (2/4) were observed in the 200 mg/kg and prostatic atrophy (1/4) was reported in the 100 mg/kg/day group. Atrophy of prostate was still present (2/2) at end of 4 week recovery period, indicating a non reversibility of its prostatic atrophy at 200 mg/kg.

In conclusion, Zileuton given orally for 6 months caused hematopoietic toxicity, ~~kidney~~ toxicity, mortality, clinical signs of toxicity, elevations of AP and GGT, renal toxicity, nonreversible prostatic atrophy but reversible testicular degeneration at 200 mg/kg/day dose level. At 100 mg/kg, it produced body weight loss, mortality, clinical signs, renal toxicity and prostatic atrophy. Transient decrease in plasma testosterone and LH were found at dose levels of 100 and 200 mg/kg at weeks 7 and 15. At 50 mg/kg, body weight loss, emesis and morphological changes in kidneys were seen. Thus, a lethal dose of 100 mg/kg and a no effect dose of 20 mg/kg were established. At 20 mg/kg, AUC was 3-4 folds the AUC of the proposed clinical dose (2400 mg/day).

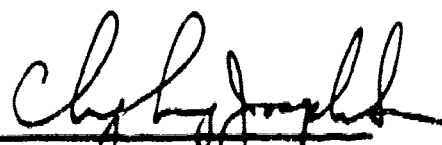
SUMMARY AND EVALUATION:

Target organs of toxicity induced by Zileuton were hematopoietic system, kidney, liver, GI tract and male reproductive organs. Enhancement of toxicity was indicated by lower lethal dose being established in the 6 month study (100 mg/kg) as compared to that in the 3 month study (400 mg/kg) and the presence of duodenal ulcer, although drug accumulation was not apparent.

Similar findings in male reproductive organs were reported in the three month study in dogs whereas histological changes in male reproductive organs were not seen in rats and monkeys. Zileuton has been shown to bind to androgen receptors from the prostate glands of dogs, rats, monkeys and man with affinity (pK_d) values of 4.94, 4.32, 4.17 and 2.98, respectively. Thus, the sponsor suggested that toxicity observed in the male reproductive organs was species specific and possibly related to greater affinity of Zileuton for dog versus rats and monkeys. Although the affinity to androgen receptors is much weaker in humans, clinical monitoring of male reproductive performance, i.e. prostatic ultrasound, semen analysis and endocrine profile could be served as a precaution.

RECOMMENDATION:

Clinical monitoring of male reproductive performance in the planned clinical trial as recommended by Dr. Fourcroy of HFD-510 could be useful (see the Interoffice Memorandum of July 29, 1992).


C. Joseph Sun, Ph.D.

Feb. 12, 1993

SUN

FEB 23 1993

Pharmacologist Review of IND
(Amendment of Feb. 9, 1990)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)

DRUG: Zileuton (Abbott-64077)

DRUG CATEGORY: Lipoxygenase inhibitor

Date of review: Feb. 23, 1993

SUBMISSION CONTENT OF PRECLINICAL STUDY: Hepatic drug-metabolizing activity in rats

Methods: Charles River Rats (6M+6F/dose group) were dosed daily via oral gavage with Abbott-64077 as a suspension in 0.2% hydroxypropyl methyl cellulose at doses of 0, 20, 80, 150 and 300 mg/kg/day or sodium phenobarbital (50 mg/kg/day) for 10 days. The animals were sacrificed and the livers were removed, weighed and frozen for the determination of P-450 contents and benzphetamine N-demethylase and 7-ethoxycoumarin O-deethylase activities.

Results:

Relative liver weight: Increased relative liver weights were observed in the 150 and 300 mg/kg and the sodium phenobarbital groups (22-39%).

N-demethylase activity: Dose-related increases were observed in all female drug-treated groups (53-399%) and the male 80-300 mg/kg groups (131-162%). Sodium pentobarbital produced increases in activity (57% in males and 371% in females).

O-Demethylase activity: Dose-related increases were observed in all drug-treated animals (59-1100%). Sodium pentobarbital caused marked increases (1400-2000%).

P-450 contents: Increases were reported in all drug-treated groups (67-328%). Positive control, sodium pentobarbital, produced 370% and 60% increases in males and females, respectively.

In conclusion, ten daily oral administrations of Zileuton (300 mg/kg/day) caused same degree of enzyme induction as that of sodium pentobarbital (50 mg/kg/day). Higher N-demethylase activity was observed in zileuton-treated female rats, however, no gender differences in increases in liver weights, O-deethylase activity and P-450 content were reported in the zileuton-treated rats.

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RECOMMENDATION: NAI.

Orig. IND
HFD-150/Division file
HFD-150/Dr. Sun *CS* 2-23-93


C. Joseph Sun, Ph.D.

Feb. 23, 1993

SUN

FEB 22 1993

Pharmacologist Review of IND
(Amendment of Nov. 16, 1990)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)
SPONSOR: Abbott
DRUG: Zileuton (Abbott-64077)
DRUG CATEGORY: lipoxygenase inhibitor
Date of review: February 22, 1993

SUBMISSION CONTENTS: (1) pk data of the 3-month MTD dietary study in rats, (2) PK data of the 6-week oral toxicity study in immature rats, (3) metabolism and disposition in mice following oral administration of Zileuton C¹⁴ and (4) protein binding to human plasma.

Rats:

Methods: Blood samples were collected via cardiac puncture from rats (5M+5F/dose group) at 7 a.m. on day 14 following dietary administration of zileuton at dose levels of 80, 170, 350 and 750 mg/kg/day. A complete study of the 3 month MTD study in rats was submitted (amendment of 6/20/89) and reviewed by Dr. Sun on February 22, 1993. The concentrations of zileuton and its N-dehydroxylated metabolite, Abbott-66193, in the plasma were determined using the HPLC procedure developed by the sponsor. The detection limit was 0.01 ug/ml.

Results: The average dose levels and plasma concentrations were as follows (extracted from sponsor table on page 6):

Treatment Group/Sex	Dose (mg/kg/day)	Plasma Concentrations (µg/mL)	
		ABBOTT-64077	ABBOTT-66193
T ₁ /M	60.4 ± 2.8	6.66 ± 1.96	2.84 ± 1.17
T ₂ /M	124.8 ± 2.4	10.83 ± 1.81	6.09 ± 0.86
T ₃ /M	245.8 ± 11.6	22.20 ± 11.14	10.38 ± 1.88
T ₄ /M	501.3 ± 16.1	35.07 ± 14.90	19.87 ± 2.11
T ₁ /F	63.9 ± 3.6	11.40 ± 3.06	2.48 ± 0.90
T ₂ /F	121.8 ± 4.1	28.96 ± 8.92	6.82 ± 3.01
T ₃ /F	249.0 ± 10.7	32.93 ± 7.63	14.38 ± 3.41
T ₄ /F	521.2 ± 22.7	54.85 ± 14.82	25.36 ± 3.28

T1: 80 mg/kg/day; T2: 170 mg/kg/day; T3:350 mg/kg/day and T4:750 mg/kg/day

Higher plasm level of parent compound (1.5 times that of males) and significant dose effect were demonstrated in the females.

The ratios of Abbott-66913 concentration over parent compound ranged from 0.43-0.67 in the male rats and from 0.22-0.48 in the female rats, indicating high degree of metabolism to Abbott-66913 in rats. Significant dose-normalized decreases in plasma Abbott-64077 was reported in females, suggesting possible increased liver weight and enzyme induction. The mean Abbott-64077 plasma concentrations in rats at actual dietary doses of 60-64, 122-125, 246-249 and 501-521 mg/kg were 1.6-2.7, 2.6-6.9, 5.3-7.8 and 8.4-13.1 times, respectively, the mean human steady-state concentration (4.2 ug/ml) at maximuan dose of 600 μ q.i.d..

Immature rats:

Methods: Plasma samples were collected at 3 hours after the third daily dose in a six-week toxicity study, involving administration of 20, 80 and 300 mg/kg doses of Zileuton to immature (2 weeks of age) rats. Zileuton were administered as a suspension in 0.2% hydropropylmethylcellulose. HPLC was used for determination of parent compound and its metabolite, Abbott-66193 in the plasma. Following PK data were obtained (extracted from sponsor's table on page 57):

Dose (mg/kg/day)	Plasma Concentrations 3 Hr. Postdosing (μ g/mL)			
	A-64077	A-66193	A-64077	A-66193
	Male Rats		Female Rats	
0	0.36 $\pm$ 0.13	0.00 $\pm$ 0.00	0.25 $\pm$ 0.14	0.00 $\pm$ 0.00
20	11.71 $\pm$ 4.06	0.12 $\pm$ 0.08	14.81 $\pm$ 2.33	0.13 $\pm$ 0.08
80	46.84 $\pm$ 16.69	0.96 $\pm$ 0.10	52.08 $\pm$ 17.31	1.55 $\pm$ 1.15
300	101.10 $\pm$ 16.58	3.17 $\pm$ 0.86	121.88 $\pm$ 9.84	3.63 $\pm$ 0.91

As compared with plasma concentrations of parent compound obtained from adults rats with 80 and 300 mg/kg in study V89-042 being 29.7 and 84.95 ug/ml, respectively and those of Abbott-66193 being 0.29 and 0.61 ug/ml, respectively, it appeared that the plasma concentrations of both compounds were higher in the juvenile (immature) rats. Although without AUC data a conclusion of higher exposure due to better absorption or

decreased elimination of compound by immature liver in immature rats is difficult to be drawn from plasma drug concentration alone, careful risk and benefit assessment and titration of dosage should be exercised in the pediatric and infant patients.

Mice:

Method: The metabolism and disposition of carbon-14 labeled zileuton were studied in mice following oral administration of a single 5 mg/kg dose of the drug (23.4 uCi/mg). At 1, 2, 4, 8 and 10 hrs after oral dose, blood samples were collected by cardiac puncture from groups of mice. In addition, all urine and feces excreted by the two groups of mice were collected daily for three days after oral dosage.

Results: For parent compound, T_{max} was 1 hr and C_{max} was 2.02 ug/ml. Very low levels (0.01-0.02 ug/ml) of abbott 66913 were detected at any time points. S-zileuton glucuronide was found in the plasma (0.49-0.68 ug/ml) at 1 and 2 hours. Only traces of R-zileuton glucuronide were detected, suggesting stereoselective glucuronidation of the S-enantiomer. Other unidentified metabolite was detected at 0.07 ug/ml at 1 hr to a maximum of 0.68 ug/ml at 8 hrs. Significant amounts of polar material were present but have not been characterized. An average of 74 % and 16% of the administered radioactivity was recovered in urine and feces, respectively. The major metabolites in the urine consisted of polar material which represented 45 % of the dose. Majority of polar material in urine consisted of conjugates of another unidentified metabolite (M-1). R and S-zileuton glucuronides accounted for 0.6 and 15.6 % of the dose, respectively. Zileuton and Abbott 66913 were not found in the urine samples. In the feces, predominant metabolites were M-1 (9.5% of the dose) and Abbott 66913 (4.8% of the dose).

Human plasma protein binding: The in vitro protein binding of ¹⁴C-zileuton in human plasma ranged from 92.7 to 93.4 % at a concentration of 5 ug/ml. The addition of theophylline (20 ug/ml), prednisone (50 ug/ml), and salbutamol (0.005 ug/ml) had no effect on the protein binding of zileuton. The addition of naproxen (100 ug/ml), salicylate (250 ug/ml), ibuprofen (50 ug/ml) and sulfasalazine (50 ug/ml) produced small reduction (0.3-2%) in the binding of zileuton. It did not affect the protein binding of warfain, salicylate, theophylline, naproxen, ibuprofen and prednisone at concentration of 5 ug/ml. The bindings of zileuton to human albumin and α_1 -acid glycoprotein were 87.5 and 34 %, respectively.

SUMMARY AND EVALUATION:

In the 3 month dietary MTD study in rats, dose-related increases in plasma parent levels were demonstrated. Higher plasma level of parent compound was indicated in female rats. Major metabolite, Abbott 66913, was present in the plasma. The PK data could be useful in determining proper employment of maximal tolerated dose in carcinogenicity study.

Higher plasma concentrations (31-66%) were observed in the immature rats of 2 weeks of age as compared with those in the adult rats. Thus, titration of dosage to be used in pediatric patients should be exercised.

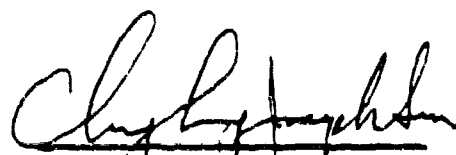
In mice, oral absorption of parent compound was rapid with T_{max} being 1 hr. Very low level of Abbott 66913 was detected in plasma. S-glucuronide was present and only traces of R-zileuton glucuronide were detected, suggesting stereoselective glucuronidation of S-enantiomer. Major route of elimination was urinary. The major metabolites in the urine consisted of polar material. None of parent compound and Abbott 6913 were detected in the urine.

Human plasma protein binding of zileuton was high (92.7-93.4%).

RECOMMENDATION: NAI.

Orig. IND
HAD-150/Division File
HAD-150/Dr. Sun

WJ, 2-22-93


C. Joseph Sun, Ph. D.

Feb. 22, 1993

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Pharmacologist Review of IND
(Amendment of June 20, 1989)

REVIEWER: C. Joseph Sun, Pharmacologist (HFD-150)

SPONSOR: Abbott

DRUG: Zileuton (Abbott-64077)

DRUG CATEGORY: lipoxygenase synthesis inhibitor

Date of review: Feb. 22, 1993

SUBMISSION CONTENTS: Three month maximal tolerated dietary dose studies in rats and mice

The studies have been reviewed by Dr. Sun (see INT. of 10/17/90).

SUMMARY AND EVALUATION:

In the rats study, Zileuton at dietary dose levels of 80, 170, 350 and 750 mg/kg was tested to determine its MTD for carcinogenicity study. At 750 mg/kg, it produced severe retardation of body weight gain, decreased WBC counts, elevations of GGT, BUN and cholesterol, nephritis as well as increased liver weight. At lower dose of 350 mg/kg, retardation of body weight gain, nephritis and increased liver weight were observed. The PK data on day 14 suggest that the plasma levels were increased dose proportionately. The sponsor considered that 170 mg/kg was the MTD. Based on the data, we conclude that the maximal tolerated dose is 350 mg/kg. Had additional effects observed at lower dose tested at end of the carcinogenicity study, lower dose could be the MTD.

In the mouse study, Zileuton at dietary dose levels of 80, 200, 450 and 1000 mg/kg/day was tested to determine its MTD for carcinogenicity study. It did not produce any toxicities except increased liver weight and hepatic cytomegaly at 450 and 1000 mg/kg, lower cholesterol level, retardation of food consumption and hepatic degenerative change (vacuolation) at 1000 mg/kg. The sponsor considered that 450 mg/kg was the MTD for carcinogenicity study. Judging from no effect on body weight gain but some liver effects, we conclude that maximal tolerated dose could be 1000 mg/kg. However, lower dose could be considered the MTD if additional effects are seen at end of the two year carcinogenicity study.

IND
Page 2

RECOMMENDATION: Final evaluation for the employment of proper dose for the carcinogenicity studies will be assessed upon submission of complete reports of the carcinogenicity studies in rats and mice.


C. Joseph Sun, Ph. D.

Feb. 22, 1993

Orig. IND
HFD-150/Division file
HFD-150/Dr. Sun
~~HFD-150/Dr. Taylor~~ ~ *JS* 2-22-93

IND:

DATE: August 5, 1988

Review # 4

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Amendment # 6 3/4/88

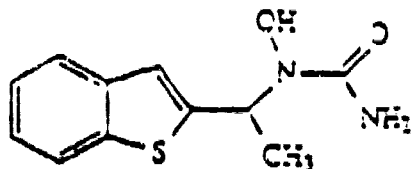
Amendment #10 5/19/83

Amendment #13 7/21/88

SPONSOR: Abbott Laboratories
Pharmaceutical Products Division
Abbott Park, IL 60064

NAME OF DRUG: ABBOTT-64077

Chemical Structure



Chemical Name

N-(1-benzo(b)thien-2-yl)ethan-1-amine N-oxide.

M.W.: 236.294

CATEGORY: Lipxygenase Inhibitor (leukotriene synthesis inhibitor)

ROUTE OF ADMINISTRATION: Oral

DOSAGE FORM: Capsule

PRECLINICAL DATA:

TOXICOLOGY:

Studies submitted in amendment #6 are the following:

Abbott-64077 Drug Metabolism Report No. 3:
"Pharmacokinetics of Abbott-64077 in Dogs After One Month
Treatment with 5, 20, 100 and 400 Mg/Kg. Day Doses (Study T887-
1347)." REVISED 1/12/88;

Abbott-64077 Drug Metabolism Report No. 9:
"High Sensitivity High-Performance Liquid Chromatographic
Procedure for the Determination of ABBOTT-64077 in Plasma."
1/28/88;

Abbott-64077 Drug Metabolism Report No. 10:
"Plasma ABBOTT-64077 Levels in Rats Treated for Three Months with
Oral 5, 20, 30 and 300 Mg/Kg. Day Doses in Toxicology Study T887-
140." 1/15/88;

Abbott-64077 Drug Metabolism Report No. 11:
"Plasma Concentrations and Plasma Protein Binding of ABBOTT-64077
after Oral Administration to Rats in Corn Oil and
Hydroxypropylmethylcellulose." 2/2/88;

Abbott-64077 Drug Metabolism Report No. 12:
"ABBOTT-64077 Levels in the Plasma of Monkeys Given a Single Oral
100, 200, 400, 600, 800 or 1000 Mg/Kg Dose." 2/24/88.

Studies submitted in amendment #10 are the following:

- o Abbott Scientific Report No. PPRd/87/047: "Abbott-64077 Drug Metabolism Report No. 13 - Plasma Abbott-64077 Levels in Monkeys Treated for One Month with Oral 20, 50, 150 and 360 Mg/Kg/Day Doses in Toxicology Study T887-428" (reports 1 through 12 previously submitted to the IND)
- o Abbott Scientific Report No. PPRd/87/281: "One-Month Oral Toxicity Study of Abbott-64077 in Monkeys"
- o Abbott Scientific Report No. PPRd/88/063: "Unscheduled DNA Synthesis in Rat Primary Hepatocytes of Abbott-64077"
- o Abbott Scientific Report No. PPRd/87/280: "Evaluation of the Effects of Orally Administered Abbott-64077 on the Embryonic and Fetal Development of the Rat (Segment II TFR)" ✓
- o Abbott Scientific Report No. PPRd/88/064: "Acute Oral Toxicity Evaluation of Abbott-64077 in Combination with Several Bronchodilator and Anti-Inflammatory Agents in Male Mice" ✓
- o Abbott Scientific Report No. PPRd/88/081: "Developmental Toxicity (Embryo-Fetal Toxicity and Teratogenic Potential) Study of Abbott-64077 Administered Orally to New Zealand White Rabbits" ✓
- o Abbott Scientific Report No. PPRd/88/017: "Abbott-64077 Drug Metabolism Report No. 11 - Plasma Concentrations and Plasma Protein Binding of Abbott-64077 after Oral Administration to Rats in Corn Oil and Hydroxypropylmethylcellulose" REVISED (originally submitted in IND Amendment 005, March 4, 1988)

REVIEW OF STUDIES:

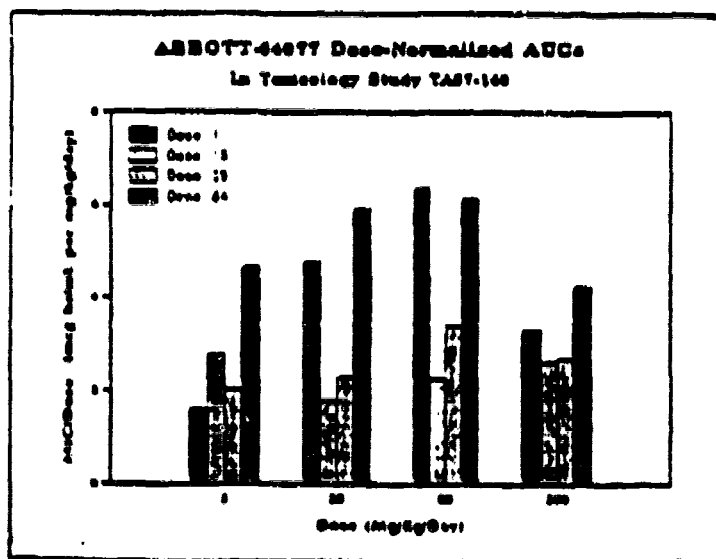
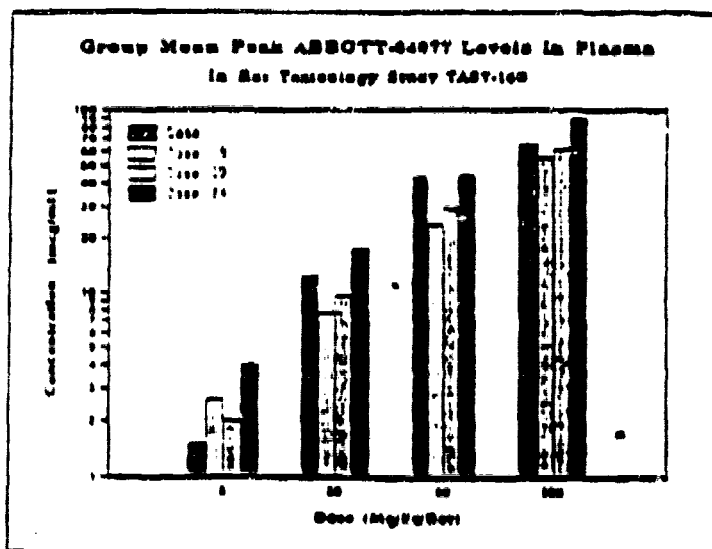
Amendment # 6:

1. Pharmacokinetics of ABBOTT-64077 in dogs after one month treatment with 5, 20, 100 and 400 mg/kg/day doses (study TB87-134)," REVISED 1/12/88;

The original study report was submitted (9/29/87) and reviewed (11/29/87). The revised report submitted here contains very minor revision which does not change the conclusion and its impact on the final recommendation established in the 11/29/87 review. Therefore, in order not to duplicate the effort, no further comment at this time.

3. Plasma ABBOTT-64077 levels in rats treated for three months with oral 5, 20, 80 and 300 mg/kg/day doses in toxicology study TA87-140

Serial plasma samples were taken after doses 1, 15, 29 and 84 of a three month toxicology study with one month interim kill involving oral administration of 5, 20, 80 and 300mg/kg/day doses to Sprague-Dawley rats. In this report, peak plasma levels and estimated AUCs of the 84th dose are provided. In order to see the whole picture, Peak plasma concentrations and dose normalized AUCs of ABBOTT-64077 are shown in the following tables:



Conclusion: The results indicated that systemic exposure in rats are dose related, however, pathology findings require a more detailed examination of the chronological patterns in AUC/Dose. High interanimal variances limit the extent of inferences derived from the data.

4. PPRD 88/017 Plasma concentrations and plasma protein binding of ABBOTT-64077 after oral administration to rats in corn oil and hydroxypropylmethylcellulose.

See review on the revised report in Amendment # 10.

5. PPRD/88/017 ABBOTT-64077 levels in the plasma of monkeys given a single oral 100, 200, 400, 600, 800 and 1000 mg/kg/day

Animal: cynomolgus monkeys, 6 of either sex, body weight of 2-4 kg.

Drug: ABBOTT-64077 was administered orally through a nasogastric tube at a concentration of 33 ml/kg in corn oil.

Dosage and Design: six doses were studied, 100, 200, 400, 600, 800 and 1000mg/kg. Animals were fasted over night and 8 hours post dosing. Venous blood samples were collected at 0.5, 1.0, 2.0, 4.0, 6.0 and 24 hours.

Analytical methodology:

Results:

Plasma concentrations of ABBOTT-64077 and estimated AUCs are summarized in the following table:

Dose (mg/kg)	Concentration (mcg/ml)						Area Under 0-24 Hr Curve (mcg hr/mL)
	Hours After Oral Administration						
	0	0.5	1	2	4	6	24
100							
200							
400							
600							
800							
1000							

Conclusions:

Erratic results prevented further pharmacokinetic analysis. The sponsor decided to select the dose, 400mg/kg/day as the maximal dose in monkey toxicity studies.

Amendment # 10:

1. PPRd/88/047 Plasma A-64077 Levels in Monkeys Treated for One Month with Oral 20, 50, 150 and 360 mg/kg/day Doses in Toxicology Study TC87-428

Animal: Cynomolgus monkeys, 4 male and 4 female/group
Body weight ranging from 2.1 to 4.2 kg.
age not specified.

Drug: ABBOTT-64077 suspended in 0.2% hydroxypropylmethylcellulose (5.0 ml/kg), administered via nasogastric tube.

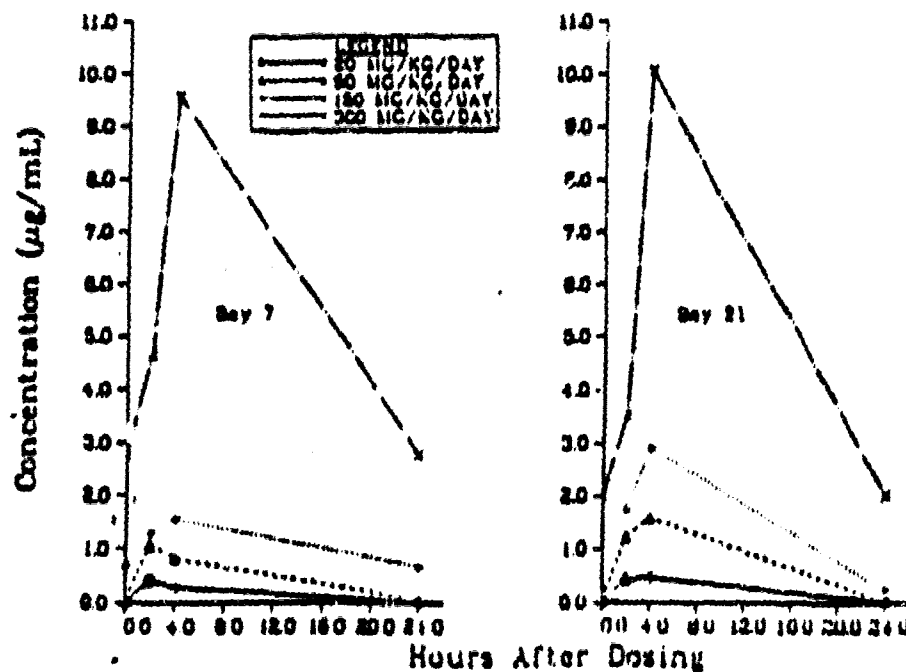
Dosage and Design: Dosages and dosing schedules are as the following:

Treatment Group	Dosage (mg/kg/day)
T0	0 (vehicle only)
T1	20
T2	50
T3	150
T4	360

At 2, 4 and 24 hours after dosing on days 7 and 21, samples of venous blood were collected from each monkey.

Results:

The plasma concentrations of ABBOTT-64077 in monkey after oral administration at Day 7 and Day 21 are summarized in the following figure:



The roughly estimated AUCs are summarized in the following Table.

Monkey No. *	20 mg/kg/day		50 mg/kg/day		150 mg/kg/day		360 mg/kg/day	
	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
1	11.49	8.93	22.77	23.96	21.91	94.98	49.53	98.31
3	3.40	3.88	9.12	17.77	39.12	55.18	55.95	64.25
5	1.74	5.92	15.77	36.94	11.17	19.27	147.45	148.19
7	1.63	2.22	7.26	21.00	22.36	29.26	195.52	91.17
2	2.52	4.36	10.28	21.95	34.41	15.07	83.40	114.54
4	3.46	7.77	5.56	9.54	8.70	16.00	348.90	169.76
6	5.98	11.40	14.00	8.72	49.90	34.05	214.88	343.54
9	1.21	4.50	8.46	22.61	28.56	42.33	67.32	99.57
Mean	3.93	6.12	11.65	20.25	27.02	38.27	145.19	140.20
S.D.	3.41	3.01	5.62	8.89	13.95	26.76	104.31	89.00
S.E.M.	1.21	1.07	1.99	3.14	4.93	9.46	36.99	31.46

* Monkey number within each dosing group

Conclusions:

The plasma concentrations of ABECTT-64077 are highly variable among the animals. The data apparently showed a trend toward higher plasma concentrations at day 21 after dosing compared with day 7.

The peak plasma concentration and the estimated AUCs showed a linear relationship to the administered dose. As for the AUC data, it is worth noting that the AUC estimates were only based on three sampling times. Should this estimate be used as an estimate of exposure, then the highest dose, 360mg/kg/day in the monkey yield an AUC of 140-145 mcg.hr/ml which is about 10x of the human 600mg (or 12mg/kg/day) dose.

2. FFR1/87/261 One month oral toxicity study of ABECTT-64077 in monkeys

Animal: same as 1.

Drug: same as 1.

Dosage and Design: same as 1.

Results:

Mortality: none

Clinical signs: summarized in the following table:

Observations

ABBOTT-64077					
Treatment Group	Dosage (mg/kg/day)	Sex	Monkey Number ^a	Day ^b	Observation
T ₀	0	F	0004	11-15	Vaginal discharge
			0006	1	Emesis
T ₁	20	M	1003	9,10	Patch of hair loss on left forearm
			1005	21	Prolonged bleeding after blood sampling
		F	1004	13-15	Vaginal discharge
			1006	25-27	Vaginal discharge
T ₂	50	M	2001	4	Clear nasal discharge
			2005	7,8	Picking hair from left forearm
		F	2004	16,17	Vaginal discharge
			2008	21	Vaginal discharge
T ₃	150	M	3005	9-11,13, 15-18,21-23, 26,27,29	Unformed, loose, mucoid or watery stool or diarrhea
			3007	18	Greenish foam with stool
			F	3004	4 24-27
		M	3006	11,15-18, 21,30 21	Loose and/or watery stool or diarrhea Vaginal discharge
			3008	21-23	Vaginal discharge
T ₄	360	M	4001	0	Slightly decreased activity, drowsy appearance, and drooping eyelids
		F	4004	15-17,21,22	Vaginal discharge

^aAnimals not listed appeared normal throughout the treatment period.

^bTreatments began on day 0. All monkeys were examined twice daily for survival and general physical condition, and detailed examinations were made on days 0, 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, 16, 18, 21, 23 and 25.

Post-mortem studies:

Organ weights: There was no biologically significant differences found among the mean organ weights of treated monkeys and controls.

Gross and microscopic observations: Immature or developing testes with larger than normal thymus, tubular microgranulomata with yellow brown birefringent crystalline material in the kidneys from several monkeys including the controls were noted.

<u>Treatment group</u>	<u>Kidney crystalline</u>	<u>Immature testes</u>
Control	4 male/4	2/4
20mg/kg/day	1 female/4	2/4
50mg/kg/day	2 female/4 1 male/4	2/4
150mg/kg/day	1 female/4 1 male/4	1/4
360mg/kg/day	1 female/4 1 male/4	1/4

Conclusions:

There was no significant findings in any of the following parameters: body weight, food consumption, hematology, bone marrow, clinical chemistry, hormone levels, organ weights and gross and microscopic observations (with the exception of tubular microgranulomata with yellow brown birefringent crystalline material in the kidneys in quite a few monkeys including the controls). However, it should be noted that the testes weight of the male monkeys are highly variable. The coefficient of variations are as high as 50% in many cases. same magnitude of variation was also observed in the serum hormone levels (attached table I and II). It is not understood why the sponsor select monkeys which are not sexually matured for this study, since one of the toxic concerns of this drug is its effect on male reproductive organs and the female estrus cycle. (see Review 2 and 3 of IND-30,661)

No effect dose: Based on the submitted study, the NOEL is 360mg/kg/day in a population of monkeys with almost 50% of them were not sexually matured.

3. PPRD/88/065 Unscheduled DNA synthesis in rat primary hepatocytes of ABBOTT-64077

Material: Primary rat hepatocytes derived from the livers of normal adult male Sprague-Dawley rats.

The procedure used for obtaining rat hepatocyte cultures was essentially that of Williams, et al., (In Vitro 13:809-817, 1977). Each rat used was sacrificed by inhalation of metorane. The animal was dissected and the liver perfused first with 0.5mM EGTA solution and then with a collagenase solution. The liver was removed from the animal and the cells were dissociated, counted, and seeded into 35 mm dishes containing coverslips (5×10^5 viable cells/dish). The cells were seeded in Williams' Medium E (WME) supplemented with 10% fetal bovine serum, 2mM L-glutamine and 50 ug/ml gentamicin. The cultures were incubated at $37 \pm 1^\circ\text{C}$ in a humidified $5 \pm 1\%$ CO_2 incubator for 90 to 150 minutes, washed and refed with serum-free medium and used in the test.

Test articles: The test article, A-64077 and the positive control compound, 7,12-dimethylbenz(a)anthracene, were dissolved in DMSO.

Unscheduled DNA synthesis test:

Based on the results of the initial cytotoxicity test, the test article, A-64077, was tested at nine dose levels. Three replicate plates seeded with 5×10^5 rat hepatocytes/plate were treated with 0.1 to 300 ug/ml of test article. DMBA, at 1 ug/ml and 3.0 ug/ml, was used as the positive control. DMSO, which was used to dissolve both the test article and DMBA, was used as the solvent control for both the test article and positive control. Each test article and control dish received ^3H -thymidine at a final concentration of 10 uCi/ml. In parallel with the test plates, three cultures per dilution were treated with the test article and control compounds for a parallel toxicity test.

The cells were treated for 18-20 hours as described earlier. The parallel toxicity plates were harvested by removal of a portion of the medium for L³H determinations as described in the initial cytotoxicity test to obtain the relative survivals and relative toxicities.

After eighteen to twenty hours of exposure, the cells in the Unscheduled DNA Synthesis assay plates were washed in serum-free WME, swilled in 1% sodium citrate and fixed in ethanol-acetic acid fixative. The coverslips were air-dried, mounted cell side up on glass slides, and allowed to dry. The slides were coated with Kodak NTB emulsion and stored for ten days at 4°C in light tight boxes with desiccant. The slides were then developed in Kodak D-19 developer, fixed in Kodak fixer and stained in hematoxylin-sodium acetate-eosin stain.

Results:

The results of the UDS assay were summarized in the following:

TREATMENT	RELATIVE SURVIVAL	SLIDE DESIGNATION	NO. OF NUCLEI COUNTED	AVERAGE NET GRAINS PER NUCLEUS	S.D.	GRAND MEAN	S.D.	PERCENT CELLS WITH 5 OR MORE NET NUCLEAR GRAINS
A-64077								
300 ug/ml	33%			Too Toxic to be Evaluated for UDS				
200 ug/ml	41%			Too Toxic to be Evaluated for UDS				
100 ug/ml	67%			Too Toxic to be Evaluated for UDS				
30 ug/ml	89%	44A	25	0.0 +/-	2.2	0.1 +/-	2.3	0%
		44B	25	0.6 +/-	2.3			
		44C	25	-0.4 +/-	2.4			
10 ug/ml	93%	42A	25	-0.2 +/-	2.3	0.1 +/-	2.3	1%
		42B	25	0.6 +/-	2.0			
		42C	25	0.0 +/-	2.6			
3.0 ug/ml	87%	47A	25	-1.6 +/-	2.7	-0.7 +/-	2.8	2%
		47B	25	0.1 +/-	2.8			
		47C	25	-0.7 +/-	2.9			
1.0 ug/ml	83%	45A	25	0.8 +/-	2.3	0.5 +/-	2.5	1%
		45B	25	1.3 +/-	2.2			
		45C	25	-0.6 +/-	2.7			
0.3 ug/ml	77%	43A	25	0.9 +/-	2.5	0.5 +/-	2.9	5%
		43B	25	1.0 +/-	3.4			
		43C	25	-0.5 +/-	2.5			
DMBA								
3.0 ug/ml	91%	55A	25	13.1 +/-	4.7	10.0*	4.8	85%
		55B	25	9.1 +/-	3.8			
		55C	25	7.7 +/-	4.3			
1.0 ug/ml	90%	56A	25	11.9 +/-	3.4	11.5*	3.9	97%
		56B	25	12.1 +/-	3.9			
		56C	25	10.5 +/-	4.3			
DMSO (Solvent Control for Test Article and DMBA)								
10 ul/ml	100%	53A	25	-1.1 +/-	1.7	-1.2 +/-	1.7	0%
		53B	25	-0.8 +/-	1.5			
		53C	25	-1.7 +/-	1.7			
WME (Media Control)								
	87%	51A	25	-1.1 +/-	1.6	-1.2 +/-	1.8	0%
		51B	25	-0.5 +/-	1.7			
		51C	25	-2.0 +/-	1.8			

S.D. Standard Deviation

* Significant (See Protocol: Section 8.0, Evaluation of Test Results)

Conclusions:

ABBOTT-64077 was tested in the rat UDS at nine dose levels ranging from 0.1 to 300ug/ml and was fully evaluated at 5 dose levels of 0.3, 1.0, 3.0, 10 and 30 ug/ml.

The results of the UDS assay indicate that under the test conditions, there was no significant increase in the unscheduled DNA synthesis according to the set criteria (5 counts over the control of net nuclear grain counts at any dose level). Whereas, 7,12-dimethylbenz(a)anthracene, the positive control for this test, induced significant increases in the mean number of net nuclear grain counts over the solvent control.

4. PPRD/87/280 Evaluation of the Effects of Orally Administered ABBOTT-64077 on the Embryonic and Fetal Development of Rat (segment II TRF)

Animal: Female Crl:CD BR rats, sperm-positive
22/group

Drug: AEBOTT-64077 suspended in 0.2% hydroxypropylmethylcellulose,

Dosage and Design: Drug administered once daily by gavage beginning on the sixth day of gestation and ending on the 15th day of gestation according to the following schedule:

Treatment Group	Dosage (mg/kg/day)	Concentration (mg/ml)	No. of Females Per Group
T ₀	0 (Vehicle) ^a	0	22
T ₁	15	1.5	22
T ₂	70	7.0	22
T ₄	150	15.0	22
T ₃	300	30.0	24

All animals were killed on day 20 and all fetus were then delivered by laparotomy and examined.

Results:

Maternal:

Mortality: 3/22 in T₃

Physical signs: Ataxia and/or flaccidness in 11/22 (T₃).

Alopecia in control and all treated groups.

Body weight and food consumption: The T₃ monkeys had significantly lower body weight and food consumption.

Fetal:

Uterus contents: Statistically, there was no significant differences from control in the number of implantation sites and viable fetuses per dam. In the statistical analysis of the percentages (relative to the number of implantation sites) of viable fetuses, degenerating fetuses, resorption sites, and the sum of the numbers of degenerating fetuses plus resorption sites neither a significant difference from the To group nor a significant trend with increasing dose were observed.

Fetuses: There was no statistical significant difference in male:female sex ratios for treatment groups from the control. Whereas, the body weights of the fetuses of the highest dose group were significantly lower than the control. For the abnormal development of the fetus, it was noted that there was significantly higher incidences of skeletal abnormalities in the fetuses of the highest dose group.

Conclusions: Oral administration of this ABBOTT-64077 to pregnant rats during the period of major organogenesis was associated with maternal and fetotoxicity at a maternal dose of 300mg/kg/day:

The maternal toxicity included signs of ataxia and flaccidness, alopecia, and decreases in body weight, weight gain and food consumption and death.

The fetotoxicity was characterized by increased incidence of minor anatomical abnormalities and ossification delays as well as by significant reductions in fetal body weight.

No effect dose: 150mg/kg/day.

5. Acute Oral Toxicity Evaluation of ABBOTT-64077 in Combination with Several Bronchodilator and Anti-inflammatory Agents in Male Mice

Animal: Male Crl:CD mice, weighing 18 to 27 g each, approximately 4 to 5 weeks old. 10/group.

- Drug:
1. ABBOTT-64077, bulk drug (Lot 06-679-AL)
 2. Theophylline, bulk drug (Lot 07-018-JR)
 3. Albuterol, powdered 4 mg Ventolin^{®4} tablet formulation (Lot W0607FA, Exp. 11/89)
 4. Prednisone, powdered 5 mg tablet formulation⁵ (Lot 69288, Exp. 11/90)
 5. Ibuprofen, bulk drug (Lot 07-711-AL)
 6. Naproxen, powdered 250 mg Naprosyn^{®6} tablet formulation (Lot 28694, Exp. 1/89)
 7. Sulfasalazine, powdered 500 mg Azulfidine^{®7} tablet formulation (Control No. 7C677C, Exp. 1/92)

Dosage and Design: Food was withheld from the mice for 30 to 60 minutes. 10/group were pretreated by gavage with one of the following doses:

80mg/kg theophylline (10 ml/kg of 0.8% suspension)
4 mg/kg albuterol (10 ml/kg of a 0.4% suspension)
25mg/kg prednisone (10ml/kg of 0.25% suspension)
350mg/kg ibuprofen (10ml/kg of a 3.5% suspension)
120mg/kg naproxen (10ml/kg of a 1.2% suspension)
400mg/kg sulfasalazine (10ml/kg of a 4.0% suspension)
10 ml/kg vehicle (0.2% HPMC)

A suspension of ABBOTT-64077 in HPMC was administered one time by gavage to the mice in each group at doses from 1.5 to 4.0 g/kg.

Statistics: LD₅₀ and 95% confidence interval were calculated by probit analysis.

Results: The toxic signs observed are generally as the following: decreased activity, ataxia, squinting, dyspnea, prostration hypothermia and even coma in the highest dose of 4.0 g/kg of ABBOTT-64077.

The LD₅₀ values and 95% confidence limits of ABBOTT-64077 in combination with the following various agents are summarized:

Pretreatment	ABBOTT-64077 LD ₅₀ in g/kg (95% Confidence Limits)
Vehicle ^b	2.42 (1.85-3.17)
Theophylline, 80 mg/kg	1.94 (1.53-2.65)
Albuterol, 4 mg/kg	2.94 (2.16-4.11)
Prednisone, 25 mg/kg	2.51 (1.83-3.44)
Ibuprofen, 350 mg/kg	>4.00
Naproxen, 120 mg/kg	3.36 (2.41-4.87)
Sulfasalazine, 400 mg/kg	2.33 (1.85-3.47)

Conclusions:

^aEstimated by parallel probit analysis
^b0.22 hydroxypropylmethylcellulose

Based on the LD₅₀ values of ABBOTT-64077, the tested bronchodilators, theophylline and albuterol did not significantly alter the values, likewise, the anti-inflammatory agents, prednisone, ibuprofen, naproxen and sulfasalazine did not alter the values significantly either.

6. Developmental Toxicity (Embryo-fetal Toxicity and Teratogenic Potential) Study of ABBOTT-64077 Administered Orally to New Zealand White Rabbits

Animal: Female Hazelton Research Animals rabbit, five month old. The selected female rabbits were artificially-inseminated with spermatozoa from untreated proven male rabbits used for breeding. Body weights of the animals ranged from 2.64 to 3.90 Kg.

Drug: ABBOTT-64077 (in 0.2% aqueous HMPC) was administered to the rabbits orally via stomach tube between 9:30 - 12:30 each day on days 6 through 12 of presumed gestation.

Dosage and Design: Dosage group assignment was as follows:

<u>Dosage Group</u>	<u>Dosage (mg/kg/day)</u>	<u>Insemination Group (Buck Number)</u>	<u>Female Rabbit Numbers</u>
I	0 (Vehicle)	A(9102)	12681 to 12685
		B(6223)	12686 to 12690
		C(9384)	12691 to 12695
		D(6222)	12696 to 12700
II	15	A(9102)	12701 to 12705
		B(6223)	12706 to 12710
		C(9384)	12711 to 12715
		D(6222)	12716 to 12720
III	50	A(9102)	12721 to 12725
		B(6223)	12726 to 12730
		C(9384)	12731 to 12735
		D(6222)	12736 to 12740
IV	150	A(9102)	12741 to 12745
		B(6223)	12746 to 12750
		C(9384)	12751 to 12755
		D(6222)	12756 to 12760

On day 29 of presumed gestation, a Caesarean section was performed, both the maternal and uterus contents were studied.

Results:

Maternal:

Mortality: None

Abortion: 3/18 in 50/mg/kg/day group aborted on days 18 (1) and 20 (2) of gestation. These abortions were not dose-related.

Necropsy did not reveal gross abnormality.

Clinical observations: summaries in the following Table:

	DOSAGE GROUP	I	II	III	IV
MAXIMUM INCIDENCE/0 RABBITS EXAMINED		480/ 20	480/ 20	451/ 20	480/ 20
RABBITS - PREGNANT	N	19	16	18	18
ABORTED AND SACRIFICED	N(%)a	0	0	3(16.7)**	0
RED SUBSTANCE IN CAGE PAN		0/ 0	2/ 1c	0/ 0	4/ 2d,e
DYSPNEA		1/ 1b	0/ 0	0/ 0	0/ 0
RALES		14/ 1b	0/ 0	0/ 0	0/ 0
SOFT OR LIQUID FECEs		0/ 0	7/4c**	0/ 0	3/ 1
DRIED FECEs		2/ 1	3/ 2	2/ 1	2/ 1e
ALOPECIA		26/ 2	19/ 3	1/ 1	21/ 2

DOSAGE GROUP:

I - 0 MG/KG/DAY II - 15 MG/KG/DAY III - 50 MG/KG/DAY IV - 150 MG/KG/DAY

Dosage occurred on days 6-18 of presumed gestation.

Maximum incidence is the number of rabbits observed multiplied by the number of days observed.

/ = # days observed/ # rabbits observed.

- a. Percentage is based on pregnant rabbits.
- b. Occurred in doe 12700 that was not pregnant.
- c. Occurred in doe 12706.
- d. Occurred in doe 12745; litter consisted of one early resorption.
- e. Occurred in doe 12578 that had numerous dark areas in gastric mucosa.
- ** Significantly different from the vehicle control group value (P < 0.01).

Maternal body weight:

Predose period, day 0 -6, Body weight of all dosage groups were comparable and not significantly different. Day 6 -19, Administration of the 50 and 150mg/kg/day inhibited average maternal body weight gain, as compared with the control group value. Postdose period, day 19 -29, no significant differences were observed.

Maternal feed consumption:

Maternal feed consumption variation showed similar pattern as the maternal body weight difference.

Caesarean-Section Data:

Section was performed on day 29 of gestation. Results are summarized in the following table:

	DOSAGE GROUP	0 MG/KG/DAY	15 MG/KG/DAY	50 MG/KG/DAY	150 MG/KG/DAY
RABBITS - TESTED	N	20	20	20	20
RABBITS - PREGNANT	N(x)	19 (95.0)	16 (80.0)	19 (95.0)	18 (90.0)
ABORTED	N	0	0	1*	0
ANIMALS PREGNANT AND DELIVERED BY CAESAREAN-SECTIONING ON DAY 29	N	19	16	15	13
CORPORA LUTEA	MEAN ± S.D.	9.9 ± 1.5	10.1 ± 2.4	9.5 ± 1.5	8.9 ± 2.3
IMPLANTATIONS	MEAN ± S.D.	7.7 ± 2.5	7.6 ± 1.4	7.1 ± 2.4	7.3 ± 2.5
LITTER SIZE	MEAN ± S.D.	7.3 ± 2.5	7.1 ± 1.5	6.8 ± 2.7	6.8 ± 2.5
LIVE FETUSES	N	138	113	98	113
	MEAN ± S.D.	7.3 ± 2.5	7.1 ± 1.5	6.8 ± 2.7	6.8 ± 2.5
DEAD FETUSES	N	0	0	0	0
RESORPTIONS	MEAN ± S.D.	0.5 ± 0.9	0.5 ± 0.6	0.6 ± 1.1	0.4 ± 0.7
EARLY RESORPTIONS	N	9	7	9	7
	MEAN ± S.D.	0.5 ± 0.9	0.4 ± 0.6	0.6 ± 1.1	0.4 ± 0.7
LATE RESORPTIONS	N	0	1	0	1
	MEAN ± S.D.	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0	0.0 ± 0.2
DOES WITH ANY RESORPTIONS	N(x)	5 (26.3)	7 (43.8)	5 (33.3)	6 (33.3)
DOES WITH ALL CONCEPTUSES RESORBING	N(x)	0	0	0	1 (5.6)
DOES WITH VIABLE FETUSES	N(x)	19 (100.0)	16 (100.0)	15 (100.0)	17 (94.4)

DAY refers to the day of gestation.

Dosage occurred on days 6-18 of gestation.

* Significantly different from vehicle control group value (P < 0.01)

Litter data:

<u>Dose</u> fetuses/liter	<u># of litters</u>		<u># of fetuses</u>
0 mg/kg	19	138	7.3
15mg/kg	16	113	7.1
50mg/kg	15	98	6.5
150mg/kg	17	118	6.9

The administration of ABBOTT-64077 did not affect fetal sex ratios, body weight, or live conceptuses/litter.

Fetal alterations:

Alterations were defined as 1) malformations (irreversible) or 2) variations. Parameters studied included: 1) fetal gross external, 2) soft tissue, 3) skeletal alterations.

There were instances of fetal cleft palate, 3/118 fetuses, in 2/17 litters of the high dosed (150mg/kg/day) group. These were significantly higher than those of the control but was within the range of the historical data. 2

Combined incidences of malformations and variations identified in fetal external, soft tissue and skeletal examinations did not demonstrate any dosage-dependent, statistically significant differences among the four groups. Combination of malformations and variations resulted in the following incidences for fetal alterations:

<u>Parameters</u>	<u>0</u>	<u>Dose (mg/kg/day)</u>		
		<u>15</u>	<u>50</u>	<u>150</u>
Total fetal alteration (litter)	18 94.7%	15 93.8%	14 93.3	12 70.6
Total fetal alteration (fetuses)	62 44.9%	44 38.9%	37 37.8%	33 28.0%
Alteration (fet/litt.)	44.85%	40.82%	37.14%	28.73%

7. PPRD/88/017 Plasma Concentrations and Plasma Protein Binding of ABBOTT-64077 After Oral Administration to Rats in Corn Oil and HPMC. (revised 4/19/88, originally submitted in amendment #006, 3/4/88)

Animal: Male CD rats, 200 -280 grams

Drug: ABBOTT-64077, either in corn oil or in 0.2% HPMC

Dosage and Design: Rats were fasted before dosing with the following schedule:

Group	Rat No.	Dose(mg/kg)	Vehicle	mL/kg
T ₁	1-4	80	Corn oil	10
T ₂	5-8, 17-18	300	Corn oil	10
T ₃	8-12	80	HPMC	10
T ₄	13-16	300	HPMC	10

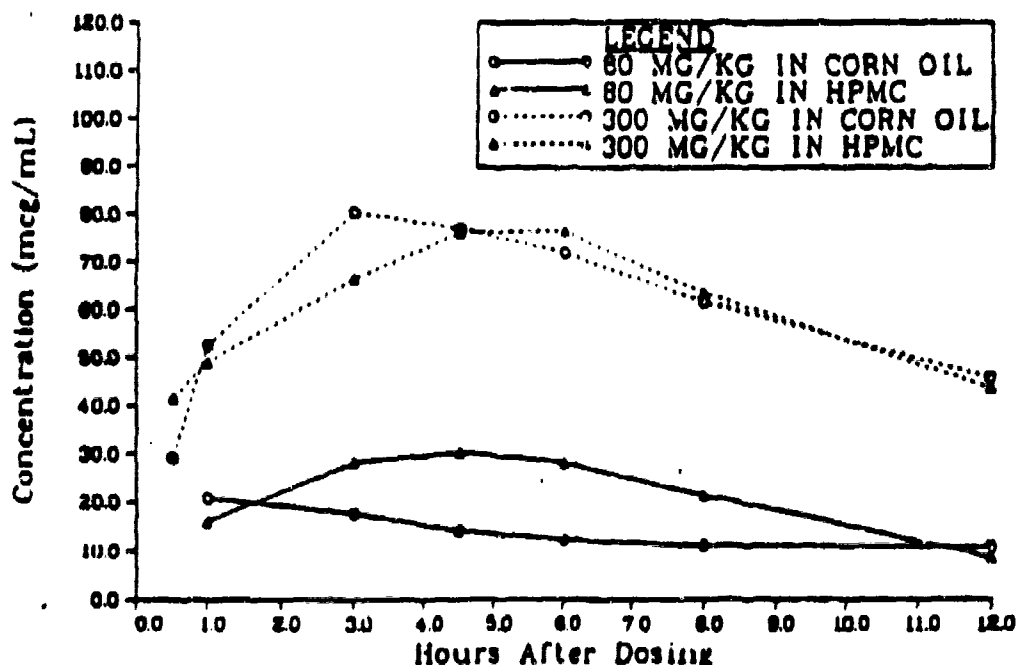
Samples: Plasma samples were collected from a tail vein at 1, 3, 4.5, 6, 8, 12, 24 and 48 hours post-dosing.

Analytical methodology: HPLC , uv detector at 260nm

Statistics: C_{max}, T_{max}, AUC₀₋₁₂ were compared by Student's T test.

Results:

Plasma concentrations of ABBOTT-64077 after oral administration in male rats are summarized in the following figure:



The average pharmacokinetic parameters of ABBOTT-64077 after oral administration are summarized in the following table:

Dose (mg/kg)	Parameter	Vehicle	
		Corn Oil	HPMC
80	C _{MAX} (µg/ml)	22.3 [5.5]*	31.3 [7.1]
	T _{MAX} (hr)	3.8 [5.5]	5.3 [0.9]
	AUC (0-12)	158.9 [62.3]	250.1 [56.6]
300	C _{MAX} (µg/ml)	82.5 [6.2]	81.2 [10.5]
	T _{MAX} (hr)	4.1 [2.0]	5.4 [2.1]
	AUC (0-12)	738.6 [89.1]	726.9 [164.9]

* Standard deviation between brackets

The plasma protein binding of ABBOTT-64077 were determined in plasma drawn from rats received ABBOTT-64077 in either corn oil or HPMC formulations, results are summarized in the following:

Rat No.	Vehicle	Concentration (mcg/mL)		% Free
		Total	Free	
19	corn oil	38.4	6.3	16.5
20		65.0	13.0*	20.0
21		54.5	7.3	13.4
average		52.6 [52.6]**	8.9 [3.6]	16.6 [3.3]
22	HPMC	48.6	8.6	17.7
23		68.2	10.7	15.8
24		47.2	7.3	16.6
average		54.7 [11.7]	9.0 [1.5]	16.7 [1.0]

Conclusions:

It was found that in the rat, after oral administration, ABBOTT-64077 showed higher toxicity when formulated in corn oil than in 0.2% HPMC. However, the present study showed that the two different formulations do not differ in their bioavailability and extent of plasma protein binding.

Amendment # 13:

In this amendment, six studies, one human and five preclinical studies were submitted. In order to evaluate the safety of this drug used in clinical studies, the pharmacokinetic properties of the drug need to be compared among the various species used in the toxicology studies and human. The following is a review of human pharmacokinetics of ABBOTT-64077.

1. PPRD/88/164, The Pharmacokinetics of ABBOTT-64077 in Man After Oral Administration of Single 25, 50, 100, 200, 400, 600 and 800mg Doses (M87-067 and M87-068)

OBJECTIVE OF THE STUDY: To characterize the pharmacokinetics of ABBOTT-64077 in human.

DESIGN OF THE STUDY:

Subject: Six healthy male volunteers.

Design: Randomized, double blind, placebo controlled, according to the following schedule:

Study M87-067:

Dose Level (mg)	Number of 25 mg Capsules	Number of 100 mg Capsules
25	1	
50	2	
100		1

Study M87-068:

Dose Level (mg)	Number of 200 mg Capsules
200	1
400	2
600	3
800	4

Sample: Plasma samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 28, 33, 48 and 60 hours post-dosing. Twenty-four hour urine samples were also collected for assaying of parent drug and metabolites.

Assay method: HPLC (Not sure whether the new method described in Amendment #10 was applied in these studies)

PHARMACOKINETIC ANALYSIS: The means of T_{max} and C_{max} were calculated for each dose level. The individual and mean $AUC_{0-\infty}$ were calculated using trapezoidal method. The apparent clearance, Cl/F was calculated as $dose/AUC$. Since in the higher dosed group, the plasma-time profile show a second peak, a one-compartment enterohepatic circulation model was used to perform the pharmacokinetic analysis. The following parameters were estimated:

Primary parameters:

1. k_a : first-order rate constant of absorption (first peak)
2. k_a' : first-order rate constant of reabsorption (second peak)
3. k_e : first-order rate constant of elimination
4. V/f : apparent volume of distribution (V) divided by the fraction of the dose absorbed (f)
5. $t-lag$: lag time for absorption (onset of first peak)
6. $t-lag_2$: lag time for reabsorption (onset of second peak relative to the onset of the first peak)
7. $f-reab$: fraction of the initial absorbed dose that is reabsorbed

Secondary parameters:

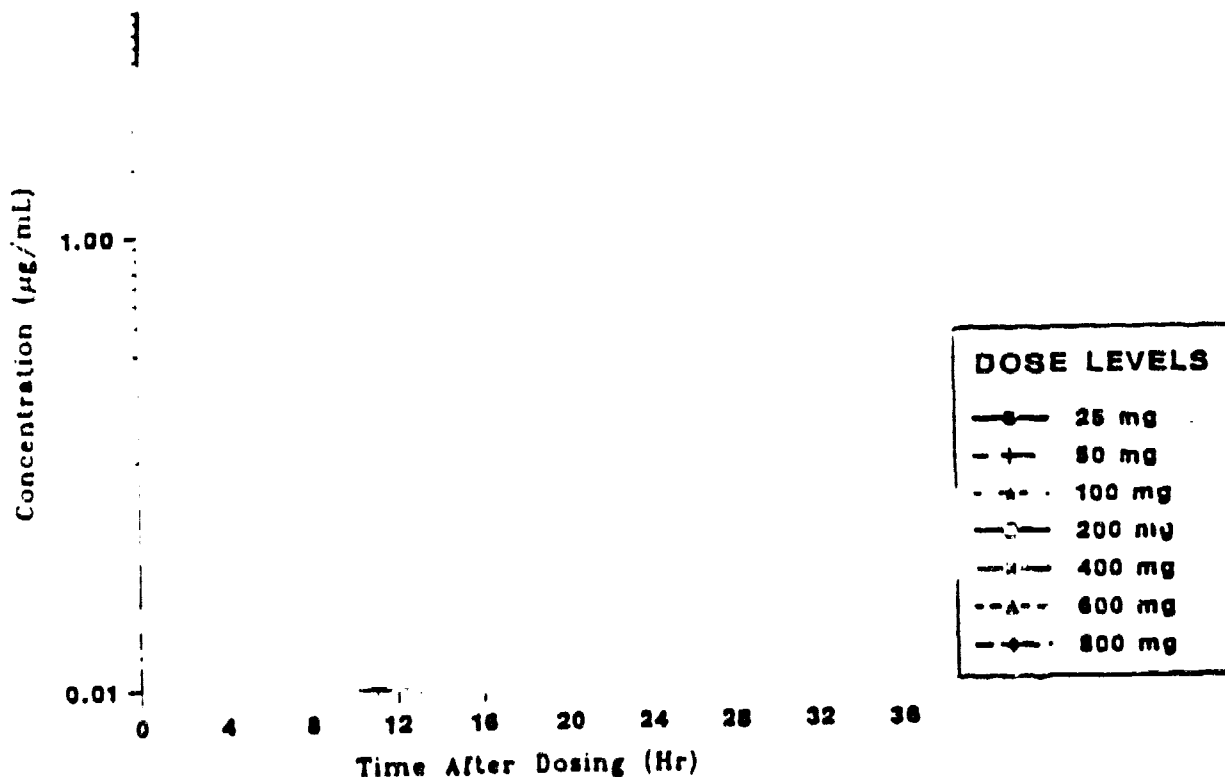
1. T_{max} : plasma peak time
2. C_{max} : plasma peak concentration
3. $t_{1/2}$: elimination half-life
4. AUC : total area under the fitted plasma concentration-time profile
5. Cl/f : apparent plasma clearance divided by the fraction of the dose absorbed
6. AUC_p : precyclic AUC ($observed\ AUC / [1 + f-reab]$) or area under the main absorption peak (8).
7. Cl_p/f : precyclic clearance ($Cl/f * [1 + f-reab]$) or apparent plasma clearance based on AUC_p (8).

STATISTICS: The equivalence among the four higher doses (M87-068) in the pharmacokinetic profile of the drug was examined for each of the following variables using ANOVA with dosage as the factor:

T_{max} , $C_{max}/dose$, $AUC/dose$, $AUC_p/dose$, Cl/f , Cl_p/f , $t_{1/2}$, V/f , $f-reab$. As for the relationship between the pharmacokinetic parameters and the dose, the parameters were subject to a linear regression against the dose, and the slopes of the lines were tested for significance.

RESULTS:

1. Plasma levels of ABBOTT-64077 in volunteers at each dose level were summarized in the following figure:



2. The observed pharmacokinetic parameters were summarized in the following table:

	<u>study M87-067</u>			<u>study M87-068</u>			
	<u>25mg</u>	<u>50mg</u>	<u>100mg</u>	<u>200mg</u>	<u>400mg</u>	<u>600mg</u>	<u>800mg</u>
t _{max} (hr)	1.96 (1.3)	1.42 (0.65)	1.25 (0.52)	2.7 (1.0)	1.7 (0.9)	2.9 (1.6)	2.29 (0.88)
C _{max} (ng/ml)	78.1 (29.7)	163.4 (32.6)	361.3 (109.6)	850 (280)	1880 (810)	1920 (790)	2740 (610)
t _{1/2} (hr)	1.58	1.44	2.14	4.36	4.15	5.68	
AUC _{total} (ug·hr/ml)	0.28	0.58	1.44	4.79	10.50	15.14	23.19

3. The pharmacokinetic parameters obtained from modeling are summarized the following:

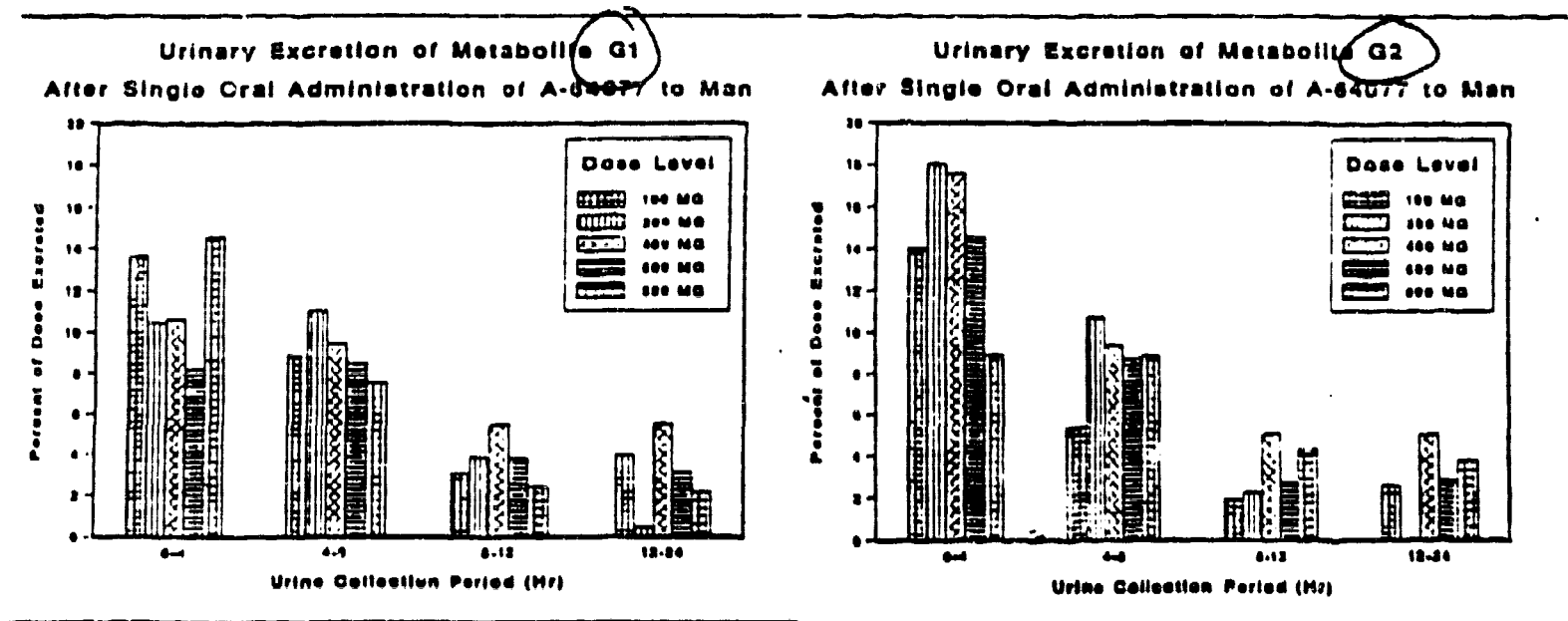
<u>Parameter</u>	<u>Dose (mg)</u>						
	<u>25</u>	<u>50</u>	<u>100</u>	<u>200</u>	<u>400</u>	<u>600</u>	<u>800</u>
T _{max}	1.89	1.34	1.44	2.42	1.51	2.2	2.29
C _{max}	0.07	0.15	0.33	0.71	1.58	1.62	2.50
k _a	1.11	2.05	3.63	1.15	4.16	1.48	1.05
k _a '	-	-	0.07	0.19	0.19	0.26	0.21
k _e	0.55	0.40	0.31	0.33	0.26	0.23	0.24
t _{1/2}	1.26	1.72	2.23	2.11	2.65	3.00	2.69
v/f	194	231	259	167	222	284	208
t-lag	0.43	0.27	0.58	0.73	0.47	0.36	0.33
t-lag ²	-	-	11.99	10.18	11.16	10.33	12.55
f-reab	0.00	0.05	0.29	0.09	0.32	0.44	0.67
AUC	0.26	0.60	1.92	4.24	10.26	15.63	27.09
Cl/f	1649	1460	1056	804	680	663	497
AUC _p	0.25	0.51	1.39	4.24	8.66	12.10	17.97
Cl _p /f	1649	1938	1333	806	868	864	766

Units: T_{max}, t-lag, t-lag² (hr); C_{max} (mcg/mL); AUC, AUC_p (mcg.hr/mL);
Cl/f, Cl_p/f (mL/min); k_a, k_a', k_e (hr⁻¹); t_{1/2} (hr); v/f (L).

ANOVA detected significant difference in AUC/dose, Cl/f but not in any other parameters.

4. ABBOTT-64077 metabolites in urine:

The results are summarized in the following:



It showed that, the majority of the metabolites are excreted during the first 4 hours, and in 24 hours, urinary recoveries of ABBOTT-64077 conjugates are 53%(100mg), 57%(200mg), 68%(400mg), 52%(600mg) and 53%(800mg). The total recoveries were independent of the dose.

CONCLUSIONS:

From the pharmacokinetic analysis by fitting data to a one compartment enterohepatic recycling model, it appeared that the precyclic clearance volume of distribution are dose independent at least for the four higher doses, the $t_{1/2}$ was also dose-independent ($t_{1/2} = 0.7 \times v/C_{1p}$), yet the extent of enterohepatic circulation appeared to be a non-linear process.

From the urine data, it appears that more than 50% of dose was recovered in the urine as conjugates of ABBOTT-64077, and urinary data suggests of dose-independent clearance.

SUMMARY:

Analytical methodology:

Pharmacokinetics, human vs. monkeys

1. Human: To this date (6/24/88), human pharmacokinetic parameters derived from two separate studies are analyzed by fitting the data to a one compartment enterohepatic recycling model (see review on p.21-25), which showed that for the total AUC (AUC of precyclic and reabsorbed phases), the drug was not dose-independent, in fact, there was a decline in the apparent clearance. The total AUCs of a single oral dose of 600mg(12mg/kg) and 800mg(16mg/kg) were 15.6mcg.hr/ml and 27.0mcg.hr/ml respectively.

2. Monkeys: To this date (3/15/88), roughly estimated pharmacokinetic parameters of ABBOTT-64077 in monkeys showed that the total AUC (determined with 3 data points) on 21 day post 360mg/kg/day dosing was 140 mcg.hr/ml (see review on p.7)

? { 3. Metabolism: To this (date 6/24/88), the sponsor reported that the metabolism pattern of ABBOTT-64077 was almost identical qualitatively and quantitatively between human and monkey. (see review on p.25)

Toxicity in monkey:

Previous submitted acute and subacute toxicology studies in rats and dog suggested an oral NOEL of 20mg/kg, the target organ in rat is liver, in dog is male reproductive organ. Based on the ADME studies in these animals, it was felt that the rat or the dog may not be the best animal model and therefore, the sponsor carried out more animal toxicity studies with monkeys. In Amendment # 10, the sponsor submitted a 30 day oral toxicity study in monkeys, the results suggested that the oral NOEL may be as high as 360mg/kg/day in a population of monkeys which is 50% sexually immature.

EVALUATION:

1. Based on the submitted 30 day toxicity study results, in monkeys dose with ABBOTT-64077 for a month, the oral NOEL may be 360mg/kg/day.
2. Human pharmacokinetic analysis based on two single dose studies indicated that there is enterohepatic recirculation of the oral drug and therefore, there may be non-linear kinetic relationship between the exposure and the dose of the drug. The apparent clearance of the drug declines when dose was increased from 200mg to 800mg.
3. When pharmacokinetic parameters obtained in human and monkey were compared, it was found that the 360mg/kg oral dose in monkey yielded an AUC of 9X and 5X of that of human oral doses of 600mg and 300mg.
4. Based on the above evaluations of the present available information, the 800mg oral dose should be the upper limit of clinical trial.

RECOMMENDATIONS:

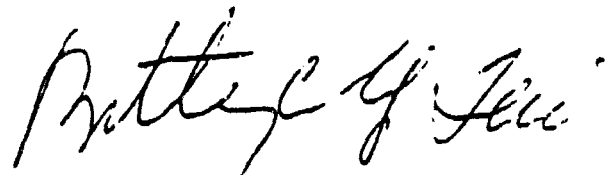
Based on the submitted ADME and toxicology studies, it is our opinion that the data support the safety of a human 800mg oral dose. If the sponsor intends to extend the human dose, additional preclinical assurance needs to be established.

DRAFT OF LETTER TO THE SPONSOR:

It is our opinion that based on the information you submitted to date, it is only sufficient to support the 800mg oral dose in clinical trial for the following reasons:

1. The oral NOEL in monkeys is 360mg/kg.
2. The roughly estimated AUC for monkey dosed with 360mg/kg is 140-150 mcg.hr/ml.
3. There appears to be enterohepatic recirculation of the orally administered drug in human, the reabsorbed fraction of the administered dose was dose-dependent and therefore it is difficult to estimate the exposure of the drug in human beyond the highest tested dose, 800mg or 20mg/kg/day.
4. Based on the pharmacokinetic analysis of the human data from the single oral rising dose study, the AUC of 800mg oral dose is 27mcg.hr/ml.
5. Therefore, the 360mg/kg/day NOEL is about only 5X the human 800mg dose based on the estimates of AUCs and the ratio may be much lower for higher human dose since the apparent clearance of the drug showed a trend to decrease from 200mg to 800mg.

#



Betty C.Y. Tai, Ph.D.
Pharmacologist, HFD-160

cc. IND
HFD-160
Doc. Rm. 160
HFD-160 JLewis
R/D BTai 8/5/88
R/D Init. by DJean for JInscoc 8/5/88
Typed by BTai

IND:

DATE: March 1, 1988

Review # 3

110
3-10-88

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

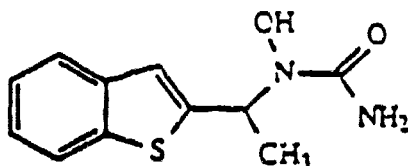
Amendment #3 1/27/77

Amendment #4 2/10/88

SPONSOR: Abbott Laboratories
Pharmaceutical Products Division
Abbott Park, IL 60064

NAME OF DRUG: ABBOTT-64077

Chemical Structure



Chemical Name

N-(1-benzo[b]thien-2-ylethyl)-N-hydroxyurea.

M.w.: 236.274

CATEGORY: Lipxygenase Inhibitor (leukotriene synthesis inhibitor)

ROUTE OF ADMINISTRATION: Oral

DOSAGE FORM: Capsule

The capsule formulations (25mg, 100mg and 200mg) are as the following:

<u>Scale</u>	<u>Item</u>	<u>Ingredient</u>	<u>Code</u>	<u>Amount</u>	<u>Amount</u>	<u>Amount</u>

PROPOSED CLINICAL STUDY: (Amendment# 4)
 Protocol# M87-046, titled "The safety and bioavailability of multiple oral doses of ABBOTT-64077"

OBJECTIVE OF THE STUDY:

To compare the safety of ABBOTT-64077 to placebo in male volunteers following oral administration once daily for 7 days. To determine pharmacokinetic parameters and the effect on LTB₄ and prostaglandin synthesis.

DESIGN OF THE STUDY:

Subjects: 40 healthy adult male volunteers.

Treatments: 4 dose groups. I 100mg/day, for 7 days.
 II 200mg/day
 III 400mg/day
 IV 600mg/day

There will be 10 subjects/group, 7 receive active drug, 3 receive placebo. This is randomized, double-blind, placebo controlled single center study.

Sampling: 5 ml of plasma samples will be collected according to the following schedule:

<u>Time Following the Morning Dose</u>	<u>Study Day</u>						
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
Prior to Dose (0 Hour)	X	X	X	X	X	X	X
15 minutes	X						
30 minutes	X						
45 minutes	X						
1 hour	X						
1.5 hours	X						
2 hours	X	X	X	X	X	X	X
3 hours	X						
4 hours	X	X	X	X	X	X	X
6 hours	X						
8 hours	X	X	X	X	X	X	X
12 hours	X	X		X			X
16 hours	X						
24 hours							X

In addition, some whole blood and urine will also be collected. Plasma biochemistry, vital signs, ophthalmologic exam, neurologic assessment, laboratory assessment, laboratory tests, EKG, ECG will be evaluated at designated time period. Pharmacokinetic analysis: not specified.

PRECLINICAL DATA: (Amendment# 3)

Studies submitted in this amendment are the following:

1. A final report of M87-067, " Summary of the safety and bioavailability of three single rising oral doses of ABBOTT-64077" (completed in W. Germany);
2. The three month final report of T827-134, " Three month toxicity study of ABBOTT-64077 administered orally to dogs (with one month interim kill)";
3. The three month final report of T827-140, "Three month toxicity study (with one month interim kill) of ABBOTT-64077 administered orally to rats."

REVIEW OF STUDIES:

1. A final report of M87-067, " Summary of the safety and bioavailability of three single rising oral doses of ABBOTT-64077" (completed in W. Germany. :

Purpose: To compare the safety of ABBOTT-64077 to placebo following oral administration of single 25, 50 and 100mg doses, to determine pharmacokinetic parameters as well as the effect of the drug on leukotriene and prostaglandin synthesis.

Subjects: 27 healthy, adult male volunteers.

Treatments: ABBOTT-64077 25mg, 50mg and 100mg, placebo. this was a phase I, randomized, double blind, single-dose, placebo controlled.

Sampling: Plasma samples of 5 ml will be collected at the following times: 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 20, 24, 28, 36, 48 and 60 hours post-dosing. Additional plasma samples will be collected at designated time points for in vitro anti-leukotriene activities of the drug or for blood biochemistry profile of the volunteers.

Results: Bioavailability/pharmacokinetics:

1. Plasma concentrations of ABBOTT-64077 for individual subjects are summarized in the following Table:

Subj.	Concentration (ng/mL)												
	Hours After Oral Administration												
	0	0.25	0.5	0.75	1	1.5	2	3	4	6	8	10	14

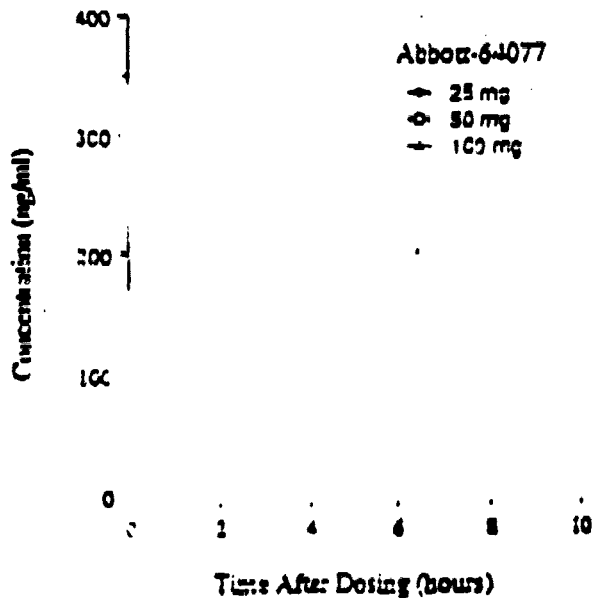
25 MC ORAL

50 MC ORAL

100 MC ORAL

NOTE: Parentheses indicate estimated values.

The plasma-time profile in the following figure.



Pharmacokinetic parameters of ABBOTT-64077 after single oral dose to man are summarized in the following:

	Dose		
	25 mg	50 mg	100 mg
t_{max} (hr)	1.96 ($\pm$ 1.27)	1.42 ($\pm$ 0.65)	1.25 ($\pm$ 0.52)
C_{max} (ng/mL)	78.1 ($\pm$ 29.7)	163.4 ($\pm$ 32.6)	361.3 ($\pm$ 109.6)
$t_{1/2}$ elim (hr)	1.58 ($\pm$ 0.46)	1.44 ($\pm$ 0.38)	2.14 ($\pm$ 0.48)
AUC (ng·hr/mL)	280.6 ($\pm$ 52.3)	579.2 ($\pm$ 99.7)	1437.3 ($\pm$ 438.3)
Cl (L/hr)	89.1	86.3	69.6

** The sponsor, in this report, mistakenly labeled the unit of AUC in mg.hr/ml, it should be ng.hr/ml.

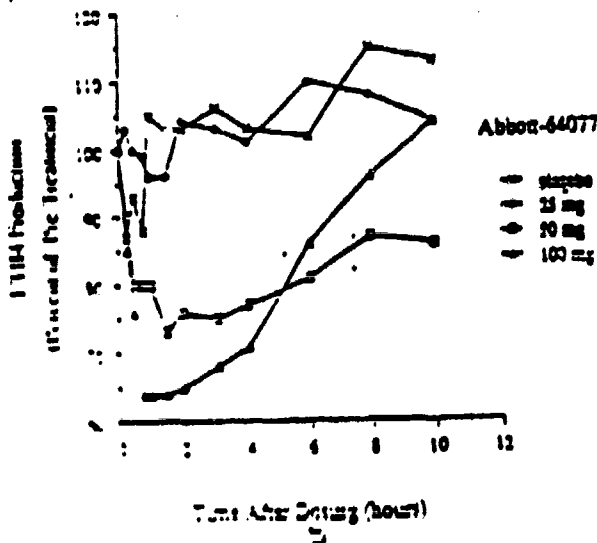
Enzyme inhibition:

1. LTB₄ synthesis:

Heparinized venous blood samples collected at different time points are incubated for 30 mins with 47 μ M of A23187 to trigger LTB₄ biosynthesis. The results are summarized in the following:

<u>Hours post dosing</u>	<u>LTB₄ synthesis (ng/ml)</u>			
	<u>Placebo</u>	<u>25mg</u>	<u>50mg</u>	<u>100mg</u>

When the results are expressed as percent pre-dose level, the inhibition of LTB₄ synthesis is shown in the following figures:



Thromboxane synthesis:
Results not available yet!

Conclusion:

1. According to a one-compartment model analysis by fitting the curve with NONLIN-84, the elimination half lives of the drug are the following:

1.58±0.46hours(25mg), 1.44±0.38hours(50mg), 2.14±0.48hours(100mg). The mean AUCs for the three doses appear to be propotional to the administered doses.

2. Single doses of 50mg and 100mg caused significant inhibition of LTB₄ production by whole blood stimulated with A23187 ex vivo. The degree of inhibition appeared to be correlated with serum drug concentration.

2. Three month final report of TB87-134, "Three month toxicity study of ABBOTT-64077 administered orally to dogs (with one month interim kill)," Scientific Report No. PPRD/87/201

Doses: 5, 20, 100 and 400mg/kg/day (gealtin capsule)

Animals: Beagle dogs, 4/sex/group.

Route of administration: oral

Treatment duration: 90 days (5/21 - 8/27)

Results:

Mortality: 400mg/kg/day, three dogs died in extremis on study days 36, 40 and 49 respectively.

Clinical signs: 400mg/kg/day all, and 100mg/kg/day 4/8, mucoid stools, emesis, emaciation, decreased activity, ataxia and decreased activity or salivation. Anomalous and transient episode of vomiting of blood in one dog treated with 5mg/kg on day 42 only.

Body Weight: 400mg/kg/day, significant body weight loss in 3/4 male and 4/4 female. 100mg/kg/day, body weight gain in male but weight loss in female.

Food consumption: 400mg/kg/day, all female showed lower food consumption.

Clinical pathology: 400mg/kg/day, significant depression of mean hematocrit, Hb in males, decreased total erythrocyte count in both males and females.

Anatomic pathology: 400mg/kg/day, mean relative liver weight, mean absolute and relative adrenal weight, mean relative brain weight were increased and the mean absolute heart weight was decreased in the male. In the female, the mean relative liver and thyroid weights were higher. It should be noted that the absolute and relative prostatic weight in the 400mg male group was lower and was related to the prostate atrophy observed microscopically.

Histological abnormalities: 400mg/kg/day, drug related testicular degeneration and/or atrophy 3/4, and prostatic atrophy found in 4/4 males. 1/4 of uterine atrophy in females. 100mg/kg/day, 1/4 males was found to have testicular degeneration and atrophy. Serum testosterone: Serum testosterone concentration was assayed in the control and drug treated dogs, the results are summarized in the following table:

SERUM TESTOSTERONE CONCENTRATION IN THREE MONTH OLD KILL DOGS - MALES
SUMMARY STATISTICS AND RESULTS OF STATISTICAL ANALYSIS

TREATMENT GROUP	N	TESTOSTERONE (ng/ml)	
CONTROL	1	145.0	124.31 ^a
	2	189.0	
	3	151.0	
	4	176.0	
100 mg/kg/day	1	182.00	-
	2	152.00	
	3	149.0	
	4	175.0	
200 mg/kg/day	1	182.0	(87.33) ^b
	2	172.0	
	3	170.0	
	4	147.0	
400 mg/kg/day	1	122.23	-
	2	14.39	
	3	258.0	
	4	301.0	
100 mg/kg/day	1	483.0	(11.8) ^b
	2	141.0	
	3	295.75	
	4	141.87	
100 mg/kg/day	1	7.6	(48.31) ^b
	2	58.8	
	3	1	
	4	1	
RESULTS OF STATISTICAL ANALYSIS		32.10	
		34.68	
RESULTS OF STATISTICAL ANALYSIS		NS ^c	

^aSingle testosterone measurements were replicated due to low values. Mean and standard deviation values were not used in the statistical analysis.

^bAnimal 100 mg/kg/day group (Day 30) died prior to the termination of the treatment group in this study. These animals represent the dogs that were not tested.

^cNS = no significant differences among treatment groups (p > 0.05).

The results are highly variable, although, statistically no significant difference can be detected, the conclusion of no differences among the treatment groups may be meaningless.

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It should be noted that prostatic atrophy was found in 2/3 males (10mg/kg/day) and 1/10 males (20mg/kg/day) in the one month study, and testicular atrophy was observed in 2/3 males (100mg/kg/day) and 1/10 males (20mg/kg/day) in the one month study. The reason for the decreased incidence and/or the absence of drug related testicular and prostatic alterations in males treated with 20 and 100mg/kg/day for three months was not investigated by the sponsor. Additional histological findings included renal hemorrhage, necrosis, gastric atrophy and degeneration, splenic thrombosis, severe bronchopneumonia, severe intestinal tubular basophilia, bone marrow hypoplasia and multifocal interstitial degeneration were found mainly in high doses. The overall condition was unbound.

Conclusion: The most effective dose appeared to be 20mg/kg/day in dog treated for three months.

3. Three month final report: "98"-140, "Three month toxicity study of ABBOTT-64077 administered orally to rats (with one month interim kill)," Scientific Report No. 98PB/87,202

Doses: 5, 20, 80, 300 mg/kg/day (oral gavage)

Animal: CDBR rat, 10 rats/group

Treatment duration: three months

Results: Mortality: One female (20mg) died of dosing accident
Clinical signs: Hyperactivity in 20, 80 and 300mg groups; females, alopecia on neck and body was more frequently seen in the females in the 20mg group. salivation was observed in both sexes in the 80 and 300mg groups.

Clinical pathology: Treatment related increases in the serum alanine aminotransferase and cholesterol values.

Vaginal cytology: Prolonged proestrus/estrus noted in 5/15(0mg), 1/10(5mg), 1/9(20mg), 3/15(80mg) and 10/15(300mg) females.

Organ weights: Significant increase in the mean absolute and relative liver weights in the 80 and 300 groups. The liver weights of the recovery rats tended to return to control values.

Anatomical pathology: microscopically treatment-related cytomegaly was observed in the hepatocytes of some of the 80mg group and all of the 300mg group. These changes regressed in the recovery rats.

Conclusion: The most effective dose appeared to be 20mg/kg/day in rat treated for three months.

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SUMMARY AND EVALUATION:

1. As of this date, human pharmacokinetic parameters derived from two separate studies; (M87-067, 25, 50, 100mg and M87-068, 200, 400, 600mg), are summarized in the following:

	<u>study M87-067</u>			<u>study M87-068</u>		
	<u>25mg</u>	<u>50mg</u>	<u>100mg</u>	<u>200mg</u>	<u>400mg</u>	<u>600mg</u>
t_{max} (hr)	1.96 (1.3)	1.42 (0.65)	1.25 (0.52)	2.7 (1.0)	1.7 (0.9)	2.9 (1.6)
C_{max} (ng/ml)	78.1 (29.7)	163.4 (32.6)	361.3 (109.6)	850 (280)	1360 (610)	1920 (790)
$t_{1/2}$ (hr)	1.58	1.44	2.14	4.36	4.15	5.68
AUC_{total} (mg·hr/ml)	0.28	0.58	1.44	4.79	10.50	15.14

These results suggested that the estimates of C_{max} and AUC_{total} appear to be linearly related to the administered doses. However, the reported $t_{1/2}$ from the two studies were quite different. This may be attributed to the fact that 1) the assay sensitivity which was not specified by the sponsor in the report could limit the detection of the plasma drug concentration, especially for the lower doses, and consequently, data from study M87-067 was fitted with an one compartment model whereas, data from study M87-068 was fitted with a two compartment model. Since, the plasma drug concentrations were in the mcg/ml range for study M87-068, the estimated $t_{1/2}$ of 4.15 - 5.68 hours were more reliable. It should be noted that, these half-lives are not that much shorter than those determined in dog (6.5-8.5 hour) as previously described, in addition, both the rat and dog pharmacokinetic studies suggested of nonproportional increase of AUC_{total} with the administered doses, the tested dose range was 5-300mg/kg/day for rat, 5-800mg/kg/day for dog, in the dog, The AUC appeared to be leveled off at 200mg/kg/day.

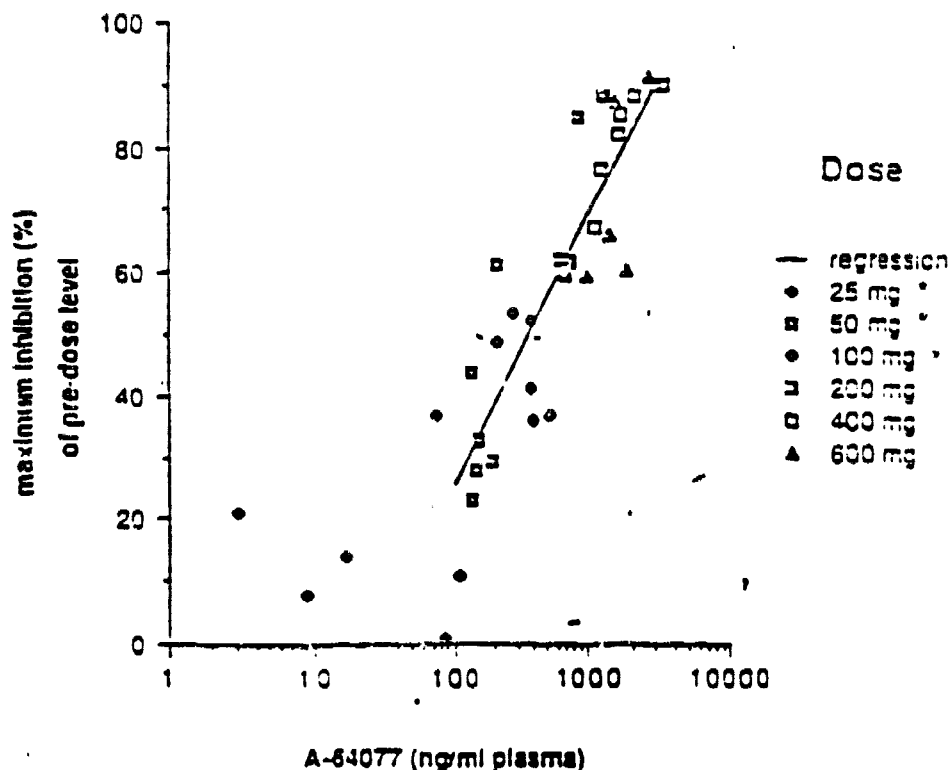
2. The maximum inhibition % of LTB₄ synthesis is defined as the maximum value of (1 - % pre dose level). The combined results are summarized in the following:

<u>Dose of ABBOTT-64077</u>	<u>Maximum inhibition %</u>
Study M87-067	
Placebo	13
25mg	4
50mg	17
100mg	37
Study M87-068	
Placebo	21 ± 15
200mg	72 ± 14*
400mg	82 ± 8*
600mg	76 ± 15*

* Values are significantly ($F < 0.05$) different from the placebo.

When a linear regression analysis performed with the above combined data, correlation coefficient was not provided:

Effect of Oral Administration of Abbott-64077 on Ionophore-Induced LTB₄ Production in Whole Blood



A linear relationship between the dose and the maximum inhibition appears existing up to 100mg; beyond 400mg, the linear relationship is not convincing.

3. The toxicity profile of ABBOTT-64077, so far, has been defined in the following:

<u>ANIMAL</u>	<u>SPECIES/ STRAIN</u>	<u>#/SEX GP</u>	<u>DOSING</u>	<u>DOSES (mg/kg/day)</u>	<u>DURATION (DAYS) DOSING/STUDY</u>
Mouse	Crl:CD ¹ BR	10M/10F	Gavage	500-3200	1/14
Rat	Crl:CD ¹ BR	10M/10F	Gavage	100-4000	1/14
	Crl:CD ¹ BR	3M/3F	Gavage	40-1200	10/10
	Crl:CD ¹ BR	10M/10F	Gavage	5, 20, 80, 300	30/30, 90/90
Dog	Beagle	1M/1F	Oral	50-300	1/14
	Beagle	3M/3F	Oral	5, 20, 100, 400	30/30, 90/90

Based on the three months study in dog, the sponsor proposed that the no-effect dose was 20mg/kg/day; however, this was higher than the the results obtained from the one month interim kill, which indicated the no-effect dose was 5mg/kg/day. The reason for the decreased incidence of testicular and prostate atrophy in the prolonged study was not provided by the sponsor. In the three month study in rat, results suggested the no-effect dose was 20mg/kg/day. At 80mg/kg/day, significant increase of mean and absolute liver weight as well as cytomegaly of hepatocytes were noted.

Results of acute toxicity, and other multidose toxicity are attached in Table 1. (summarized from the original summary, submitted 9/29/67)

<u>ANIMALS</u>	<u>TREATMENT DURATION</u> <u>IN DAYS</u>	<u>NOEL DOSE</u> <u>(mg/kg)</u>	<u>TOXIC DOSE</u> <u>(mg/kg)</u>	<u>TOXIC EFFECTS</u>
Mouse	1		LD ₅₀ = 1070 - >4000	Sedation
Rat	1		LD ₅₀ = 410 - >4000	Sedation
	10	300	600	Reduced activity, death, weight gain reduction, reduced food consumption, increased serum ALT & cholesterol
	30/90 90	80 20	300	Hyperactivity, reduced weight gain, increased serum ALT & cholesterol
Dog	10	200	400	Emesis, weight loss, ataxia, renal tubular vacuolation
Dog	30/90 90	5 20	20 100	Emaciation, reduced activity, sporadically low weight & food consumption, prostatic & testicular atrophy, renal tubular vacuoles, focal gastritis, hepatocellular degeneration

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CONCLUSIONS:

1. Based on the human pharmacokinetic data derived from the ongoing clinical study, M87-05, 25, 50, 100, 200, 400, 800mg oral dose of ABBOTT-64077, the absorption of the drug is proportional to the administered dose and the half life of the drug is in the range of 4.15 - 5.14 hours, which was considerably longer than those (1.44 - 2.14 hours) reported from a previous report, M87-05, with 25, 50, 100mg oral dose. This discrepancy may be explained by the limitation of the assay method or the assay method.
2. Based on the three months toxicity study, the no effect dose appeared to be 20mg/kg and up, increased liver weight as well as cytomegaly was observed.
3. Based on the three months toxicity study, the no effect dose appeared to be 20mg/kg, which is higher than the previously determined no effect dose of 10mg/kg in the report of one month interim kill for the first dose of 100mg/kg and up, degenerated testicles was observed.
4. Based on conclusions 1, 2, and 3, we have serious concerns of safety for the proposed multiple rising dose tolerance clinical study. The doses which are proposed for this clinical trials are 2, 4, 8, 15mg/kg. The animal toxicity study data provided very little if not at all, margin of safety.

RECOMMENDATION:

Based on conclusions 1, 2, and 3, the sponsor is advised to proceed the clinical trial with the first two doses, 100mg, 200mg with great caution, obtain safety data, submit such information to the agency for evaluation before proceeding further. In the meantime, the sponsor is urged to further characterize the metabolism and pharmacokinetics of the drug in the different animal species as to define the most relevant animal model for future toxicity studies.

DRAFT LETTER TO THE SPONSOR:

Based on the data of the three months toxicity study in rat and dog, we have come to the conclusion that the no-effect-dose of ABBOTT-64077 is 20mg/kg in both the rat and dog; therefore, we have serious concerns of safety for the proposed multiple rising dose tolerance clinical study. We recommend that you proceed the clinical trial with the first two doses, 100mg and 200mg, obtain safety data, submit such information to the agency for evaluation before continuing ~~proceeding~~ to the higher doses. In the mean time, you are urged to characterize the metabolism patterns of the drug in human and the laboratory animals and developing the toxicity profile of the drug. Additional pharmacokinetic analysis with the ~~IV~~ ^{intravenous} data from the various species of laboratory animals used in the toxicity study, such as the parameter ~~of~~ would also help in defining the most relevant animal model for the toxicity studies.

cc: INL
HFN-160, Doc. Rm. 160
R/D:B. Tai:3/1/88
R/D init. by:Dr. In...

Betty C Y Tai
Betty C Y Tai, Ph.D.

IND: -

DATE: February 25, 1968

Handwritten: 2-27-68

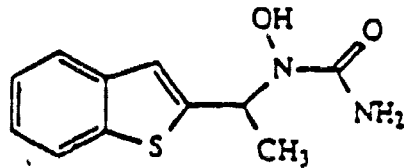
Review No. 2

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Amendment 2, 1/12/68

SPONSOR: Abbott Laboratories
Pharmaceutical Products Division
Abbott Park, IL 60064

NAME OF DRUG: ABBOTT-64077

Chemical Structure



Chemical Name

N-(1-benzo[b]thien-2-ylethyl)-N-hydroxyurea.

M.W. : 236.294

/CATEGORY: Lipoxxygenase Inhibitor (leukotriene synthesis inhibitor)

ROUTE OF ADMINISTRATION: Oral

DOSAGE FORM: Capsule

The capsule formulations (25mg, 100mg and 200mg) are as the following:

<u>Scale</u> mg/cap	<u>Item</u>	<u>Ingredient</u>	<u>Code</u>	<u>Amount</u>	<u>Amount</u>	<u>Amount</u>

BACKGROUND:

In the original summary of IND , animal ADME study results showed signs of non-linear pharmacokinetics of the drug in rat and dog. The sponsor concluded that the reduced absorption of the drug at higher dose was due to the low solubility of the drug. In a human clinical study carried out in Germany, the C_{max} of the drug seems to be linearly related to the oral drug dose from 25 - 100mg.

In the protocol of a rising dose tolerance study, the sponsor intended to study doses from 200mg, 400mg, 600mg, 800mg, 1200mg all the way to 1200mg in human. The Division requested the sponsor to proceed the clinical trial with the first three doses until more human pharmacokinetic data is generated, submitted and evaluated, due to 1) indication of non-linearity pharmacokinetics of the drug in animal and 2) the lack of pharmacokinetic data in human.

This amendment No.2 is the interim report on the clinical study with the first three doses, 200mg, 400mg and 600mg.

STUDY SUBMITTED: Interim report of safety and bioavailability of ABBOTT-64077 in single rising or ; Protocol MS7-068

PURPOSE: This is a phase I, double-blind, randomized, parallel trial, single 200mg, 400mg and 600mg doses were compared to placebo in young, healthy, male volunteers. This study were designed to determine the 1) safety, 2) pharmacokinetics and the 3) ability of ABBOTT to inhibit LTS_4 production in calcium-ionophore stimulated whole blood.

This review will be focused on evaluating the 2) Pharmacokinetics and the 3) In vitro inhibition of LTS_4 .

Pharmacokinetics:

Subject: 9 human volunteers/group, three parallel test groups

Treatments: 200, 400, 600mg of ABBOTT-64077 capsules

Samples: Plasma samples of 5 ml were collected at 0, 1/4, 1/2, 3/4, 1, 1 1/2, 2, 3, 4, 6, 6 1/2, 7, 8, 10, 12, 16 and 24 hours post-dosing

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Pharmacokinetic parameters: Data on plasma concentrations of ABBOTT-64077 in man after oral administration is attached (Table 1). The estimated pharmacokinetic parameters estimated from these data is summarized in the following:

	Dose*		
	200 Mg	400 Mg	600 Mg
t_{max} (hr)	2.7 [1.0]**	1.7 [0.9]	2.9 [1.6]
C_{max} (mcg/mL)	0.35 [0.28]	1.38 [0.31]	1.92 [0.79]
$t_{1/2}$ elim (hr)	4.36	4.15	5.68
AUC(0-24 hr) (mcg·hr/mL)	4.53 [0.86]	10.06 [2.05]	14.4 [1.68]
AUC(total) (mcg·hr/mL)	4.79	10.50	15.14

* Five different subjects for 200 mg dose level; 6 for 400 mg and 600 mg dose.

** Standard deviation between brackets.

/ Conclusion:

Mean C_{max} and AUC increased linearly with increased oral dose of the drug from 200 to 600mg. $t_{1/2}$ of the drug is from 4.15 - 5.68 hours.

In Vitro inhibition of LTB_4 synthesis:

Methods: Heparinized venous blood samples obtained before and at various times after dosing were incubated for 30 minutes at 37°C with 47mM A23187 to trigger LTB_4 synthesis. Following incubation, samples were rapidly cooled in an ice bath and then centrifuged at 3°C for 10 minutes at 2500g. Plasma samples were collected and assayed for LTB_4 with a commercially available RIA. (which has a linear range of 10 to 200 pg LTB_4 per tube.)

*Inhibition of LTB₄
is not dose related
200 = 600 ug
400 = 600 ug/kg*

Results:

1. Ionophore-induced LTB₄ formation in whole blood:

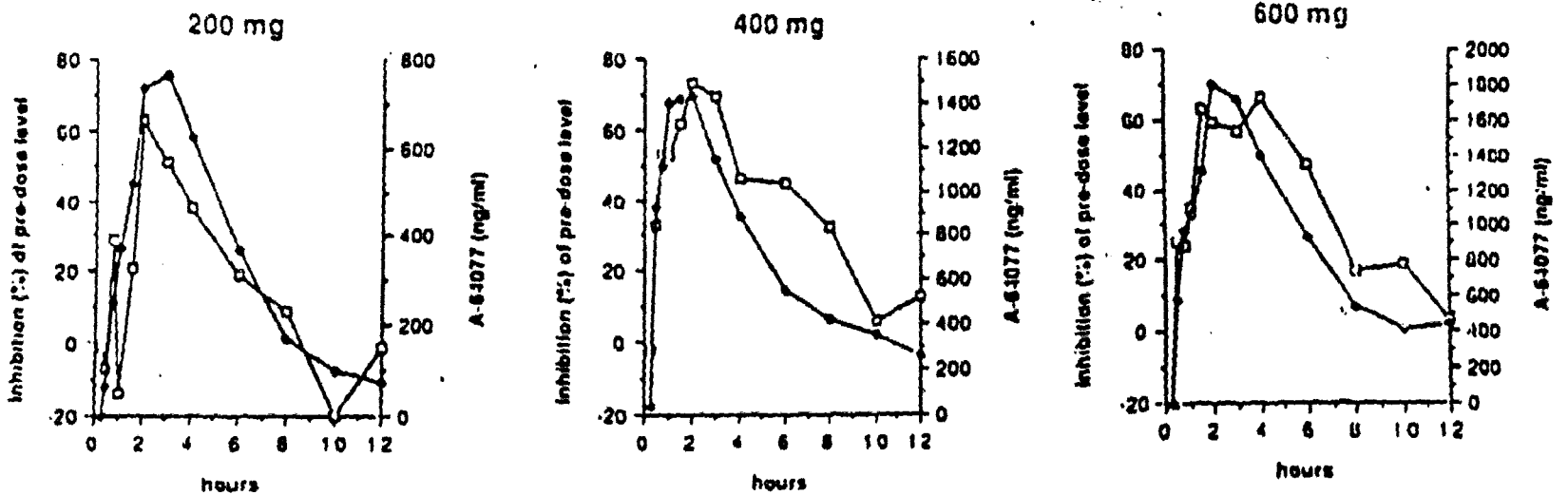
Hours post-dosing	LTB ₄ formation (ng/ml plasma)			
	Placebo	200mg	400mg	600mg
0				
0.25				
0.50				
0.75				
1.0				
1.5				
2.0				
3.0				
4.0				
6.0				
8.0				
10.0				
12.0				

* values differ significantly (P 0.05) from placebo for the corresponding time.

2. The % inhibition of LTB₄ synthesis and corresponding plasma concentrations following oral administration of ABBOTT-64077 is shown in the following figures:

Open Square = Inhibition

Filled Square = Plasma Concentration

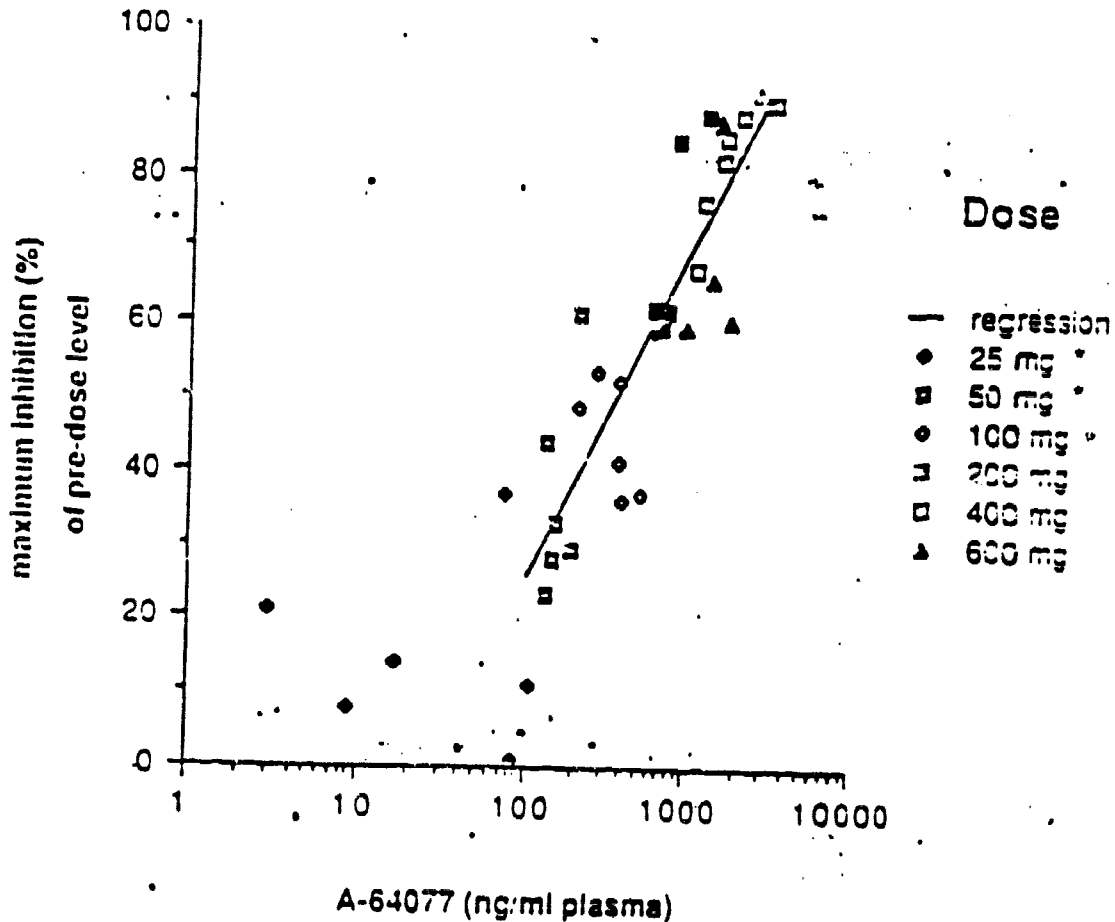


3. The maximum inhibition % of LTB_4 synthesis is defined as the maximum value of $(1 - \% \text{ pre dose level})$. The results are summarized in the following:

<u>Dose of ABBOTT-64077</u>	<u>Maximum inhibition %</u>
Placebo	21 ± 15
200mg	$72 \pm 14^*$
400mg	$82 \pm 8^*$
600mg	$76 \pm 15^*$

* Values are significantly ($P < 0.05$) from the placebo.

4. A linear regression analysis performed with the above data as well as the data from protocol M87-067, illustrated in the following figure, indicated that the IC_{50} of ABBOTT-64077 is 375ng/ml or 1.6 μ M against ionophore-induced LTB_4 biosynthesis in whole blood.



* DATA FROM PROTOCOL M87-067 (APPENDIX A).

Conclusion:

1. ABBOTT-64077 was demonstrated to be able to inhibit the ionophore-induced LTB_4 biosynthesis in the in vitro blood. This inhibition corresponds well with the plasma concentration of ABBOTT-64077.

2. The mean maximum inhibition of LTB₄ synthesis for ABBOTT-64077 was 72% ± 14 for 200mg, 82% ± 8 for 400mg and 76% ± 15 for 600mg. The present data do not support the claim of a dose-related inhibition from 200-600mg dose range made by the sponsor.

EVALUATION:

The submitted interim report on the single rising dose (200mg, 400mg, 600mg) tolerance study suggested that the Mean AUC of ABBOTT-64077 increased linearly with the increased oral dose. The elimination half life was approximately 4 to 5 hours based on a two compartment model.

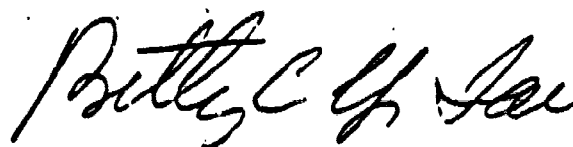
The in vitro study suggested that ABBOTT-64077 is an inhibitor of the ionophore-induced biosynthesis of LTB₄ in the ex-vivo whole blood. The magnitude of this inhibition was directly related to serum concentration of ABBOTT-64077. The claim that there is a dose related inhibition of the LTB₄ synthesis is not conclusive. A review with the combined data from protocol MB7-067 (a rising dose tolerance study with doses of 25, 50 and 100mg), The maximum inhibition (%) of the synthesis of LTB₄ seems to be leveled off at 400mg dose level.

CONCLUSION:

A linear kinetics of ABBOTT-64077 has been established in human from 200mg to 600mg oral dose. However, in the in vitro study, a linear relationship between the inhibition of the ionophore induced LTB₄ biosynthesis in the whole blood and the absolute oral dose of ABBOTT-64077 was not well established from 200mg to 600mg.

DRAFT OF LETTER TO THE SPONSOR:

None at this time.



Betty Chung-yui Tai, Ph.D.
Pharmacologist, HFN-160

CC: IND
✓ HFN-160
Doc. Rm. 160
R/D: B. Tai: 2/25/88
R/D init. by: Dr. Inscoc: 2/29/88

IND:

DATE: November 29, 1987

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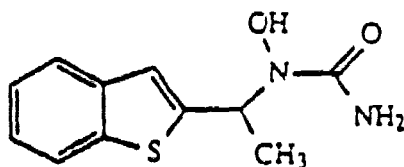
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

SUBMISSION DATED: Sept. 29, 1987

SPONSOR: Abbott Laboratories
Pharmaceutical Products Division
Abbott Park, IL 60064

NAME OF DRUG: ABBOTT-64077

Chemical Structure



Chemical Name

N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea.

M.w.: 236.294

CATEGORY: Lipoxygenase Inhibitor (leukotriene synthesis inhibitor)

ROUTE OF ADMINISTRATION: Oral

DOSAGE FORM: Capsule

The capsule formulations (25mg, 100mg and 200mg) are as follows:

<u>Scale</u>	<u>Item</u>	<u>Ingredient</u>	<u>Code</u>	<u>Amount</u>	<u>Amount</u>	<u>Amount</u>

PROPOSED CLINICAL STUDY:

Protocol # M87-068, Titled " Phase I study, Safety and Bioavailability Six Single Rising Oral Doses of Abbott-64077"

OBJECTIVE OF THE STUDY:

1. To compare the safety of Abbott-64077 to placebo in healthy male volunteers following oral administration of single 200mg, 400mg, 600mg, 800mg, 1000mg and 1200mg doses.
2. To determine pharmacokinetic parameters
3. To determine the effect on leukotriene and prostaglandin synthesis following each dose.

DESIGN OF THE STUDY:

Subjects: 54 healthy male volunteers. (9 subjects/group)

Treatments:	<u>Dosing period</u>	<u>Group</u>	<u>Dose(mg)</u>	<u>Week</u>
	I	A	200	1
	II	B	400	2
	III	C	600	3
	IV	D	800	4
	V	E	1000	5
	VI	F	1200	6

This will be a randomized, double-blind, placebo-control study. Safety will be monitored via continuous cardiac monitoring for the first 24 hours post dose.

Sampling: 5 ml of blood samples will be collected at 0, 15min, 30min, 45min, 1 hour, 1.5 hour, 2 hour, 3 hour, 4 hour, 6 hour, 6.5 hour, 7 hour, 8 hour, 10 hour, 12 hour, 16 hour and 20 hour.

Urine samples will also be collected collectively for 24 hour period. volumes recorded, samples saved for drug assay.

Analysis: Plasma drug concentrations will be assayed. Whole blood samples will be analyzed for LTB4 by negative ion mass spectroscopy. Selected samples will also be assayed for TXB2 by radioimmunoassay.

PRINCIPLE INVESTIGATOR/SITE:

PRECLINICAL DATA:

PHARMACOLOGY

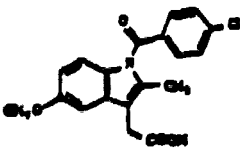
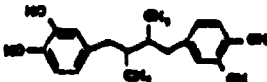
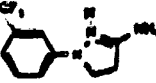
Studies submitted for review: (Title, report number)

1. The evaluation of ABBOTT-64077 for its: (1) In Vitro inhibitory activity against RBL-1 cell lysate 5-lipoxygenase; (2) In Vitro inhibitory activity against intact cell 5-lipoxygenase; and (3) In Vitro inhibitory activity against other related enzymes. (PPRD/87/162)
2. The evaluation of ABBOTT-64077 for: (1) 5-lipoxygenase inhibitory activity in vivo, (2) Antiinflammatory activity, (3) Preliminary pharmacokinetic properties and (4) Preliminary toxicity. (PPRD/87/163)
3. Effects of orally and intraperitoneally administered ABBOTT-64077 in neuropharmacological profile test in mice and effects of oral administration on the spontaneous motor activity and rotarod performance of mice and rats. (PPRD/87/165)
4. Hemodynamic evaluation of ABBOTT-64077 following intragastric administration in conscious dogs (PPRD/87/167)

(I) Biochemical Pharmacology:

The Evaluation of ABBOTT-64077 for its: (1) In Vitro Inhibitory Activity Against RBL-1 Cell Lysate 5-Lipoxygenase; (2) In Vitro Inhibitory Activity Against Intact Cell 5-Lipoxygenase; and (3) In Vitro Inhibitory Activity Against Other Related Enzymes.

Reference compounds:

<u>Compound</u>	<u>Structure</u>	<u>Chemical Formula</u>	<u>Molecular Weight</u>
Pseudoephedrine (Abbott-08847)		$C_{10}H_{15}NO$	165.2
Indomethacin (Abbott-29838)		$C_{20}H_{25}ClO_2$	357.4
NDGA (Abbott-29833)		$C_{20}H_{27}O_4$	328.4
BW735c (Abbott-50899)		$C_{10}H_{15}N_2O$	228.2

Test Species:

The broken cell 5-lipoxygenase preparation was derived from cultured rat basophilic leukemia (RBL-1) cells maintained at Abbott Laboratories.

The broken cell 12-lipoxygenase preparation was derived from human platelets (purchased from Interstate Blood Bank, Chicago Illinois).

The isolated cyclo-oxygenase preparation was derived from sheep seminal vesicles.

Rat polymorphonuclear leukocytes (PMNL) were obtained from Sprague Dawley derived rats (SASCO, Oregon, WI).

Human PMNL and whole blood were obtained from normal human volunteers.

Results:

(1) RBL-1 Cell Lysate 5-lipoxygenase:

a. Potency:

The IC₅₀s of the inhibitory effects on 5-lipoxygenase by ABBOTT-64077 and a host of reference compounds are listed in the following Table:

<u>Compound</u>	<u>IC₅₀^a (μM)</u>	<u>IC₅₀ (μM) with added 13-HPOD^b and ATP</u>
Abbott-64077	0.53 (0.43-0.63)	3.7 (3.2 - 4.3)
Phenidone	1.9 (1.5-2.8)	2.8 (2.6 - 3.0)
BW755c	2.0 (1.8-2.3)	8.8 (7.4 - 11)
NDGA	0.27(0.21-0.32)	0.68 (0.40-0.91)
Indomethacin	12% at 100 μM ^c	not tested

b. Time Dependence:

The effect of varying preincubation time on the inhibitory potency of ABBOTT-64077 is summarized in the following Table:

<u>Preincubation minutes</u>	<u>5-HETE (ng/ml)^b</u>		<u>% Inhibition</u>
	<u>Control</u>	<u>0.6 μM Abbott-64077</u>	
0	2724 ± 117	1606 ± 34	41
2	2544 ± 82	1238 ± 20	51
4	2679 ± 86	1423 ± 66	47
20	2765 ± 84	1493 ± 107	46

c. Reversibility:

The reversibility of the inhibitory effect of ABBOTT-64077 on 5-lipoxygenase is shown in the following Table:

<u>Pre- Incubation</u>	<u>Assay Post Dilution</u>	<u>Abbott-64077 (μM)</u>	
		<u>ng/ml^c</u>	<u>% Inhibition^d</u>
0	0	395 ± 34 ^e	-
0	1	62 ± 25 ^f	84
0	0.2	271 ± 26 ^g	31
1	1	56 ± 7 ^f	86
1	0.2	256 ± 16 ^g	35
0.2	0.2	191 ± 64 ^{f,g}	51

(2) Inhibition on Intact Cell 5-Lipoxygenase:

a. Human intact PMNL and Whole Blood 5-Lipoxygenase:

The IC50s of ABBOTT-64077 and a host of other compound on the inhibition of ionophore-induced formation of LTB₄ in isolated human PMNL and whole blood are summarized in the following Table:

<u>Compound</u>	<u>IC₅₀(μM).</u>	
	<u>Human PMNL LTB₄^a</u>	<u>Human Blood LTB₄^b</u>
Abbott-64077	0.7 (0.3-1.0)	0.6 (0.3-1.2)
Phenidone	1.3 (1.1-1.6)	0.9 (0.7-1.1)
BW755c	4.9 (3.8-6.0)	7.3 (5.0-9.6)
NDGA	1.0 (0.7-1.2)	1.4 (0.5-2.4)
Indomethacin	49 (39-62)	NS at 30 μ M ^c

b. Rat intact PMNL:

The IC50s of ABBOTT-64077 and a host of reference compounds on the inhibition of ionophore-induced formation of LTB₄ and PGE₂ in rat PMNL are summarized in the following Table:

<u>Compound</u>	<u>IC₅₀(μM)^a</u>		
	<u>Rat PMNL LTB₄</u>	<u>Rat PMNL PGE₂</u>	<u>Selectivity Ratio^b</u>
Abbott-64077	0.16 (0.11-0.25)	4.5 (2.4-8.6)	28
Phenidone	0.52 (.33-.74)	13 (11-16)	25
BW755c	12 (10-14)	0.8 (0.6-1.0)	0.07
NDGA	0.24 (0.16-0.53)	6.3 (5.1-7.5)	26
Indomethacin	NA ^c	0.02 (0.01-0.02)	<0.002

(C) Effect on Other Related Enzymes:

The interactions of ABBOTT-64077 and a host of reference compounds with human platelet 12-lipoxygenase, 2) Soybean 15-lipoxygenase and 3) sheep seminal vesicle cyclo-oxygenase are studied, the inhibitory IC₅₀s are summarized in the following Table:

<u>Compound</u>	<u>IC₅₀^a (μM)</u>		
	<u>Human platelet 12-Lipoxygenase</u>	<u>Soybean 15-Lipoxygenase</u>	<u>Sheep Seminal Vesicle Cyclo-oxygenase</u>
Abbott-64077	19% at 100 μM ^b	NS at 100 μM ^c	10% at 100 μM ^b
Phenidone	0.10 (0.09-0.10)	0.42 (0.34-0.51)	94 (78-115)
BW755c	5% at 100 μM ^b	213 (113-535)	97 (85-111)
NDGA	0.24 (0.19-0.30)	2.0 (1.7-2.3)	45% at 200 μM ^b
Indomethacin	NS at 100 μM ^c	NS at 100 μM ^c	0.63 (0.57-0.74) ^d

(II) Animal Pharmacology:

The Evaluation of ABBOTT-64077 for: (1) 5-lipoxygenase Inhibitory Activity In Vivo, (2) Antiinflammatory Activity, (3) Preliminary Pharmacokinetic Properties and (4) Preliminary Toxicity.

(1) In vivo inhibition of Leukotriene Biosynthesis:

(Rat Peritoneal Anaphylaxis Model)

Rats were passively sensitized by intraperitoneal (i.p.) injection of rabbit anti-bovine serum albumin (anti-BSA) in phosphate buffered saline (PBS), pH 7.1. Three hours after sensitization, the rats were injected i.p. with 4 mg BSA (fraction V., Miles Scientific, Naperville, IL.) in 5 ml of PBS containing 30 mM l-cysteine. Test compound or control vehicle was administered p.o. 1 hour prior to the antigen challenge unless otherwise stated.

The rats were sacrificed 15 minutes after challenge with carbon dioxide, the peritoneal cavity opened and the fluid contents collected with a plastic trocar and disposable pipets. The cavities were rinsed with 5 ml of cold PBS containing 0.1% gelatin, 0.1% sodium azide, 10 mM tripotassium ethylenediaminetetraacetic acid (EDTA) and 30 mM l-cysteine. The fluids were transferred to 20 ml. of ice cold methanol, allowed to stand for 20 minutes, vortexed and then centrifuged at 1000xg for 15 minutes. Fluid volumes were measured and the samples stored frozen until analyzed for leukotrienes by radioimmunoassay (RIA).

a. The inhibitory effects of ABBOTT-64077 and a host of reference agents in the Rat Peritoneal Anaphylaxis Model are summarized in the following Table:

Compound	Oral ED50 ^a mg/kg
Abbott-64077	3.1 (2.2 - 4.1)
BW755C	35.7 (14.2 - 52.2)
Phenidone	16.9 (10.4 - 27.5)
Indomethacin	b
NDGA	b

b. The dose response data of the orally administered ABBOTT-64077 in the Rat Peritoneal Anaphylaxis Model is summarized in the following Table:

Treatment ^b	Dose mg/kg	N ^c	(-Leukotrienes) ^a Mean (ng/rat)	Inhibition
Vehicle	(4 ml/kg)	24	344.4 ± 42.5	-
Abbott-64077	1.2	16	230.0 ± 26.5	33.2%
Abbott-64077	3.5	16	143.8 ± 16.3 ^d	58.3%
Abbott-64077	11.8	16	91.2 ± 10.0 ^d	73.5%
Abbott-64077	35.4	14	20.3 ± 6.6 ^d	94.1%

ED₅₀ = 3.1 mg/kg. p.o. (2.2-4.1)^e

c. Duration of Activity in the Rat Peritoneal Anaphylaxis Model:
 The duration of effects of orally administered ABBOTT-64077 at 10mg/kg in the Rat Peritoneal Anaphylaxis Model is summarized in the following Table:

Treatment ^a	Time (Hours)	N	(-Leukotrienes ^b Mean (ng/rat)	Inhibition
Vehicle	0.5	7	207.9 ± 45.3	-
Abbott-64077	0.5	8	90.4 ± 34.3 ^c	56.5%
Vehicle	1.0	8	215.3 ± 104.0	-
Abbott-64077	1.0	8	96.0 ± 39.9 ^c	55.4%
Vehicle	2.0	8	253.3 ± 76.8	-
Abbott-64077	2.0	8	114.7 ± 62.2 ^c	54.7%
Vehicle	4.0	8	200.8 ± 38.2	-
Abbott-64077	4.0	7	119.3 ± 22.2 ^c	40.6%
Vehicle	8.0	8	229.1 ± 50.8	-
Abbott-64077	8.0	8	188.4 ± 83.4	17.8%

c. Activity after repeated daily dose of ABBOTT-64077
 The activity of ABBOTT-64077 following daily (5 day) dosing in the Rat Peritoneal Anaphylaxis Model is summarized in the following Table:

Oral Treatment	N	(-Leukotrienes ^a Mean (ng/rat)	Inhibition
Vehicle (5 Doses) 4 ml/kg/day	10	195.7 ± 21.4	-
Abbott-64077 (5 Doses) 10 mg/kg/day	10	77.0 ± 11.2 ^b	60.7%
Abbott-64077 (1 Dose) ^c 10 mg/kg	10	85.7 ± 8.8 ^b	56.2%

4 Pages

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(4). Inhibition of Monkey Whole Blood 5-lipoxygenase Activity Following Oral Administration:

The inhibition of ionophore-induced LTB₄ biosynthesis within 30 minutes after oral dosing of 20mg/kg ABBOTT-64077 is shown in the following Table:

Time After Administration (Hours)	Plasma Concentration (uM)	Percent Inhibition ^{a,b}
-0.25	0.0	0.0 (23.3)
0.5	15.5	94.1 (2.9) ^c
1.0	5.6	98.6 (2.5) ^c
2.0	1.7	71.0 (3.8) ^c
3.0	0.8	3.7 (14.9)

(III) Aucillary Pharmacology

Effects of Orally And Intraperitoneally Administered ABBOTT-64077 in Neuropharmacological Profile Test in Mice and Effects of Oral Administration on the Spontaneous Motor Activity and Rotarod Performance of Mice and Rats

Reference Compounds And Dosages:

ABBOTT-64077

Neuropharmacological profile (mice): 10.0, 30.0, 100.0 and 300.0 mg/kg, i.p. and p.o. Spontaneous motor activity and rotarod (mice and rats): 10.0, 30.0 and 100.0 mg/kg, p.o.

Methamphetamine

Spontaneous motor activity (mice and rats): 2.0 mg/kg., p.o.

Chlorpromazine HCl

Spontaneous motor activity (mice and rats): 30.0 mg/kg, p.o.

Diazepam

Rotarod (mice and rats): 1.0, 3.0 and 10.0 mg/kg, p.o.

Vehicle (0.2% Hydroxypropyl Methylcellulose)

Neuropharmacological profile (mice) and spontaneous motor activity and rotarod (mice and rats): a volume dose (10.0 ml/kg, i.p. or p.o.) of 0.2% Hydroxypropyl methylcellulose.

Test Species:

Mice, Male Albino Charles River CD-1

Rats, Male Albino Charles River CD

Results:

a. ABBOTT-64077 was evaluated in a neuropharmacological profiling test in mice, the effects of intraperitoneally and orally administered ABBOTT-64077 are summarized in the following:

<u>Treatment</u> ^o	<u>Dose, Mg/Kg</u>	<u>Temperature, °C</u>	<u>Fatalities</u>	<u>Predominant Signs</u>
Control	-	37.7	-	-
ABBOTT-64077	10.0, i.p.	37.5	0/4	Very slight hypothermia
	30.0, i.p.	37.1	0/4	Slight hypothermia
	100.0, i.p.	37.3	0/4	Slight hypothermia, slight increase in activity followed by a slight decrease, slight ptosis reduced grip strength, muscle relaxation, piloerection, bright yellow urine, dyspnea
	300.0, i.p.	34.8	3/4	Same, fatalities 18-48 hours

Estimated LD₅₀ = 200.0 mg/kg, i.p.

Control	-	37.3	-	-
ABBOTT-64077	10.0, p.o.	37.6	0/4	Asymptomatic
	30.0, p.o.	37.1	0/4	Slight hypothermia
	100.0, p.o.	33.8	0/4	Hypothermia, dyspnea, bright yellow urine, piloerection, decreased muscle tone, reduced grip strength.
	300.0, p.o.	35.0	0/4	Same, plus slight increase in motor activity followed by a slight decrease

Estimated LD₅₀ = Beyond 300.0 mg/kg, p.o.

^o Four (4) mice per dose level. ABBOTT-64077 was suspended in the 0.25 hydroxypropylmethylcellulose vehicle. Seventy-two (72) hour observation.

b. ABBOTT-64077 was evaluated for its effects on the spontaneous motor activity of mice and rats, metaamphetamine (2.0mg/kg po) used as a CNS stimulant reference and chlorpromazine (30mg/kg po) used as a CNS depressant reference. Results showed that ABBOTT-64077, administered at doses of 10, 30 and 100mg/kg, po produced increase in spontaneous motor activity which was not statistically significant from the control.

c. The results of the rotarod studies in mice are summarized in the following Table:

Treatment ^a	Dose, mg/kg, p.o. ^b	Mean Time on Rotarod (± SEM) in Seconds at			
		Control	0.5	1.0	4.2 hours
Vehicle	-	100	100	100	100
ABBOTT-64077	10.0	100	96.3 (± 3.8)	100	100
	30.0	100	91.2 (± 4.3)	99.9 (± 0.9)	100
	100.0	100	76.8 (± 9.3)	91.1 (± 9.0)	96.9 (± 1.1)
ABBOTT-24147	1.0	100	88.4 (± 6.0)	96.3 (± 1.3)	100
	3.0	100	72.6 (± 11.1) ^{**}	80.0 (± 9.4)	100
	10.0	100	18.1 (± 9.9) ^{**}	49.8 (± 14.1) ^{**}	77.3 (± 11.7)

^a Ten (10) mice per dose level. Mice were trained to remain on a rod rotating at 16 revolutions per minute for 100 seconds (Omni-Rotor, Omnicron Electronics, Inc., Columbus, OH).

^{**} $p \leq 0.05$, one-way analysis of variance versus vehicle.

The Results of the rotarod studies in rats are summarized in the following Table:

Treatment	Dose, mg/kg, p.o. ^a	Mean Time on Rotarod (± SEM) in Seconds at			
		Control	0.5	1.0	4.2 hours
Vehicle	-	30	30	30	30
ABBOTT-64077	10.0	30	30	30	30
	30.0	30	30	30	30
	100.0	30	30	30	30
ABBOTT-24147	1.0	30	29.1 (± 0.7)	28.2 (± 1.4)	30
	3.0	30	27.3 (± 2.3)	23.3 (± 2.3)	30
	10.0	30	16.3 (± 3.7) ^{**}	16.9 (± 3.2) ^{**}	28.1 (± 1.3)

^a Ten (10) rats per dose level. Rats were trained to remain on a rod rotating at 5 revolutions per minute for 30 seconds (Omni-Rotor, Omnicron Electronics, Inc., Columbus, OH).

^{**} $p \leq 0.05$, one-way analysis of variance versus vehicle.

NDA 020471

FIRM: ABBOTT LABS

7 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

Hemodynamic Evaluation of ABBOTT-64077 following Intra-gastric Administration in Conscious Dogs

Reference compounds:

ABBOTT-64077 5mg/kg, 50mg/kg (intra-gastric)

Vehicles: 0.2% hydroxypropylmethylcellulose + 0.7% Tween 80g

Animals: Beagle dogs, male, 5/group.

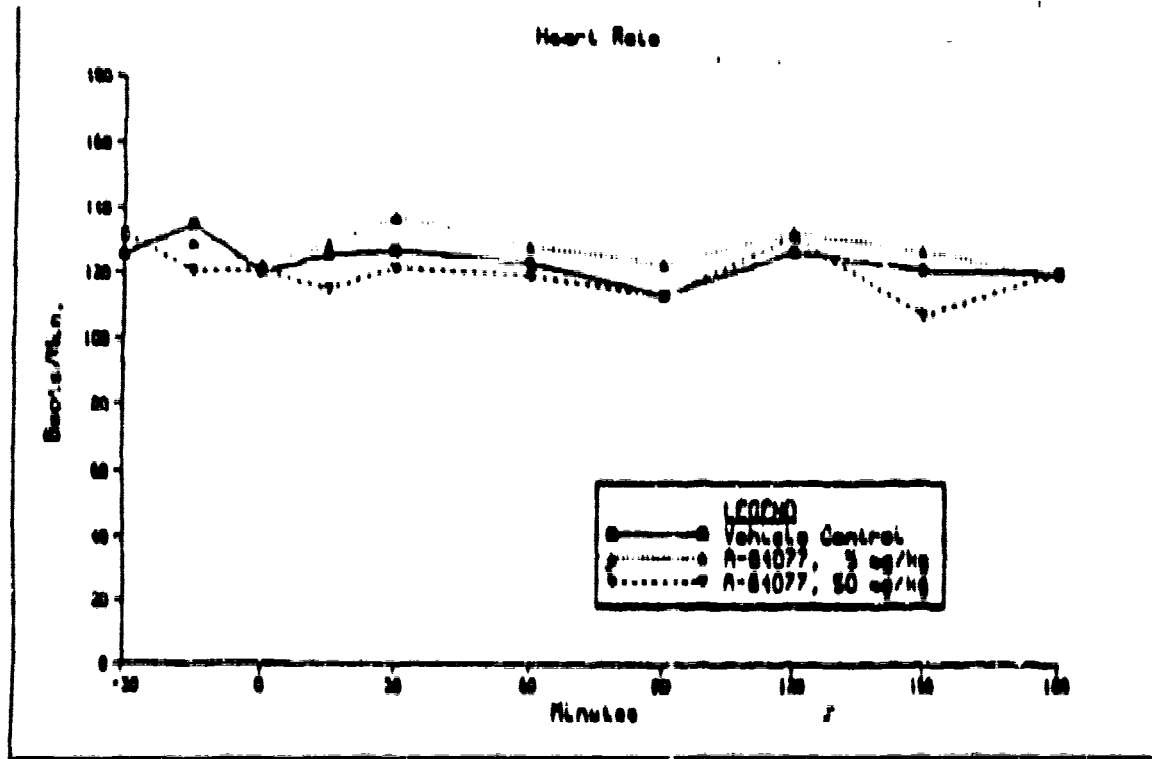
Results:

Heart rate (beats/min), mean arterial pressure (mmHg), pulmonary arterial pressure (mmHg), and plasma level of ABBOTT-64077 (ug/ml) were monitored and analyzed.

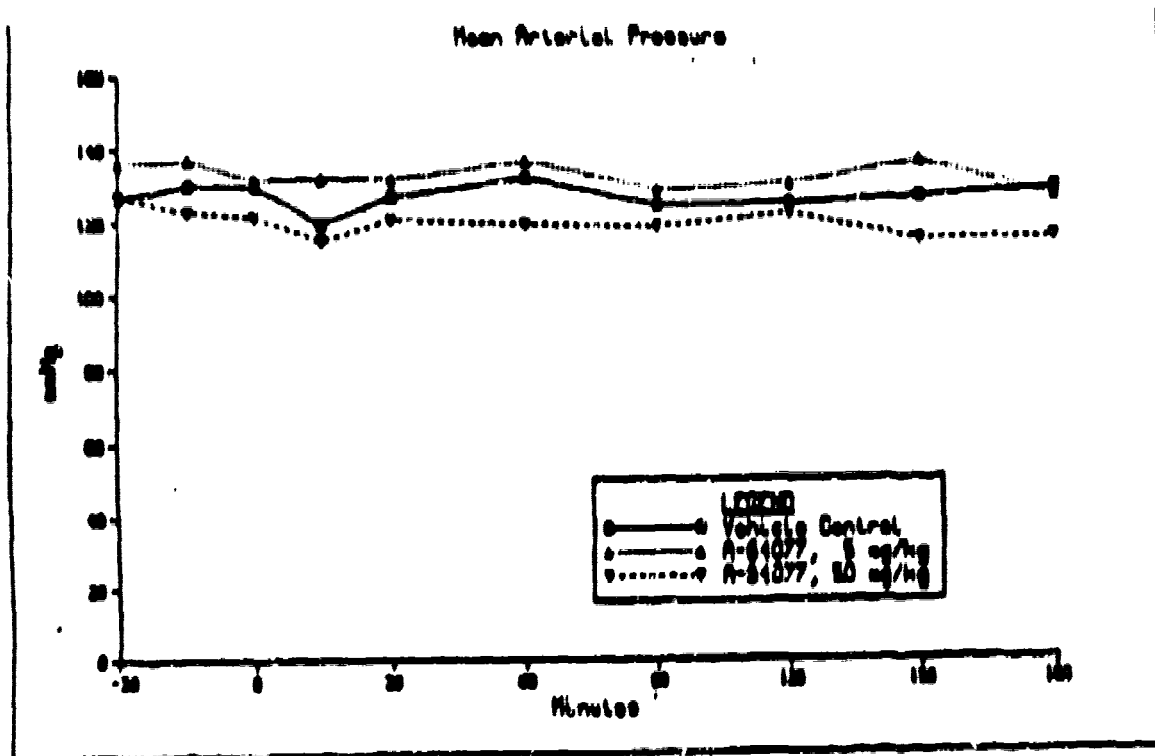
The plasma levels of ABBOTT-64077 in dogs are summarized in the following Tables:

Dog	Concentration (mcg/mL)					Area Under 0-3 Hr Curve (mcg/mL)
	Hours After Dosing					
	0	0.5	1	2	3	
5 Mg/Kg						
7/07	0.0	3.0	3.3	3.0	2.2	8.1
7/17	0.0	2.2	3.1	4.1	3.7	9.4
7/21	0.0	3.7	3.7	3.2	3.0	9.3
7/28	0.0	0.8	1.2	1.9	2.6	4.5
8/05	0.0	4.5	5.0	3.9	3.9	11.9
Mean	0.0	2.8	3.3	3.2	3.1	8.6
S.D.	0.0	1.4	1.4	0.9	0.7	2.7
S.E.M.	0.0	0.6	0.6	0.4	0.3	1.2
50 Mg/Kg						
7/01	0.0	11.5	15.4	15.0	13.1	38.9
7/15(a)	0.0	4.2	5.3	12.2	14.5	25.5
7/15(b)	0.0	8.6	11.7	18.1	20.3	41.3
7/24	0.0	16.1	19.8	18.1	19.7	50.9
7/28	0.0	2.9	5.3	10.2	17.5	24.4
Mean	0.0	8.7	11.5	14.7	17.0	36.2
S.D.	0.0	5.4	6.3	3.5	3.2	11.2
S.E.M.	0.0	2.4	2.8	1.6	1.4	5.0

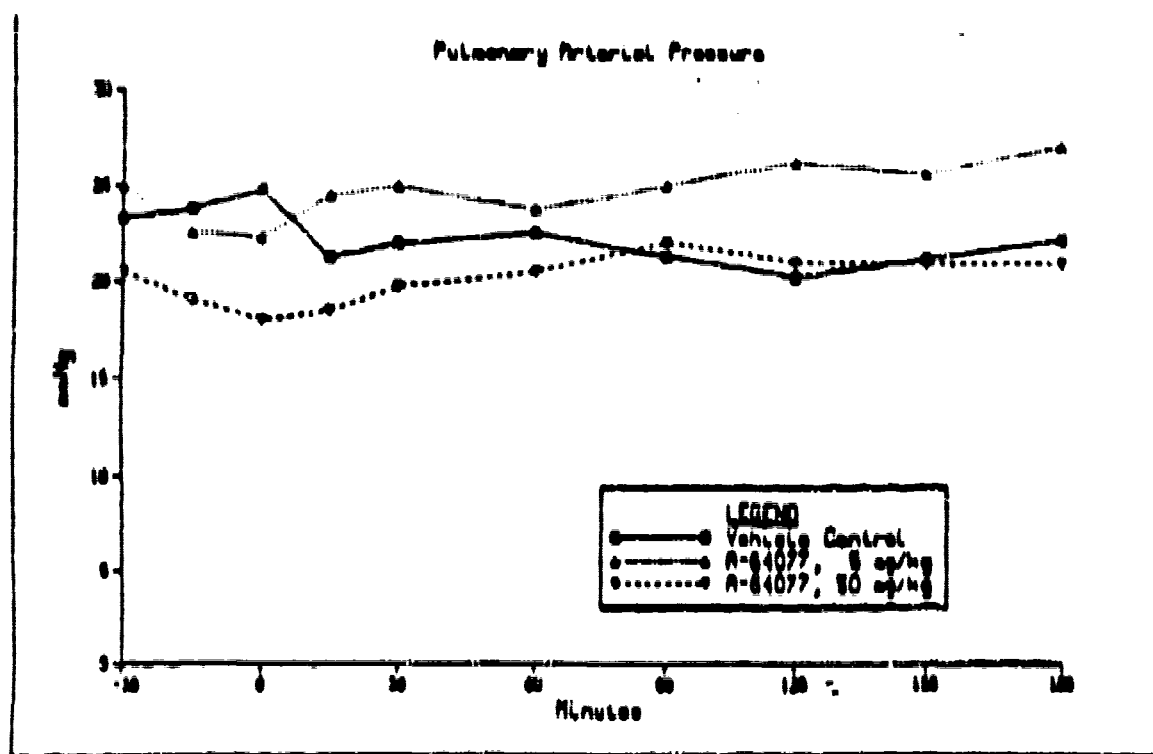
The effect of ABBOTT-64077 on the heart rate of the dog is summarized in the following:



The effect of ABBOTT-64077 on the mean arterial pressure is summarized in the following:



The effect of ABBOTT-64077 on the pulmonary arterial pressure is summarized in the following:



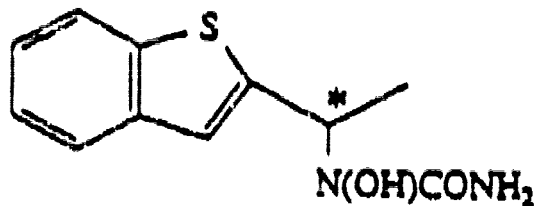
ADME

The following studies were submitted: (Title, Report No)

1. Synthesis of ABBOTT-64077-14C, PPRD/87/140
2. High-performance Liquid Chromatographic Procedure for the Determination of ABBOTT-64077 in plasma, PPRD/87/165
3. Pharmacokinetics of ABBOTT-64077 in dogs after one month treatment with 5, 20, 100 and 400 mg/kg/day doses (study T887-134), PPRD/87/171
4. Plasma ABBOTT-64077 levels in rats treated for one month with oral 5, 20, 80 and 300mg/kg/day doses (study T887-140), PPRD/87/178
5. In vitro plasma protein binding of ABBOTT-64077-14C, PPRD/87/168
6. Metabolism of orally administered ABBOTT-64077-14C in Cynomolgus monkeys, PPRD/87/169
7. Overview of pharmacokinetic and metabolism studies with ABBOTT-64077, PPRD/87/177

(1) Synthesis of ^{14}C -ABBOTT-64077

The compound was labeled with carbon-14 in the position indicated by the asterisk in the structure below:



ABBOTT-64077- ^{14}C

About 8.3 mCi of ABBOTT-64077- ^{14}C was obtained, which was shown to have a specific activity of 23.4 $\mu\text{Ci/mg}$ and >98% chemical and radiochemical purity. It was assigned Lot No. 15294-29.

REVISED 8/28/87: About 8.3 mCi of ABBOTT-63004- ^{14}C should have been ABBOTT-64077- ^{14}C .

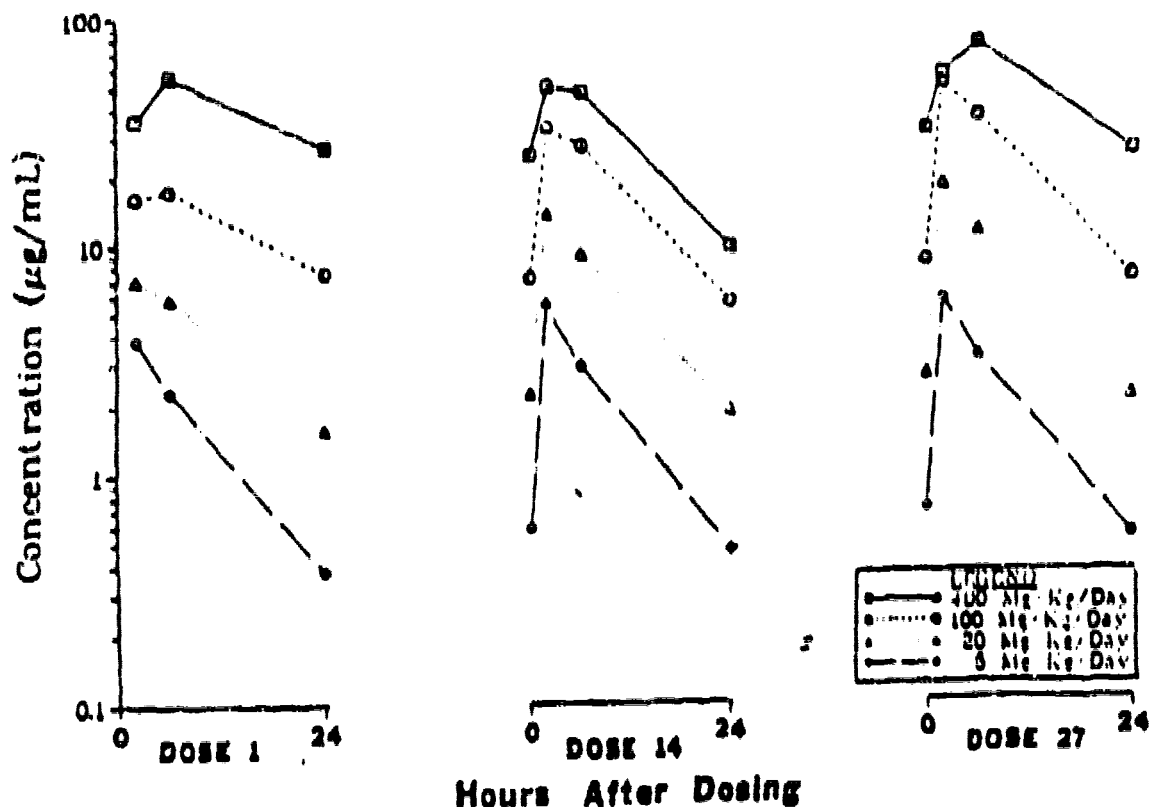
(2) Pharmacokinetics of ABBOTT-64077 in dogs

Dose tested: Oral doses of 5, 20, 100 and 400mg/kg/day in capsules

Samples: Serial plasma samples were taken after doses 1, 14 and 27
three month toxicology study

Results:

a. The plasma levels of ABBOTT-64077 in the dogs are summarized in the following Figure.



b. The estimated AUCs and elimination constants in dogs treated with ABBOTT-64077 for one month are summarized in the following:

DOSE (mg/kg/day)	AUC (ug.hr/ml)			K _e (hr ⁻¹)			*CSF
	days: <u>1</u>	<u>14</u>	<u>21</u>	<u>1</u>	<u>14</u>	<u>21</u>	
5	40.3	52.4	55.0	0.1058	0.1101	0.1053	0.0
20	99.8	159.4	196.9	0.0878	0.1037	0.0968	0.0
100	308.2	451.3	607.8	0.0740	0.0901	0.0982	0.0
400	968.2	776.2	1224.6	0.0616	0.1004	0.0820	0.0

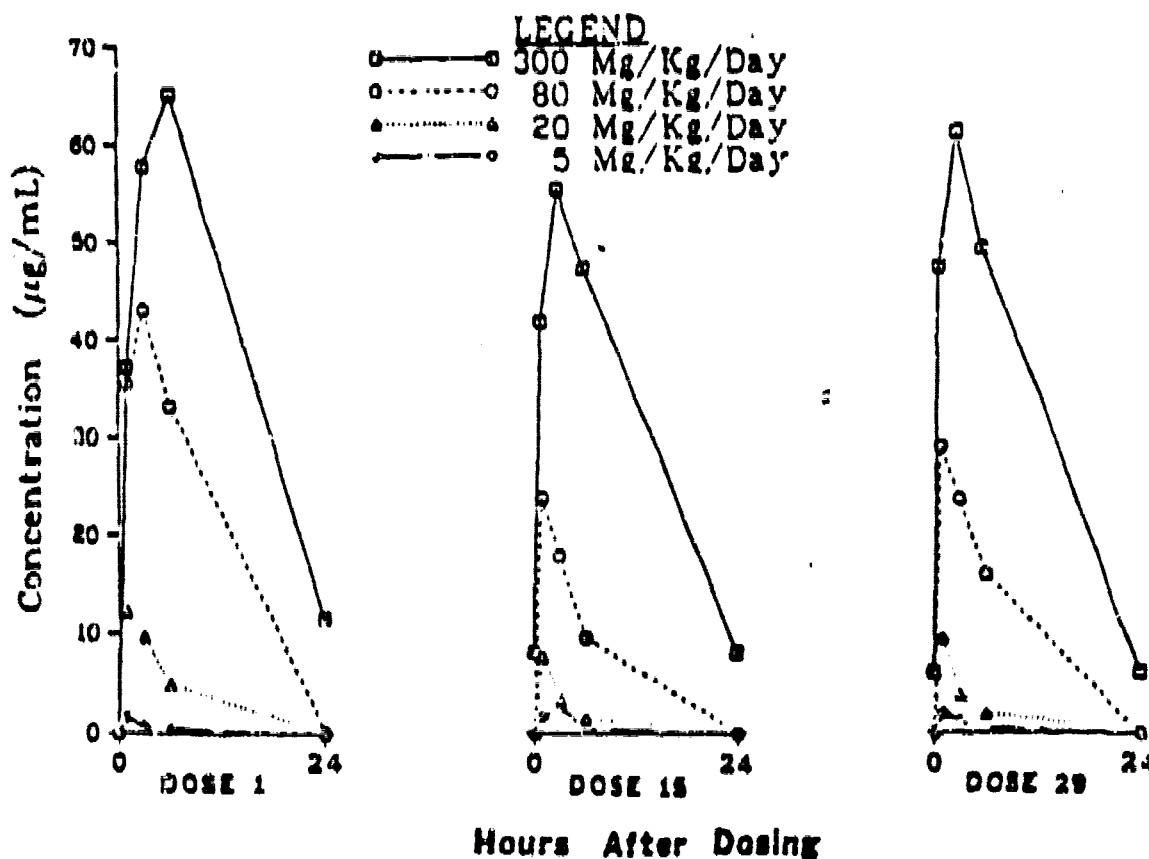
* Ratio of CSF and plasma ABBOTT-64077 levels at one month of treatment.

(4) Plasma levels of ABBOTT-64077 in rats

Doses: 5, 20, 80 and 800 mg/kg/day were administered by gavage.

Samples: Serial samples were collected from rats after doses 1, 15, 29 of a three month toxicology study.

Results: The group mean plasma ABBOTT-64077 levels in rats are summarized in the following Figures:



The estimated AUCs/dose is summarized in the following Table:

Group	Dose No:	AUC/Dose		
		1	15	29
T ₁		1.7 ± 0.8	2.8 ± 0.8	2.0 ± 0.4
T ₂		4.8 ± 4.1	1.8 ± 0.5	2.3 ± 0.5
T ₃		6.4 ± 2.2	2.3 ± 0.7	3.4 ± 1.7
T ₄		3.3 ± 0.2	2.6 ± 0.7	2.7 ± 0.7

(5) In vitro Plasma protein binding of ABBOTT-64077

The in vitro plasma protein binding of ABBOTT-64077-14C was determined with an ultrafiltration technique.

Blood (50ml) was obtained from male and female CD rats, beagle dogs, cynomolgus monkeys and healthy human volunteers that had been fasted overnight.

Results:

The protein binding of ABBOTT-64077 was determined at concentrations ranging from 0.1-100mcg/ml. The results are summarized in the following Table:

Species	Sex	Percent Protein Bound			
		0.1 mcg/mL	1 mcg/mL	10 mcg/mL	100 mcg/mL
Rat	M	90.5	91.3	90.6	87.2
	F	92.3	92.0	92.0	89.3
	Mean	91.4	91.6	91.3	88.2
Dog	M	87.0	88.8	87.8	83.3
	F	90.9	89.9	88.4	85.2
	Mean	89.0	89.4	88.1	84.2
Monkey	M	88.3	89.5	89.1	87.3
	F	90.1	90.1	89.4	87.1
	Mean	89.2	89.8	89.2	87.2
Human	M	94.1	93.4	93.6	91.3
	F	93.5	93.4	93.8	91.8
	Mean	93.8	93.4	93.7	91.6

(6) Metabolism of ABBOTT-64077 in monkeys

Animals: 6 female cynomolgus monkeys

Dose: monkeys were given a 5mg/kg oral dose of 14C-ABBOTT-64077

Samples: Blood samples were obtained from the monkeys at 0, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 and 24 hours after oral administration.

Urine samples were collected from 0-7, 7-24, 24-48 and 48-72 hours after dosing.

Feces were collected daily for three days after dosing.

(7) Overview of Pharmacokinetic and metabolism studies with ABBOTT-6407

Summary of Pharmacokinetics:

The pharmacokinetics of ABBOTT-64077 were studied in dogs from a range-finding toxicity study (50-800mg/kg/day) and during the first month of three-month toxicity studies in dogs (5-400mg/kg/day) and rats (5-300mg/kg/day).

The results obtained from the dog range-finding toxicity study are summarized in the following:

<u>Dose</u> (mg/kg/day)	<u>C_{max}</u> (ug/ml)	<u>AUC</u> (ug.hr/ml)
50	43	361
100	89	1006
200	94	1510
400	102	1573
800	124	1842

The half life of the drug ranged from 6.5 - 8.3 hours in the dog. The results obtained from the three-month toxicity study of dog and rat were reviewed under (3) and (4) of the ADME section.

Further analysis of these results indicated that in the dog as well as the rat, the absorption of ABBOTT-64077 did not increase linearly with dose administered.

The pharmacokinetics of ABBOTT-64077 were also determined in male human volunteers. the results are summarized in the following:

<u>DOSE</u> (mg)	<u>C_{max}</u> (ng/ml)	<u>T_{max}</u> (hr)	<u>AUC</u> (ug.hr/ml)
25	78	2.0	4.79
50	163	1.4	10.50
100	361	1.2	15.14

The half life of the drug in human ranged from 1.4 to 2.1 hour.

The metabolism of the drug was reviewed under (6), the protein binding of the drug was reviewed under (5) of ADME section.

TOXICOLOGY

the following studies were submitted:

Acute Toxicity:

1. # T87-156 Acute Oral Toxicity Evaluation of Abbott-64077 in Mice and Rats

Multidose Toxicity:

2. # T87-119 Ten Day Oral Dosage-Range-Finding Study of Abbott-64077 in Rats (Exploratory Research Report)

3. # T87-140 Three Month Toxicity Study of Abbott-64077 Administered Orally to Rats One Month Interim Report

4. # T87-121 Abbott-64077 Oral Dosage-Range-Finding Study in Dogs (Exploratory Research Report)

5. # T87-134 Three Month Toxicity Study of Abbott-64077 Administered Orally to Dogs (With One Month Interim Kill); One Month Interim Report

Mutagenicity Studies:

6. # T87-165 Salmonella/Mammalian Microsome Mutagenicity Test (AMES test) of Abbott-64077

Acute Toxicity:

Acute oral toxicity of ABBOTT-64077 was evaluated in mice and rats using two different vehicles, corn oil and 0.2% hydroxypropylmethylcellulose (HPMC).

Formulation: ABBOTT-64077, Lot 05-661-AL was suspended in either corn oil or 0.2% HPMC.

Animals: Male and female CD-1(ICR)BR mice, 10/sex/group
Male and female CDBR rats, 10/sex/group

Treatment: Single dose was administered by gavage, 4-9 groups of each sex and species were treated with different dose levels. Animals were observed for two weeks post dose.

Results: The oral LD50s of ABBOTT-64077 in mice and rats are summarized in the following Table:

ABBOTT-64077 LD ₅₀ Value in g/kg (95% Confidence Limits)				
Vehicle	Species	Male	Female	Combined ^b
Corn Oil, N.F.	Mouse	1.04 (0.71 - 1.94)	1.07 (0.91 - 2.41)	1.31 (0.93 - 3.41)
	Rat	0.41 (0.16 - 0.66) ^c	0.63 (0.33 - 1.92) ^d	0.47 (0.13 - 1.10) ^e
0.25 10% ^f	Mouse	14.00 ^g	14.00 ^g	14.00 ^g
	Rat	14.00 ^g	4.00 (1.51 - 10.73) ^h	14.00 ^g

The mortality ratio and toxic signs observed are summarized in the following two Tables:

Observations - ABBOTT-64077 in Corn Oil

Species	Dose (g/kg)	Mortality Ratio ^a		Signs of Toxicity
		Males	Females	
Mouse	0.30	0/10	1/10	Decreased activity, ataxia, dyspnea, prostration and hypothermia
	1.00	0/10	0/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia and urine-stained coat
	1.31	9/10	9/10	Decreased activity, ataxia, dyspnea, prostration, coma and hypothermia
	1.79	0/10	0/10	Decreased activity, ataxia, dyspnea, prostration, coma and hypothermia
	2.39	7/10	7/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia and squinting ^b
	3.20	4/10	0/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, squinting and urine-stained coat
Rat	0.10	0/10	0/10	Diarrhea
	0.30	0/10	1/10	Decreased activity, ataxia, dyspnea, prostration, hypothermia, diarrhea, lacrimation ^c and ataxia ^d
	0.40	4/10	— ^b	Decreased activity, ataxia, dyspnea, coma and hypothermia
	0.50	0/10	7/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, diarrhea ^e , lacrimation, urine-stained coat ^f and red substance (presumably porphyrin) around face ^g
	0.71	10/10	9/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia and lacrimation
	1.30	0/10	— ^b	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation and red substance (presumably porphyrin) around face ^h
	2.00	10/10	0/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation and red substance (presumably porphyrin) around face
	3.00	10/10	10/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation and red substance (presumably porphyrin) around face
4.00	10/10	7/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation, red substance (presumably porphyrin) around face and urine-stained coat	

^aNumber of deaths/number of animals treated.

^bFemale rats not treated with this dose.

^cObserved in only one animal.

Observations - ABBOTT-64077 in 0.25 Hydroxypropylmethylcellulose

Species	Dose (g/kg)	Mortality Ratio ^a		Signs of Toxicity
		Males	Females	
Mouse	1.00	0/10	1/10	Decreased activity, ataxia, dyspnea, prostration and hypothermia
	1.30	0/10	0/10	Decreased activity, ataxia, dyspnea, prostration, hypothermia, squinting and coma ^b
	2.37	0/10	0/10	Decreased activity, ataxia, dyspnea, prostration, hypothermia and squinting
	4.00	1/10	0/10	Decreased activity, ataxia, dyspnea, prostration, hypothermia, squinting and coma ^b
Rat	1.00	2/10	1/10	Decreased activity, ataxia, dyspnea, squinting, prostration, coma, hypothermia, lacrimation, red substance (presumably porphyrin) around eyes ^c and ocular opacity ^d
	1.30	0/10	3/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation, red substance (presumably porphyrin) around eyes ^e and ocular opacity
	2.37	2/10	1/10	Decreased activity, ataxia, dyspnea, prostration, coma, hypothermia, lacrimation and ocular opacity ^f
	4.00	2/10	0/10	Decreased activity, ataxia, dyspnea, squinting, prostration ^g , coma, hypothermia, lacrimation, red substance (presumably porphyrin) around eyes, ocular opacity ^h and ataxia ⁱ

^aNumber of deaths/number of animals treated.

^bObserved in only one animal.

Conclusion:

1. CNS seems to be the target organ.
2. LD50s for ABBOTT-64077 suspended in corn oil were several times higher in mice than in rats.
3. Similar toxic signs were observed regardless of which vehicle was used, but, lower doses of ABBOTT-64077 were found to cause death when suspended in oil than in 0.2% HPMC.

Multidose Toxicity:

(I) Ten-day range finding studies in rats and dogs;

RAT

Doses: 40, 75, 150, 300, 600 and 1200mg/kg/day, vehicle control is in 0.2% HPMC.

Animals: CDBR rats, 3/sex/treatment

Results: Clinical signs: decreased activity in 1200 group
Clinical pathology: Serum ALT increased in 1/3 female 600 group and 1/1 female 1200 group. At necropsy, mean liver weights were increased in both sexes in groups dosed with 150, 300, 600 and 1200, histological studies revealed cytomegaly and granularity.

DOG

Doses: 50, 100, 200, 400, 800 mg/kg/day.

Animals: Beagle dog, 1/sex/group

Results: Clinical signs: sporadic emesis was observed in 400 and 800 dosing group, the female of 800 dosing group appeared to be ataxic and with decreased activity.
Clinical pathology: No treatment related gross pathologic changes were noted. Mild kidney cortical tubular vacuolation was revealed in the 800 dosing group.

(II) Three-month studies (with one month interim kill) in rats and dogs

RAT

Doses: 5, 20, 80 and 300mg/kg/day (oral gavage)

Animal: CDBR rat, 10-13/sex/group

Results: Clinical Sign: The 300 group, particularly the female ones are more active

Clinical pathology: Treatment related increases in the serum alanine aminotransferase and cholesterol values. Significant increase in the mean absolute and relative liver weights in the 80 and 300 groups. Microscopically treatment-related cytomegaly was observed in the hepatocytes of the 80 and 300 group.

Conclusions: The no-toxic-effect dose appeared to be 80mg/kg/day in rat.

DOG

Doses: 0, 20, 80 and 300mg/kg/day (gelatin capsule)

Animals: Beagle dogs, 3/sex/group.

Results: Clinical signs: emaciation, decreased activity in 80 and 300 dosing group.

Anatomic pathology: Mean absolute and relative prostatic weights for 100 and 400 male dogs, were decreased. Treatment related depression of absolute testicular weights was found in 20 to 400 group.

Histological abnormalities: diffuse prostatic atrophy and focal to multifocal testicular atrophy were found to be treatment related. Renal cortical tubular vacuolation in 80 group was noted.

Conclusions: The no-toxic-effect dosage appeared to be 5mg/kg/day in dog.

Mutagenicity Test:

Test compound: ABBOTT-64077, Lot No. 05-661-AL at the following concentrations: 0, 5, 10, 50, 100, 500, 1000, 2000, 5000ug/petri plate

Test organism: Salmonella typhimurium histidine auxotroph strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100.

Test methods: Agar overlay plate incorporation method, nonactivated and rat liver microsome S-9 activated tests.

Incubation duration: 48 hours

Results: No dose related increase in revertant frequencies. Toxicity at 5000ug.

SUMMARY AND EVALUATION:

ABBOTT-64077 is proposed as an orally active 5-lipoxygenase inhibitor. This enzyme converts arachidonic acid to 5-hydroperoxy-eicoso-6,8,11,14 tetra enoic acid (5-HPETE) in the pathway leading to the production of leukotrienes (LK). Leukotrienes have been implicated in the pathophysiology of numerous disease processes which include; inflammatory bowel disease, psoriasis, rheumatoid arthritis and asthma. The therapeutic rationale of ABBOTT-64077, a 5-lipoxygenase inhibitor was that it may be useful in the treatment of the above mentioned conditions by inhibiting the synthesis of leukotrienes.

PHARMACOLOGY

(I) Biochemical Pharmacology

ABBOTT-64077 has been demonstrated to be a specific 5-lipoxygenase inhibitor in the following systems:

<u>SYSTEMS</u>	<u>IC50</u>
20,000xg supernatant of sonicated rat basophilic leukemia-1 cells	0.53uM
Intact polymorphonuclear leukocytes	
RAT	0.20uM
HUMAN	0.7uM
Whole blood	
HUMAN	0.6uM

It was also demonstrated that ABBOTT-64077 was rather specific to 5-lipoxygenase than other enzymes such as human platelet 12-lipoxygenase, soybean 15-lipoxygenase, sheep seminal vesicle cyclo-oxygenase.

(II) Animal Pharmacology

1. In Vivo inhibition of leukotrienes(LT) synthesis

RAT: ABBOTT-64077 inhibits LT synthesis in a dose related manner with a calculated ED50 of 3.1mg/kg po in the rat peritoneal cavity model. The duration of inhibition at 10mg/kg lasts at least four hours and no tolerance developed when given daily for 5 days.

3. Inhibition of blood 5-lipoxygenase activity

MONKEY: ABBOTT-64077 when given to monkey orally, has been shown to inhibit the 5-lipoxygenase activity in blood samples. A clear dose (ABBOTT-64077 plasma concentration) response (inhibition of whole blood 5-lipoxygenase) was demonstrated.

4. Ancillary activity

RAT and MOUSE: In the dose range of 10-100mg/kg, ABBOTT-64077 had no significant stimulant or depressant effects on the CNS.

DOG: At dose of either 0.5 or 50 mg/kg, ABBOTT-64077 produced no effects on cardiopulmonary hemodynamic parameters in anesthetized dog. In conscious dog, at dose either 5 or 50mg/kg, ABBOTT-64077 had no effect on pulmonary arterial pressure, mean arterial pressure or heart rate.

ADME

1. Metabolism

MONKEY: When C14 labeled ABBOTT-64077 was given orally, the radioactivity was well absorbed, but it underwent extensive first-pass conjugation with glucuronic acid, thus resulting in low parent drug levels. The metabolites were rapidly excreted in the urine and accounted for about 90% of the dose. No unchanged drug was found in the urine.

2. Pharmacokinetics

RAT: The peak plasma concentration and the AUC of the drug increased with increasing dose from 5-300mg/kg/day, but the increase was not strictly linear.

DOG: The peak plasma concentration and the AUC of the drug increased with increasing dose from 5-800mg/kg/day, but, when dose exceeds 200mg/kg, the AUC seems to be leveled off. The sponsor suggested that the decreased absorption was due to the low solubility of the drug. Should that be the cause, then toxicity tests in dogs with doses higher than 200mg/kg/day provided little meaning.

It was also shown that CSF concentration of the drug was about the same as the free drug concentration in the plasma.

HUMAN: The Cmax of the drug seems to be linearly related to the oral drug dose from 25-100mg.

3. The half life of the drug

Dog- 6.5-8.3 hour
Human- 1.4-2.1 hour

4. In vitro Plasma Protein binding

SPECIES	% PROTEIN BOUND (ug/ml)			
	0.1	1.0	10.0	100.0
rat	91.4	91.6	91.3	88.2
dog	89.0	89.4	88.1	84.2
monkey	89.2	89.8	89.2	87.2
human	93.8	93.4	93.7	91.6

TOXICOLOGY

1. In Vivo toxicity

The in vivo toxicity of ABBOTT-64077 was defined in an acute study and one month interim report of a three month study. Three species were tested and summarized in the following Table:

<u>ANIMAL</u>	<u>SPECIES/ STRAINS</u>	<u>♂/SEX OF</u>	<u>ROUTING</u>	<u>DOSES (mg/kg/day)</u>	<u>DURATION (DAYS) ROUTING/STUDY</u>
Mouse	Crl:CD ¹ BR	10M/10F	Gavage	500-3200	1/14
Rat	Crl:CD ¹ BR	10M/10F	Gavage	100-4000	1/14
	Crl:CD ¹ BR	3M/3F	Gavage	40-1200	10/10
	Crl:CD ¹ BR	10M/10F	Gavage	5, 20, 80, 300	30/30
Dog	Beagle	1M/1F	Oral	50-800	1/14
	Beagle	3M/3F	Oral	5, 20, 100, 400	30/30

The results of these toxicity studies are summarized in the following Table:

<u>ANIMALS</u>	<u>TREATMENT DURATION</u> <u>IN DAYS</u>	<u>NOEL DOSE</u> <u>(mg/kg)</u>	<u>TOXIC DOSE</u> <u>(mg/kg)</u>	<u>TOXIC EFFECTS</u>
Mouse	1		LD ₅₀ = 1070 - >4000	Sedation
Rat	1		LD ₅₀ = 410 - >4000	Sedation
	10	300	600	Reduced activity, death, weight gain reduction, reduced food consumption, increased serum ALT & cholesterol
	30	80	300	Hyperactivity, reduced weight gain, increased serum ALT & cholesterol
Dog	10	200	400	Emesis, weight loss, ataxia, renal tubular vacuolation
Dog	30	5	20	Emasiation, reduced activity, sporadically low weights & food consumption, prostatic & testicular atrophy, renal tubular vacuoles, focal gastritis, hepatocellular degeneration

(2) In Vitro mutagenicity test

The results of Ames test was negative.

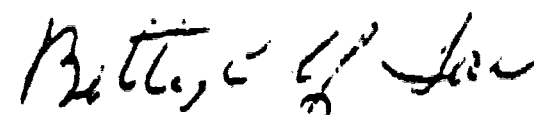
CONCLUSION AND RECOMMENDATION:

Based on the submitted 1) toxicology profile of this drug developed in mouse (acute), rat (acute, 10days, 30days) and dog (10days, 30days) which indicated that the no-toxic-effect dose in the 30 day dog study was 5mg/ and toxic effects shown at 20mg/kg, and 2) Pharmacokinetic properties of this drug in rat, dog, cynomolgus monkey and human which suggested non-linear kinetics, we have reservations about the safety of the proposed clinical single oral rising dose tolerance study. We have the following recommendations:

- 1) The sponsor should proceed the clinical trial with the first three doses, i.e., 200mg, 400mg and 600mg with caution. Further human pharmacokinetic information on this drug should be obtained and submitted to the Division for better understanding and possible prediction of the disposition and toxic potential of this drug in human.
- 2) The dog, as indicated by the pharmacokinetic data, may not be the most relevant animal model in predicting the disposition and toxicity of the drug in human. Therefore, the sponsor is urged to explore another species perhaps another strain of monkey, to further characterize the pharmacokinetics and toxicity of the drug.

DRAFT LETTER TO THE SPONSOR

Copy recommendation (1) and (2).


Betty C Y Tai, Ph.D.
Pharmacologist, HFN-160

cc: IND .
HFN-160, Doc. Rm. 150
R/D: B. Tai: 11/29/87
RD init. by: Dr. Inscos: 12/7/87

JUN 10 1993

PHARMACOLOGIST'S REVIEW OF IND
(Amendment Dated May 12, 1993)

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

Sponsor & Address: Abbott Laboratories
Abbott Park, IL

Drug: Zileuton (Abbott-64077)

Category:

Date of Review: 6/8/93

Date of Submission: 5/12/93

Date of HFD-180 Receipt: 5/13/93.

Submission Contents: Reanalysis of the incidence of hemangiomas/hemangiosarcomas in mouse carcinogenicity study.

I have reviewed the mouse carcinogenicity study earlier, in which Zileuton produced hepatocellular adenomas/carcinomas in mice of both sexes, and renal adenomas/carcinomas and hemangiomas/hemangiosarcomas in multiple organs of treated females. In female mice statistically significant increased incidence of hemangiomas and hemangiosarcomas in multiple organs (control = 10%, low dose = 6.7%, mid dose = 8.3% and high dose = 31.6%, p value = 0.0000 [Peto trend test, using Haseman adjustment rule]) were seen. Pairwise comparisons of the incidence rates of hemangiomas and hemangiosarcomas in multiple organs of control and high dose treated females also gave statistically significant results (p = 0.0015, Fisher exact test). Furthermore, in the Amendment dated February 10, 1993 (see my review dated 5/10/93) sponsor also reported increased incidence of hemangiosarcoma in the ovary of treated females (control = 0%, low dose = 0%, mid dose = 0% and high dose = 8.8%, p value = 0.0003 [Peto trend test]). Pairwise comparisons of the incidence of rate of hemangiosarcoma in ovary of control and high dose treated females also gave statistically significant result (p = 0.0199, Fisher exact test). In this Amendment sponsor indicated that calculated p-values for hemangiosarcoma in the ovaries were not adjusted for multiplicity. Once the p-values were adjusted by the method of Westfall and Young then in the ovaries the incidence of hemangiosarcoma still gave a statistically significant trend test (p = 0.0056) but pairwise comparison of high dose versus control gave a statistically non-significant result (p = 0.0845). According to NTP "neoplasm of the same histomorphogenic type may

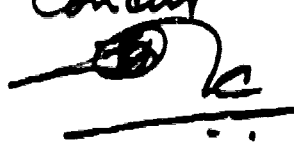
be combined if they occur in different anatomic sites" (Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation, August 17, 1984, page 226). In treated female mice statistical significance was achieved for the incidence of hemangiosarcoma and combined hemangiomas and hemangiosarcoma in multiple organs even when p-values were adjusted according to Westfall and Young method (p-value per Peto test = 0.0003, pairwise comparison of control versus high dose: p = 0.0033). Hence our conclusion remains the same. Zileuton induced statistically significant dose related increases in hepatocellular adenomas/carcinomas in mice of both sexes and renal adenomas/carcinomas and hemangiomas/hemangiosarcomas in multiple organs in treated females.

RECOMMENDATIONS: None

Tanveer Ahmad 6/8/93

Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

cc:
Orig. IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Ahmad
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-150/Dr. Taylor
HFD-007/Dr. Mukherjee

Concur


6/10/93

TA/hw/6/8/93
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PHARMACOLOGIST'S REVIEW OF IND
(Amendment # 071 Dated June 18, 1993)

ADD TO IND
Sun

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

Sponsor & Address: Abbott Laboratories
Abbott Park, IL

Drug: Zileuton (Abbott-64077)

Category:

Date of Review: 8/12/93

Date of Submission: 6/18/93

Date of HFD-180 Receipt: 6/21/93

Submission Contents: (1) One-Month Oral Toxicity Study in Dogs

One-Month Oral Toxicity Study in Dogs
Followed by 1-, 3- and 6- Month Recovery Periods
(Study # TB 92-310)

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: June 30, 1992

Study Completed: April 30, 1993 (date reported)

GLP Requirements: A Statement of Compliance with GLP Regulation was included.

Animals: Male beagle dogs (9-13 kg and 7-8 month old)

Drug Batch No.: 46-097-AL

Methods: Twenty male beagle dogs were given orally (in capsules) 100 mg/kg/day of Zileuton for 4 weeks. Control group animals (n=8) were given empty gelatin capsules in similar fashion. Two dogs from control group and 5 dogs from treated group were sacrificed at the end of treatment period, and after one, three and six months of the recovery period. All dogs were observed twice daily for clinical signs and mortality during the study period. Body weights and food consumptions were recorded once or twice weekly. Blood samples (1-2 ml) were collected from each dog at 0 (pre-dose), 1, 3, 6, 10, 16 and 24 hours after drug administration during week 4 of the study period for measuring

plasma levels of Zileuton. At the end of treatment/recovery period, dogs were sacrificed and subjected to complete necropsy. Testes, epididymides and prostate were examined histologically.

Results:

1. **Observed Effects:** Diarrhea, abnormal stool, emesis, interdigital cysts, dehydration and emaciation were seen in treated dogs.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** At the end of treatment period, body weights of control group dogs were increased by 5% over pretest values, while treated dogs lost 11% of their initial weights. During the treatment period treated dogs consumed about 21-69% less food than control dogs. During recovery period the food consumptions were comparable in all dogs. At the end of 6-months recovery period body weights of treated dogs were still about 9% less than the corresponding control values.
4. **Plasma Drug Levels (report # 68):** The mean plasma $AUC_{0-24 \text{ hr}}$ was 630.3 mcg.hr/ml. The mean T_{max} and plasma $t_{1/2}$ values were 4.0 ± 2.25 hr. and 5.2 ± 1.24 hr. respectively. The apparent mean volume of distribution was 1.33 ± 0.47 L/kg and mean total plasma clearance was 2.79 ± 0.69 ml/min/kg.
5. **Organ Weights:** The relative weights of prostate in treated dogs were decreased by 68%, 57%, 35% and 7% at the end of treatment period, and at the end of 1-, 3- and 6-months of recovery period. No statistically significant effect on the mean weights of testes were seen in treated dogs. However, testes of two treated dogs (#1009 [sacrificed at the end of treatment period], #3003 [sacrificed at the end of 1-month of recovery period]) weighed significantly less than the weights of corresponding control values.
6. **Gross Pathology:** "Small prostate" were seen in 5/5, 4/5, 3/5 and 1/5 drug treated dogs at the end of one-month treatment period, and at the end of 1-, 3- and 6-months of recovery period respectively.
7. **Histopathology:** Histopathological examinations revealed atrophy of prostate in 4/5, 4/5, 2/5 and 3/5 drug treated dogs at the end of one-month treatment period, and at the end of 1-, 3- and 6-months of recovery period respectively. Additionally, testicular degeneration along with spermatogenic arrest was evident in 1/5 dogs (# 1009) at the end of treatment period

and 1/5 dogs (# 3003) at the end of one-month recovery period. Sponsor claimed that effects of Zileuton on prostate and testes are reversible since no testicular degeneration was seen at the end of 3-month or 6-month recovery period and severity of prostatic atrophy decreased as the recovery period was increased.

SUMMARY AND EVALUATION:

Zileuton is a benzothiophene N-hydroxyurea and it is a known inhibitor of 5-lipoxygenase. It is being developed for the treatment of ulcerative colitis, asthma and various inflammatory disease.

In the present submission sponsor has submitted the results of a one-month oral toxicity study in male dogs with 1-, 3- and 6-month recovery period. In this dogs were given 100 mg/kg/day of Zileuton for 4-weeks. Histopathological examinations revealed atrophy of prostate in 4 out of 5 treated males. Additionally, testicular degeneration along with spermatogenic arrest was seen in 1 out of 5 treated dogs. All treated dogs lost their body weight by 11% while an increase in body weights were recorded in untreated dogs. Hence, it is difficult to judge whether histopathological findings seen in prostate and testes of treated dogs were due to Zileuton or due to general deterioration of the health of these dogs. Zileuton has not been tested for anti-androgenic effect in conventional bioassay. However, Zileuton has weak affinity to cytosolic androgen receptors of prostate of rat, dog, monkey and human (see review of Amendment # 014, 006 and 007 of IND 35,097 by Dr. Mukherjee; HFD-007).

RECOMMENDATIONS: None

Tanveer Ahmad 8/14/93

Tanveer Ahmad, Ph.D.
Pharmacologist, HFD-180

CC:
Orig. IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Ahmad
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-502
HFD-150/Dr. Taylor
HFD-007/Dr. Mukherjee
TA/hw/8/12/93
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Congey
8/15/93

MAY 10 1993

**PHARMACOLOGIST'S REVIEW of IND
Amendments Dated February 10, 1993 (# 060), February 24, 1993
(# 062), March 5, 1993 (# 064) March 29, 1993 (# 066)
and April 26, 1993 (# 068).**

Reviewer: Tanveer Ahmad, Ph.D.
Pharmacologist (HFD-180)

Sponsor & Address: Abbott Laboratories
Abbott Park, IL

Drug: Zileuton (Abbott-64077)

Category:

Date of Review: 5/10/93

Date of Submission: Amendment # 060: 2/10/93
Amendment # 062: 2/24/93
Amendment # 064: 3/05/93
Amendment # 066: 3/29/93
Amendment # 068: 4/26/93

Date of HFD-180 Receipt: Amendment # 060: 2/12/93
Amendment # 062: 2/25/93
Amendment # 064: 3/08/93
Amendment # 066: 3/31/93
Amendment # 068: 4/27/93

Submission Contents: (1) Two-year Carcinogenicity Study of
Abbott-64077 in Rats,
(2) Two-year Carcinogenicity Study of
Abbott-64077 in Mice.

(3) Genotoxicity Studies: Ames test and
EX VIVO UDS assay in kidney cells of mouse
and rat.

(4) Special Toxicology Studies: Renal cell
proliferation study in mice and rats,
Zileuton Proliferating Cell Nuclear Antigen
(PCNA) studies in rodent kidneys,
effect of Zileuton on peroxisome
proliferation in liver and kidney of mice
and rats and effect of Zileuton on
hypothalamic-pituitary-gonadal axis in male
rats and over view of testicular and
prostate changes in dog.

Two-Year Dietary Carcinogenicity Study of Abbott-64077 in Mice
(Study No. TD 89-276)

COVERSHEET FOR CARCINOGENICITY STUDY IN MOUSE

1. Study No.: TA 89-276
2. Name of Laboratory: Abbott Laboratories, Abbott Park, IL
3. Strain: Crl: CD BR Mice (Charles River, U.S.A.)
4. No./sex/group: 60
5. Doses (O, L, M, H): 0 (group T₀), 0 (group T₁), 50, 150 and 450 mg/kg/day. In the statistical analysis group T₀ was used as control, and group T₁ was declared the "non-primary control group". However, kidneys and livers from all T₁ mice were examined microscopically.
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 104
9. Week/site for first tumor:

	<u>Male</u>	<u>Female</u>
O	64/Hepatocellular Carcinoma (Liver)	61/Malignant Alveolar/Bronchiolar Carcinoma (Lung)
L	71/Benign Meningioma (Cerebrum)	73/Alveolar/Bronchiolar Adenoma (Lung)
M	72/Alveolar/Bronchiolar Adenoma (Lung)	44/Histocytic Sarcoma (Multiple Organ)
H	45/Malignant Alveolar/Bronchiolar Carcinoma (Lung)	61/Hemangioma (Multiple Organ)

10. No. alive at termination:

	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
O	26/60	43.3	29/60	48.3
O	25/60	41.7	20/60	33.3
L	27/60	45.0	22/60	36.7
M	25/60	41.7	26/60	43.3
H	18/60	30.0	17/60	29.3

11. Statistical Methods Used: Method of Cox, D.R., (J. Royal Stat. Soc. Series B, 1972; 34:187-220) and Tarone, R.E., (Biometrika 1975, 62: 679-682) to analyze the survival data. Tumor incidence data were analyzed by Peto method (IARC monograph, supplement 2, 1980: 311-426).

12. Attach tumor and non-tumor data for each tissue (i.e., benign; malignant; hyperplastic): See Appendix 1.

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: October 31, 1989

Study Completed: January 20, 1993

GLP Requirement: A Statement of Compliance with GLP Regulation was included

Testing Species & Strain: Crl: CD-1 BR mice (Charles River, U.S.A.)

No. of Animals: 60/sex/group (Approx. 6 weeks old, 18-34 g)

Route of Administration: Via diet

Dose Levels: 0, 0, 50, 150 and 450 mg/kg/day

Drug Batch No.: 28-932-AL and 41-047-AL

Methods: Groups of 60 males and 60 females were given Zileuton (via diet) at daily doses of 0 (group T₀), 0 (group T₁), 50, 150 and 450 mg/kg/day for 2 years. The dose selection was based on 3-month maximum-tolerated dose study in mice in which Zileuton was given via diet at daily dose levels of 80, 200, 450 and 1000 mg/kg/day. Decreased food consumption was seen in 1000 mg/kg treated mice compared to control mice. Hepatic cytomegaly was observed in 450 mg/kg/day and 1000 mg/kg/day treated mice. Additionally hepatocellular vacuolation was also seen in mice of 1000 mg/kg/day dose group. Based on these findings, 450 mg/kg/day was considered to be maximum tolerated dose. Hence the highest dose level selected in the main carcinogenicity study was 450 mg/kg/day and which is appropriate. Additionally, 20 mice/sex/treatment group and 20 mice/sex/on of the control group were also included for monitoring plasma levels of the drug and its metabolite (Abbott-66193) at various time intervals. All mice were observed twice daily for clinical signs and mortality. Physical examinations of all animals excluding satellite groups were performed weekly. Body weights and food consumptions were recorded weekly for the first 13 weeks of the treatment and bi-weekly thereafter. From 6th month onward all animals were examined for the presence of palpable tissue mass weekly. During 12th and 18th months of the study, blood smear was obtained from the tail of each surviving mice in the main carcinogenicity groups and examined for differential count. Just before sacrifice blood samples were also collected by cardiac puncture from each surviving mice for hematological tests. All surviving mice were sacrificed at the end of study period and subjected to complete necropsy. All tissues from all mice (excluding satellite groups and one of the control group [T₁]) which died/killed were examined histopathologically. Only kidneys and livers from the control group T₁ were examined microscopically. Furthermore, electron microscopic examinations were conducted on liver tissues and kidney cortex tissues of several mice. In the

statistical analysis group T₀ was used as control and group T₁ was declared the "non-primary control group". Sponsor used methods of Cox, D.R. (J. Royal Stat. Soc. Series B 1972; 34: 187-220) and Tarone, R.E. (Biometrika 1975, 62: 679-682) to analyze the survival data and the tumor incidence data were analyzed using the Peto method (IARC Monograph, supplement 2, 1980: 311-426).

Results:

1. **Observed Effects:** No treatment related effects were seen.
2. **Mortality:** At termination survival rates were comparable in all groups.

Table 1*
Survival Rates

Sex	Control (C) ^a	Low (L)	Middle (M)	High (H)	Tread Test ^d	Pairwise Comparison ^e
Male	25/60 ^b (41.7%)	27/60 (45.0%)	23/60 (41.7%)	18/60 (30.0%)	NS	NS
Female	20/60 (33.3%)	22/60 (36.7%)	26/60 (43.3%)	17/60 ^c (29.3%)	NS	NS

Notes:
 a C = Control; L = Low Dose (50 mg/kg day); M = Mid Dose (150 mg/kg day); H = High Dose (450 mg/kg day)
 b No. survived to terminal necropsy / No. entered into the study
 c Excludes 2 escaped animals #3048 and #3116 (on Day 1 and Day 41, respectively).
 d NS = Not Significant (p>0.05).
 e Bonferroni adjustment applied.

* = Sponsor's table 7, Page 008-00093, Vol. 1.8

3. **Body Weight/Food Consumption/Water Consumption:** Body weight gains of high dose treated males were decreased by 10% compared to the control values. In females effect on body weight gains were highly variable. During first 6 weeks of treatment mid and high dose treated male mice consumed about 5-12% less food than the control group mice. Similar magnitude of reduction in food intakes were seen in mid and high dose treated females during weeks 41-95 of the study.

4. **Achieved Doses:** The mean intakes of the drug were within 10% of the intended doses (data presented graphically).

5. **Plasma Levels of Abbott-64077 and its Metabolite (Abbott-66193):** Plasma concentrations of Zileuton and its metabolite increased with increasing dose.

Table 2*

Plasma Levels of Abbott-64077 and Its Metabolite (n=10)

Duration of Dosing	Mean Plasma Concentration (µg/mL) [†]		Mean 24-hr AUC (µg-hr/mL) ^{††}	
	Zileuton	Metabolite	Zileuton	Metabolite
	50 mg/kg/day			
Week 4	1.17±0.71	0.05±0.11		
Week 52	1.82±0.78	0.14±0.16		
Week 93/94			50.46±13.07	0.18±0.33
	150 mg/kg/day			
Week 4	2.96±1.22	0.21±0.16		
Week 52	4.06±3.34	0.24±0.17		
Week 93/94			153.69±26.88	4.80±2.24
	450 mg/kg/day			
Week 4	8.52±5.39	0.59±0.34		
Week 52	11.43±4.49	0.63±0.27		
Week 93/94			395.44±172.61	18.84±8.10

† Single plasma sample obtained in the morning.

†† Calculated from plasma samples collected at four different times on four separate days in the same mice.

* - Sponsor's Table, Page 008-00149, Vol. 1.8

6. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

7. Gross Pathology: No tabulated summary was provided.

8. Histopathology:

Non-neoplastic Findings: Hepatocytomegaly were seen in mid and high dose treated mice. Additionally, unspecified renal hyperplasia was also seen in high dose treated mice. The incidence of the above mentioned findings were as follows:

Table 3
Non-neoplastic Findings

Site/Type	Sex (M/F)	Dose (mg/kg/day)				
		0	0	50	150	450
Liver/ Hepatocytomegaly	M	0/60	0/60	0/60	10/60	37/60
	F	0/60	0/60	0/60	4/60	25/58
Kidneys/ Hyperplasia	M	0/60	0/60	0/60	0/60	3/60
	F	0/60	0/60	0/60	0/60	4/58

a) Electron-microscopic Examinations of Liver Tissues from Several Mice: Liver samples from 2 control (#0001 & #0070), 4 low dose (#1007, #1019, #1026 and #1078), 4 mid dose (#2019, #2027, #2054 and #2108) and 4 high dose (#3069, #3087, #3054 and #3108) treated mice were examined electron-microscopically. Slight to marked proliferation of peroxisomes were seen in mid and high dose treated mice, and no such effects were seen at low dose. This conclusion should be taken with caution due to the following reasons: 1) liver slides were of poor quality, 2) the increase in peroxisome number was not only restricted to enlarged cells but "occurred randomly in hepatocytes," and 3) smooth endoplasmic reticulum proliferation could not be determined due to "poor quality of fixation".

b) Electron-microscopic Examinations of Kidney Tissues from Several Mice: Kidney samples from 2 control (#0007 & #0012) and 4 high dose (#3013, #3024, #3058 and #3095) treated mice were examined electron-microscopically. No significant difference in the morphology, number and/or distribution of the peroxisomes were seen between control and high dose treated groups.

Sponsor also used immunohistostaining technique (specific antibody for proliferating cell nuclear antigen: PCNA) to assess the percent of cells in S-phase. Kidney slides from control animals (males: #0007, #0025, #0029, #0035 and #0055 and females: #0008, #0046, and #0078) and high dose treated animals (males: #3021, #3033, #3047, #3061 and #3117 and females: #3014, #3068, #3088, #3100 and #3118) were examined. In male and female mice, a 2.5 fold and 4.8 fold increase in renal cell proliferation was seen. This increase in S-phase cell proliferation was statistically significant in males. Sponsor suggested that an increase in renal tumors (see below) seen in high dose treated mice may be due to increase in cellular proliferation.

Neoplastic Findings: In female mice, statistically significant increased incidence of hepatocellular adenomas (control = 0%, low dose = 3.3%, mid dose = 0% and high dose = 18.3%; p value = 0.000076 [Peto trend test, using Haseman adjustment rule]) and hepatocellular carcinomas (control = 0%, low dose = 1.7%, mid dose = 0% and high dose = 5.0%; p value = 0.0224 [Peto trend test, using Haseman adjustment rule]) were seen. The combined incidence of hepatocellular adenomas plus carcinomas were also statistically significant in treated mice (males: p value = 0.0077, females: p value = 0.0000; Peto trend test using Haseman adjustment rule). Pairwise comparisons of the incidence rates of hepatocellular adenomas plus carcinomas in control and high dose treated group gave a statistically significant results in female mice (p = 0.00005, Fisher exact test) but not in male mice (p = 0.0833, Fisher exact test). Additionally, statistically significant increased incidence of renal adenomas plus carcinomas in treated mice (males: p value = 0.0276, females: p value = 0.0029; Peto trend test using Haseman adjustment rule). However, pairwise comparisons of the incidence rates of renal adenomas plus carcinomas in control and high dose treated group gave a statistically

significant result in only female mice ($p = 0.048$, Fisher exact test) but not in male mice ($p = 0.1694$, Fisher exact test). Vascular tumors (hemangiomas and hemangiosarcomas in skin, ovary, vagina, uterus, kidney, liver and spleen) were seen in all groups including controls. In female mice statistically significant increased incidence of hemangiomas and hemangiosarcomas in multiple organs (control = 10%, low dose = 6.7%, mid dose = 8.3% and high dose = 31.6%, p value = 0.0000 [Peto trend test, using Haseman adjustment rule]) were seen. Pairwise comparisons of the incidence rates of hemangiomas and hemangiosarcomas in multiple organs of control and high dose treated females also gave statistically significant results ($p = 0.0015$, Fisher exact test).

Table 4
Neoplastic Findings

Site/Type	Sex (M/F)	Dose (mg/kg/day)					p Value Trend Test	
		0 ¹	0	50	150	450	Sponsor ²	FDA ⁴
# Examined		60	60	60	60	60		
Liver/ Hepatocellular Adenoma	M F	8 0	13 1	9 2	7 0	13 8	0.0005 (0.0017)	0.000076
Hepatocellular Carcinoma	M F	6 0	3 1	1 1	2 0	8 3	0.0434 (0.4280)	0.0224
Hepatocellular Adenoma + Carcinoma	M F	14 0	16 2	10 3	9 0	21 11	0.0342 (0.3506) 0.0001 (0.0001)	0.0077 0.0000
Kidney/ Tubular Adenoma	M F	0 0	0 0	0 0	1 0	1 3	0.0187 (0.1144)	0.0143
Tubular Carcinoma	M F	0 0	0 0	0 0	0 0	1 1		
Combined Tubular Adenoma & Carcinoma	M F	0 0	0 0	0 0	1 0	2 4	0.0335 (0.2121) 0.0037 (0.0194)	0.0276 0.0029
Multiple Organ/ Hemangioma	M F	2 1	ND ND	2 3	1 3	2 3		
Hemangiosarcoma	M F	1 5	ND ND	4 1	2 2	6 16	0.0270 (0.3445) 0.0002 (0.0003)	0.0000
Combined Hemangioma & Sarcoma	M F	3 6	ND ND	6 4	3 5	8 19	0.0491 (0.6471) 0.0001 (0.0003)	0.0000

1 = Used for statistical analysis

2 = Only 58 females were examined

ND = Not done

3 = Sponsor's Peto trend test and number in parenthesis represents the adjusted P-value using the method of Westfall and Young

4 = Peto exact trend test using Haseman's rule of p-value adjustment conducted by FDA

In this study dose selection was appropriate. Furthermore, according to sponsor, plasma concentration of Zileuton in high dose treated mice was about 5.7 times higher than that seen in human after administering the clinical dose (2400 mg/day). The comparison of AUC values is not appropriate, because in calculation of AUC in mice, sponsor used plasma samples from the same mice on 4 separate days at 4 different times during the day. This kind of sampling method can only give a rough approximation of the daily time course of Zileuton and AUC values can only provide a rough estimation of the exposure to mice. Zileuton produced hepatocellular adenomas/carcinomas, renal adenomas/carcinomas and hemangiomas/hemangiosarcomas in multiple organs of treated females.

Two-year Dietary Carcinogenicity Study of Abbott-64077 in Rats
(Study No TA 89-279)

COVERSHEET FOR CARCINOGENICITY STUDY IN RAT

1. Study No.: TA 89-279
2. Name of Laboratory: Abbott Laboratories, Abbott Park, IL
3. Strain: CrI: CD BR Rats (Charles River, U.S.A.)
4. No./sex/group: 60
5. Doses (0, L, M, H): 0 (group T₀), 0 (group T₁), 40, 80 and 170 mg/kg/day
In the statistical analysis group T₁ was used as control, and group T₀ was declared the "non-primary control group".
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 104
9. Week/site for first tumor:

	<u>Male</u>	<u>Female</u>
O	60/Benign Adenoma (pituitary)	46/Benign Fibroadenoma (mammary gland)
L	59/Malignant Meningioma (meninges)	53/Benign Adenoma (pituitary)
M	46/Malignant Squamous Cell Carcinoma (skin)	51/Benign Adenoma (pituitary)
H	41/Malignant Lymphoma (multiple organ)	66/Malignant Leukemia (multiple organ)

10. No. alive at termination:

	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
O	23/60	38.3	19/60	31.7
0	30/60	50	29/60	48.3
L	23/60	38.3	32/60	53.3
M	29/60	48.3	30/60	50.0
H	36/60	60.0	38/60	63.3

11. Statistical Methods Used: Method of Cox, D.R., (J. Royal Stat. Soc. Series B, 1972; 34:187-220) and Tarone, R.E., (Biometrika 1975, 62: 679-682) to analyze the survival data. Tumor incidence data were analysed by Peto method (IARC monograph, supplement 2, 1980: 311-426).

12. Attach tumor and non-tumor data for each tissue (i.e., benign, malignant, hyperplastic): See Appendix 1.

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: December 5, 1989

Study Completed: January 15, 1993

GLP Requirements: A Statement of Compliance with GLP Regulation
was included.

Testing Species & Strain: Cri: CD BR Rats (Charles River,
U.S.A.)

No. of Animals: 60/sex/group [Approximately 6 week old]

Route of Administration: Via diet

Dose Levels: 0, 0, 40, 80 and 170 mg/kg/day

Drug Batch No.: # 28-932-AL, # 41-047-AL and # 52-133-AL

Methods: Groups of 60 males and 60 females were given Zileuton (via diet) at daily doses of 0, 0, 40, 80 and 170 mg/kg/day for 2 years. The dose selection was based on 3-month oral (via diet) toxicity study in rats in which doses of 80, 170, 350 and 750 mg/kg/day were used. Decreased body weight gain, elevations of BUN, serum cholesterol and protein, proteinuria, increases in liver, kidney, prostate, uterus and ovary were seen in 350 and 750 mg/kg/day treated rats. Increased liver and kidney weights compared to control rats were also seen in 170 mg/kg/day treated rats. Cytomegaly of liver were seen in all treated rats and chronic nephritis was seen in 350 and 750 mg/kg/day treated rats. Based on these findings 170 mg/kg/day was considered to be maximum tolerated dose. Hence the highest dose level selected in the main carcinogenicity study was 170 mg/kg/day and which was appropriate. Additionally, 10 rats/sex/treatment group and 10 rats/sex/one of the control group were also included for monitoring the plasma levels of the drug and its metabolite Abbott-66193 at various intervals during the study period. All rats were observed daily for clinical signs and mortality. Physical examinations of all animals excluding satellite groups were performed weekly. Body weights and food intakes were recorded weekly for the first 13 weeks of the treatment and then bi-weekly thereafter. From the 6th month onward all animals were examined for the presence of any palpable tissue mass weekly. During 12th and 18th months of the study, blood smear was obtained from the tail of each surviving rat in the main carcinogenicity groups and examined for differential count. Furthermore, just before sacrifice blood samples were collected by cardiac puncture from each rat for hematological tests. All surviving rats were sacrificed at the end of the study period and subjected to complete necropsy. All tissues from all animals (excluding satellite groups) which died/killed during the study period were examined for histopathological changes. The histopathological examinations were conducted by
In the statistical analysis group

T₁ was used as control and group T₀ was declared the "non-primary control group". Sponsor used methods of Cox, Dr. (J. Royal Stat. Soc., Series B 1972; 34: 187-220), and Tarone, R.E. (Biometrika 1975; 62: 679-682) to analyze the survival data, and the tumor incidence data were analyzed using Peto method (IARC Monograph, supplement 2, 1980: 311-426). Sponsor also analyzed tumor incidence data after adjusting p value according to Westfall and Young method.

Results:

1. **Observed Effects:** No treatment related effects were seen.
2. **Mortality:** At termination the survival rates were comparable in all groups (see below). It should be noted that only one control group (T₁) was used for statistical analysis because 2nd control group (T₀) has significant mortality rate and survival rate at the end of the study period for females were only 32% and for males were only 38%. Sponsor considered it as "chance", and declared this group as "non-primary control group".

Table 5*

Sex	Control (C) ^a	Low (L)	Middle (M)	High (H)	Trend Test ^b	Pairwise Comparisons ^d
Male	30/60 ^b (50.0%)	23/60 (38.3%)	29/60 (48.3%)	36/60 (60.0%)	NS	NS
Female	29/60 (48.3%)	32/60 (53.3%)	30/60 (50.0%)	38/60 (63.3%)	PT (p=0.0438)	NS

Notes:
 a C = Control; L = Low Dose (40 mg/kg/day); M = Mid Dose (80 mg/kg/day); H = High Dose (170 mg/kg/day)
 b No. survived to terminal necropsy / No. entered into the study
 c NS = Not Significant (p>0.05); PT = Positive Trend
 d Bonferroni adjustment applied.

* = Sponsor's Table 8, Page 004-00039, Vol. 1.4

3. **Body Weight/Food Consumption/Water Consumption:** At the end of treatment period, body weight gains were reduced by 7%, 11% and 38% compared to the control values in males treated with low, mid and high dose respectively, the corresponding reductions in females were 16%, 30% and 44% respectively. In high dose treated rats (both sexes), the food intakes were significantly reduced (6-24%) through out the treatment period. At mid dose, food intakes were also reduced compared to the control values but most of the time these reductions were not statistically significant.

4. Achieved Doses: The mean intakes of the drug were within 10% of the intended doses except during the early phase of treatment (week 1-10) period the intakes of the drug were 12-30% less than the intended doses (data presented graphically).

5. Plasma Levels of Abbott-64077 and its N-Dehydroxylated Metabolites: Plasma concentrations of Zileuton and its metabolite increased with increasing dose. There was an indication that levels in females were consistently higher than males. In calculation of AUC_{0-24hr} sponsor used plasma samples from the same rat on 4 separate days at 4 different times during the day. This kind of sampling method can only give a rough approximation of the daily time course of Zileuton and its metabolite and AUC values can only provide a rough estimate of the exposure to mice.

Table 6*

Duration of Dosing	Mean 24-hr AUC of Zileuton ($\mu\text{g}\cdot\text{hr}/\text{ml}$) ^a	
	Males	Females
40 mg/kg/day		
Week 57/58	125.8±46.0	131.2± 46.1
Week 75-76	187.1±51.0	250.2± 57.8
Week 99-100	278.8±45.5	345.2±103.4
80 mg/kg/day		
Week 57/58	292.6±166.5	369.8±124.2
Week 75-76	284.6± 78.8	456.2±101.7
Week 99-100	432.7±121.3	611.9±114.3
170 mg/kg/day		
Week 57/58	483.9± 69.6	883.8±187.0
Week 75-76	498.3± 67.2	1042.8±209.0
Week 99-100	640.9±179.1	913.3± 82.2

^a Estimated from plasma samples collected at four different times on four separate days in the same rat (n = approximately 10 rats per dose group), except values for week 57/58 that were estimated from plasma samples collected at three different times on two separate days.

Table 7*

Duration of Dosing	Mean Plasma Concentration (µg/mL) [†]		Mean 24-hr AUC (µg·hr/mL) ^{††}	
	Zileuton	Metabolite	Zileuton	Metabolite
40 mg/kg/day				
Week 4	3.961±1.845	1.018±0.321		
Week 52	8.963±2.413	1.956±0.457		
Week 58			128.48±43.29	22.65±10.81
Week 76			218.60±61.21	40.50±11.77
Week 100			308.30±79.20	56.54±15.57
80 mg/kg/day				
Week 4	9.772±2.955	2.964±0.601		
Week 52	18.256±7.350	5.198±2.045 [#]		
Week 58			331.22±144.32	80.95±34.17 [#]
Week 76			370.38±124.60	82.05±30.00
Week 100			522.29±145.83	127.34±37.69
170 mg/kg/day				
Week 4	20.645± 8.930	6.907±1.664		
Week 52	33.669±15.201	9.230±3.497		
Week 58			683.58±249.43	170.62±56.92
Week 76			770.55±322.15	184.02±61.00
Week 100			792.24±189.78	260.41±96.88

[†] Single plasma sample obtained in the morning (n=approximately 20 rats per dose group)

^{††} Estimated from plasma samples collected at four different times on four separate days in the same rat (n=approximately 10 rats per dose group)

[#] Calculated from estimated values

* = Sponsor's Table, page 004-00164, Vol 1.4

6. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.

7. Gross Pathology: No tabulated summary was provided.

8. Histopathology:

Non-neoplastic Findings: A statistically significant increase in the incidence of cytomegaly of the liver was seen in treated rats (males: control = 0/60, low dose = 14/60, mid dose = 40/60, and high dose = 48/60; p value = 0.0001, females: control = 0/60, low dose = 2/60, mid dose = 27/60 and high dose = 49/60, p value = 0.0001, [Peto exact/trend test]).

Neoplastic Findings: Dose related increased incidences of interstitial cell tumor (Leydig cell tumor) of the testes were seen in treated males (control = 5%, low dose = 20%, mid dose = 20% and high dose = 40%; p value = 0.000007 [Peto trend test using Haseman adjustment rule]). Pairwise comparisons of the incidence rates of interstitial cell tumor of the testes in control and high dose group also gave a statistical significant result (p = 0.0000, Fisher exact test). Additionally, increased incidences of renal tubular adenoma plus carcinoma were seen in treated rats (P values per Peto trend test: male = 0.0301 and female = 0.0125). However, when sponsor used adjusted P values (obtained by using the method of Westfall and Young (J. Amer. Stat. Assoc. 1989; 84: 780-786) then the trend test for renal tumor becomes non-significant. I requested Dr. Chen (HFD-715: Division of Biometrics) to conduct a statistical analysis of the tumor data. According to him the incidence rates of renal tubular adenoma plus carcinoma in males (control = 5%, low dose = 3.3%, mid dose = 1.7%, high dose = 15%, p value = 0.0062) and females (control = 0%, low dose = 1.7%, mid dose = 3.3% and high dose = 8.3%, p value = 0.011324) were statistically significant. In his analysis he used Peto exact/trend test and adjusted p value according to Haseman rule. Pairwise comparisons of the incidence rates of renal tubular adenomas plus carcinomas in control and high dose group also gave statistically significant results (males: p value = 0.02645, females: p value = 0.045; Fisher exact test).

Table B

Neoplastic Findings

Site/Type	Sex (M/F)	Dose (mg/kg/day)					P Values Trend Test	
		0	0 ¹	40	80	170	Sponsor ²	FDA ³
<u>Testis/</u>								
Interst. Cell Tumor	M	3/60	3/60	12/60	12/60	24/60	0.0001 (0.0001)	0.000007
<u>Kidney/</u>								
Tubular (Adenoma)	M		3/60	1/60	1/60	7/60	NS	0.0017
	F		0/60	0/60	2/60	5/60	0.0036 (0.036)	0.00399
Tubular (Adenocarcinoma)	M		0/60	1/60	0/60	2/60	NS	
	F		0/60	1/60	0/60	0/60	NS	
Tubular Adenoma + Carcinoma	M		3/60	2/60	1/60	9/60	0.0301 (0.1690)	0.0062
	F		0/60	1/60	2/60	5/60	0.0125 (0.1263)	0.011324

NS = Not significant (P>0.05) per Peto test

1 = Used for statistical analysis

2 = Sponsor's Peto trend test and number in parenthesis represents the adjusted p-value using the method of Westfall and Young

3 = Peto exact/trend test using Haseman's rule of p-value adjustment conducted by FDA

In this study dose selection was appropriate. Furthermore, according to sponsor mean plasma concentration of Zileuton in high dose treated male and female rats were 9.2 and 13.1 fold higher than that seen in human after administration of a clinical dose (i.e. 600 mg q.i.d.). It should be noted here that in calculation of AUC values in rats, sponsor used plasma samples from the same rats on 4 separate days at 4 different times during the day. This kind of sampling method can only give a rough approximation of the daily time course of Zileuton and AUC values can only provide a rough estimation of the exposure to rats. Zileuton produced benign Leydig cell tumors in the testes of male rats and renal tubular adenoma plus carcinoma in treated rats (both sexes).

GENETIC TOXICOLOGY

Ames Test
(Study # TX 92-224)

Testing Laboratories: Abbott Laboratories

Dates Studies Started and Completed: April - July 1992

Strain Employed: Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538

Concentration Employed: 100 - 5000 mcg/plate in the absence of S-9 mix and 1 - 5000 mcg/plate in the presence of S-9 mix

Solvent Control: DMSO

Positive Control: 2-aminoanthracene (2.5 mcg/plate), 2-nitrofluorene (2-10 mcg/plate), N-Methyl-N-nitro-N-nitrosoguanidine (10 mcg/plate) and quinacrine mustard (5 mcg/plate)

Source of Metabolic Activation: Rata liver microsomal enzymes

Drug Batch No.: 41-047-AL

Criteria of Positivity: Two-fold increase in the number of revertant colonies above the solvent control value in strains TA-98 and Ta-100 and three-fold increase in the number of revertant colonies above the solvent control value in strains TA-1535, TA-1537 and TA-1538 are considered as positive provided if the effect is also dose related.

Results: The drug inhibited the growth of all of the strains at 5000 mcg/plate. Drug was not mutagenic in any of the tester strains, irrespective of the treatment with metabolic activation system (S-9 Mix). Increase in mutant colonies was noted in all microbial strains employed in the presence of positive control (with or without S-9 mix).

Unscheduled DNA Synthesis in Sprague-Dawley Rat and CD-1 Mouse
Kidney Cells Following In Vivo Treatment with Zileuton
(Study # TX 92-341)

Testing Laboratories:

Date of Study Started: June 30, 1992

Date of Study Completed: December 22, 1992

Animals: Seven-weeks old male (210-243 g) and female (174-232 g) SD rats and 7-weeks old male (28-36 g) and female (24-29 g) CD-1 mice

Doses Used: Male rats: 0, 400, 800 and 1600 mg/kg
Female rats: 0, 225, 450 and 900 mg/kg
Male & female mice: 0, 450, 900 and 1800 mg/kg

Drug Batch No.: 46-097-AL

Solvent Control: Hydroxypropyl methylcellulose (HPMC: 0.2% in water)

Positive Control: Streptozocin (STZ: 400 mg/kg; dissolved in saline)

Methods: In this study dose selection was based on acute toxicity study. Rats (n=3/dose/group) and mice (n=3/dose/group) were given a single oral (gavage) dose of Zileuton, positive control or negative control. Groups of animals were sacrificed at 2 and 16 hours after the drug administration. Kidney cells were isolated and [³H] thymidine incorporation was measured. Incorporation was followed by autoradiography of the kidney cells and grains were counted over the nuclei of kidney cells, and UDS and DNA replication (SPS:S-phase synthesis) were assessed. A total of 450 cells/dose group were evaluated for UDS and 9000 cells/dose group were evaluated for SPS. The test compound is considered positive for UDS when the mean net grain count is greater than zero and 20% or more of the cells responding. SPS for each Zileuton dose group were compared with SPS value for the negative control group, if percent of cells in S-phase in treated groups is significantly greater than negative control then the positivity is established.

Results: Zileuton did not induce UDS or S-phase DNA synthesis in primary cultures of kidney cells obtained from rats and mice (both sexes). Sponsor did not include a positive control in S-phase DNA synthesis tests. It should also be noted that no UDS/S-phase DNA synthesis have been previously conducted using primary cultures of kidney cells from mice or rats.

SPECIAL TOXICOLOGY

Cell Proliferation Study in Mice Treated with Zileuton
(Study # TD 92-303)

Testing Laboratories:

Study Started: July 9, 1992

Study Completed: January 21, 1993 (report date)

GLP Requirements: A Statement of Compliance with GLP regulation was included.

Testing Species & Strain: Crl: CD BR Mice (about 10 weeks old and about 20g in weight).

No. of Animals: 10 mice/sex/group

Route of Administration: Via Diet

Dose Levels: 0, 50, 150 and 450 mg/kg/day

Drug Batch No.: 46-097-AL

Methods: This study was conducted to assess the potential of Zileuton to induce cell proliferation in kidneys of mice. The dose selected in this study was the same as used in 2-year carcinogenicity study in mice. Animals were given Zileuton via diet for 26 days. All animals were observed for clinical signs and mortality daily. Body weights and food intakes were recorded weekly. Five mice/sex/group were sacrificed on day 5 of the study and the remaining 5 mice/sex/group were sacrificed on day 26 of the study. Alzet minipumps containing 20 mg/ml BrdU were implanted s.c. in each animal four days prior to sacrifice. Liver and right kidney of each animal were examined histologically. Additionally, renal cell proliferation in control and high dose treated mice were evaluated by immunohistochemical technique using BrdU uptake into the nuclei of replicating cells.

Results:

- 1. Mortality:** One female of mid dose group died following the surgical implantation of Alzet minipump on day 22 of the study.
- 2. Body Weight/Food Consumption/Water Consumption:** No treatment related effects were seen.
- 3. Gross Pathology:** No treatment related effects were seen in livers and kidneys.

4. **Histopathology:** Results of histopathological examinations of livers and kidney were not reported. Only BrdU-labeling indices in kidneys of control and high dose groups were reported. No biological significant changes in labeling index (%) were observed in the proximal or distal tubules of male or female treated mice. In high dose treated group 3 out of 5 females at day 5 and 2/5 females at day 26 showed a BrdU-labeling "pattern" that was concentrated in the Cortico-medullary junction, and increases in labeling indices (%) were significantly greater than that found in corresponding control mice. Sponsor concluded that Zileuton significantly increased cell proliferation in the Cortico-medullary junction of the proximal tubules of female mice. This conclusion is not well founded because the results of BrdU-labeling index were highly variable as can be seen by large standard deviations, on day 26 statistically significant decrease in cell proliferation was observed in the proximal and distal tubules of male mice and no positive control was included in the study. Hence, there is no evidence that Zileuton induced cell proliferation in kidneys of mice. The results at best is equivocal. The BrdU-labeling index in kidneys of male and female mice were as follows:

Table 9*

BrdU-LABELING INDEX: KIDNEY				
MALE MICE				
Animal Number	Days on Test	Dose Group	Proximal Tubule (%)	Distal Tubule (%)
2681	5	0 mg/kg	4.9	3.7
2682	5	0 mg/kg	5.0	12.2
2683	5	0 mg/kg	6.2	3.1
2684	5	0 mg/kg	8.3	3.0
2685	5	0 mg/kg	6.1	2.7
Mean ± S.D. (%)			5.7 ± 1.6	5.0 ± 6.1
Fold Increase Over Control			1.0	1.0
2711	5	450 mg/kg	17.1	2.3
2712	5	450 mg/kg	9.8	2.4
2713	5	450 mg/kg	16.6	3.3
2714	5	450 mg/kg	20.3	1.2
2715	5	450 mg/kg	5.3	2.6
Mean ± S.D. (%)			15.4 ± 5.9*	2.6 ± 0.8
Fold Increase Over Control			2.6	0.5
2686	26	0 mg/kg	2.4	0.7
2687	26	0 mg/kg	3.3	0.6
2688	26	0 mg/kg	1.5	0.6
2689	26	0 mg/kg	5.6	0.8
2690	26	0 mg/kg	6.7	2.5
Mean ± S.D. (%)			3.5 ± 1.7	1.0 ± 0.9
Fold Increase Over Control			1.0	1.0
2716	26	450 mg/kg	0.3	0.6
2717	26	450 mg/kg	0.4	0.3
2718	26	450 mg/kg	0.6	0.2
2719	26	450 mg/kg	2.4	0.2
2720	26	450 mg/kg	0.4	0.5
Mean ± S.D. (%)			0.8 ± 0.9*	0.3 ± 0.1*
Fold Increase Over Control			0.2	0.3

* Significantly different from control at $p \leq 0.05$ as determined by Kruskal-Wallis test and Mann-Whitney U test.
S.D. = Standard Deviation

Table 9 Cont.*

BROW-LABELING INDEX: KIDNEY FEMALE MICE					
Animal Number	Days on Test	Dose Group	Proximal Tubule (%)	Distal Tubule (%)	Corticomedullary Junction (%)
2721	5	0 mg/kg	1.7	4.2	1.7
2722	5	0 mg/kg	4.4	6.3	4.0
2723	5	0 mg/kg	4.0	5.6	2.1
2724	5	0 mg/kg	8.3	4.2	3.0
2725	5	0 mg/kg	5.4	3.0	2.2
Mean ± S.D. (%)			4.8 ± 2.5	5.7 ± 1.3	2.6 ± 1.0
Fold Increase Over Control			1.0	1.0	1.0
2731	5	450 mg/kg	10.4	2.7	28.5
2732	5	450 mg/kg	9.2	1.7	33.6
2733	5	450 mg/kg	4.2	3.5	2.6
2734	5	450 mg/kg	3.6	3.0	2.3
2735	5	450 mg/kg	5.3	3.6	11.3
Mean ± S.D. (%)			6.5 ± 3.1	2.9 ± 0.8	15.7 ± 16.6
Fold Increase Over Control			1.4	0.6	6.0
2726	26	0 mg/kg	3.0	2.0	2.1
2727	26	0 mg/kg	1.0	0.3	1.3
2728	26	0 mg/kg	2.9	2.0	3.6
2729	26	0 mg/kg	2.2	1.3	1.5
2730	26	0 mg/kg	0.2	0.7	2.8
Mean ± S.D. (%)			1.9 ± 1.2	1.3 ± 0.8	2.3 ± 1.0
Fold Increase Over Control			1.0	1.0	1.0
2736	26	450 mg/kg	1.5	0.2	5.3
2737	26	450 mg/kg	1.7	0.0	4.3
2738	26	450 mg/kg	10.0	0.1	26.6
2739	26	450 mg/kg	5.1	1.2	20.3
2740	26	450 mg/kg	2.4	1.0	3.7
Mean ± S.D. (%)			4.1 ± 3.6	0.5 ± 0.6	12.0 ± 10.7
Fold Increase Over Control			2.2	0.4	5.2

* Significantly different from control at $p \leq 0.05$ as determined by Kruskal-Wallis test and Mann-Whitney U test.
S.D. = Standard Deviation

* = Sponsor's Table 3, Pages 010-00033 and 010-00034, Vol. 1.10

Cell Proliferation Study in Rats Treated with Zileuton
(Study # TA 92-302)

Testing Laboratories:

Study Started: July 2, 1992

Study Completed: December 9, 1992 (report date)

GLP Requirements: A Statement of Compliance with GLP Regulation was included.

Testing Species & Strain: Cr1: CD BR Sprague-Dawley rats (about 10 weeks old and 200g)

No. of Animals: 10 rats/sex/group

Route of Administration: Via Diet

Dose Levels: 0, 40, 80 and 170 mg/kg/day

Drug Batch No.: 46-097-AL

Methods: This study was conducted to assess the potential of Zileuton to induce cell proliferation in kidneys of rats. The dose selected in this study was the same as used in 2-year carcinogenicity study in rats. Animals were given Zileuton via diet for 26 days. All animals were observed for clinical signs and mortality daily. Body weights and food intakes were recorded weekly. Five rats/sex/group were sacrificed on day 5 of the study and the remaining 5 rats/sex/group were sacrificed on day 26 of the study. Alzet minipumps containing 20 mg/ml BrdU were implanted s.c. in each animal four days prior to sacrifice. Liver, right kidney and testis of each animal were examined histologically. Additionally, renal cell proliferation in control and high dose treated mice were evaluated by immunohistochemical technique using BrdU uptake into the nuclei of replicating cells. Furthermore, liver, kidney and testis were also examined under electron microscope.

Results:

1. Mortality: One male rat from low dose group died following the surgical implantation of Alzet minipump on day 1 of the study.
2. Body Weight/Food Consumption/Water Consumption: Significant body weight gain reductions were seen in low and high dose treated rats (male: 16-22% and female: 17-24%). However, body weight gains in mid dose treated rats (both sexes) were comparable to control values. No treatment related effects were seen in food intakes.
3. Gross Pathology: No treatment related effects were seen in livers and kidneys.
4. Histopathology: Results of histopathological findings in livers and testis were not reported. Only results of histopathological examinations of kidneys from control and high dose were reported. Minimal proximal tubule regenerations were seen in 1 out of 5 male rats which were sacrificed on day 5 of the treatment and 4 out of 5 male rats which were sacrificed on day 26 of the treatment. This tubular regeneration was "characterized by an increase in cell size along with cytoplasmic basophilia". However, there is no hyperplastic changes in this study. No biological significant increase in labeling index (%) was observed in proximal or distal tubule cells of male or female high dose treated rats at day 5 or day 26. On day 26, the increase in labeling index of the proximal tubules of high dose treated male rats over the control value became statistically significant because labeling index in the control rats was

unusually low. Hence, no evidence was provided that Zileuton increases cell proliferation in kidney. The BrdU-labeling index in kidneys of male and female rats were as follows:

Table 10*

BRdU-LABELING INDICES: KIDNEY
MALE RATS

Animal Number	Days on Test	Dose Group	Proximal Tubule Labeling Index (%)	Distal Tubule Labeling Index (%)
2601	5	0 mg/kg bwt.	11.2	3.0
2602	5	0 mg/kg bwt.	14.3	3.0
2603	5	0 mg/kg bwt.	7.6	1.9
2604	5	0 mg/kg bwt.	12.2	3.1
2605	5	0 mg/kg bwt.	9.6	1.5
Mean ± S.D.(%)			11.0 ± 2.5	2.6 ± 1.4
Fold Increase Over Control			1.0	1.0
2631	5	170 mg/kg bwt.	9.6	6.2
2632	5	170 mg/kg bwt.	14.9	4.7
2633	5	170 mg/kg bwt.	10.1	3.9
2634	5	170 mg/kg bwt.	12.6	4.5
2635	5	170 mg/kg bwt.	8.9	4.1
Mean ± S.D.(%)			11.2 ± 2.5	4.4 ± 1.3
Fold Increase Over Control			1.0	1.6
2606	26	0 mg/kg bwt.	4.3	3.1
2607	26	0 mg/kg bwt.	3.3	1.1
2608	26	0 mg/kg bwt.	7.3	2.6
2609	26	0 mg/kg bwt.	9.9	3.3
2610	26	0 mg/kg bwt.	5.1	1.9
Mean ± S.D.(%)			5.2 ± 1.5	2.4 ± 0.9
Fold Increase Over Control			1.0	1.0
2636	26	170 mg/kg bwt.	15.6	8.3
2637	26	170 mg/kg bwt.	10.5	2.3
2638	26	170 mg/kg bwt.	9.6	8.7
2639	26	170 mg/kg bwt.	13.9	1.3
2640	26	170 mg/kg bwt.	7.7	1.8
Mean ± S.D.(%)			11.5 ± 3.2*	1.1 ± 0.8
Fold Increase Over Control			2.2	NA

* Significantly different from control at $p \leq 0.05$ as determined by Kruskal-Wallis test and Mann-Whitney U test.
S.D. = Standard Deviation

* = Sponsor's Table 3, Page 010-00114 and 101-00115, Vol. 1.10

Table 10 Cont.*

GROUP-LABELLING INDICES: KIDNEY
FEMALE RATS

Animal Number	Days on Test	Dose Group	Proximal Tubule Labeling Index (%)	Distal Tubule Labeling Index (%)
2641	5	0 mg/kg bwt.	4.9	3.0
2642	5	0 mg/kg bwt.	3.4	3.6
2643	5	0 mg/kg bwt.	4.7	2.1
2644	5	0 mg/kg bwt.	4.2	3.3
2645	5	0 mg/kg bwt.	2.9	2.5
Mean ± S.D. (%)			4.0 ± 0.9	3.1 ± 0.7
Fold Increase Over Control			1.0	1.0
2671	5	170 mg/kg bwt.	5.4	1.5
2672	5	170 mg/kg bwt.	5.7	2.7
2673	5	170 mg/kg bwt.	3.6	2.6
2674	5	170 mg/kg bwt.	4.0	4.5
2675	5	170 mg/kg bwt.	4.1	3.9
Mean ± S.D. (%)			4.6 ± 0.9	3.4 ± 1.0
Fold Increase Over Control			1.2	1.1
2646	26	0 mg/kg bwt.	7.2	0.7
2647	26	0 mg/kg bwt.	3.9	1.3
2648	26	0 mg/kg bwt.	5.2	0.4
2649	26	0 mg/kg bwt.	3.4	0.9
2650	26	0 mg/kg bwt.	1.8	1.6
Mean ± S.D. (%)			4.1 ± 2.2	1.0 ± 0.5
Fold Increase Over Control			1.0	1.0
2676	26	170 mg/kg bwt.	2.3	0.4
2677	26	170 mg/kg bwt.	b	b
2678	26	170 mg/kg bwt.	3.0	0.5
2679	26	170 mg/kg bwt.	1.1	0.6
2680	26	170 mg/kg bwt.	b	b
Mean ± S.D. (%)			2.1 ± 1.0	0.5 ± 0.1
Fold Increase Over Control			NA	NA

* Significantly different from control at p ≤ 0.05 as determined by Kruskal-Wallis test and Mann-Whitney U test.
 b Failed to demonstrate immunohistochemical proliferation in the duodenum.
 S.D. = Standard Deviation

* = Sponsor's Table 3, Pages 010-00114 and 010-00115, Vol. 1.10

Examination of Kidney Tissues by Using a Specific Antibody for Proliferating Cell Nuclear Antigen (PCNA)

a. **Kidney Tissues From 3-Month Maximum Tolerated Dose Study in Mice (Study # TD 88-173)**: This study was previously submitted under IND and reviewed by Dr. Sun on 10/16/90. In the present submission (page 010-00164-T, Vol. 1.10) sponsor reperformed the histopathological examinations of kidneys and quantified renal cell proliferation by immunohistochemical technique (PCNA) and submitted the summary report of cell proliferation in proximal/distal tubule epithelial cells of control, low and high dose treated mice (per Dr. Sun's review low dose = 80 mg/kg/day and high dose = 1000 mg/kg/day). No treatment related histological lesions were evident in kidneys and PCNA data did not demonstrate renal cell proliferation in mice treated with up to 1000 mg/kg/day of Zileuton.

b. Kidney Tissues From 3-Month Maximum Tolerated Dose Study in Rats (Study # TA 88-172): This study was previously submitted under IND and reviewed by Dr. Sun on 10/16/90. In the present submission (page 010-00164-L-O, Vol. 1.10) sponsor reperformed the histopathological examinations of kidneys and quantified renal cell proliferation by immunohistostaining technique (PCNA) and submitted the summary report of cell proliferation in proximal/distal tubule epithelial cells of control, low and high dose treated rats (per Dr. Sun's review low dose = 80 mg/kg/day and high dose = 750 mg/kg/day).

Table 11

PC NA-Labeling Index (%)

	<u>Dose (mg/kg/day)</u>				
	0	80	170	350	750
Males	0.17	0.25	ND	ND	0.38
Females	0.092	0.24	ND	ND	0.21

ND = not done

No significant treatment related histological findings were evident in kidneys of male or female rats. Significantly increased renal cell proliferation was evident in high dose treated males (2.2 fold over control) and low and high dose treated females (2.3-2.6 fold over control). Sponsor has not assessed the renal cell proliferation in rats treated with 170 mg/kg/day of Zileuton, which was the highest tested dose in rat carcinogenicity study. Table 10 on page 001-00017, Vol. 1.1 is misleading with respect to dose i.e. 170 mg/kg/day should be 750 mg/kg/day and whole analysis of the data contained in this table is also misleading.

c. Kidney Tissues From 1/3-Month Oral Toxicity Study in Rats (Study # TA 87-140): This study was previously submitted under IND and reviewed by Dr. Tai (see his review dated 11/29/87 & 3/1/88). In the 1-3 month oral (gavage) toxicity study doses of 0, 5, 20, 80 and 300 mg Zileuton/kg/day were used. This study also included interim sacrifice at the end of 1-month of treatment. In the present submission sponsor reperformed the histopathological examinations of kidneys and quantified renal cell proliferation by immunohistostaining technique (PCNA) and submitted the summary report of cell proliferation in proximal/distal tubule epithelial cells in control and high dose treated rats (page 010-00164 A-J).

Table 12*

Dosage (mg/kg/day)	PCNA-Labeling Index (%)			
	One Month		Three Months	
	0	300	0	300
Males	0.17	0.8	0.14	0.81
Females	0.28	0.23	0.082	0.33

* - Sponsor's Table 9, Page 001-00017, Vol. 1.1

Significantly increased renal cell proliferation was evident in high dose treated males at 1 & 3-months of treatment and in high dose treated females a significant increase in renal cell proliferation was observed at the end of the treatment period (i.e. 3-months). No significant treatment related histological findings were evident in kidneys of male or female rats.

Hepatic and Renal Peroxisomal and Hepatic Drug
Metabolizing Activity in Mice After 14-Days of Oral
Treatment with Racemic Zileuton (Abbott-64077)
(Study # V 922-008)

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: March 10, 1992

Study Completed: December 22, 1992

Testing Species & Strain: Crl: CD-1 BR Mice (Charles River,
U.S.A.)

No. of Animals: 10-13/sex/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 50, 150 and 450 mg/kg/day

Drug Batch No.: 53-144-AL

Positive Control: Clofibrate (462 mg/kg/day which is equimolar to 450 mg/kg/day of Zileuton; solvent for clofibrate was corn oil) and phenobarbital (50 mg/kg/day suspended in 0.2% hydroxypropylmethyl-cellulose [HPMC]).

Negative Control: 0.2% HPMC

Methods: Sponsor had conducted three separate experiments. In the first experiment, groups of mice (10-13/sex/group) were given a single oral (gavage) dose of HPMC (negative control), 50, 150 and 450 mg/kg/day of Zileuton, corn oil (solvent control for clofibrate), 250 mg/kg/day of clofibrate, or phenobarbital (50 mg/kg/day). The negative control, Zileuton and phenobarbital were given for 14 consecutive days, while corn oil and clofibrate were given for 3 consecutive days. In the second experiment, only two groups of mice (10-13/sex/group) were included. One group received the negative control and the other group received 450 mg/kg/day of Zileuton for 14 days. In the third experiment, also only two groups of mice (10-13/sex/group) were included. One group received corn oil (solvent control for clofibrate) and the other group received 462 mg/kg/day of clofibrate (equimolar to 450 mg/kg/day of Zileuton) for 14 days. The volume of administration was fixed at 10 ml/kg for negative control, phenobarbital and Zileuton. Corn oil and clofibrate were administered at 5 ml/kg. Body weights of all animals were recorded weekly. About 24 hr after the last dose, animals were sacrificed, livers and kidneys were collected. Peroxisomal marker enzymes (catalase and cyanide-insensitive palmitoyl CoA oxidase [PCoA]) activities in liver and kidneys were monitored. Additionally, levels of liver microsomal total cytochrome P-450, cytochrome P-450 dependent monooxygenase activities (including 12-hydroxylauric acid hydroxylase activity), qualitative immunoassay analysis of mouse liver microsomal cytochrome P-450 (using Western immunoblot method), quantitative immunoassay of cytochrome P-450 (using indirect non-competitive enzyme linked immunosorbent assay [ELISA] method) were also monitored.

Results:

1. **Effects on Body Weights:** No treatment related effects on body weight were evident.
2. **Effects on Relative Liver and Kidney Weights:** The relative liver weights were increased by 25-34% compared to control values in mice (both sexes) treated with 450 mg/kg/day of Zileuton. No significant effect on liver weights were seen in 50 and 150 mg/kg/day of Zileuton treated mice. Clofibrate (462 mg/kg/day, equimolar to 450 mg/kg/day of Zileuton) also increased the relative weights of livers by 26% in both sexes of mice. Phenobarbital (50 mg/kg/day) only increased the liver weights by 6-9% (both sexes). Lower dose of clofibrate (250 mg/kg/day for only 3 days) resulted in 9-10% increase in relative liver weights compared to control values. In this study relative weights of kidneys were not affected by the treatment.

3. Effects on Peroxisomes, Microsomes and Cytochromes:

Liver

In experiment I, Zileuton induced dose related increase in liver PCoA and catalase activities. However, the effect of 450 mg/kg/day of Zileuton could not be reproduced in the experiment II. Furthermore, the positive control (clofibrate) in experiment I was not appropriate for comparison purposes because animals were treated for only 3 days and dosage used was equivalent to about 243 mg/kg/day of Zileuton. However, if one compares with results obtained in experiment III with equimolar (462 mg/kg/day) dose of clofibrate then the inductive effect of Zileuton on PCoA and catalase activities will be negligible. In experiment III, clofibrate increased the activities of PCoA and catalase by 358% and 44% over control values respectively (see table 13). Phenobarbital (50 mg/kg/day for 14 days) had no effect on liver PCoA and catalase activities.

Liver microsomal total protein and total cytochrome P-450 levels were increased dose dependently in Zileuton treated male and female mice. Phenobarbital gave the expected results. However, on equimolar bases (450 mg/kg of Zileuton = 1903 micro-moles/kg, 50 mg/kg of phenobarbital = 197 micro-moles/kg) Zileuton potency as cytochrome P-450 inducer is about one-tenth of phenobarbital.

Cytochrome P-450 dependent N-demethylase (using substrates: benzphetamine, N,N-dimethylnitrosamine [DMN], or erythromycin), O-dealkylase (using substrate: 7-ethoxyresorufin or 7-pentoxyresorufin) and hydroxylase (using substrate: 12-hydroxy lauric acid) activities in mice liver microsomes were increased significantly and dose dependently with few exceptions: 1) in females, Zileuton had no effect on erythromycin metabolism and 2) in females, 12-hydroxy lauric acid hydroxylase activity (a marker of induction of cytochrome P-450 IV A) in liver was greatly induced by Zileuton compared to males. Phenobarbital produced similar effect as Zileuton except, it had no significant effect of DMN-N-demethylase and 12-hydroxy lauric acid hydroxylase activities. Over all Zileuton was much weaker inducer of cytochrome P-450 dependent mixed function oxygenases activities than phenobarbital on equimolar basis (clofibrate effects can not be compared with that of Zileuton, because animals were treated for only 3 day as opposed to 14-day treatment for the rest of the animals). Qualitative (Western immunoblotting) and quantitative (enzyme-linked immunosorbent assay [ELISA]) immuno-assays have also indicated that Zileuton dose dependently induced cytochrome P-450 II B (which is involved in 7-pentoxyresorufin-o-dealkase activity) and this effect was about one-fifth of phenobarbital on molar basis. In a separate experiment (# III) clofibrate (462 mg/kg/day for 14 days) had highly variable effect on cytochromes P-450 II B levels (could be considered negligible), but it significantly induced cytochrome P-450 IV A in male (979% over control) and female (753% over control) mice. Clofibrate is not only peroxisome proliferator

but also induces cytochrome-P-450 IV A. In experiment I, Zileuton dose dependently induced cytochrome P-450 IV A in female mice only (no effect were evident in treated males). The induction of cytochrome P-450 IV A at high dose (450 mg/kg/day of Zileuton for 14 days) was 255% in females which was about one-third of that seen with equimolar clofibrate in experiment III. Phenobarbital depressed the level of cytochrome P-450 IV A despite observed induction of cytochrome II B.

On equimolar basis, peroxisome proliferating activity of Zileuton is negligible compared to clofibrate. Zileuton potency as cytochrome P-450 inducer is about one-tenth of phenobarbital. Furthermore Zileuton was much weaker inducer of cytochrome P-450 dependent mixed function oxygenases activities than phenobarbital, even though the response profile was some what similar. It can be said that Zileuton shares some of the properties of clofibrate as well as phenobarbital but its potency is much weaker than the two respective positive controls on equimolar basis.

Table 13
Effects of 14 Day Oral Zileuton Pre-treatment on CD-1 Mouse Liver

Parameter	Sex (M/F)	% Change Over The Control						
		Experiment 1			Phenobarbital (mg/kg/day) 50	Experiment 2 Zileuton (mg/kg/day) 450	Experiment 3 Clofibrate (mg/kg/day) 462	
		Zileuton (mg/kg/day) 50	150	450				Clofibrate (mg/kg/day) 250*
Peroxisomes:								
PCoA	M	27	43	77	111	- 6	16	385
	F	- 8	45	121	170	- 45	97	358
Catalase	M	0.7	19	54	30	- 8	- 11	58
	F	18	25	51	21	7	15	44
Mitochondria:								
Total Protein	M	32	57	82	1.3	57	4	17
	F	19	33	49	6	51	69	67
Total Cytochrome P-450	M	2.2	22	56	1.1	25	78	23
	F	26	82	147	- 4	85	94	20
Cytochromes P-450 II B (sub family)	M	101	430	1164	ND	758	ND	77
	F	105	248	627	ND	280	ND	- 58
Cytochromes P-450 IV A (sub family)	M	- 41	- 39	- 25	ND	- 66	ND	979
	F	- 42	40	255	ND	- 47	ND	753
BZ Nase	M	23	46	81	- 1	54	ND	ND
	F	46	77	104	- 11	99	ND	ND
DM Nase	M	- 7	3.8	35	33	6	ND	ND
	F	- 6	19	40	- 7	- 14	ND	ND
ER Nase	M	14	6.5	61	- 17	53	ND	ND
	F	- 35	- 11	11	4	- 7	ND	ND
7-ERO Dase	M	16	34	126	- 2	62	122	13
	F	45	184	184	- 32	145	208	- 24
7-PRO Dase	M	131	354	1214	- 37	902	1324	- 34
	F	96	586	1264	- 45	1354	1399	- 50
LA Nase	M	4	32	111	456	- 4	109	963
	F	51	279	851	499	- 44	396	1240

* = Treatment was for 3 days only

Activities are abbreviated as follows: PCoA, KCN-insensitive palmitoyl CoA oxidase; 7-ERODase, 7-ethoxyresorufin O-deethylase; BZNase, benzphetamine N-demethylase; 7-PRODase, 7-pentoxeresorufin O-dealkylase; DMNase, N,N-dimethyl-nitrosamine N-demethylase; ERNase, erythromycin N-demethylase; LANase, lauric acid 12-hydroxylase.

Kidney

In male mice, Zileuton inhibited PCoA activity in kidneys, while clofibrate (462 mg/kg/day) increased kidney PCoA activity by 59% compared to the control values. In female mice the basal kidney PCoA activity was about 31% of that found in corresponding males (1.09 unit vs 3.46 unit). However, Zileuton induced kidney PCoA activity by 26% and 22% (in experiment II by 76%) in 150 and 450 mg/kg treated female mice respectively, clofibrate at 250 mg/kg (for 3 days in experiment I) and 462 mg/kg (for 14 days, in experiment III) increased kidney PCoA activities by 73% and 255% respectively. Again here the data indicates that effects of Zileuton of kidney PCoA activities were marginal, variable and much less than that seen with clofibrate at equimolar dose. Phenobarbital had no significant effect of kidney PCoA activity.

Table 14
Effects of 14 Day Oral Zileuton Pre-treatment on CD-1 Mouse Kidney

Parameter	Sex (M/F)	% Change Over The Control						
		Experiment 1			Phenobarbital (mg/kg/day) 50	Experiment 2 Zileuton (mg/kg/day) 450	Experiment 3 Clofibrate (mg/kg/day) 462	
		Zileuton (mg/kg/day)						
		50	150	450	Clofibrate (mg/kg/day) 250*			
<u>Peroxisomes:</u>								
	M	-10	-9	-2	-8	-31	-45	59
PCoA	F	5	26	22	73	-15	76	255

PCoA = KCN-insensitive palmitoyl CoA oxidase activity

**The Effect of Zileuton on Peroxisome Proliferation in Liver
and Kidney of Rat Following 14 Days of Oral Treatment**
(Study # TA 92-229)

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: Not given

Study Completed: January 12, 1993 (report date)

Testing Species & Strain: Crl: CD-BR Sprague Dawley male &
female rats (120g)

No. of Animals: 10/sex/group

Route of Administration: Oral (gavage)

Dose Levels: 0, 40, 80, 170 and 300 mg/kg/day

Negative Control: 0.2% HPMC

Positive Control: Clofibrate (308 mg/kg/day)

Solvent for Positive Control: Corn oil

Drug Batch No.: 52-133-AL

Methods: Groups of rats were given a single oral (gavage) dose of HPMC (negative control), 40, 80, 170 and 300 mg/kg/day of Zileuton, corn oil (solvent for positive control), or 308 mg/kg/day of clofibrate (positive control) for 14 consecutive days. The volume of administration was fixed at 10 ml/kg for negative control and for Zileuton. Corn oil and clofibrate were administered at 5 ml/kg. About 24 hr after the last dose animals were sacrificed, livers and kidneys were collected. Peroxisomal palmitoyl-CoA oxidase and 12-lauric acid hydroxylase activities in the liver and peroxisomal palmitoyl-CoA oxidase activity in kidney cortex were monitored. Additionally, liver and kidney cortex from 2 rats/sex/group were examined under electron-microscope.

Results:

- 1. Effects on Body Weight:** No treatment related effects on body weight were evident (data presented graphically).
- 2. Effects on Organ Weights (Liver, Kidney & Testis):** No significant treatment related effects on relative weights of kidneys and testis were evident. However, relative liver weights were significantly increased in Zileuton or clofibrate treated rats (data presented graphically) compared to their respective control values.

3. Effects on Peroxisomes: Induction palmitoyl-CoA oxidase activity has been used a marker of peroxisome proliferation. Zileuton had no inductive effect on palmitoyl-CoA oxidase activity in rats liver, while clofibrate increased PCoA oxidase activity by 443% in males and by 78% in females. In kidney cortex, Zileuton had no significant effect on PCoA oxidase activity in males but in females the PCoA oxidase activity was increased up to 58%, but this increase in induction was not dose related. Clofibrate increased PCoA oxidase activity in kidney cortex by 82% in males and by 76% in females.

Induction of liver 12-hydroxy lauric acid hydroxylase activity has also been used as a marker for peroxisome proliferation. In males, Zileuton had no effect on liver 12-hydroxy lauric acid hydroxylase activity while clofibrate increased the activity by 384% compared to control value. In females, liver 12-hydroxy lauric acid hydroxylase activities were reduced by 79%, 15%, 65% and 69% at 40, 80, 170 and 300 mg/kg/day of Zileuton while a increase of 57% observed in clofibrate treated females. Thus Zileuton is not peroxisomal proliferator in rats. Additionally, sponsor has also monitored liver 11-hydroxy lauric acid hydroxylase activity (inducible by phenobarbital and linked to cytochrome P-450 II B activity) in above experiment. Zileuton had no significant effect on this enzyme in females but in males, a dose dependant induction was observed (up to 94% over control value) but unfortunately no phenobarbital control was included in this study (see review of study # V 92-008).

4. Electron Microscopic Examinations of Liver and Kidney Cortex: No significant difference in number of peroxisomes were observed in livers of control and high dose treated male rats. Similarly, no significant difference in number of peroxisomes were observed in the kidney cortexes of control and high dose treated rats. Clofibrate induced peroxisomal proliferation in the rat livers had no effect on peroxisomes in kidney cortexes of rats. Hence there was no evidence of peroxisome proliferation by electron microscopic examination in livers and kidneys of rats treated with Zileuton (300 mg/kg/day for 14 days).

Two-Week Study of Orally Administered Zileuton in Male Rats:
Hypothalamic - Pituitary - Gonad Challenge
(Study # TA 92-313)

Testing Laboratories: Abbott Laboratories
Abbott Park, IL

Study Started: June 23, 1992

Study Completed: October 29, 1992

Testing Species & Strain: Crl: CD BR Male Sprague-Dawley rats
(Charles Rivers, U.S.A.; 256-312g, 8-9 weeks old)

No. of Animals: 15/group

Route of Administration: . Oral (gavage)

Dose Levels: 0, 170 mg/kg/day

Drug Batch No.: 46-097-AL

Methods: A total of 90 male rats were included in this study, 45 rats were given vehicle (0.2% hydroxy propyl methyl cellulose [HPMC]) and the remaining 45 rats were given 170 mg/kg/day of Zileuton for 14 consecutive days. The volume of administration was fixed at 10 ml/kg. Fifteen rats/group received either hCG (human chorionic gonadotropin: 100 I.U./rat, s.c., 0.2 ml/kg), GnRH (gonadotropin releasing hormone: 3 mcg/kg, I.P., 1 ml/kg or naloxone (2 mg/kg, s.c., 0.5 ml/kg) one hour after the last vehicle or Zileuton dose. All animals were observed for mortality and clinical signs twice daily. Body weights were recorded weekly during treatment period. Samples of blood (about 2 ml) were collected from orbital sinus on day 14 at 0 (just prior to receiving challenges), 0.5, 1, 3 and 6 hours after the administration of either hCG, GnRH or naloxone. Levels of LH and testosterone were monitored in serum samples using radioimmunoassay methods. About 6.5 hours after hCG, GnRH or naloxone administration, rats were sacrificed, both testes were removed and testicular levels of testosterone were monitored.

Results:

1. Observed Effects: No treatment related effects were seen.
2. Mortality: One rat (# 2011) died due to dosing accident. In addition, 4 rats (# 3005, # 4007, # 4017 and # 5001) died on day 14 prior to sacrifice but after receiving appropriate challenge. These deaths were not considered to be treatment related.
3. Body Weights: Body weight gains were reduced by 4% in Zileuton treated rats compared to control rats.
4. Serum Hormone Levels: Zileuton had no significant effect on the levels of serum LH or testosterone in male rats in different set of experiments. In view of this lack of effect challenging rats with hCG, GnRH or naloxone was not needed.

Table 15

Serum Hormones Level on Day 14 Prior Challenge

<u>Treatment Groups</u>	<u>LH (ng/ml)</u>	<u>Testosterone (ng/ml)</u>
<u>hCG Challenge Group</u>		
Control	0.899 ± 0.269	1.508 ± 0.694
Zileuton (170 mg/kg/day)	0.856 ± 0.308	1.954 ± 1.101
<u>GnRH Challenge Group</u>		
Control	1.061 ± 0.387	1.950 ± 1.207
Zileuton (170 mg/kg/day)	0.915 ± 0.371	1.703 ± 0.809
<u>Naloxone Challenge Group</u>		
Control	0.930 ± 0.395	1.467 ± 0.725
Zileuton (170 mg/kg/day)	1.088 ± 0.437	1.784 ± 0.916

5. Testicular Testosterone and Organ Weights: No treatment related effects were evident.

Thus the data indicated that Zileuton had no altering effect on hypothalamic - pituitary - gonadal axis in rats.

Overview of Testicular and Prostatic Changes in the Dog
(Amendment # 062 Dated 2/24/93)

This Amendment discusses the results of 4 oral toxicity studies of Zileuton in dogs. These studies are as follows:

Study # TB 87-134 (3-month oral toxicity study in dogs with 1-month interim kill).

Study # TB 90-284 (6-month oral toxicity study in dogs)

Study # TB 91-219 (1-year oral toxicity study in dogs)

Study # TB 92-310 (1-month oral toxicity study in male dogs with 1 -, 3 - and 6 - month recovery period)

Study # TB 87-134 was submitted in the initial submission dated June 7, 1990. In this study sexually immature dogs (6-9 months old) were given 0, 5, 20, 100 and 400 mg/kg/day of Zileuton orally for 3-months. In this study 7 dogs/sex/group were used, 3 dogs/sex/group were used for 1-month interim kill and the remaining dogs (4/sex/group) were used for the main study. Only testicular and prostatic changes in male dogs will be discussed here. 2/4 male dogs (# 4007 and # 4009) died on 36 and 40 days of treatment respectively. Histopathological examinations revealed decreased spermatogenesis with "diminished" seminiferous tubular cellularity in 1/4 dogs (# 3001) which received 100 mg/kg/day and testicular degeneration in 3/4 dogs (# 4003, # 4007

and # 4009) treated with 400 mg/kg/day. According to sponsor foot note (table 1, page 000-00011) these 3 dogs lost their respective body weights by 20%+. Additionally, prostate atrophy (multi-focal to diffuse) were seen in all high dose treated dogs. Some of these findings (decreased spermatogenesis and prostate atrophy) were also present in the dogs which were sacrificed at 1-month after the drug administration. The no effect dose in this study was considered to be 20 mg/kg. Based on sponsor statement on page 2 of the Amendment, the no effect dose is only about 2 times higher than the recommended clinical dose (600 mg q.i.d.) when plasma exposures were compared (AUC [mcg.hr/ml]: Dog at 20 mg/kg/day = 155 mcg.hr/ml, human at 600 mg q.i.d. = 70 mcg.hr/ml).

Six-month (Study # TB 90-284) and 1-year (Study # TB 9-219) oral toxicity studies in dogs are being reviewed by Dr. Williams. The information regarding these two studies submitted in this Amendment was discussed with Dr. Williams.

In 6-month oral toxicity study mature dogs (13-15 months old) were given 0, 20, 50, 100 and 200 mg/kg/day of Zileuton. Decreased spermatogenesis with decreased seminiferous tubular cellularity was seen in 1 out of 6 high dose treated dogs (# 4005). Additionally, prostate atrophy (multi-focal to diffused) were seen in 1 out of 6 dogs (# 3001) which received 100 mg/kg and 3/6 dogs (# 4003, # 4005 & # 4007) which received 200 mg/kg/day of Zileuton. All dogs which had testicular changes and/or prostate atrophy lost their body weight (about 20%+) and had to be killed prior to the termination of the study. Thus it is difficult to judge whether these reproductive toxicities are due to Zileuton or due to general deterioration of the health of these dogs.

In 1-year oral toxicity study in immature dogs (7-10 months old) were given 0, 10, 25 and 50/75 mg/kg/day of Zileuton. In this study no drug related histopathological abnormalities in testis and prostate were evident.

Full report of study # TB 92-310 has not been submitted to us. Hence no comment shall be made at this time.

Sponsor indicated in the summary portion (page 000-00008) of this Amendment that effect of Zileuton on testis and prostate is seen only in dogs at very high plasma levels and associated with severe systemic toxicity and mortality. Furthermore, the plasma levels of Zileuton (368 mcg.hr/ml [?]) which is associated with the above findings is many folds higher than that seen in human (70 mcg.hr/ml).

SUMMARY AND EVALUATION:

Zileuton is a benzothiophene N-hydroxyurea and it is a known inhibitor of 5-lipoxygenase. It is being developed for the treatment of ulcerative colitis, asthma and various inflammatory disease.

In the present submission sponsor has submitted the results of carcinogenicity studies in mice and rats, genotoxicity Studies: Ames test and ex vivo UDS assay in kidney cells of mouse and rat and special toxicology studies: renal cell proliferation study in mice and rats, Zileuton PCNA studies in rodent kidneys, effect of Zileuton on peroxisome proliferation in liver and kidney of mice and rats and effect of Zileuton on hypothalamic-pituitary-gonadal axis in male rats, and overview of testicular and prostate changes in the dog.

In two years carcinogenicity study in mice, Zileuton was given via diet at daily doses of 50, 150 and 450 mg/kg/day. The dose selection was appropriate. Sponsor has used two concurrent control groups in this study. In statistical analysis only one group was used and the other group was declared "non-primary control group" without providing any reasoning. This is not acceptable practice. Sponsor should have used both controls in statistical analysis. However, the study was conducted properly. At termination survival rates were comparable in all groups. Non-neoplastic changes included hepatocytomegaly in mid and high dose treated mice (males: control = 0%, low dose = 0%, mid dose = 16.7% and high dose = 61.7%; females: control = 0%, low dose = 0%, mid dose = 6.7% and high dose = 43.1%). Additionally, unspecified renal hyperplasia was seen only in high dose treated mice (males = 5% and females = 6.9%). Dose dependent and statistically significant increased incidence of hepatocellular adenomas plus carcinomas were seen in treated mice. In males incidences were: control 23.3%, low dose 16.6%, mid dose 15% and high dose 36.2%, (p value = 0.0077). These incidences are above the sponsor's historical control incidence rate of 14.5%, (range 7-20%). In females incidences were: control 0%, low dose 5%, mid dose 0% and high dose 19%, (p value = 0.0000). These incidences were above the sponsor's historical control incidence rate of 5.2%, (range 2-9%). Pairwise comparisons of the incidence rates of hepatocellular adenomas plus carcinomas in control and high dose treated group gave a statistically significant results in female mice (p = 0.00005, Fisher exact test) but not in male mice (p = 0.0833, Fisher exact test). Additionally, statistically significant increased incidence of renal adenomas plus carcinomas were seen in treated mice. In males incidences were: control 0%, low dose 0%, mid dose 1.7% and high dose 3.4%, (p value = 0.0276). These incidences were above the sponsor's historical control incidence rate of 0.3%, (range 0-1%). In females incidences were: control 0%, low dose 0%, mid dose 0% and high dose 6.9%, (p value = 0.0029). These incidences were above the sponsor's historical control incidence rate of 0.3%, (range 0-1%). However, pairwise comparisons of the incidence rates of renal adenomas plus carcinomas in control and high dose treated group gave a statistically significant result in only female mice

($p = 0.048$, Fisher exact test) but not in male mice ($p = 0.1694$, Fisher exact test). In female mice statistically significant increased incidence of hemangiomas and hemangiosarcomas in multiple organs (hemangiomas and hemangiosarcomas in skin, ovary, vagina, uterus, kidney, liver and spleen) were seen. Incidences were control 10%, low dose 6.7%, mid dose 8.3% and high dose 31.6%, (p value = 0.0000). These incidences were above the sponsor's historical control incidence rate of 13.1%, (range 6.9-16.3%). Pairwise comparisons of the incidence rates of hemangiomas and hemangiosarcomas in multiple organs of control and high dose treated females also gave statistically significant results ($p = 0.0015$, Fisher exact test). Zileuton induced statistically significant dose related increases in hepatocellular adenomas/carcinomas, renal adenomas/carcinomas and hemangiomas/hemangiosarcomas in multiple organs in treated females.

In two years carcinogenicity study in rats, Zileuton was given via diet at daily doses of 40, 80 and 170 mg/kg/day. The dose selection was appropriate. Sponsor has used two concurrent control groups in this study. In statistical analysis only one group was used and the other group was declared "non-primary control group" because female of this group had a lower survival rate than any other female group in this study. However, the study was conducted properly. At termination survival rates were comparable in all groups. Dose related and statistically significant increased incidences of interstitial cell tumor (Leydig cell tumor) of the testes were seen in treated males. The incidences were: control 5%, low dose 20%, mid dose 20% and high dose 40%, (p value = 0.000007). These incidences were above the sponsor's historical control incidence rate of 3.6%, (range 0-7%). Pairwise comparisons of the incidence rates of interstitial cell tumor of the testes in control and high dose group also gave a statistical significant result ($p = 0.0000$, Fisher exact test). Additionally, increased incidences of renal tubular adenoma plus carcinoma were seen in treated rats (P values per Peto trend test: male = 0.0301 and female = 0.0125). However, when sponsor used adjusted P values (obtained by using the method of Westfall and Young, *J. Amer. Stat. Assoc.* 1989; 84: 780-786) then the trend test for renal tumor becomes non-significant. According to Dr. [redacted] (RFD-715: Division of Biometrics) review dated April 27, 1993 the incidence rates of renal tubular adenoma plus carcinoma in males were: control 5%, low dose 3.3%, mid dose 1.7%, high dose 15%, (p value = 0.0062). These incidences were above the sponsor's historical control incidence rate of 0.2%, (range 0-1%). In females the incidences were: control 0%, low dose 1.7%, mid dose 3.3% and high dose 8.3%, (p value = 0.011324). These incidences were also above the sponsor's historical control incidence rate of 0.2%, (range 0-2%). Pairwise comparisons of the incidence rates of renal tubular adenomas plus carcinomas in control and high dose group also gave statistically significant results (males: p value = 0.02645, females: p value = 0.045; Fisher exact test). Zileuton induced statistically significant dose related increases in

benign Leydig cell tumors in the testes of male rats and it also produced renal tubular adenoma plus carcinoma in treated rats of both sexes.

In Ames test, Zileuton was not mutagenic in any of the tester strains. Zileuton did not induce UDS or S-phase DNA synthesis in primary cultures of kidney cells obtained from rats and mice (both sexes). Renal cell UDS assay or SDS assay are not commonly used in mutagenicity tests.

Since Zileuton is not genotoxic (see above and Dr. Tai review of IND dated 11/29/87), sponsor suggested that increased incidences of tumors seen in mouse and rat carcinogenicity studies might be related to epi-genetic or non-genotoxic mechanism (cell proliferation, peroxisomal proliferation or microsomal enzymes induction). Each of the above mechanisms will be discussed sequentially.

Renal tumors were seen in female mice and male and female rats. When mice were given 450 mg/kg/day of zileuton for 26 days, no biological significant changes in BrdU labeling index (%) were observed in the proximal or distal tubules of male or female treated mice (renal cell proliferation were evaluated by immunohistochemical technique using BrdU uptake into the nuclei of replicating cells). Similarly, when rats were given 170 mg/kg/day of zileuton for 26 days, no biological significant changes in BrdU labeling index (%) were observed in the proximal or distal tubules of male or female treated rats. Hence there is no evidence that Zileuton increases cell proliferation in kidney.

Kidney tissues from previously conducted toxicity studies in mice (# TD 88-173), and rats (TA 88-172 and Ta 87-140) were also evaluated to determine renal cell proliferation using PCNA methods. In study # TD-88-173 (3-month MTD study in mice) no renal cell proliferation was observed in mice treated with up to 1000 mg/kg/day of Zileuton. In study # TA 88-172 (3-month MTD study in rats), significantly increased renal cell proliferation was evident in high dose treated males (2.2 fold over control) and low and high dose treated females (2.3-2.6 fold over control). It should be noted here that in this study the high dose was 750 mg/kg/day and not 170 mg/kg/day as stated by the sponsor in table 10 on page 001-00017, Vol. 1.1. Thus the highest tested dose in this study is about 4.4 fold higher than the highest tested dose in rat carcinogenicity study. In study # TA 87-140 (3-month oral toxicity study in rats) significantly increased renal cell proliferation was evident in high dose (300 mg/kg/day) treated males at 1 & 3-months of treatment and in high dose treated females a significant increase in renal cell proliferation was observed at the end of the treatment period (i.e. 3-months). No significant treatment related histological findings were evident in kidneys of male or female rats in the above mentioned studies.

In mouse carcinogenicity study, statistically significant increased incidence of hepatocellular adenomas/carcinomas were seen in female mice. To elucidate the mechanism of

tumorigenicity sponsor conducted 3 experiments. The experimental designs were flawed. In the first experiment, mice were treated with Zileuton or phenobarbital for 14 days while positive control clofibrate were given for only 3 days. In the second experiment, no positive control group was included. In the third experiment, only positive control (clofibrate: 462 mg/kg/day equimolar to 450 mg/kg/day of Zileuton) was given. In liver, on equimolar basis, peroxisome proliferating activity of Zileuton is negligible compared to clofibrate. Zileuton potency as cytochrome P-450 inducer is about one-tenth of phenobarbital. Furthermore Zileuton was much weaker inducer of cytochrome P-450 dependent mixed function oxygenases activities than phenobarbital, even though the response profile was some what similar. It can be said that Zileuton shares some of the properties of clofibrate as well as phenobarbital but its potency is much weaker than the two respective positive controls on equimolar basis. Effects of Zileuton of kidney PCoA activities were marginal, variable and much less than that seen with clofibrate at equimolar dose. Phenobarbital had no significant effect of kidney PCoA activity. Electron microscopic examinations of livers and kidneys from Zileuton treated mice were not done. Zileuton (up to 300 mg/kg/day for 14 days) is not peroxisomal proliferator in rats and there was no evidence of peroxisome proliferation by electron microscopic examination in livers and kidneys of rats treated with Zileuton (300 mg/kg/day for 14 d /s).

In rats carcinogenicity study, statistically significant increased incidence of leydig cell tumors in testis were seen in male rats. Sponsor attributed this effect to the disruption of hypothalamic-pituitary-gonadal axis. to prove this hypothesis, sponsor treated male rats with 170 mg/kg/day of Zileuton for 14 days and monitored the levels of LH and testosterone before and after the challenge with hCG, GnRH or naloxone on day 14 of the study. Zileuton had no significant effect on the levels of serum LH or testosterone in male rats and in view of this lack of effect, challenging rats with hCG, GnRH or naloxone was not even needed.

In Amendment # 062 dated 2/24/93, sponsor discussed the testicular and prostatic changes induced by Zileuton in 4 oral toxicity studies (study # TB 87-134 [3-month oral toxicity study in dogs with 1-month interim kill], study # TB 90-284 [6-month oral toxicity study in dogs], study # TB 91-219 [1-year oral toxicity study in dogs] and study # TB 92-310 [1-month oral toxicity study in male dogs with 1 -, 3 - and 6 - month recovery period]) in dogs.

Study # TB 87-134 is not appropriate to assess the effect of Zileuton on prostate and testis because in this study sexually immature dogs (6-9 months old) were used and duration of treatment was short i.e. 3-months.

Six-month (Study # TB 90-284) and 1-year (Study # TB 9-219) oral toxicity studies in dogs are being reviewed by Dr. Williams. The information regarding these two studies submitted in this Amendment was discussed with Dr. Williams. In 6-month oral

toxicity study, dogs which had testicular changes and/or prostrate atrophy lost their body weight (about 20%+) and had to be killed prior to the termination of the study (approximately 2-3 months into the study). Thus it is difficult to judge whether these reproductive toxicities are due to Zileuton or due to general deterioration of the health of these dogs. In 1-year oral toxicity study no drug related histopathological abnormalities in testis and prostate were evident. Furthermore, it should be noted that testicular tumors were seen in rats of carcinogenicity study and no histopathological changes in testis were seen in 3-month or 6-month oral toxicity studies.

Liver and kidneys are the target organs of toxicities in mice and rats. Zileuton produced renal tumors in mice (females) and rats (males and females). It also produced hepatocellular adenomas/carcinomas in female mice and Leydig cell tumors in testis of male rats. Sponsor attributed the renal tumors due to increased renal cell proliferation and liver tumors due to peroxisome proliferation in the livers of treated animals. Increased renal cell proliferation was evident in high dose treated mice (both sexes) of carcinogenicity study. No renal cell proliferation assessment was done in rat carcinogenicity study. However, kidney tissues from previously conducted toxicity study in rat were examined. The data indicated that in 3-month MTD study a dose of 750 mg/kg/day (about 4.4 fold higher than the highest tested dose in rat carcinogenicity study) induced renal cell proliferation in rats (both sexes) similar results were seen in another 3-month oral toxicity study when a dose level of 300 mg/kg/day was used.

With respect to liver tumors in female mice, no valid evidence of peroxisomal proliferation in the liver by electronmicroscopic examination was provided. In liver, on equimolar basis, peroxisome proliferating activity of Zileuton is negligible compared to clofibrate. Zileuton potency as cytochrome P-450 inducer is about one-tenth of phenobarbital. Furthermore, Zileuton was much weaker inducer of cytochrome P-450 dependent mixed function oxygenases activities than phenobarbital, even though the response profile was some what similar. It can be said that Zileuton shares some of the properties of clofibrate as well as phenobarbital but its potency is much weaker than the two respective positive controls on equimolar basis.

With respect to the safety concerns in extending clinical trial beyond six month. Following findings should be kept in mind. Zileuton is not mutagenic. Irrespective of the mechanism of tumorigenicity, it produced renal tumors (adenomas plus carcinomas) in female mice and male and female rats, leydig cell tumors in testis of male rats and hepatocellular adenomas/carcinomas and vascular tumors (i.e hemangiomas and hemangiosarcomas) in female mice. Furthermore, on mg/sq. meter basis the highest tested dose in mouse carcinogenicity study (450 mg/kg/day = 1350 mg/sq.m.) was only 0.76 times the human proposed clinical dose (600 mg q.i.d. = 1776 mg/sq.m) and the highest

tested dose in rat carcinogenicity study (170 mg/kg/day = 1003 mg/sq.m) was only 0.56 times the human proposed clinical dose (50 kg body weight assumed). Therefore long term administration of Zileuton is not recommended.

RECOMMENDATION: On the basis of preclinical findings, long term administration of Zileuton is not recommended.

Tanveer Ahmad 5/10/93
Tanveer Ahmad, PH.D.
Pharmacologist, HFD-180

cc:
Original IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Ahmad
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-502
HFD-150/Dr. Taylor
HFD-007/Dr. Mukherjie
HFD-007//Dr. Chen

Concur
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5/10/93

TA/hw/5/10/93
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150/514

IND

REVIEWER: Ching-Long Joseph Sun, Pharmacologist (HFD-180)

SPONSOR: Abbott Laboratories
Abbott Park, Illinois

Date of submission: June 7, 1990

Date of receipt by HFD-180: June 14, 1990

Date of review: Oct. 16, 1990

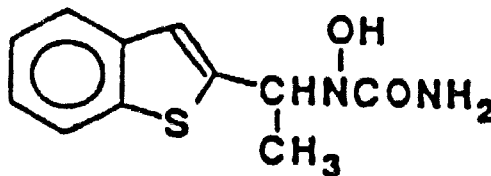
Date of preliminary safety review: July 9, 1990

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
(Original Summary)

DRUG: Zileuton (ABBOTT-64077) 200 mg Capsules

Chemical name: N-(1-Benzo[b]thien-2-ylethyl)-N-hydroxyurea

Structure:



Empirical Formula: $C_{11}H_{12}N_2O_2S$

Molecular weight: 236.29

Each capsule contains:

Ingredient	(mg/cap)

DRUG CATEGORY: Leukotriene synthesis inhibitor (lipoxygenase inhibitor)

PROPOSED CLINICAL STUDY:

This will be a randomized, double blind, placebo-controlled phase II study in patients with moderate ulcerative colitis to examine the safety and efficacy of a range of dosage regimens of zileuton in the control of symptoms and mucosal inflammation following oral administration. Two hundred patients will be eligible.

PROPOSED DOSE LEVELS:

or 600 mg q.i.d. (48 mg/kg/day) for 8 weeks.

RELATED DRUG: IND (Zileuton, Abbott)

PRECLINICAL STUDIES AND TESTING LABORATORIES: Most preclinical studies were performed by sponsor. Otherwise, testing laboratories are listed in each individual study.

Study	Report no.	Batch no.
Pharmacology *	--	--
ADME *, **, ***, ****, +, ++	--	--
Acute toxicity study	T87-127, T87-144, T87-156* T-88-170, TB-88-171	05-661-AL 06-679-AL
Acute oral toxicity study in combination with bronchodilator and anti-inflammatory agents in male mice ***	TD87-47	06-679-AL1
Acute oral toxicity study in combination with acetaminophen in male mice	TD88-124	06-679-AL
Acute oral toxicity study in combination with indomethacin in rats	TA88-232	06-679-AL
3 month maximum-tolerated dose study in mice	TD88-173	15-797-AL
10 day oral range-finding in rats *	TA87-119	15-797-AL
6 week oral toxicity study in immature rats	TA88-470	15-797-AL
3 month oral toxicity study in rats *, **	TA87-140	15-797-AL
3 month maximum-tolerated dose study in rats	TA88-172	
6 month oral toxicity study in rats	TA88-028	10-753-AL
10-11 Day Oral dosage range-finding study in dogs *	TB87-121	98-983-AX 99-015-AX 99-077-AX
3 month oral toxicity study in dogs *, **	TB87-134	05-661-AL
1 month oral toxicity study in monkeys ***	TC88-152	06-679-AL

6 month oral toxicity study in monkeys	TC87-428	15-797-AL
Oral segment I fertility and reproductive performance in female rats	TA87-375	06-079-AL
Oral segment I fertility and reproductive performance in rats	TA97-375 TA88-001	10-753-AL 15-679-AL
Oral Segment II teratology study in rats ***	TA87-339	06-679-AL
Oral segment II teratology study in rabbits ***	TE87-358	46F-0579
Oral segment III peri- and postnatal development in rats	TA87-426	06-679-AL
Ames test *	T87-165	05-661-AL
Reverse mutation assay	T87-454	10-753-AL
Unscheduled DNA synthesis in rat hepatocytes ***	T87-457	06-679-AL
CHO/HGPRT mutation assay	T87-457	06-679-AL
Micronucleus test in mice	TD-87-403	10-753-AL
Chromosome aberration in human lymphocytes	T87-455	06-679-AL
Passive cutaneous sensitization in guinea pigs	TF89-061	06-079-AL
Active systemic anaphylaxis in guinea pigs	TF89-184	06-079-AL
Antigenicity test in guinea pigs	TF89-184	06-679-AL

*: Submitted in the original IND (9/29/87) and reviewed by
Dr. Tai (11/29/87)

** : Submitted in the IND amendment (1/27/88) and reviewed by
Dr. Tai (3/1/88)

***: Submitted in the IND amendment (5/19/88) and reviewed by
Dr. Tai (8/5/88)

****: Submitted in the IND amendment (7/21/88) and reviewed by
Dr. Tai (8/5/88)

+: Submitted in the IND amendment (3/4/88) and reviewed by
Dr. Tai: (8/5/88)

++: Submitted in the IND amendment (1/12/88) and reviewed by
Dr. Tai (2/25/88)

Dr. Tai's reviews are enclosed (see attached Appendix).

PHARMACOLOGY

Pharmacological studies have been submitted for _____ and reviewed (see attached IND original review of IND _____ of 11/29/87 by Dr. Tai).

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION: Part of ADME studies have been submitted for IND _____ and reviewed (see attached IND 30661 reviews of 11/29/87, 2/25/88 & 8/5/88).

Absorption: Pharmacokinetic studies were conducted in conjunction with dose-finding and toxicology studies in rats (5-300 mg/kg), mice (60-500 mg/kg via diet), dogs (5-400 mg/kg) and monkeys (50-800 mg/kg).

Rats: Oral absorption (n=3) was moderate (T_{max} = 1-6 hr after single administration or 84 daily administrations and 1-3 hrs after 15 or 29 daily administrations). Drug accumulation was not present after 29 daily administrations but was evident after 84 daily administration. Decreases in dose-normalized AUCs were observed at 20 and 80 mg/kg dose levels but not at 5 and 300 mg/kg/day dose levels after 29 daily administrations. Thus, data may have limited the strength of inferences derived from the studies in regard to enzyme induction. Elimination- $T_{1/2}$ was 2-3 hours after oral administration and was 0.3 hrs after i.v. administration. Plasma levels of zileuton were generally higher in females (1.6-2.2x). The higher acute toxicity of zileuton found after administration in corn oil compared with the drug in 0.2% HPMC cannot be explained by a difference in the pharmacokinetic behavior of the compound nor by an interaction of the vehicle with plasma proteins. ABBOTT-66193 was present in the plasma following administration. Ratio of zileuton/ABBOTT-66193 was 1.8-4.5.

Dogs: Oral absorption was rapid following single, 14, 27 and 84 daily oral administrations of 5, 20, and 100 mg/kg/day in dogs (n = 8-15) with T_{max} of 2 hour but was moderate (T_{max} = 2-6 hr) at dose levels of 400 mg/kg/day. Drug accumulation was present. Elimination $T_{1/2}$ life was 6.5-8.3 hours.

Mice: Plasma levels of zileuton and ABBOTT-66193 (N-dehydroxyiated metabolite) were measured at 7pm, 9pm, 12am and 7am in mice (n = 5) after daily oral administration at dose levels of 50-450 mg/kg/day in diet for 5 days. T_{max} was reached at the 12 am except the 300 mg/kg/day male group (9pm). AUC values for parent drug were significantly greater than that of the N-dehydrated metabolite. The AUC values for metabolite as a percentage of parent AUC values ranged form 2.8 to 5.7%. No significant pattern was established to differentiate between the male and female groups.

Monkeys: Oral absorption for zileuton was fast (T_{max} = 2 hours) but was slow for ABBOTT 66193 (T_{max} = 5-12 hrs) in female monkeys (n = 3) following oral administration of zileuton at dose levels of 360, 600 and 800 mg/kg/day for one week. Elimination $T_{1/2}$ life was 1-3 hours.

However, the terminal phase T_{1/2} could not be estimated because of substantial high level of ABBOTT-66193 were still present 24 hour after dosing. The C_{max} and AUC for parent compound were smaller than those of the metabolite (ABBOTT-66193). There were negative dose-linear relationships of C_{max} level and AUC. This may be due to the low aqueous solubility of zileuton at high dose levels. In another 6 month oral toxicity study, pharmacokinetics were studied in monkey (n = 8-12) following 65 or 149 daily administrations at dose level of 50-500 mg/kg/day. Oral absorption was moderate with T_{max} of 3 hours. Elimination was fast with plasma levels at 8 hours postdosing being negligible. Comparison from this data with the previous-mentioned data is difficult because plasma sampling was restricted to 3, 8 and 24 hour in the study. The plasma concentrations of metabolites were several-fold higher than the levels of the parent drug.

Man: The oral absorption was fast (T_{max}=2.2 hours) following administration of 800 mg in capsules (n=16). T_{max} was 0.9 hr for dosing with the suspension. Elimination T_{1/2} was 3.67-4.11 hr after dosing with the solid formulation (2.28 hr with the suspension). Volume of distribution was 87-222 liter after single oral doses of 100-800 mg. Interspecies comparison of AUC_{0-24hr} of zileuton and ABBOTT-66193 after oral administration during clinical and preclinical studies is summarized below (extracted from sponsor's submission p. 21-46 of vol. 1.21):

Species	24hr Dose (mg/kg/day)	Dosage Regimen*	Sampling Period	Number of Animals/Sex	AUC 0-24hr (ug-hr/mL)		AUC ratio m/p [†]
					A-44077	A-66193	
MONKEY	360	q24hr	Day 7	2	29.5	236.6	8.01
	600	q24hr	Day 7	3	18.4	154.1	8.37
	800	q24hr	Day 7	3	9.4	132.6	14.11
	50	q24hr	Day 84-93	4	6.2	4.3	0.70
	150	q24hr	Day 84-93	4	18.4	71.1	3.86
	500	q24hr	Day 84-93	4	17.4	113.6	6.55
DOG	5	q24hr	Day 0††	3	47.9	1.0	0.02
			Day 6	3	42.0	1.3	0.03
	20	q24hr	Day 0	3	140.6	2.2	0.02
			Day 6	3	133.1	4.3	0.03
	100	q24hr	Day 0	2	369.8	2.3	0.01
			Day 6	3	367.7	3.3	0.01
RAT	300	q24hr	Day 4†††	6/M	644.7	151.9	0.24
	80	SD	Day 1	4/M	338.2	30.7	0.09
	300	SD	Day 1	4/M	1294.2	133.6	0.10
MOUSE	59	via diet	Day 6	5/M	22.9	<1.0	<0.04
	58	via diet	Day 6	5/F	28.8	<0.8	<0.03
	169	via diet	Day 6	5/M	98.9	5.6	0.06
	172	via diet	Day 6	5/F	73.7	2.6	0.04
	320	via diet	Day 6	5/M	173.8	9.8	0.06
	333	via diet	Day 6	5/F	172.1	9.4	0.05
	493	via diet	Day 6	5/M	248.9	11.9	0.05
MAN**	8	600mg SD	Day 1	6	13.78	4.41	0.32
	8	600mg q24hr	Day 2-7	7	9.33	6.31	0.70
	8	600mg SD	Day 1	6	10.64	1.54	0.14
	24	600mg q8hr	Day 3-14	6	36.36	13.62	0.37
	9	600mg SD	Day 1	7	13.47	1.85	0.14
	35	600mg q6hr	Day 3-14	7	69.48	26.12	0.38
	10	800mg SD	Day 1	6	20.41	15.69	0.76
	11	800mg q24hr	Day 2-7	7	14.5	6.58	0.45
	21	800mg q12hr	Day 4-14	7	26.66	10.66	0.39

* SD denotes single dose
 ** Actual group mean body weights were used for 24hr-doses
 AUC(0-24hr) = mean AUC per dosing interval times number of daily doses
 † Metabolite AUC over parent drug AUC
 †† Day 0 = Dose 1; Day 6 = Dose 7
 ††† Day 4 = Dose 5
 § Final report in preparation

Distribution:

Rats: The tissue distribution of radioactivity in male rats (n = 15) given a 5 mg/kg oral dose of C¹⁴-zileuton was studied. Radioactivity was found in the following organs or tissues: stomach (7 times the plasma level), small intestine, bladder and thyroid (3-4X), liver (2x), adrenal and kidney (1.5x), and pancreas and lung (1x). By 24 hours, the levels of radioactivity in the plasma and most tissues had decreased to about 2-10 % of their respective peak levels.

Dogs: The distribution of C¹⁴-zileuton was studied in two dogs 1 and 8 hours after an oral dose of 5 mg/kg. Radioactivity was found in liver (3-4X), kidney (1x), lung, testis, prostate and brain (0.7-0.9x) at 1 hr. The radioactivity in the tissues generally decreased between 1 and 8 hours. It appeared to have slightly greater affinity for the pigmented than the nonpigmented tissues of the eye.

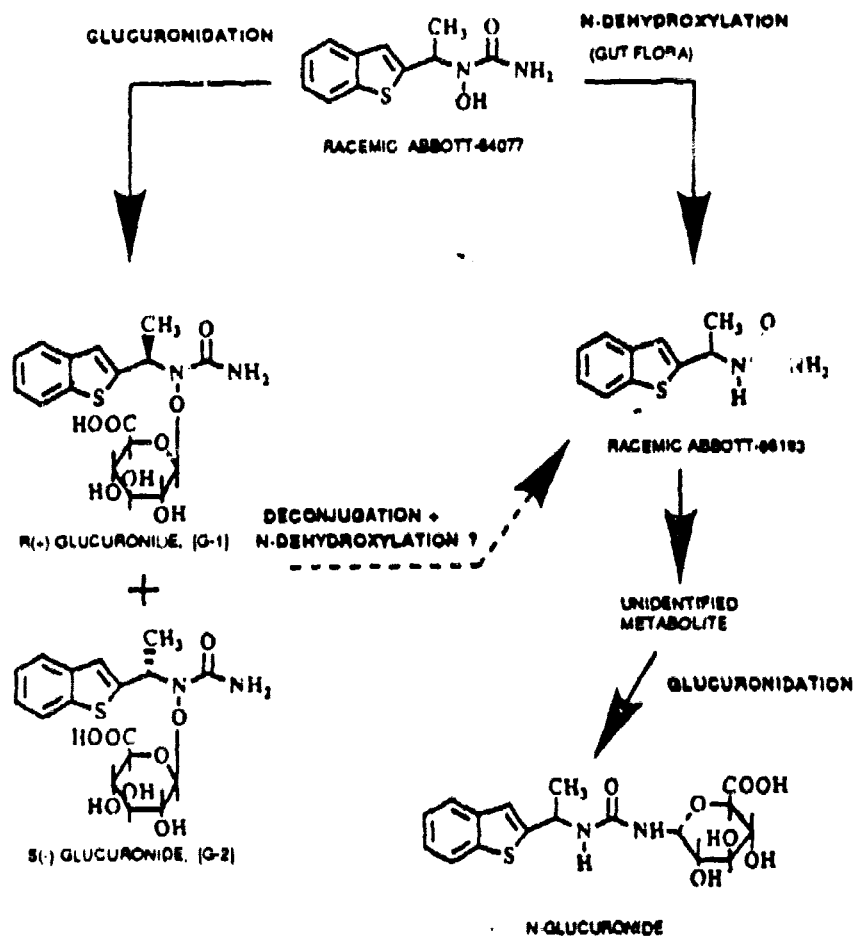
Metabolism:

Rats: Metabolic fate of C¹⁴-zileuton was studied in rats (n = 4) following oral and i.v. administration of a single 5 mg/kg dose. It was extensively metabolized in rats, with about 0.1-11% being recovered in the urine as free parent drug. Higher urinary levels of zileuton in females than males was observed. Higher levels of parent drug (5-11%) were seen after intravenous administration and probably attribute to the absence of first-pass metabolism. The major urinary metabolites (38-60%) consisted of polar material (conjugates of an unidentified urinary metabolite). The [R]- and [S]-zileuton glucuronide (G-1 and G-2) were minor metabolites (0.5-5% of the dose). The major circulating component was zileuton.

Dogs: Metabolism of C¹⁴-zileuton (5 mg/kg) was studied (n = 4) following single oral and i.v. administration. It was extensively metabolized as evidenced by the lack of parent drug in urine. The major metabolite in urine (60%) and bile (80%) was [S]-zileuton glucuronide (G-2). About 21% of the dose in urine consisted of unidentified polar and other metabolites which could be products of [R]-zileuton metabolism. The major circulating component was zileuton while the major plasma metabolite was [S]-zileuton glucuronide (G-2). Circulating component following intravenous administration were comparable to those after oral dosage.

Monkeys: Metabolism following oral and intravenous administration (5 mg/kg) was studied in female monkeys (n = 3). It was subjected to rapid and complete first pass metabolism. The major metabolites in urine and plasma were two glucuronide acid conjugates of parent compound. Both of the glucuronides accounted for 87% of the dose in the 24 hours. Parent compound was not detected in urine samples. The plasma concentration of the N-dehydrated metabolite (ABBOTT-66193) exceeded that of parent compound by at least 19-fold.

Man: The metabolism of C^{14} -zileuton were studied in four adult males after oral administration of a single 400 mg dose. The major circulating metabolites were O-glucuronide of zileuton (G-1 and G-2). The concentrations of G-1 and G-2 were 1.4-2-fold greater than that of parent drug, suggesting significant first pass metabolism. The major urinary metabolites were the G-1 (35% of th dose) and G-2 (43%) glucuronides. Free parent drug was not found in the urine samples, suggesting extensive metabolism. Polar and other metabolites comprised only 8 and 0.4% of the dose. The proposed metabolic pathway is given below:



Excretion: Excretion was studied in animals following oral or i.v. administration of ^{14}C -zileuton (5 mg/kg) and in adult male volunteers following oral administration of ^{14}C -zileuton (400 mg)

Rats: Within three days after oral administration, about 73% of the ^{14}C dose was excreted in the urine and about 21% was recovered in the feces. Similar results were obtained after intravenous administration with 71% of the dose being eliminated in the urine and 20% in the feces.

Dogs: In orally dosed dogs, an average of 88.1% of the ^{14}C -dose was excreted in the urine after 5 days and 14.2% in the feces. After

intravenous administration, urinary and fecal excretion accounted for 83.0% and 13.1% of the dose, respectively. Biliary secretion was a minor excretory route. Within 8 hours, 15.2% of the ¹⁴C-dose was recovered in the bile of intravenously-dosed animals and 6.0% in intraduodenally-dosed dogs. About 35% of the dose was found in the 8-hour urine.

Monkeys: An average of 92% of the administered radioactivity was found in the 0-24 hour urine, 86% of which was recovered after 7 hours. Only 0.3% of the dose was found in the feces.

Man: An average of 94.5% of the administered radioactivity was recovered in the urine after 7 days, and only 2.2% was found in the feces. Total recoveries ranged from 89 to 102%.

Comparison of plasma and urinary levels of parent compound and its metabolites and excretion in man and animals is given below (extracted from sponsor's submission p. 21-44 of Vol. 1.21):

Species Oral Dose	HUMAN 5 mg/kg	MONKEY 5 mg/kg	DUG* 5 mg/kg	RAT* 5 mg/kg
PLASMA				
ABBOTT-64077				
C _{max} (µg Eq/mL)	2.3	0.3	6.3**	3.6**
T _{max} (hr)	3.0	0.5	1.0	1.0
ABBOTT-66193				
C _{max} (µg Eq/mL)	0.1	-	-	-
T _{max} (hr)	8.0	-	-	-
G-1(R)†				
C _{max} (µg Eq/mL)	1.9	3.1	0.1	-
T _{max} (hr)	3.0	0.5	12.0	-
G-2(S)†				
C _{max} (µg Eq/mL)	2.3	3.0	1.6	-
T _{max} (hr)	2.0	0.5	1.0	-
G-1 + G-2†				
C _{max} (µg Eq/mL)	4.2	6.1	1.7	-
T _{max} (hr)	3.0	0.5	1.0	-
EXCRETION				
Urine (% Dose)	94.5	96§	88.0(83.0)§#	72.8(70.6)§#
Feces (% Dose)	2.2	0.3	14.2(13.1)	20.7(19.7)
METABOLISM				
	Urine (% Dose)			
ABBOTT-64077				1.8(8.2)**
G-1(R)†	35.5	38.2	3.1(2.8)	1.1(0.9)
G-2(S)†	43.2	48.8	60.4(56.7)	3.2(2.0)
G-1 + G-2†	78.8	87.0	63.5(59.5)	4.3(2.9)
Polar Material	8.2	4.3	15.2(14.5)	53.9(45.9)

- * Mean values for male and females
- ** May also contain ABBOTT-66193
- † G-1 = (R)-ABBOTT-64077-O-glucuronide
G-2 = (S)-ABBOTT-64077-O-glucuronide
- § Including cagewash
- # Values in parenthesis obtained after I.v. administration

Enzyme induction: The effect on hepatic drug-metabolizing enzymes was determined in rats following oral administration of 0, 20, 80, 150 and 300 mg/kg/day for ten days. Phenobarbital (50 mg/kg/day) was used as a positive control. Liver weights increased (22-39%) notably at the 150 and 300 mg/kg/day dose levels than those of the vehicle control group. The effects were similar to those obtained with the positive controls. Increases in N-demethylase, 7-ethoxycoumarin O-deethylase activities and P-450 content were dose-related as listed below (expressed as of % of control, n = 6):

Treatment (mg/kg/day)	N-demethylase Activity		O-deethylase Activity		P-450 Content nmoles per g Liver	
	Percent Liver Wt.	Per g. Liver	Per mg Protein	Per g Liver		Per mg Protein
<u>Males</u>						
20	106	95.7	100	159	166	247
80	109	131	131	269	269	286
150	124	153	160	307	318	299
300	137	162	169	929	964	428
50*	132	157	171	1,530	1,570	470
<u>Females</u>						
20	107	153	160	208	152	167
80	105	237	244	355	256	175
150	122	361	365	438	308	220
300	139	499	510	1,231	885	416
50*	130	471	560	2,110	1,740	160

*: Na pentobarbital

The induction of demethylase activity was similar to that found in Na phenobarbital group in both sexes at the 300 mg/kg/day dose level and at the 150 mg/kg/day dose level in the males. The 7-ethoxycoumarin O-deethylase activity at the 300 mg/kg/day dose level was half that of the positive control group. Thus, it would be considered a moderate cytochrome P-450 inducer at 300 mg/kg/day dose level.

TOXICOLOGY

Acute Toxicity Study:

The results are summarized below:

Species/ strain	No./Sex/ dose/ level	Route	Dose range (g/kg)	LD50	Time to death (days)	Maximum nonlethal dose	Toxic signs
Mouse/ICR	10 M	p.o.	1, 2, 5*	>1, <2.	1-3	1	(1)
Mouse/ICR	10	p.o.	1- 3.17*	1.8	1-3	1	(2)
Mouse/ICR	10	p.o.	0.5-3.2*	1.33	2	< 0.5	(3)
Mouse/ICR	10	p.o.	0.1-4.0**	4.0	2	< 1.0	(4)
Rat/Crl: CDBR	10	p.o.	0.1-4.0*	0.47	2	0.1	(5)
Rat/Crl: CDBR	10	p.o.	1.0-4.0**	>4.0	2	< 1.0	(6)
Mouse/ICR	10	i.p.	0.6-1.35**	0.89 - 1.03	3	0.73	(7)
Rat/Crl: CDBR	10	i.p.	0.6-1.10**	0.72 - 0.81	2-4	0.60	(8)
Mouse/ICR	10	s.c.	5.0**	5.0	1	5.0	(9)
Rat/Crl CDBR	10	s.c.	5.0**	5.0	--	5.0	(10)
Dog/beagle	1 M or 1 F	p.o. (capsules)	0.5 & 1.0	1.0	--	1.0	(11)

*: zileuton dissolved in corn oil.

** : zileuton dissolved in 0.2% hydroxypropylmethylcellulose (HPMC) observation period, 14 days.

(1)-(8): Squinting, dyspnea, unconsciousness, decreased activity, ataxia, rough coat, prostration, hypothermia and coma..

(9): Decreased activity, ataxia, squinting as dyspnea.

(10): Scab and masses in the area of the injection site.

(11): Ataxia, emesis, loose stool & mucoid stool.

The data suggests that higher toxicity was observed after administered drug in corn oil as compared with drug in 0.2% HPMC.

Acute Oral Toxicity Study in Combination with
Acetaminophen in Male Mice
(Study No. TD 88-124)

Date of the study: April 26, 1988 to Oct. 4, 1988.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Male Cr1: CD-1 mice weighing 19 to 27 g and age of 4-6 weeks were used.

Methods: Eight to nine groups of animals each consisting of 10 males were pretreated by gavage with 10 ml/kg of the vehicle (0.2% HPMC) or 150 mg/kg acetaminophen thirty minutes before treatment with zileuton at dose range of 1.08 - 4 g/kg in phase 1 study and 1.30 - 4 g in phase 2 study.

Results:

Clinical signs: Similar signs of CNS depression, such as decreased activity, ataxia, prostration and coma were observed, regardless of the pretreatment.

Mortality: LD50 value (2.11 g/kg) for male mice pretreated with acetaminophen was statistically lower than the LD50 value (3.32 g/kg) for male mice pretreated with vehicle in phase I study. In the phase II study, LD50 values were > 4 and ≥ 1.80 g/kg for mice pretreated with vehicle and acetaminophen, respectively. Thus, the data indicates that interaction between zileuton and acetaminophen may occur when both are simultaneously present.

Acute Oral Toxicity Study in Combination with
Indomethacin in Rats
(Study No. TA 88-232)

Date of the study: July 6, 1988 to Oct. 5, 1988

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Cr1: CD rats weighing 92-157 g were used.

Methods: Twelve groups of animals each consisting of 5 males and 5 females were pretreated with vehicle (corn oil) or 8 mg/kg indomethacin in corn oil thirty minutes before treatment with zileuton at dose range of 0.2 - 4 g/kg. Observation period was 14 days.

Results:

Clinical signs: Similar clinical signs of decreased activity, dyspnea, ataxia, diarrhea, prostration, urine-stained abdomen, ocular discharge, unconsciousness and sleep were observed; regardless of pretreatment.

Mortality: The LD50 values for zileuton in rats pretreated with either indomethacin or corn oil alone were greater than 4 g/kg (the largest dose of zileuton could be administered).

Thus, the data may suggest that there were no drug interaction between zileuton at dose of 4 g/kg and indomethacin at dose of 8 mg/kg.

SUBACUTE/SUBCHRONIC TOXICITY:

Three Month Maximum-tolerated Dose Study in Mice
(Study No. TD 88-173)

Date of the study: July 8, 1988 to May 30, 1989.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: CD-1 mice 21-23 days of age were used.

Methods: Five groups of animals each consisting of 10 males and 10 females were given zileuton (Lot 15-797-AL) via diet at dose levels of 0, 80, 200, 450 and 1000 mg/kg/day for 3 months to determine a maximum tolerated dose for an anticipated chronic assay according to sponsor.

Results:

Clinical signs (daily): There were no drug-related changes.

Mortality: One mouse in the control group was sacrificed in a moribund condition on day 56.

Body weight (weekly): There were no significant changes in treated mice when compared to controls.

Food consumption (weekly): Lower food consumption (10-16%) was noted in the 1000 mg/kg/group.

Hematology (terminal): Slightly high (6%) mean corpuscular hemoglobin concentration was found in the 1000 mg/kg group.

Blood chemistry (terminal): Decrease in cholesterol (47%) was observed in the males of 1000 mg/kg group.

Organ weight: Higher liver weight (37-95%) was seen in the 200, 450 and 1000 mg/kg/day groups. Lower adrenal weights (46-66%) were observed in all drug-treated males but were not dose related.

Gross pathology: Normal.

Histopathology: Hepatic cytomegaly was noted in the 450 and 1000 mg/kg/day groups.

In conclusion, zileuton given in diet at doses up to 1000 mg/kg did not produce any clinical signs of toxicity. At 200 mg/kg and above, it produced increase in liver weight and decrease in adrenal weight. Decrease in cholesterol level was seen in the 1000 mg/kg/day male group only. Hepatic cytomegaly was observed at the 450 mg/kg/day and above dose groups. Although a no effect dose was not established, at 200 mg/kg it produced only increased liver weight and decreased adrenal weight. At 80 mg/kg, only decrease in adrenal weight was observed.

6 Week Oral Toxicity Study in Immature Rats
(Study No. TA88-470)

Testing laboratory:

Date of the study: Dec. 13, 1988 to Aug. 4, 1989

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Sprague-Dawley rats weighing 26.7-37.9 g and 14 days of age were used.

Methods: Four groups of animals each consisting of 15-20 males and 15-20 females were given zileuton (Lot no. 15-797-AL) suspended in 0.2% hydroxypropylmethylcellulose at dose levels of 0 (vehicle), 20, 80 and 300 mg/kg/day for 6 weeks. Five males and 5 females for each group were killed after the first 3 daily doses for blood level determination of zileuton. Following the six weeks of dosing, 5 males and 5 females from the control and 300 mg/kg/day groups were retained untreated for a 2-week recovery period.

Results:

Clinical signs (daily): Salivation, uncoordinated body movements, thin body, yellow staining around the urogenital area, nasal staining, tail scab and alopecia were observed separately in 1-2 animals of the 300 mg/kg/day group.

Mortality: Two rats in the 300 mg/kg/day group found dead at weeks 1 and 4. Additional 4 rats in the 300 mg/kg group and 1 rat in the 80 mg/kg/day group were replaced. Autopsy of these dead animals revealed lung congestion and the finding was probably related to dosing error.

Body weight (daily or twice weekly): Retardation of body weight was observed in the 300 mg/kg group (15%). It returned to normal at recovery period.

Food consumption (weekly): The food consumption for the 300 mg/kg/day groups was lower (10%) than the controls but it returned to normal during recovery period.

Ophthalmoscopy (weeks 1, 6 and 8): There were no drug-related findings.

Hematology (weeks 6): Slightly higher hemoglobin (5%) level was observed in the 300 mg/kg group and persisted during recovery period.

Blood chemistry (week 6): Reduced (30%) SPOT and elevated cholesterol (73%) were observed in the females of the 300 mg/kg/day group. Elevations of protein (6%), globulin (9-13%) and albumin (5%) were seen in the 300 mg/kg/day group. They returned to normal at recovery period.

Urinalysis (week 6): Normal.

Organ weight: Increase in absolute liver weight (33%) was observed in the female of the 300 mg/kg group. At end of recovery period, increase in relative liver weight (9%) was still present. Decrease in adrenal weight (16-22%) was observed in all drug-treated groups. However, it returned to normal at end of recovery period except that in the female decrease in adrenal weight (22%) was observed.

Gross pathology: No treatment-related finding were observed.

Histopathology: Minimal centrilobular hypertrophy of hepatocytes with granulated cytoplasmic basophilia were observed in the livers of the 300 mg/kg/day group. Livers of the other groups and recovery group were not examined histopathologically.

In conclusion, zileuton given orally by gavage in immature rats starting at age of 14 days for 6 weeks produced minimal clinical signs, retardation of body weight gain, increase in hemoglobin, increase in liver weight and liver hypertrophy at 300 mg/kg. Thus, a no effect dose of 80 mg/kg/day was established.

3 Month Maximum-tolerated Dose Study in Rats (Study No. TA 88-172)

Date of the study: July 13, 1988 to May 31, 1989.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Crl: CDBR (Sprague-Dawley) rats weighting 50-75 g and 7 weeks of age were used.

Methods: Five groups of animals each consisting of 10 males and 10 females were given zileuton (Lot no. 15-797-AL) via diet at dose levels of 0, 80, 170, 350 and 750 mg/kg/day. Histopathological examinations of all tissues were conducted in the control and 750 mg/kg/day groups. The livers, kidneys and testes of the 80, 170 and 350 mg/kg/day groups were examined.

Results:

Clinical signs (daily): Sedation and thinness were observed in the 750 mg/kg group.

Mortality: None.

Body weight (weekly): Decreases (14-56%) in body weight gain were observed in the 350 and 750 mg/kg/day group.

Food consumption (weekly): Decreases (11-38%) in food consumption were noted in the 350 and 750 mg/kg/day groups.

Hematology (terminal): Increase (4-10%) in RBC was observed in the 750 mg/kg/day group.

Blood chemistry (terminal): BUN (29-43%), cholesterol (81-100%), total protein (12-13%) were increased in the 350 and 750 mg/kg/day groups. Elevation of SGOT (from 0-0.4 to 10.5 - 22.5 IU/L) and reduction of glucose (38-54%) were seen in the 750 mg/kg/day group.

Urinalysis (terminal): Presence of protein was noted in the 350 and 750 mg/kg/day groups.

Organ weight: Increases (25-75%) in liver weight were seen in the 170, 350 and 750 mg/kg/day groups. Increases in (11-22%) relative kidney weight were observed in the 170, 350 and 750 mg/kg/day groups. Increases in prostate (36%), uterus (38%) and ovary (28%) were found in the 750 mg/kg/day group.

Gross pathology: Pale discoloration of livers was noted in the 750 mg/kg/day group.

Histopathology: Cytomegaly of liver were found in all drug treated groups. Chronic nephritis was seen in the 350 and 750 mg/kg/day groups.

In conclusion, zileuton via diet produced clinical signs of toxicity, retardation of body weight gain, elevations of BUN, cholesterol and protein, proteinuria, increases in liver, kidney, prostate, uterus and ovary weights as well as cytomegaly and chronic nephritis at 750 mg/kg/day. Thus, target organs of toxicity were liver, kidney, prostate, uterus and ovary. A no effect dose was not established, however, at 80 mg/kg it produced only cytomegaly.

CHRONIC TOXICITY:

6 Month Oral Toxicity in Rats
(Study no. TA 88-028)

Date of the study: March 3, 1988 to April 28, 1989.

GLP requirement: A statement of compliance with GLP regulation was included.

Animals: Crl: CDBR (Sprague-Dawley) rats 4 weeks of age were used.

Methods: Five groups of animals each consisting of 20 males and 20 females were given zileuton (Lot no. 10-753-AL) suspended in 0.2% hydroxypropylmethylcellulose by gavage at dose levels of 0, 20, 80, 150 and 300 mg/kg/day. Additional 5 males and 5 females for the control and the 300 mg/kg/day groups were allowed for recovery period of 1 month before they were sacrificed. All tissues from the control and 300 mg/kg/day groups as well as from all rats that died or sacrificed in moribund conditions were examined histologically. The livers, kidneys, hearts, uteri from other groups were also examined histologically.

Results:

Mortality: Nineteen animals died and were attributed to esophageal injury. None were drug-related.

Clinical signs (daily): Salivation was observed in the 150 and 300 mg/kg/day groups.

Body weight (weekly): Retardation (11%) of body weight gain was observed in the 300 mg/kg/day group. However, it gained more weight than in the control during the recovery period.

Food consumption (weekly): Increases in food consumption was observed in all drug treated group.

Ophthalmology (terminal): Corneal dystrophy was observed in one each from the control, 20, 80 and 300 mg/kg/day groups. Epiphora was observed in one 300 mg/kg/day rat and a juvenile cataract was found in another 300 mg/kg/day rat.

Hematology (terminal): Lower hemoglobin level (5%) in the 300 mg/kg/day female group was observed. At the end of recovery period, no changes were seen.

Blood chemistry (terminal): Increases in cholesterol (22-32%), protein (5-7%) and phosphorus (13-24%) levels were observed in the 150 and 300 mg/kg/day groups. Elevation of protein (10%) was still present in the 300 mg/kg males at end of recovery period.

Organ weight: Increases (25-63%) in liver weight were observed in all drug treated groups. Increases in kidney weights (16-27%) were seen in the 150 and 300 mg/kg/day groups. Increases in thyroid (28%) weights were reported in the 300 mg/kg/day group. They returned to normal at end of recovery period.

Gross pathology: Hydrometra and/or pyometra (infection) were seen in the 300 mg/kg/day female group.

Histopathology: Cytomegaly and/or hypertrophy of livers were observed in the 80 mg/kg/day and above groups. Hepatocellular vacuolation were seen in the 150 and 300 mg/kg/day groups. Chronic nephritis were observed with higher frequency in the 150 and 300 mg/kg/day groups. Severe uterine luminal dilatations and endometritis was observed in the 300 mg/kg/day groups. All were absent in the recovery group.

In conclusion, zileuton given by gavage at doses up to 300 mg/kg/day for 6 months produced salivation, retardation of body weight gain, elevations of cholesterol, protein and phosphorus, increases in liver, kidney and thyroid gland weights and histopathological changes in liver, kidney and uterus. Thus, target organs of toxicity of liver, kidney, thyroid and uterus were identified. A no effect dose of 20 mg/kg/day was established.

6 Month Oral Toxicity Study in Monkeys

Date of the study: May 27, 1988 to April 28, 1989.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Cynomolgus monkey weighing 1.9 to 3.2 kg were used.

Methods: Four groups of animals each consisting of 4 males and 4 females were given zileuton (Lot no. 15-797-AL) suspended in 0.2% hydroxypropylmethylcellulose via nasogastric tube at dose levels of 0 (vehicle), 50, 150 and 500 mg/kg/day. Additional two males and two females each were assigned to the control and the 500 mg/kg/day groups for an additional 4 week recovery period.

Results:

Clinical signs (daily): Normal.

Mortality: None.

Body weight and food consumption (once or twice weekly): Normal.

Ophthalmology (weeks 0, 7, 14 and 25): Normal.

Plasma drug levels (weeks 10 and 22): Drug accumulation was not apparent.

Hematology (weeks 0, 6, 13 and 26): Statistically significant decreases in MCV (9%) and WBC (33-40%) were observed in the 500 mg/kg female group.

Blood chemistry (weeks 0, 6, 13 & 26): Normal.

Urinalysis (terminal): Normal.

Organ weight: Normal.

Gross pathology: Normal.

Histopathology: Normal.

In conclusion, zileuton given orally for 6 months were well tolerated at dose of 500 mg/kg except decrease in MCV and WBC of female. A no effect dose of 150 mg/kg/day was established.

REPRODUCTIVE TOXICITY:

Segment I Fertility and General Reproductive Performance
in Female Rats
(TA 97-375)

Date of the study: Nov. 13, 1987 to March 22, 1988.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Crl: CDBR (Sprague-Dawley) rats weighing 200 g were used.

Methods: Four groups of animals each consisting of 24 or 30 females were given zileuton (Lot no. 06-679-AL) suspended in 0.2% hydroxypropylmethylcellulose by gavage at dose levels of 0, 15, 70 and 300 mg/kg/day two weeks prior to mating, dosing continued during mating with untreated males and gestation. Dose selection was based on the 3 month oral toxicity study that it depressed body weight gain at 300 mg/kg/day (Study no. TA87-140). Dams were sacrificed on day 13 or day 21.

Results:

Clinical signs: Labored breathing, hypothermia, red discharge from eyes or nose, prostration preceded death in two females of 300 mg/kg/day group were observed. Gavage-related esophageal perforation in one death and erosive gastritis in other were the causes of death. Ataxia and alopecia were observed in the surviving 300 mg/kg/day group.

Body weight: Dams in the 300 mg/kg/day gained only half as much weight as the control group during the first week of treatment.

Mating and fertility indices: Normal

Day 13 interim kill: A statistically significant increase in post implantation loss and decreases in number of viable young were observed only in the 70 mg/kg/day group.

Parturition and progeny data: Longer gestation period (23 vs 22 days) was observed in the 300 mg/kg/day group. Eight out of 122 in the 70 mg/kg and 15/152 in the 300 mg/kg were "stillborn", but they were not

statistically significant as compared to those of the controls. However, significant dose-response trends were noted for increased still-birth and decreased live-birth with increasing dose. Sex ratio was normal. Pup of the 300 mg/kg group weighed less (13%) than the control.

In conclusion, zileuton at daily oral dosage up to 300 mg/kg did not produce any effect on the fertility of females. However, it produced stillbirth and decrease in live-births.

Segment I Fertility and General Reproductive Performance
(Study No. TA 88-001)

Testing laboratory:

Date of the study: Feb. 16, 1988 to June 15, 1989.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Rats received from Charles River Breeding Laboratories were used.

Methods: Five groups of animals each consisting of 45 males and 45 females were given zileuton (Lot no. 10-753-AL & 15-797-AL) in 0.2% HPMC by gavage at dose levels of 0, 15, 75, 150 and 300 mg/kg/day. The F0 males were dosed daily for 100 days prior to mating with the treated females and 80 days prior to mating with untreated females. Dosing continued throughout the study until one day prior to sacrifice. The treated F0 females were dosed for 14 days before mating and continued throughout gestation, and in 1/3 of the females through parturition and lactation period. All untreated female were sacrificed on day 13 of gestation for evaluation of pregnancy status and viability of embryo. Two thirds of treated female were necropsied on day 20 of gestation. The remaining treated females were allowed to litter normally. The offspring were evaluated for postnatal survivals, growth and developmental and behavioral indices. F1 generation were mated to assess their reproductive performance. The F2 fetuses were evaluated for viability and external development.

Results:

F0 observation:

Clinical signs: Salivation were noted in the 75 mg/kg/day and above dose groups.

Mortality: No compound related death occurred. A single death occurred in the 300 mg/kg/day female group. Lethargy and hypothermia were observed. The death of this female was not considered treatment-related because necropsy finding indicated a small cervix and small vagina. These anatomic abnormalities probably prevented normal parturition resulting in death. No treatment-related effect was apparent in males.

Body weight: No treatment-related effect was apparent in male. Retardation of body weight gain were observed in the 150 and 300 mg/kg/day female groups during the initial 3 days of dosing (1.5-2%) and during gestation (9-12%). Food consumption was diminished during the lactation period in the 150 and 300 mg/kg/day groups.

Fertility: Normal.

Estrous cycle: Extended period (3 or more consecutive days of estrus) of estrus phase in the 150 and 300 mg/kg/day groups (56-64% vs 29% in control) were observed.

Gestation day 13 uterine examination data of untreated female: The mean numbers of viable fetuses, pre- and postimplantation losses, implantation sites and corpora lutea were comparable to the vehicle control.

Gestation day 20 cesarean section and fetal evaluation:

Gestation period: It was increased in the 300 mg/kg (22.9 days) in comparison with the control group (21.9 days). One, 2, 6 females in the 15, 150 and 300 mg/kg groups, respectively, had total litter loss between lactation day 0 and 3.

Fetuses: Fetal weight in the 300 mg/kg/group was lower but was not statistically significant.

Fetal examination: Unossification of the skeletal structure was observed in the 300 mg/kg (majority of these variations were observed in one litter).

F₁ litter data:

Live litter size and viability indices: Statistically significant increases in number of dead pups on lactation day 0 occurred in the 75, 150 and 300 mg/kg/day groups. The live litter size in the 300 mg/kg/day group was reduced (7.1 vs. 13.5 pup/dams in control group). Although at the 75 and 150 mg/kg/day levels, decreases in viable litter size (10.8 and 9.6 pup/dam) were not statistically significant, the incidences were outside the range of the WIL historical control data (11.9-13.9). Pup survival indices in the 300 mg/kg/day were reduced during lactation day 4 (56.5%) and days 7-21 (93%). Sex ratio was not affected. Pup weights were decreased in the 150 and 300 mg/kg/day groups.

Development indices: Normal.

F₁ generation: There were no effect on all parameters measured except lower body weight were observed in the 150 and 300 mg/kg/day groups (8-17%).

The above-mentioned findings are listed below:

Dose groups (mg/kg)	0	15	75	150	300
FO					
gestation period (days)	21.9	21.8	22.1	22.0	22.9
total litter loss	0	1	0	2	6
fetal weight (g)	3.4	3.2	3.3	3.5	3.1
F1					
number of dead pups on lactation day 0	0	1	0	2	6
Live litter size (pups/dams)	13.5	12.3	10.8	9.6	7.1
Pup survival (%) on lactation day 4	100	92.6	98.5	84	56.5
lactation days 7-21	100	98-100	99	97	93.3
Pup weight (g) on lactation day 7	15.9	15.6	16.1	12.8	12.0
on lactation day 14	33.1	31.9	32.8	28.1	25.9
Fetal examination					
No. of fetuses examined	268	238	274	180	142
Ischium unossified	0	2	0	2	8
Hyoid unossified	0	2	0	0	5
Reduced ossification of skull	0	0	0	0	2
Sternum unossified	0	2	0	1	6
Reduced ossification of the 13 rib(s)	0	2	0	1	3

In conclusion, all maternal and fetal parameters were comparable between the treated groups and the vehicle group in phase I study (treated males mated with untreated females). Thus, data suggests that it had no effect on male in fertility and reproductive performance. Zileuton at doses 150 mg/kg/day and above produced retardation of maternal body weight gain, occurrence of protracted estrus period, decreases in the number of implantation sites and viable fetuses, decrease in pup body weight as well as depression of body weight gain in F1 generation. At 300 mg/kg it produced lower fetal weight, increases in unossification of fetal skeletal structure, decrease in pup viability and prolongation of gestation period as well. Significant increases in the number of dead pups on lactation day 0 and decreases in live litter size were observed in

the 75 mg/kg/day and above. Since maximum tolerated dose appears to be 150 mg/kg, the effects produced at higher dose level (300 mg/kg) could be attributable to its maternal toxicity. Thus, zileuton at its maximum tolerated dose had no antifertility effect but produced fetal toxicity and developmental toxicity (increase in perinatal death and decrease in litter size).

Segment II Teratology Study in Rabbits
(Study No. TE 87-358)

Testing laboratory

Date of the study: Nov. 17, 1987 to April 7, 1988.

GLP requirement: Statement of compliance with GLP regulations was included.

Animals: Female New Zealand white rabbits were used.

Methods: Four groups of animal each consisting of 20 pregnant rabbits were given zileuton (Lot no. 46F-0579) suspended in 0.2% hydroxypropylmethylcellulose by gavage at dose levels of 0, 15, 50 and 150 mg/kg/day during days 6-18 of gestation period. Dams were sacrificed on day 29 of gestation. All fetuses were subjected to visceral and skeletal examinations.

Results:

Mortality and abortion: Three dams of the 50 mg/kg aborted.

Clinical signs: Normal.

Body weight: Retardation of body weight gain (24%) occurred in the 150 mg/kg/day group.

Food consumption: No statistically significant effect was observed.

Cesarean sectioning data: Dose-related decreases in number of implantation were seen in the 50 and 150 mg/kg/day groups but were not statistically significant.

Litter data: No differences in fetal sex ratio and fetal weight were observed.

Fetal examinations: Cleft palate occurred in three of 118 fetuses (or 2 of 17 litters) of the 150 mg/kg/day group. There were no incidences of this major malformation in the concurrent controls. Sponsor indicated that it was within the range observed historically. However, we disagree with sponsor's conclusion because incidence of cleft palate (2.5%) in the study is beyond upper range (2%) of the historical control (see page 19-100 of Vol. 1.19). In addition to the observed cleft palate, two fetuses (1.7%) had domed head. Hydrocephalus occurred in two fetuses (1.7%) of the 150 mg/kg/day group. None of the above-mentioned findings were reported in the concurrent control group.

In conclusion, zileuton at oral dose of 150 mg/kg/day produced cleft palate in the rabbits. It may possess a teratogenic potential.

Segment III Peri- and Postnatal Development Study in Rats
(Study no. TA 87-426)

Date of the study: Feb. 9, 1988 to August 16, 1988.

GLP requirement: A statement of compliance with GLP regulations was included.

Animals: Female Cr1: CDBR (Sprague-Dawley) rats were used.

Methods: Four groups of animals each consisting of 24 pregnant rats were given zileuton (Lot no. 06-679-AL) suspended in 0.2% HPMC by gavage starting gestation day 15 and continuing through parturition and lactational period at dose levels of 0, 15, 70 and 300 mg/kg/day.

Results:

Mortality: Two and one dams in the 15 and 70 mg/kg/day groups, respectively, were found dead or killed in poor condition. No drug-related mortality was observed.

Clinical signs: Decreased activity and ataxia were seen in the 300 mg/kg/day group.

Body weight and food consumption: Retardation of body weight gain (38%) and reduction of food consumption were observed in the dams of the 300 mg/kg/day group.

Length of gestation, litter size, live births and stillbirths: Length of gestation was prolonged (22.1 days) in the 300 mg/kg/day group as compared with the control group (21.6 days). There were no effects on litter size, live births and stillbirths.

Pup: Low pup weight (8-13%) was observed in the 300 mg/kg/day group.

Postnatal survival of pups: Lower survival rate (55-74%) was observed in the 300 mg/kg/day group. Gross examination of pup revealed ony hematoma.

In conclusion, zileuton given orally to female rats starting 14 days of gestation period continuing till lactation produced maternal toxicity, prolongation of gestation period, lower pup weight and lower pup survival rate.

MUTAGENICITY STUDIES:

CHO/HGPRT Mutation Assay
(Study No. T87-456)

Date of the study: Dec. 4, 1987 to July 19, 1988.

Testing laboratory:

Methods: Zileuton (lot no. 06-679-AL) at concentrations of 20 to 320 ug/ml was evaluated for its ability to induce forward mutations at the HGPRT locus in CHO cells in the presence and absence of a metabolic activation. DMSO was used as the solvent control and ethylmethanesulfonate (0.2 ug/ml) and benzopyrene (4 ug/ml) were used as the positive control. Sponsor's criteria of positive result were (1) the mutant frequency must exceed 20 mutant per 10⁶ colonable cells and (2) must be at least twice that the solvent control.

Results: In the absence of S-9, significant increases in thioguanine-resistant mutants were observed in the first assay. However, the increase was not dose-dependent and could not be reproduced in the confirmation assay. No increase in mutants were observed in the presence of S-9. Thus, it was concluded that it had no mutagenicity. Positive control exhibited a typical positive result.

Plate Incorporation Reverse Mutation Assay
(Study no. T87-454)

Testing laboratory:

Date of the study: Dec. 22 1987 to May 11, 1988

Methods: Zileuton (lot no. 10-753-AL) dissolved in DMSO at concentrations of 67 - 1000 ug/plate was tested on E. coli strain WP2 Trpvra in the presence and absence of a metabolic activation. Following a 48 hour incubation at 37°C, revertant colonies per plate were counted. 2-Aminoanthracene (10 mg/plate) and methylmethanesulfonate (1 mg/plate) were used as the positive controls in the presence and absence of S-9, respectively. DMSO was used as the vehicle control. For a test article to be considered positive, it must produce at least two times the revertants over the vehicle control and must be accompanied by a dose-related response to increasing concentration of the test article.

Results: There were no positive increase in mutant colonies either in the presence or absence of metabolic activations. Positive controls exhibited typical positive results.

Micronucleus Test in Mice
(Study no. TD87-403)

Testing laboratory:

GLP requirement: A statement of compliance with GLP regulation was included.

Date of the study: Dec. 22, 1987 to July 11, 1988.

Methods: CD-1 mice (5 males and 5 females at each dose level) were given a single i.p. injection of zileuton (Lot no. 10-753-AL) dissolved in 1% carboxymethylcellulose at dose levels of 170, 850 and 1700 mg/kg for males and 94, 466 and 930 mg/kg for females. Lower dosages were chosen on the basis of a toxicity range-finding study that greater toxicity of zileuton was observed in female mice. Bone marrow was collected 24, 48 and 72 hours after treatment and examined for any increase in micronuclei in polychromatic erythrocytes. Triethylenemelamine (0.25 mg/kg) was used as the positive control and 1% CMC was used the negative control. The criteria for positive response were (1) statistical increase must be observed as compared to the vehicle control and (2) the positive response must be dose-dependent or must be observed at a single dose level at adjacent sacrifice time.

Results: Zileuton failed to increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow at i.p. dose up to 1700 mg/kg for male and 940 mg/kg for females. Positive control induced a positive result. In conclusion, it had no mutagenic potential in the test.

Chromosome Aberrations in Human Lymphocytes
(Study no. T87-455)

Testing laboratory:

Date of the study: Dec. 4, 1987 to July 13, 1988.

Methods: Zileuton (lot no. 06-679/AL) dissolved in DMSO at concentrations of 24-185 ug/ml and incubated for 24 hours in the absence of metabolic activation and of 47-370 ug/ml for 4 hours in the presence of metabolite activation was tested for detection of any chromosome aberrations in human lymphocytes. Mitomycin (25 ug/ml) and cyclophosphamide (5 mg/ml) were used as the positive controls. The criteria for positive result was statistical increase in a dose-responsive manner.

Results: No significant increases in chromosome aberration were found in the zileuton-treated groups. Positive controls yielded a typical positive result. This, it did not produce any mutagenicity in the test.

SPECIAL STUDIES:

Passive Cutaneous Sensitization in Guinea Pigs
(Study No. TF89-061)

Date of the study: May 8, 1989 to Aug. 31, 1989

GLP requirement: A statement of compliance with GLP regulations was included.

Methods: Five male Hartley guinea pigs per group (groups T₀-T₃) were given 6 subcutaneous sensitization injections of either 0.2% hydroxypropylmethylcellulose, egg albumin in 0.2% HPMC (1 mg/animal/injection) or zileuton (Lot no. 06-079-AL) in 0.2% HPMC (30 mg/animal/injection). One group of animals remained untreated (group T₃). Following a two-week period after the sensitization injections, blood was withdrawn from these animals and pooled for each group, and dilutions (1/16, 1/64 and 1/256) of antiserum were prepared. Each guinea pig in test groups T₄, T₅, T₆ and T₇ was given intradermal injections (0.1 ml) of each dilution of antiserum from groups T₀, T₁, T₂ and T₃, respectively. Intravenous challenge doses (1 ml/animal) were given 4-5 hours later with either 2 parts DMSO: 1 part saline (T₄), 1 mg/animal egg albumin in saline (T₅) or 30 mg/animal zileuton in parts DMSO: 1 part saline (T₆ and T₇). Immediately following each injection, each animal was given a 1 ml intravenous injection of 0.5% Evans's Blue dye in saline. Approximately thirty and ninety minutes following injection PCA reactions were measured. The PCA reaction was determined in Grading Index of 1-4 according to the size of reaction from 5 to greater than 20 mm.

Results: Guinea pigs given egg albumin developed severe PCA reactions which varied as a function of the concentration of antiserum given. Animals challenged with vehicle or zileuton did not develop PCA reactions. Therefore, zileuton was negative in the passive cutaneous anaphylaxis test.

Active Systemic Anaphylaxis In Guinea Pigs
(Study No. TF 89-266)

Date of the study: July 14, 1989 - September 1, 1989.

GLP requirement: A statement of compliance with GLP regulations was included.

Methods: Six male Hartley guinea pigs were assigned to each of four treatment groups (T₀-T₃). Group T₀ received vehicle for both sensitization and challenge. Group T₁ received zileuton (Lot no. 06-679-AL) at doses of 50 mg/animal/treatment for sensitization and 25 mg/animal for the challenge. Group T₂ was not treated during the sensitization period, but was challenged with zileuton at a dose of 25 mg/animal. The vehicle for group T₀-T₂ was 0.2% hydroxypropylmethylcellulose for sensitization and 2 parts dimethyl sulfoxide (DMSO): 1 part 0.9% NaCl for injection for the challenge. Group T₃ received egg albumin (positive control) in 0.9% NaCl for injection at doses of 1 mg/animal/treatment for sensitization and 5 mg/animal for the challenge. The routes of administration were intraperitoneal for sensitization and intravenous for challenge.

Sensitization consisted of six injections given three times weekly over a two week period. Approximately two weeks after the last sensitization treatment, animals were given a single challenge injection. All guinea pigs sensitized and challenged with egg albumin convulsed and died shortly after the intravenous challenge.

Results: No anaphylactic reactions were observed in guinea pigs challenged with the vehicle or zileuton. Therefore, zileuton was negative in the active systemic anaphylaxis test.

Antigenicity Study in Guinea Pigs
(Study no. TF89-184)

Date of the study: July 25, 1989 to Oct. 5, 1989.

Methods: Three groups of Hartley guinea pigs each consisting of five males were treated during sensitization with 0.2% hydroxypropylmethylcellulose (HPMC) mixed with either complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (IFA); 2 mg/animal/treatment egg albumin in 0.9% NaCl mixed with either CFA or IFA and 50 mg/animal/treatment zileuton (Lot no. 06-679-AL) in 0.2% HPMC mixed with either CFA or IFA. CFA was used in formulations for the first sensitization period, and IFA was used in formulations for the second and third sensitizations. The sensitization injections were given one day each week over a three week period. Two weeks after the last sensitization injection, blood samples were collected from each guinea pig, and antiserum was prepared. Antiserum from each animal from each test group was tested in the gel diffusion test for antigen-antibody precipitation reactions.

Results: No antigen-antibody precipitation was observed in test discs containing antiserum from guinea pigs sensitized with vehicle or zileuton. However, precipitation bands were observed in all discs containing antiserum from guinea pigs sensitized with egg albumin. Therefore, zileuton produced no immunological response in guinea pigs.

SUMMARY AND EVALUATION:

Zileuton is a benzothiopehane N-hydroxyurea and is an active 5-lipoxygenase inhibitor and consequently a leukotriene synthesis inhibitor. Leukotriene have been implicated in the pathophysiology of several disease processes. Inhibition of leukotriene synthesis has many potential benefit for conditions in which leukotriene synthesis is elevated. Thus, zileuton may have many potential therapeutic benefits in condition such as inflammatory bowel disease, psoriasis, rheumatoid arthritis and asthma.

Sponsor proposed a randomized, double-blind, placebo-controlled study in patients with moderate ulcerative colitis to examine the safety and efficacy of a range of dosage regimens of zileuton. The proposed dose levels are 400 mg b.i.d. (16 mg/kg/day based on a 50 kg body weight), 800 mg b.i.d. (32 mg/kg/day) or 600 mg q.i.d. (48 mg/kg/day) for 8 weeks.

Supportive preclinical documents for the IND included reports pertaining to (1) pharmacological studies; (2) absorption, distribution, metabolism and excretion; (3) study of acute toxicity after a single oral dose in ICR mice, Sprague-Dawley rats and beagle dogs or a single i.p. or s.c. dose in ICR mice and Sprague-Dawley rats; (4) Acute oral toxicity in combination with bronchodilator and anti-inflammatory agents in male Crl: CD-1 mice; (5) acute oral toxicity in combination with acetaminophen in male Crl:CD-1 mice; (6) acute oral toxicity in combination with indomethacin in Sprague-Dawley rats; (7) 3 month maximum-tolerated dose study in mice; (8) 10 day oral range-finding in Sprague-Dawley rats; (9) 6 week oral toxicity study in immature Sprague-Dawley rats; (10) 3 month oral toxicity study in Sprague-Dawley rats; (11) 3 month maximum-tolerated dose study in Sprague-Dawley rats; (12) 6 month oral toxicity study in Sprague-Dawley rats, (13) 10-11 day oral dosage range-finding study in beagle dogs; (14) 3 month oral toxicity study in beagle dogs; (15) 1 month oral toxicity study in cynomolgus monkeys; (16) 6 month oral toxicity study in cynomolgus monkeys; (17) Segment I oral fertility and general reproductive study in female Sprague-Dawley rats; (18) Segment I fertility and general reproductive study in Sprague-Dawley rats; (19) Oral segment II teratology study in Sprague-Dawley rats; (20) Oral segment II teratology study in New Zealand rabbits; (21) Segment III peri- and postnatal development in Sprague-Dawley rats, (22) Mutagenicity studies (Ames test, reverse mutation assay, unscheduled DNA synthesis in rat hepatocytes, CHO/HGPRT mutation assay, micronucleus test in mice and chromosome aberration in human lymphocytes) and (25) special studies (passive cutaneous sensitization in guinea pigs, active systemic anaphylaxis in guinea pigs and antigenicity study in guinea pigs).

Most of the above mentioned studies are reviewed at this time. However, evaluation for the following previously submitted studies is based on Dr. Tai's reviews (see attached appendix): pharmacological effects, acute oral toxicity study in combination with bronchodilator and anti-inflammatory agents in male mice, 3 month oral toxicity study in rats, 3 month oral toxicity study in dogs, 1 month oral toxicity study in monkeys, Segment II teratology study in rats, Ames test and unscheduled DNA synthesis in rat hepatocytes.

Zileuton has been demonstrated to be a 5-lipoxygenase inhibitor in rat basophilic leukemia-1 cells with IC₅₀ of 0.53 μ M and a leukotriene synthesis inhibitor in the several in vitro systems (intact polymorphonuclear leukocytes in rats and human and human whole blood) with IC₅₀ of 0.16-0.7 μ M). S-Enantiomer was more potent than racemate and its R-enantiomer in the inhibitions of 5-lipoxygenase of the broken RBL-1 cells and formation of LTB₄ in the human whole blood. It reduced the LTD₄-induced guinea pig tracheal contraction. It weakly inhibited the binding of LTB₄ to human polymorphonuclear leukocyte membranes. It was more active than BW755c, phenidone, indomethacin and NDGA in inhibition of leukotriene synthesis in rats following oral administration with ED 50 of 3.1 mg/kg. It possessed anti-inflammatory activity in

arachidonic-induced edema in mice (ED 50=31 mg/kg, p.o.), passive Arthus reaction (ED50=4-10 mg/kg, p.o.) and acute endotoxemic reaction (ED50=21 mg/kg, p.o.) in rats. Inhibition of ex vivo LTB₄ biosynthesis was demonstrated in dog, rat and monkey whole blood following oral administration (ED50 were 0.7 and 1.4 mg/kg for dogs and rats, respectively). It prevented the antigen(ovalbumin)- or non antigen (arachidonic acid)-induced bronchoconstrictive response with oral ED 50 of 12-24 mg/kg in the guinea pigs. In the oral dose range of 10-100 mg/kg, it had no effect on the CNS. At oral dose of 0.5 or 50 mg/kg, it did not produce any effects on cardiopulmonary hemodynamic parameters in anesthetized dogs. In conscious dog, it had no effect on pulmonary arterial pressure, mean arterial pressure and heart rate at oral doses of 5 and 50 mg/kg.

Pharmacokinetics of zileuton have been studied in dog, rat, monkey, mouse and man. Oral absorption was moderate to slow. T_{max} were 1-6 hr for rats, 2-6 hr for dogs, 2 hr for monkeys and 2.2 hr for man. Elimination T_{1/2} was variable among species and man: 2-3 hours in rats, 6.5-8.6 hrs in dog, 1-3 hrs in monkey and 1-4 hr in man. The shorter half-life (0.3 hr) after intravenous administration was reported in monkeys.

Major organs of distribution following oral administration in rats were stomach, small intestine, bladder, thyroid, liver, adrenal kidney, pancreas and lung. In dogs, radioactivity was found in liver and kidney. It had a slightly higher affinity for the pigmented than the nonpigmented tissues of the eye in dogs.

Metabolism was extensive after both oral and intravenous administration in rats, dogs, monkeys and man as minimum amount in unchanged form were present in urine. Major metabolites consisted of [R]- and [S]-zileuton glucuronide in monkeys and man and predominantly [S]-zileuton in dogs. In rats, polar metabolite (conjugates of an unidentified metabolite) was the major urinary metabolite while glucuronide were the minor metabolite. Parent drug was the major circulating component in rats, dogs and man whereas ABBOTT-66193 (N-dehydrate metabolite) was the major circulating component in monkeys.

The excretion of radioactivity was rapid and urinary excretion is a major route of elimination in all species.

Although it appeared that monkey is a better choice of preclinical study based on its pharmacokinetic studies (metabolism and elimination t_{1/2}), AUCs do not increase when doses above 500 mg/kg are given and the large amount of ABBOTT-66193 was present in plasma. In addition, based on the dose-normalized 24 hr AUC (expressed as ug.hr/ml per mg/kg/day) at the

dose level for which the largest AUCs were measured were 0.08, 3.5, 4.4, 0.6 and 2.0 for monkey, dog, rat, mouse and man, respectively. The data indicate that man is intermediate among the species, with monkey and mouse at the low end and dog and rat at the high end.

In acute toxicity studies, when compounds were prepared in corn oil the highest nonlethal oral doses were less than 0.5 g/kg in mice and 0.1 g/kg in rats, and the LD 50 were 1.33 g/kg in mice and 0.47 g/kg in rats. However, the highest nonlethal oral dose was 1g/kg in mice and rats and the LD 50 were 4 g/kg in mice and > 4 g/kg in rats when compounds were prepared in 0.2 % hydroxycellulose. The data suggests that higher toxicity of zileuton was observed after administration in corn oil compared with the drug in 0.2 % HPMC. In dog, the highest nonlethal dose was 1 g/kg and the LD50 was > 1 g/kg. The highest nonlethal i.p. doses were 0.73 g/kg in mice and 0.6 g/kg in rats and the LD 50 were 0.89-1.03 g/kg in mice and 0.72-0.81 g/kg in rats. Clinical signs of decreased activity, ataxia, dyspnea, prostration, hypothermia, squinting, unconsciousness, coma were noted in mice and rats following oral or i.p. administration. In dogs, ataxia, emesis, loose and mucoid stool were observed. Following s.c. administration, scab and masses in the area of the injection were seen in mice and rats. The highest nonlethal s.c. dose was <5 g/kg in mice and 5 g/kg in rats and the LD50 were > 5 g/kg in mice and 5 g/kg in rats.

No drug interaction with bronchodilator and anti-inflammatory agents was present in the acute toxicity study in male mice. However, potentiation of lethality of zileuton by acetaminophen was indicated. There was no effect of indomethacin on lethality of zileuton in the acute oral study in rats.

In the 3 month maximum tolerated dose study in mice, zileuton given in diet at dose levels of 0, 80, 200, 450 and 1000 mg/kg/day was tested. It produced no clinical signs of toxicity at dose up to 1000 mg/kg. However, it produced increase in liver weight and decrease in adrenal weight at doses of 200 mg/kg and above. Hepatic cytomegaly was observed at 450 mg/kg/day and above. Although a no effect dose was not established, at 200 mg/kg it produced only increase liver and decreased adrenal weights.

In the 6 week oral toxicity study in immature rats, zileuton was given by gavage starting at age of 14 day for 6 weeks at dose levels of 0, 20, 80 and 300 mg/kg/day. It produced minimal clinical signs of salivation, uncoordinated of body movement and alopecia, retardation of body weight gain, increase in hemoglobin, increase in liver weight and liver hypertrophy at dose level of 300 mg/kg. A no effect dose of 80 mg/kg was established.

In the 3 month oral toxicity study in rats, zileuton at gavage dose levels of 0, 5, 20, 80 and 300 mg/kg/day was tested. It produced clinical signs of toxicity (alopecia and salivation), elevation of alanine aminotransferase and cholesterol, increase in liver weight and hepatic cytomegaly at 80 mg/kg and above. It prolonged estrus at 300 mg/kg. Thus, a no effect dose of 20 mg/kg was established.

In the 3 month maximum-tolerated dose study in rats, zileuton at dietary doses of 0, 80, 170, 350 and 750 mg/kg/day was tested. It produced clinical signs of toxicity, retardation of body weight gain, elevations of BUN and protein, proteinuria, increases in liver, kidney, prostate, uterus and ovary weights as well as cytomegaly and chronic nephritis at 750 mg/kg/day. At 350 mg/kg, it also exhibited retardation of body weight gain, elevation of BUN, cholesterol and protein, proteinuria, increases in liver, kidney, cytomegaly and chronic nephritis. Thus, target organs of toxicity of liver, kidney, prostate, uterus and ovary were identified. A no effect dose was not established. At 80 mg/kg, it produced only cytomegaly.

In the 6 month oral toxicity study in rats, zileuton was given by gavage at dose levels of 0, 20, 80, 150 and 300 mg/kg/day. It produced salivation, retardation of body weight gain, elevations of cholesterol, protein and phosphorus, increases in liver, kidney and thyroid weights and histopathological changes in liver, kidney and uterus at 300 mg/kg. Thus, target organs of toxicity were liver, kidney, thyroid and uterus. A no effect dose of 20 mg/kg/day was established.

In the 3 month oral toxicity study in dogs, zileuton at dose levels of 0, 5, 20, 100 and 400 mg/kg/day was tested. It produced mortality, body weight loss, clinical signs of toxicity, hematological changes, increases in liver and adrenal weights, decrease in heart and prostate weights and histopathological changes in prostate, testis, and uterus at 400 mg/kg. At 100 mg/kg, it exhibited clinical signs of toxicity, retardation of body weight gain and histological changes in testis. However, prostate atrophy and testicular atrophy were found also at 20 mg/kg/day in the one month interim study. Thus, a no effect dose of 20 mg/kg was established, lethal dose was 400 mg/kg.

In the one month oral toxicity study in monkeys, zileuton at gavage dose levels of 0, 20, 50, 150 and 360 mg/kg/day was tested. It produced no toxicity at dose up to 360 mg/kg/day. However, the study was conducted with almost 50% of the monkeys being not sexually matured.

In the six month oral toxicity study in monkey, zileuton was tested at gavage dose levels of 0, 50, 150 and 500 mg/kg/day. It produced only decreases in MCV and WBC in females at 500 mg/kg/day. Thus, a no effect dose of 150 mg/kg/day was established.

Enhancement of toxicity in rats and monkeys was not evident with increase duration of administration based on the no effect or tolerated doses. This has been reflected by the pharmacokinetic studies that drug accumulation was not present. Common target organs of toxicity were liver, kidney and reproductive system in both rats and dogs. Monkey appeared to be well tolerated with no effect doses of 360 mg/kg in the one month study and 150 mg/kg in the 6 month study and tolerated dose of 500 mg/kg in the 6 month study being reported.

In the segment I fertility and general reproductive performance study in rats, zileuton was given at dose levels of 0, 15, 75, 150 and 300 mg/kg. It had no antifertility effects on males. It induced occurrence of protracted estrus period, decreases in the number of implantation sites and viable fetuses, decrease in pup body weight as well as depression of body weight gain of F1 generation. At 300 mg/kg, it produced lower fetal weight, increase in unossification of fetal structure, decrease in pup viability, prolongation of gestation period and stillbirth. However, 300 mg/kg/day appears to exceed its maximal tolerated dose (150 mg/kg). Thus, it had no antifertility effects on females but produced fetal toxicity.

In the oral segment II teratology study in pregnant rats, zileuton was administered by gavage from days 6-15 of gestation period at dose levels of 0, 15, 70, 150 and 300 mg/kg/day. It produced minimal maternal toxicity (clinical signs and retardation of body weight gain) and fetal development retardation (delay in ossification and low fetal body weight) at dose of 300 mg/kg/day. It did not induce any teratogenic effect.

In the oral segment II teratologic a study in pregnant rabbits, zileuton was given by gavage at dose levels of 0, 15, 50 and 150 mg/kg/day during days 6-18 of gestation period. It produced cleft palate at 150 mg/kg/day. Thus, it may possess a teratogenic potential.

In the segment III peri- and postnatal development study in rats, zileuton given by gavage at dose levels of 0, 15, 70 and 300 mg/kg/day produced prolongation of gestation period lower pup weight and decreased pup survival rate.

Zileuton did not induce any mutagenicity in the Ames test at concentrations up to 5 mg/plate. It did not induce any increase in revertant colonies in *E. coli* WP2 Trpuvra at concentrations up to 1 mg/plate. It did not produce a reproducible dose-responsive increase in forward mutation in CHO/HGPRT assay. There was no significant increase in the unscheduled DNA synthesis at concentration up to 30 ug/ml in rat primary hepatocytes. It did not induce any mutagenicity in the micronucleus test in mice after i.p. doses up to 1700 mg/kg for males and 940 mg/kg for females. No mutagenic potential was detected in chromosome aberration test in human lymphocytes at concentrations up to 370 ug/ml in the presence of metabolic activation and 185 ug/ml in the absence of metabolic activation.

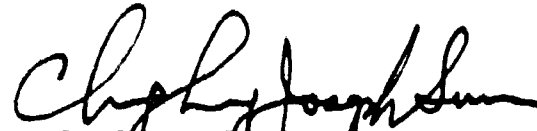
It was negative in the passive cutaneous anaphylaxis test and active systemic anaphylaxis test in guinea pigs. It did not induce any immunological response in the antigenicity study in guinea pigs.

Safety for the proposed clinical dose levels (12-48 mg/kg) is not supported by the mice, rat and dog studies since no effect dose level was 20 mg/kg in both rat and dog studies and tolerated dose level was 80 mg/kg in the mouse study. Although it was well tolerated up to 500 mg/kg day in monkeys following 6 month of oral administration, we can not

assess the safety margin of the proposed clinical dose levels from the no effect dose established from the study because of its shorter elimination half-life and different plasma metabolic profile in the monkey. However, by comparing the pharmacokinetic data in monkey and man, systemic exposure of 500 mg/kg in monkeys (AUC = 17.4 ug.hr/ml) was approximately equivalent to 800 mg (16 mg/kg) in humans (AUC = 14.5-20.6 ug.hr/ml).

RECOMMENDATION:

From the preclinical toxicity studies and pharmacokinetic studies in animals and humans, we concluded that the safety of the proposed clinical doses is not supported by the existing data. We, therefore recommend clinical hold for this IND. However, safety of a clinical dose of 400 mg, b.i.d. (16 mg/kg/day) is supported by the toxicity and pharmacokinetic studies in monkeys.


Ching-Long Joseph Sun, Ph.D.

Oct. 16, 1990

cc:
Orig. IND
HFD-180
HFD-181/CSO
HFD-180/Dr. Sun
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd
HFD-502/Dr. Contrera
HFD-345/Dr. James
CJS/IDK/9/19/90;10/12/90/w2134c

Concur



10/17/90

Attachment: Appendix - Dr. B.-C.-Y. Tai's Pharmacology
Reviews of IND.

Pharmacology Review
(supplement, serial #061)

JUL 12 1993

IND:

Sponsor: Abbott

Date Submitted: June 18, 1993

Date Received by HFD-7: June 21, 1993

Date Receive by Reviewer: June 28, 1993

Date of Review: July 8, 1993

Drug: Zileuton

Category: 5-L0 Inhibitor

Category:

NDA 020471

FIRM: ABBOTT LABS

8 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

Zileuton treatment in dogs for 1-3 months at 100 mg/kg/oral and higher doses showed prostatic atrophy and testicular degeneration. In this report the reversibility of the changes after one month of treatment was monitored for 6 months.

Study design:

Pure bred beagle dogs 7-8 months of age and 9-13 kg body weight were dosed at 100 mg/kg/oral with zileuton for one month. After the treatment histological changes in testes and prostate were monitored for 1, 3 and 6 months. These changes were compared to that observed immediately following one month dosing of dogs. Several groups of dogs were employed in the study with appropriate controls. Each drug untreated groups had 2 dogs.

Results:

No mortality was reported in the study. Major clinical signs were diarrhea and emesis which were reversible upon discontinuation of the treatment.

Body weight gain during the treatment was 0.52 kg in the control dogs and treated dogs lost about 1.18 kg during the same period. During recovery period, zileuton treated dogs recovered to the control level of weight gain. This was evident in the 3 and 6 month period post dosing. Food consumption was also reduced during the treatment period. At the end of the treatment area under the plasma concentration/time curve was about 630 ug hr/ml. It should be noted that inactive isomer of zileuton has higher plasma level and may account for most of the drug found in the plasma compared to the active form. Therefore, kinetics of enantiomers should have been determined to determine whether the toxicity was related to the inactive enantiomer of zileuton.

Blood chemistry and hematological analyses were not done in this study.

Weight of prostate in treated dogs was lower than that of 2 control dogs when absolute weights or normalized weights been compared at the end of 4 weeks dosing, testicular weights in the treated dogs were also smaller than control dogs although it was not statistically significant. It is difficult to judge whether this change in the weight of prostate and testes were treatment related or due to the sexual immaturity of dogs because 6 to 7 months old dogs often have smaller testes and prostate. However, three month recovery data did not show differences between control and treated groups with respect to weight of prostate and testes.

Microscopic Findings:

At the end of one month, hypospermia (40%), atrophy of prostate (80%), testicular degeneration (20%) and spermatogenic arrest (20%) were noted in treated dogs. None of above changes were noted in the control dogs. Degenerative changes in testes and atrophy of prostate continued to recover slowly following 3 and 6 months of withdrawal of the treatment. However, at the end of 6 months 60% of dogs showed degenerative changes in testes. Degenerative changes in testes in parallel control dogs were also noted at the end of the treatment. Therefore, atrophy of prostate was the major histological changes in the study which continued after withdrawal of the treatment for 6 months.

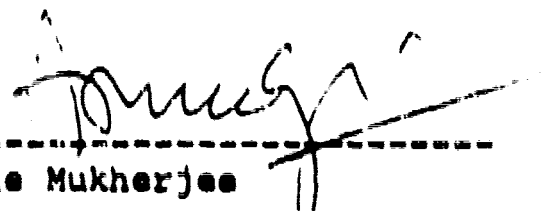
Discussion:

The result of this study suggest that zileuton treatment at 100 mg/kg oral for one month is associated with testicular atrophy in 6-7 months old beagle dogs which persisted even after 6 months of withdrawal of the treatment. Although the severity of prostate atrophy was reduced after withdrawal of the treatment yet it should not be considered as a completely reversible phenomena. This information should be included in the investigator brochure and patient consent form. Mechanism of this effect is not clearly known. However, one can speculate that this might have resulted from antiandrogenic effect. My previous review dated July 22, 1991 mentioned that both R(+) and S(-) inhibited testosterone binding in prostate e.g. pK_i around 5.07 to 5.24 in dogs. For human, pK_i was 3.21 - 3.93. These data would suggest that atrophy of prostate in dogs was related to antiandrogenic effect. Stereoselective metabolism of S(-) enantiomer also builds up inactive R(+) enantiomer in dogs which could be responsible for the atrophy of prostate. Anyway, if the antiandrogenic mechanism is involved, than it is unlikely that zileuton would show similar problem in humans.

The age of the animals was another factor. This type of study should be carried out in sexually mature dogs to avoid artefact.

Recommendation:

1. The patient consent form and investigator brochure should indicate that in dogs at 100 mg/kg/oral dose for 4 weeks developed atrophy of prostate which did not recover completely upon withdrawal of the treatment for six months.
2. Medical reviewer should note this finding
3. The sponsor should provide reasons why they included sexually immature dogs (6 month) as opposed to the sexually mature dogs (2-3 years of age).



Asoke Mukherjee

CC:
Orig. IND
HFD-007/Division File
HFD-007/Asoke Mukherjee
HFD-007/Dotti Pease
HFD-340
HFD-155/Alan Taylor
HFD-180/J. Choudary
R/D init. Lucy Jean 7/12/93
F/T by: J.Veach 7/28/93
WPI15097

61 472 150 10y111
JUL 23 1991

Food and Drug Administration
Center for Drug Evaluation and Research
Pilot Drug Evaluation Staff

PHARMACOLOGY REVIEW

(Amendment #014, 006 007)

IND "

Sponsor: Abbott Lab

Date Submitted: May 24, 1991

Date Received by CDER: May 30, 1991

Date Received by Reviewer: May 31, 1991

Date of Review: July 23, 1991

Drug: Zileuton

Category: 5-Lipoxygenase Inhibitor

Indication:

Related IND:

One year and oral toxicity to zileuton in rats:

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
TA 89-230 1. One year toxicity to rats. According to GLP. Cr1:CD-BR rats (Charles River). Doses: 20, 50 and 150 mg/kg/day/oral as 0.2% HPMC suspensions by gavage. Volume: 2, 5, 15 mg/ml Animals: 20/gr/sex 5/gr/sex for pharmacokinetics. Stability of the Formulations: Particle size: 200 microns The stability was confirmed on days 1, 21, 63, 126, 175 and 273. Plasma Zileuton levels: 3, 9, 24 hours after treatment on 2, 12, and 50 week. Zileuton and N-deOH zileuton (A-66193) levels were determined in the	<u>Mortality and Moribund condition:</u> Control: 1 Male 50 mg/kg 1 Male 50 mg/kg 3 (2 females, 1 male) 150 mg/kg 2 Male <u>Clinical signs:</u> Days 0-90: 1. Red discharge from eyes, mouth and nose at 150 mg/kg in 18 out of 40 rats. 2. Eye opacity in one male rat at 150 mg/kg dose. 3. Increased salivation at 150 mg/kg dose. Days 91-180: Same as above. Days 181-270: Same as above. Days 271-377: Same as above, one male at 50 mg/kg showed prostate sign.	Effects of zileuton on menstrual cycle should be monitored in the human trials since the animal data are not conclusive. Investigator's Brochure should point out the incidence of opacity in eyes in the male rat.

Expt

plasma. Livers and kidneys from control and 150 mg/kg dose group were examined electron microscopically.

Observations

Vaginal cytology:

No conclusive statement can be made from the observations.

Body weight gain:

There was no significant change in the body weight gain among male and female rats. Food consumption was also unchanged.

Remarks

MALE	Body wt. (g) on Day 367	% Gain (0-365 days)
Control	767 ± 114.2 (SD)	209.98
20 mg/kg	805 ± 115.0	224.31
50 mg/kg	760 ± 124.0	211.61
150 mg/kg	746 ± 101.0	205.08
FEMALE		
Control	519.65 ± 65	168.5
20 mg/kg	525.0 ± 73	177.5
50 mg/kg	542.0 ± 96	192.2
150 mg/kg	514.0 ± 100	170.65

Expt

Observations

Remarks

Blood chemistry:

Alanine and Aspartate amino transferase activities were reduced at 20, 50 and 150 mg/kg doses in male rats. Gamma glutamyl transferase activity at 150 mg/kg was higher than control male rats. Cholesterol levels were high and TG levels were low at 150 mg/kg in male rats.

(Ask the sponsor whether they determined blood or plasma or serum cholesterol).

Female rats at 150 mg/kg dose showed decrease in alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase activities. Cholesterol levels were higher and TG levels were lower than control female rats at 150 mg/kg.

Hematology:

Major changes were in the methohemoglobin

Expt

Observations

Remarks

parameter in female rats, increased at 150 mg/kg. Also hemoglobin and hematocrit values were lower than control in female rats. No Heinz bodies were found. Male rats did not show any significant change. Myeloid to erythroid cell ratios in the bone marrow did not show any significant changes among treated rats.

Urine analyses:

Proteinuria was present in male and female rats treated at 150 mg/kg dose.

Organ weight data:

Liver: Dose related increase in the wet weight of liver at 20, 50 and 150 mg/kg doses in male and at 50 and 150 mg/kg doses for female rats.

Kidney: Wet weights of kidneys were increased at 150 mg/kg

IND

ExptObservationsRemarks

for male and female rats.

Ovary: Wet weight of ovary and uteri were increased at 150 mg/kg, which was statistically not significant.

Gross changes:

? 5% of male rats showed prostate and testicular atrophy at 150 mg/kg. Ovarian cyst was present in 15% of rats treated at 150 mg/kg, control had 5%. No other gross changes were visible in this study.

? } ?
one male
rats
only

1/20

HISTOLOGICAL CHANGES

	<u>CONTROL</u>	<u>20 MG/KG</u>	<u>50 MG/KG</u>	<u>150 MG/KG</u>
<u>MALE RATS: (n=20):</u>				
<u>Pheochromocytoma:</u>				5%
<u>Nephropathy:</u>	65%	85%	80%	95%
<u>Hepatocytomegaly:</u>	0%	0%	70%	95%
<u>Carcinoma:</u>	0%	0%	0%	5%
<u>Atrophy of Pancreas:</u>	0%	0%	0%	15%
<u>Hyperplasia of Pancreas:</u>	0%	0%	0%	5%
 <u>FEMALES:</u>				
<u>Nephropathy:</u>	65%	65%	50%	80%
<u>Hepatocytomegaly:</u>	0%	0%	50%	100%
<u>Ovarian Cyst:</u>	0%	0%	0%	15%
<u>Parovarian Cyst:</u>	0%	0%	0%	5%
<u>Thymus Involution:</u>	60%	---	---	75%
<u>Uterine Cyst:</u>	0%	---	---	20%

↓ liver weight, no B proteinuria

Zileuton Pharmacokinetics (Males)

C _{Max} (µg/ml) ± SD			
	20 mg/kg	50 mg/kg	150 mg/kg
Week 2	4.9 ± .9	14.7 ± 5.4	28.9 ± 8.5
Week 12	8.2 ± .7	22.6 ± 4.4	50.2 ± 14.8
Week 50	12.2 ± 3.9	28.0 ± 8.8	70.0 ± 14.5
AUC (µh/ml)			
Week 2	29.0 ± 6.0	113.0 ± 29.0	356.0 ± 170.0
Week 12	53.0 ± 6.0	164.0 ± 31.0	460.0 ± 66.0
Week 50	102.0 ± 14.0	263.0 ± 60.0	701.0 ± 183.0

Zileuton Pharmacokinetics (Female Rats)

C _{Max} (µg/ml ± SD)			
	20 mg/kg	50 mg/kg	150 mg/kg
<u>Week 2</u>	6.5 ± 1.8	25.2 ± 2.2	57.2 ± 11.3
<u>Week 12</u>	11.0 ± 5.4	37.7 ± 18.8	75.9 ± 16.1
<u>Week 50</u>	26.5 ± 11.1	60.1 ± 6.5	95.8 ± 18.1
AUC µg/h/ml			
<u>Week 2</u>	33.0 ± 7.0	204.0 ± 46.0	692.0 ± 208.0
<u>Week 12</u>	77.0 ± 31.0	401.0 ± 239.0	847.0 ± 190.0
<u>Week 50</u>	202.0 ± 53.0	597.0 ± 79.0	1263.0 ± 380.0

A-66198 Levels (Male Rats)

C _{Max} (µg/ml) ± SD			
	20 mg/kg	50 mg/kg	150 mg/kg
<u>Week 2</u>	.59 ± .07	2.25 ± .85	4.64 ± 2.70
<u>Week 12</u>	1.28 ± .47	3.21 ± 1.69	9.29 ± 2.04
<u>Week 50</u>	1.73 ± .38	5.09 ± 2.36	15.51 ± 4.90
AUC µg/h/ml			
<u>Week 2</u>	7.11 ± .93	28.18 ± 10.14	52.73 ± 31.19
<u>Week 12</u>	14.78 ± 4.79	38.81 ± 18.28	125.42 ± 33.05
<u>Week 50</u>	20.08 ± 3.81	59.71 ± 26.55	188.0 ± 57.18

A-66193 Levels (Female Rats)

C _{MAX} (µg/ml)			
	20 mg/kg	20 mg/kg	150 mg/kg
<u>Week 2</u>	.37 ± .07	2.28 ± .32	8.69 ± 3.73
<u>Week 12</u>	.88 ± .34	4.01 ± 2.0	10.97 ± 2.15
<u>Week 50</u>	2.28 ± .85	8.39 ± 1.43	19.33 ± 7.27
AUC µg/ml/h			
<u>Week 2</u>	5.12 ± 1.36	30.7 ± 4.5	111.1 ± 49
<u>Week 12</u>	11.2 ± 3.7	49.0 ± 22.9	145.95 ± 27.4
<u>Week 50</u>	28.9 ± 12.4	100.9 ± 16.0	249.92 ± 92.0

Remarks

The drug has a chiral center, however, both enantiomers have equal 5-L0 inhibitory activity. C_{MAX} and AUC levels of A-66193 were also increased dose proportionately — over the time.

Discussion to One year Toxicity Study in Rats:

Although male and female rats showed similarity in the toxicity profiles, female rats had higher accumulation of the drug in the plasma compartment which may be due to the lower rate of metabolism in

? How can he say this?

where is the evidence?

female rats compared to male. The AUC seems to be dose related. ~~Smooth endoplasmic reticular proliferation~~ was observed at 50 and 150 mg/kg doses. Normally proliferation of smooth endoplasmic reticular (SER) enhances metabolic activity by inducing oxidative enzymes. However, such an event was not observed in ~~female~~ rats as far as zileuton kinetics were concerned. Three explanations can be provided for this effect:

- 7 male
- A. SER proliferation might have proteins which inhibited zileuton metabolism.
 - B. Due to hepatotoxicity of zileuton, it was not metabolized at the normal rate in the liver.
 - C. Kidney toxicity impaired the excretion of unchanged drug.

no

From the results of 2 week data at 25 mg/kg, it seems that the possibility of inhibition of zileuton metabolism by the biosynthesis of inhibitory proteins in SER is possible.

The N-dehydrozylated metabolite (A-66193) was considered to be resulted from the first pass metabolism in intestinal flora because treatment of rats to antibiotics reduced the levels of above metabolite. A-66193 AUC was about 20% to the parent compound in rats at 300 mg/kg/day.

7

The ~~selection of the~~ doses was made on the basis of the 6-month study where hepatotoxicity and retardation of the body weight gain at 300 mg/kg/day p.o. was observed. In the present study the lowest dose was selected so as to gain 2-3 times the plasma concentrations achieved in the clinical settings.

Expt

TA97-256

2. Immunotoxicity in
Female Rats.

Cr1:CD BR female rats
screened for hepatitis
and sendai antibodies,
at the time of the
experiment, rats were
about 6 weeks old.

Expt design: 80,
150, 300 mg/kg/day
p.o. for 14 days,
controls received
saline and vehicle.
Comparison was made
with cyclophosphamide
(CPS) at 25 mg/kg/day
(days 11-14). Each
group had 8 rats.
The expt was done
according to GLP.
Rats were immunized
with sheep RBC on the
day 11 and killed on
day 15 to examine in
vitro hemolysis of
SRBC in the presence
of complement and IgM
M secreted by anti-
body forming spleen
cells (AFC).

Observations

No mortality and loss
of body weight gain
was observed.

Zileuton did not
change the wet weight
of the spleen and its
cellularity, whereas
CPS showed decrease
in the body weights.
Cellularity was de-
termined by mincing
spleen between two
frosted slides and
suspending into 6 ml
buffer. Similar
changes to CPS were
observe in the thymus
also. The results
suggest that oral
zileuton did not af-
fect macrophage, B
and T lymphocyte
functions.

Results

This test needs to be
repeated in mouse
considering the fact
that leukotriene re-
ceptors are more sen-
sitive in mouse than
in rats.

?

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
Study V89-061 3. Single and multiple doses of ¹⁴C-zileuton (±) in monkeys to determine metabolism. Doses: 1. Single 400 mg/kg/oral. 2. Multiple 400 mg/kg/oral X 5 days. Animals: Cynomolgus monkeys 2-4 kg body weight, female, n=6. <u>Blood Samples:</u> 0.5, 1, 2, 4, 6, 8, 12, 16 and 24 hours after single dose. <u>Urine:</u> 0-24, 24-78, 48-72, 72-96, 96-120, 120-144, 144-168, 168-192, 192-216 and 216-240 hours.	R- and S- glucuronides of zileuton were eliminated in the urine. N-dehydroxy zileuton co-eluted with zileuton. Two peaks of total radioactivity were noted e.g., 2 and 8 hours. N-dehydroxy zileuton appeared 16 hours after the single dose. After multiple doses two peaks disappeared. Single peak concentrations were found between 4-8 hours after dosing. AUC for radioactivity after single and multiple doses were 1295 and 1321 µg ml/h, respectively.	About 10% of the dose undergoes first-pass metabolism in the intestinal flora. At high dose absorption is incomplete after both single and multiple doses.

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>

Feces:

Collected for 5 days.
All these were done
after single dose.

Same monkeys except
animal #15 were used
for multiple dose
study after 11 days
from the single dose
study. Time points
for blood, urine and
feces collection were
same as described
above

This suggests that
zileuton ~~did not ac-~~
~~cumulate after multi-~~
~~ple exposures.~~ Mass
balance data show
that 67% of total
radioactivity excret-
ed in urine, 19-20%
in feces, about 90-
92% of the total ra-
dioactivity was elim-
inated within 10 days
after single or mul-
tiple dosing. About
55% excreted within
24 hours through
urine.

About 60% of the drug
was absorbed from the
intestine after sin-
gle or multiple dos-
ing.

AUC (0-24 hr) for G1
and G2 = 922

N-dehydroxy zileuton
= 142

Zileuton = 106

Total = 1295

Above data related to
single dose of
zileuton.

One Year Oral Toxicity to Zileuton in Monkeys:

Expt

Observations

Remarks

#TC 88-858

Mortality:

One year toxicity to monkeys according to GLP duration 9/22/88 to 7/26/90. Cynomolgus monkeys, male and females, 2-4 kg, supplied by Hazelton Research products and Charles River Primates.

One male at 500 mg/kg dose died on day 344 cause of death was reported to be dosing related accident.

Clinical signs:

Local bruises, red discharge from face, nose and limbs. Swelling in the face and arm. Above changes were present in high dose male and female monkeys. Female monkeys had vaginal discharge which was present in the control animal also.

Doses: 50, 150, 500 mg/kg/day by gavage as 0.2% HPMC suspensions.

Volume: 5, 15, 50 mg/ml.

Animals: 4/gr/sex

Recovery: 2/sex from control and 500 mg/kg gr for one month.

Body weight gain:

Formulations: prepared on weekly basis. Dosing formulations were assayed on weeks 1, 6, 14, 27, 41.

Control: Received vehicle.

Male	Body Weight Day 358 Kg (SD)	% Gain (0-358 Days)
Control	4.6 ± 0.7	64
50 mg/kg	4.2 ± 0.6	46
150 mg/kg	3.6 ± 0.49	33
500 mg/kg	3.9 ± 0.75	39
<u>Female</u>		
Control	2.9 ± 0.37	17.2
50 mg/kg	2.7 ± .47	8.8
150 mg/kg	2.9 ± .49	15.2
500 mg/kg	3.0 ± .51	19.6

Expt

Plasma zileuton lev-
els: days 86, 93 for
male, days 90, 97 for
female. Samples were
collected at predose,
0.5, 1, 1.5, 2, 3, 4,
6, 8 hours after dos-
ing. Days 153, 365
at 3, 8 and 24 hrs.
after dosing.

Electron microscopic
studies:

Testes, prostate,
liver and kidney.

Observations

The sponsor suggested
that above changes
were not significant.

The decrease in the
body weight gain in
males was not due to
the decrease in food
consumption.

Ophthalmology:

No remarkable change
was noted.

Blood Chemistry:

Alanine amino trans-
ferase activity was
30% higher at 500
mg/kg in male
monkeys. Total bili-
rubin levels in fe-
male monkeys at 500
mg/kg was about 30%
higher. Alaline ami-
no transferase activ-
ity was higher at all
doses. However, it
was not statistically
significant. Total
cholesterol levels at
high dose was reduced
in female animals.
This blood chemistry
report suggests that
there may be some
changes in the liver
due to zileuton

Remarks

SGPT
↑ bilirubin

Expt

Observations

Remarks

treatment which needs to be confirmed in the histology finding.

Hematology:

Male monkeys did not show any remarkable change in this parameter. Female monkeys showed about 30% reduction in the platelet counts. Myeloid and erythroid ratios were unchanged.

Urine analyses:

No remarkable change in this parameter.

Organ weights:

Male monkeys showed about 30% increase in the wet weight of livers at 500 mg/kg dose. Similarly, weight of spleen was increased two-fold. Weight of prostate was reduced by about 24%.

4 liver weights

ExptObservationsRemarks

Female monkeys showed 30% increase in the liver weight.

Weight of spleen was increased by 50%. No other changes were noticed in this study.

The study results suggest that at 500 mg/kg dose in male and female monkeys, there were biochemical and histological changes in the liver. Increased pigmentation of the spleen was evident in male and female monkeys. Treatment related nephritis was also present.

Gross observations:

No change in male and female animals was observed.

Histological findings:

Inflammatory changes in the liver in male and female monkeys at high dose were present. Chronic nephritis was present in high dose female monkeys.

Higher incidences of pigmentation of spleen were evident in male and female monkeys.

Expt Observations Remarks

E.M. findings:

Liver specimen from control and high dose group were examined for peroxisomal proliferation. Volume of peroxisomes relative to cytoplasm was 0.015 for both control and the high dose group.

Pharmacokinetics (Zilauton)

<u>C_{Max} µg/ml ± SD)</u>			
	<u>50 mg/kg</u>	<u>150 mg/kg</u>	<u>500 mg/kg</u>
<u>3 Months</u>	2.1 ± 1.2	5.9 ± 1.9	3.7 ± 2.0
<u>5 Months</u>	0.8 ± .6	3.6 ± 2.4	7.1 ± 5.4
<u>12 Months</u>	1.0 ± .7	5.3 ± 3.8	5.0 ± 4.4
<u>AUC µg/h/ml</u>			
<u>3 Months</u>	6.2 ± 2.3	18.6 ± 5.3	17.4 ± 5.7
<u>5 Months</u>	4.1 ± 3.2	16.2 ± 9.7	32.9 ± 22.1
<u>12 Months</u>	4.6 ± 3.3	29.4 ± 22.2	26.7 ± 18.5
<u>T_{1/2}</u>			
<u>hr</u>	1.52	1.09	1.75

A-66193

C_{Max} ($\mu\text{g/ml}$)			
	50 mg/kg	150 mg/kg	500 mg/kg
<u>3 Months</u>	4.0 $\pm$.2	6.8 $\pm$ 5.3	7.5 $\pm$ 2.7
<u>5 Months</u>	1.0 $\pm$.6	7.7 $\pm$ 2.1	18.3 $\pm$ 2.0
<u>12 Months</u>	1.8 $\pm$ 1.0	9.0 $\pm$ 4.3	15.6 $\pm$ 6.4
AUC $\mu\text{g/h/ml}$			
<u>3 Months</u>	4.3 $\pm$ 1.5	71.1 $\pm$ 63.9	113.0 $\pm$ 37.0
<u>5 Months</u>	12.0 $\pm$ 6.6	95.0 $\pm$ 25.0	263.0 $\pm$ 38.0
<u>12 Months</u>	20.0 $\pm$ 11.0	111.0 $\pm$ 47.0	239.0 $\pm$ 93.0

Expt

#PPRD/68/299

Effect on intraocular pressure in rabbits and yeast-induced hyperthermia in rats.

Observations

There was no appreciable change in the IOP in rabbits except about 27% decrease in the IOP at 0.2% dose within 5 minutes.

Remarks

Solubility problem of zilauton posed problem to interpret the study results.

Rabbits:

Male, 2.2 to 3 kg,
Drug was instilled into conjunctival sac, 0.1 ml of 0.03, 0.1 and 0.3% suspensions. Vehicle was 0.2% HPMC. IOP was measured by tonometer, test drug or vehicle was instilled into one eye and the contralateral eye was used as the control. IOP was measured up to 150 mins.

Expt

Rats:

Male SD rats 120-200 gm were used. Hyperthermia was induced by 2 ml s.c. injection of 15% yeast suspension within 18 hours. Zileuton doses were 10, 30 and 100 mg/kg/oral as 0.2% HPMC suspensions. Aspirin 80 mg/kg was used as reference standard. Rectal temperatures were noted. Room temp was not provided.

Observations

Febrile temp of rats was about 2° C above control temp. Zileuton showed antipyretic effect at all doses and reduced the body temp by about 1° C whereas aspirin reduced by about 2°C.

Remarks

Data suggest that zileuton has antipyretic effect up to 3 hours of observations.

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
#PPRD/88/302 Effect of i.v. A-64077 on the size of the myocardial infarction in dogs. Dogs were surgically prepared and left circumflex of the coronary artery was blocked for 90 minutes to induce ischemic infarction. At 120 minutes following occlusion, the stenosis was removed to start reperfusion. The mapping of infarcted areas was accomplished by evans blue and tetrazolium staining. Area under infarction was determined by planimeter. Zileuton pretreatment was given 30 minutes before the occlusion of the coronary artery at 30 mg/kg. Control dogs received PEG-400. LTB₄ and zileuton levels were measured at several time points.	Zileuton was given at 20 mg/kg i.v. Six dogs were used for the treatment and another six for vehicle control (PEG-400). About 15-20 µg/ml plasma levels of zileuton were achieved up to 300 minutes. Mean arterial blood pressure did not alter during the experiment. Similarly HR was unaffected. Coronary blood flow also restored similarly in treated and control dogs. Data suggest that zileuton treatment did not change the potential for ischemic and reperfusion injury.	Zileuton pretreatment had no effect on the reperfusion induced myocardial injury despite inhibition of LTB₄ synthesis.

Expt

Observations

Remarks

#PPRD/90/066

Zileuton binding to prostatic androgen receptors in dog, monkey, rat and man, in vitro. Both racemic, R(+) and S(-) enantiomers were tested. Prostate gland were obtained 24-48 hours post castration and tissue homogenates were prepared for the binding to cytosolic androgen receptors. Human prostates were obtained at autopsy and prepared from flash-frozen tissue. Endogenous testosterone was removed by dextran and activated charcoal treatment.

Zileuton has weak affinity to androgenic receptors which was not stereoselective.

	pki (±)	R(+)	S(-)	IAR
Dog	4.94	5.07	5.24	1.5
Rat	4.17	4.18	4.57	2.5
Monkey	4.32	4.41	4.59	1.5
Human	2.98	3.21	3.93	5.2

pki = log [ki]

IAR = Isomeric Activity Ratio

Assay mixtures contained 18,000 dpm of ³H-testosterone, zileuton in DMSO, prostatic androgen receptor protein. Incubation period was 18 hours at 4° C. Nonspecific binding was determined by 10⁻⁶ M unlabelled androgen. Binding of tes

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>

tosterone was determined by liquid scintillation spectrometry. K_i for binding was determined by displacement of specific binding by zileuton using following formula:

$$K_i = IC_{50} / (1 + [L] / K_D)$$

IC_{50} = 50% displacement

L = ligand conc.

K_D = Dissociation constant of the ligand.

Expt

Observations

Remarks

#PPRD/90/115

Plasma levels of zileuton after oral dosing in dogs.

Animals: Male beagle dogs, each group had three dogs. Unmilled zileuton filled capsules were given.

Doses: 5, 20, 100, 400 mg/kg/day/oral. Venous blood samples were collected for 24 hours at several time points after the first and last doses. Plasma levels of zileuton and A-66193 levels were determined.

Data After First Dose:

Zileuton:	5 mg/kg	20 mg/kg	100 mg/kg	400 mg/kg
T _{max} h	1.7	2.0	6.5	11.3
C _{max} (µg/ml)	4.6	9.7	18.5	89.8
AUC (0-24) (µg/h/ml)	48	141	270	1398
T _{1/2}	8.4	10.2		11.8
A-66193:				
T _{max}	7.7	8.7	7.8	11.3
C _{max}	0.98	0.13	0.13	0.96
AUC (0-24)	0.94	2.18	2.33	7.96

Data After Dose 7:

Zileuton:	5 mg/kg	20 mg/kg	100 mg/kg	400 mg/kg
T _{max}	2.7	2.7	8.7	8.0
C _{max}	4.2	11.4	24.5	85.5
AUC (0-24)	42	155	368	1240
T _{1/2}	6.2	9.07	12.6	12.3
A-66193:				
T _{max}	2	8.7	8.7	9.3
C _{max}	0.1	.34	.17	.63
AUC (0-24)	1.27	4.25	3.4	10.8

Expt

Observations

Remarks

These data along with the normalized AUC with the dose suggest that the absorption of the drug after oral administration in dogs was incomplete even after the first dose. However, when data for the first dose and the last dose were compared, it seems that the drug does not accumulate in the plasma compartment.

EXPT

Observations

Remarks

#PPRD/90/130

Plasma levels of zileuton and A-66193 after single administration of zileuton in corn oil or HPMC in rats.

Rats: Male CD rats, 200-280 gm body weight, 4 rats/group.

Doses: 80 and 300 mg/kg/oral for each formulation. Suspensions were prepared from 40-80 mesh power in 8 and 30 mg/ml concentrations in corn oil or 0.2% HPMC. Heparinized blood samples were collected from the tail vein up to 48 hours and assayed for zileuton and A-66193.

Zileuton		
Corn Oil	C _{max}	AUC
80 mg/kg	13	191
300	80	1363
HPMC		
80 mg/kg	36	338
300	96	1294

A-66193		
Corn Oil	C _{max}	AUC
80 mg/kg	0.76	13.7
300 mg/kg	4.63	117
HPMC:		
80 mg/kg	2.9	30.7
300 mg/kg	6.4	133.6
T _{1/2} :		
Corn Oil: 11.30 hours		
HPMC: 2-3 1/2 hours		

Expt

Observations

Remarks

These data suggest that the HPMC formulation showed greater bioavailability but shorter half life of elimination compared to corn oil formulation. However, corn oil formulation showed absorption problem at 80 mg/kg dose.

A-66193 levels were 9-10% to that of zileuton at 80 and 300 mg/kg, respectively. In the acute tox study, corn oil formulation showed higher incidences of toxicities which might be unrelated to the bioavailability of the drug.

Female rats showed about 10% higher plasma levels in adult rats in report. —
#PPRD/90/130; 29
µg/ml at 80 mg/kg, 85
µg/ml at 300 mg/kg, 2
week old pups showed higher plasma levels.

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>												
#PPRD/90/157	Plasma levels $\mu\text{g/ml}$ for zileuton:													
Six week oral dosing and PK of zileuton in two week old rats.	<table border="1"> <thead> <tr> <th></th> <th>Male</th> <th>Female</th> </tr> </thead> <tbody> <tr> <td>20mg/kg</td> <td>11.7</td> <td>14.8</td> </tr> <tr> <td>80</td> <td>47</td> <td>52</td> </tr> <tr> <td>300</td> <td>101</td> <td>121</td> </tr> </tbody> </table>		Male	Female	20mg/kg	11.7	14.8	80	47	52	300	101	121	
	Male	Female												
20mg/kg	11.7	14.8												
80	47	52												
300	101	121												
<u>Rats:</u> two week old pups, 5 male and 5 female in each group.	For A-66193													
<u>Treatment:</u> 20, 80 and 300 mg/kg/oral/- day for six weeks using 0.2% HPMC sus- pensions. Blood sam- ples were collected after 3rd dose, three hours after the last dose.	<table border="1"> <thead> <tr> <th></th> <th>Male</th> <th>Female</th> </tr> </thead> <tbody> <tr> <td>20mg/kg</td> <td>.12</td> <td>.13</td> </tr> <tr> <td>80</td> <td>.96</td> <td>1.55</td> </tr> <tr> <td>300</td> <td>3.2</td> <td>3.6</td> </tr> </tbody> </table>		Male	Female	20mg/kg	.12	.13	80	.96	1.55	300	3.2	3.6	
	Male	Female												
20mg/kg	.12	.13												
80	.96	1.55												
300	3.2	3.6												

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
#PPRD/90/240 ¹⁴C-zileuton racemate metabolism in mice after oral administration. <u>Mice:</u> CD mice, female, at a dose of 5 mg/kg/oral containing about 1 μci/mouse. Plasma from 6 animals/sex was pooled at 1, 2, 4, 8 and 10 hours. Urine and feces were collected up to 72 hours in 3 male and 3 female mice.	Within 24 hours 76% and 24% radioactivity was found in urine and feces, respectively in male mice. The above data for female mice were 86% and 14%. Both sexes excreted about 90% radioactivity in urine and feces within 3 days. About 68% of the total dose was excreted as metabolite in the urine. R(+), S(-) glucuronides N-deOH zileuton, a polar metabolite and unidentified metabolite RPM were present in the plasma.	Glucuronidation was stereo-selective, mostly for S(-) form. Free and N-deOH-zileuton were not present in 0-24 hour urine. M₁ metabolite excreted in the feces and urine was not identified. Similarly, plasma showed an unidentified metabolite RMP which had a retention time very similar to M₁. Therefore, M₁ and RMP may be same metabolite which were also present in rats. Urinary M₁ = 2% of the dose. Urinary polar metabolite = 46% of the dose. Fecal M₁ = 10% of the dose. Fecal polar metabolite = 64% of the dose. Polar metabolites in urine — mostly conjugated product of M₁.

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
#R&D/90/263 1. Interactions between zileuton and salicylate, theophylline, naproxen, ibuprofen, prednisone, sulfasalazine, salbutamol and warfarin at plasma protein binding sites in human albumin and α_1-acid glycoprotein were detected. [^{14}C] zileuton at 5 ug/ml in 50% alcohol or water and: 1. Salicylate 250 μg/ml 2. Theophylline 20 μg/ml 3. Naproxen 100 μg/ml 4. Ibuprofen 50 μg/ml 5. Prednisone 50 μg/ml 6. Sulfasalazine 50 μg/ml 7. Salbutanol .005 μg/ml were mixed. Protein binding to zileuton in the presence of above drugs was determined by centrifugation.	Sulfasalazine, ibuprofen, naproxen showed significant inhibition of ^{14}C-zileuton binding to plasma protein in the presence of alcohol. Control binding was 92.7-93.4%. Salicylate also showed significant inhibition in ^{14}C-zileuton binding when water was used as vehicle. Theophylline, salbutanol and prednisone had no effect on the zileuton binding to plasma protein.	Binding of zileuton to plasma albumin was greater than that of α_1-acid glycoprotein.

<u>Expt</u>	<u>Observations</u>	<u>Remarks</u>
2. Effect of 5 $\mu\text{g/ml}$ zileuton on the plasma protein binding of ^{14}C -warfarin, ^{14}C -salicylate, ^{14}C -theophylline, ^3H -naproxen, ^3H -ibuprofen, ^3H prednisone protein binding was done by equilibrium dialysis and centrifugation techniques.	Data suggest that zileuton did not displace radiolabelled drugs from its binding sites. Probably binding of zileuton to plasma protein is more reversible than other therapeutic agents, e.g., sulfasalazine, ibuprofen, naproxen and salicylate.	
3. Zileuton binding to α_1 -acid glycoprotein binding. 500 $\mu\text{g/ml}$ stock solution was added to give a final conc of 5 $\mu\text{g/ml}$ and assayed by equilibrium dialysis method to determine protein binding.	Data suggest that 87.5% of zileuton bound to human albumin in contrast to 33.9% for α_1 -acid glycoprotein.	

SUMMARY:

This submission extends the data on the chronic toxicity studies in rats and monkeys. It is evident from the data that the compound is incompletely absorbed from the gut and induces hepatotoxicity. Zileuton has weak cyclooxygenase inhibitory activity as shown in the anti-pyretic assays. The compound has been shown to be devoid of any effect on peroxisomes. However, assay of marker enzymes from peroxisomes and regional heterogeneity of peroxisomal proliferation in the liver were not investigated. Regarding my previous request to demonstrate LTD₄-induced contractions to rat uterus, the sponsor provided reference to a paper published by Chimura, T. in Nippon Sanka Fujinka Gakkai Zasshi, 1985, 37(11);2309-14 to support that LTC₄ and LTD₄ induce significant contractions to rat uterus. However, the data as appears to me do not support that LTC₄ and LTD₄ induced significant contractions to uterus so as to account for the cause of dystocia in the Seg III reproductive study in rats. Also, the concentrations of LTC₄ and LTD₄ used were much higher than that necessary to induce biological activities in other organs. I think it is necessary to address that dystocia to rats was caused by the weak cyclooxygenase inhibitory activity rather than its effects on 5-LO system. Since the sponsor is not willing to provide data as requested I would request them to discuss why they think that the dystocia in rats resulted from 5-LO inhibition despite the fact that contractility to LTC₄ and LTD₄ in rat uterus was almost negligible.

Recommendations:

1. Data from the rat toxicity on the estrus cycle are inconclusive. If zileuton is used for women in their childbearing age its effect on menstrual cycle needs to be observed.
2. The investigator's brochure should indicate the incidence of opacity in eyes in the male rats.
3. It has been considered in the literature that zonal heterogeneity exists in liver with respect to peroxisomal proliferation. It is not clear which portion of the liver was taken for EM study to show that zileuton does not induce peroxisomal proliferation. The sponsor should provide that information to the reviewer. It is also suggested that the sponsor repeat a one week study in rats using a high dose and assay for enzymes which could be markers for peroxisomes, e.g., peroxisomal beta oxidase and catalase activities.
4. Immunotoxicity study should be repeated in mice because leukotriene receptors in mouse are more sensitive than that of the rat.

5.

Statistical Review

Statistical Review and Evaluation

(Addendum)

IND:

Date: Jan. 12, 1994

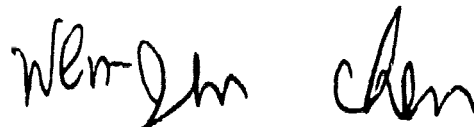
Applicant: Abbot Laboratories

Name of Drug: Zileuton.

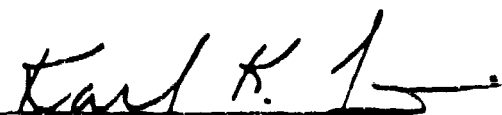
Documents Reviewed: IND Submission volumes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of 10 dated February 10, 1993.
Data on floppy diskette supplied by the sponsor.

1. Corrections of reviewer's p-values

A report of statistical review and evaluation of the mouse and rat carcinogenicity studies of this IND was issued by the Division of Biometrics on April 21, 1993. Due to misreading of a p-value from PC screen, on page 6 of the review, the p-value ($p=0.358$) of the pairwise comparison of the control vs. the high dose group for tumor type Kidney/Tubular Adenoma in female mouse study should be changed to 0.1043.


Wen-Jen Chen, Ph.D.
Mathematical Statistician

Concur:


Karl K. Lin, Ph.D.
Group Leader

1/13/94

cc: Original IND

HFD-150/Dr. Burke

HFD-150/Dr. Sun

HFD-710/Chron

HFD-715/Dr. K. Lin

HFD-715/Dr. Chen

HFD-715/DRU 2.1.1 IND

Zileuton Mouse and Rat
carcinogenicity studies.

HFD-401/Dr. Contrera

HFD-502/Dr. Osterberg or Dr. Taylor

Statistical Review and Evaluation

(Addendum)

IND:

Date: May 14, 1993

Applicant: Abbot Laboratories

Name of Drug: Zileuton.

Documents Reviewed: IND Submission volumes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of 10 dated February 10, 1993.

Data on floppy diskette supplied by the sponsor.

I. Corrections of reviewer's p-values

A report of statistical review and evaluation of the mouse and rat carcinogenicity studies of this IND was issued by the Division of Biometrics on April 21, 1993. Due to misreadings of a row of data in a table and of some p-values from PC screen, the following p-values need to be modified:

1. On pages 5 and 15 of the review, the p-value ($p=0.0276$) of the trend test for tumor type Kidney/Tubular Adenoma and Adenocarcinoma in male mouse study should be changed to 0.0335.
2. On pages 5 and 15 of the review, the p-value ($p=0.0077$) of the trend test for tumor type Liver/Hepatocellular Adenoma and Carcinoma in male mouse study should be changed to 0.0021.
3. On page 6 of the review, the p-value ($p=0.0833$) of the pairwise comparison of the control vs. the high dose group for tumor type Liver/Hepatocellular Adenoma and Carcinoma in male mouse study should be changed to 0.03.
4. On page 19 of the review, the p-value ($p=0.14$) of the trend test for tumor type Kidney/Tubular Adenocarcinoma in male rat study should be changed to 0.104.
5. On page 9 and 19 of the review, the p-value ($p=0.0017$) of the trend test for tumor type Kidney/Tubular Adenoma in male rat study should be changed to 0.026.

Since this tumor type becomes non-significant, the corresponding pairwise comparison of control vs. high dose group needs to be removed from page 10. Moreover, by the same reason, this tumor type should also be deleted from the summary of the significant tumor types for trend tests in rat study described on page 11.

II. Corrections of reviewer's misused words

The word mice at the 1st and 4th rows from the bottom of the page 8 should be changed to rats.

Wen-Jen Chen, Ph.D.
Mathematical Statistician

Concur: Karl K. Lin, Ph.D.
Group Leader

cc: Original IND
HFD-150/Dr. Burke
HFD-150/Dr. Sun
HFD-710/Chron
HFD-715/Dr. K. Lin
HFD-715/Dr. Chen
HFD-715/DRU 2.1.1 IND 30-661 Zileuton Mouse and Rat
carcinogenicity studies.
HFD-401/Dr. Contrera
HFD-502/Dr. Osterberg or Dr. Taylor

Statistical Review and Evaluation

(Addendum)

IND:

Date: May 5, 1993

Applicant: Abbot Laboratories

Name of Drug: Zileuton.

Documents Reviewed: IND Submission volumes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of 10 dated February 10, 1993.

Data on floppy diskette supplied by the sponsor.

I. Corrections of reporting sponsor's p-values

A report of statistical review and evaluation of the mouse and rat carcinogenicity studies of this IND was issued by the Division of Biometrics on April 21, 1993. On page 2 of that review, the second p-value ($p=0.05465$) of the trend test for tumor type Multiple Organs/Hemangiosarcoma in male mouse study should be changed to 0.5465.

Similarly, on page 8, the first p-value ($p=0.0036$) of the trend test for the tumor type Kidney/Combined Adenoma & Adenocarcinoma in female rat needs to be changed to $p=0.0125$.

II. Correction of a footnote

On page 8 of the original review, the footnote a. "# tumor counts are out of 60 animals in each group, except female high dose group(out of 58)" should be changed to "# tumor counts are out of 60 animals in each group".

III. Adding another tumor type to the sponsor's analysis

On page 3 of the previous review report, the following p-values of a trend test for tumor type Liver/Carcinoma in female mouse should be added:

Liver/Carcinoma	($p=0.0434$)
(0,1,0,3)	($p=0.428$).

Sun

Statistical Review and Evaluation

IND:

Date: April 21, 1993

Applicant: Abbot Laboratories

Name of Drug: Zileuton.

Documents Reviewed: IND Submission volumes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 of 10 dated February 10, 1993.

Data on floppy diskette supplied by the sponsor.

I. Background: In this IND submission two animal carcinogenicity studies, one in mice and one in rats, were included. These two studies were intended to assess the carcinogenic potential of Zileuton in mice and rats when administered via diet using some selected dose levels for two years. Dr. Joseph Sun, HFD-150, who is the reviewing pharmacologist requested the Division of Biometrics to perform the statistical review and evaluation of these studies. The results of the review have been discussed with Dr. Joseph Sun.

II. The mouse study

IIa. Design: Two separate experiments, one in male and one in female mice, were conducted. In each of these experiments there were three treated groups known as low, medium, and high, and one control group. For each sex, two hundred and forty CD-1(R)BR mice were randomly divided into equal groups of 60 animals each to form the treatment groups, but in female high dose group, two animals escaped. The dose levels for the treated groups were 50, 150, and 450 mg/kg/day for the low, medium, and high dose groups, respectively. The control group received meal only.

The animals were checked twice daily for mortality and moribundity. A detailed physical examination was performed on each animal once a week for palpable masses, and gross signs of toxic and pharmacological effects. A complete histopathological examination was conducted on all animals found dead, killed moribund during the experiment or sacrificed at the end of the experiment.

IIb. Sponsor's analysis

Survival analysis: Tables for survival rates of male and female mice were constructed. Percentages of animals survived were plotted against time for male and female mice, separately. The survival data were analyzed using the National Cancer Institute Package, which includes the test of homogeneity of survival distributions of treatment groups and the linear positive trend in mortality using

the methods described in the paper of Cox (Regression models and life tables, Journal of the Royal Statistical Society, B, 34, 187-220, 1972), and of Tarone RE. (Tests for trends in life tables analysis, Biometrika 1975), respectively.

The tests showed that no significant differences in survival distribution were detected among the treatment groups, nor was the test of trend statistically significant for either sex.

Tumor data analysis: The sponsor used the prevalence method described in the paper of Peto et al. (Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments, Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, Annex to supplement, World Health Organization, Geneva, 311-426, 1980) to analyze the tumor incidence data. The "ad hoc runs" procedure also described in the paper was used to generate the time intervals in the prevalence analysis. Tumors found at terminal necropsy were treated as occurring in a separate time interval. For each individual tumor site, two analyses were performed: the pairwise comparison for the high dose vs. control group and the linear positive trend test among four treatment groups. For the pairwise comparison, the asymptotic normal Z with the continuity correction was conducted; for the trend test, the ordinal scale of dose 0,1,2, and 3 was used as the independent variable for the control, low, median, and high dose groups, respectively. If the tumor incidence across treatment groups was fewer than 10, the exact permutation trend test was used to compute p-value, otherwise the normal approximation was employed.

Moreover, the sponsor also used the resampling-based multiple testing technique developed by Westfall and Young to compute the adjusted p-values. The adjusted p-values were obtained by using the bootstrap procedure of Westfall and Young in conjunction with the Cochran-Armitage test stratified over five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, weeks 93-104, and terminal necropsy (proposed by National Toxicology Program).

The tumor types having the p-values computed by Peto's method less than 0.05 along with their adjusted p-values are listed below.

<u>Sex</u>	<u>Tumor Type</u> <u>Tumor Count #</u>	<u>Trend</u>	<u>Pairwise</u> <u>(H. Vs. C.)</u>
Male	Multiple Organs/Hemangiosarcoma (1, 4, 2, 6)	(p=0.027) ^b (p=0.05465)	(p=0.041) (p=0.4084)
	Multiple Organs/Combined Hemangioma & Hemangiosarcoma (3, 6, 3, 8)	(p=0.0491) (p=0.6671)	

<u>Sex</u>	<u>Tumor Type</u> <u>Tumor Count #^a</u>	<u>Trend</u>	<u>Pairwise</u> <u>(H. Vs. C.)</u>
Male	Liver/Combined Adenoma & Carcinoma (14,10,9,21)	(p=0.0342) ^b (p=0.3506)	
	Kidney/Combined Adenoma & Adenocarcinoma (0,0,1,2)	(p=0.0335) (p=0.2121)	
Female	Multiple Organs/Hemangiosarcoma (5,1,2,16)	(p=0.0002) (p=0.0003)	(p=0.0054) (p=0.0033)
	Multiple Organs/Combined Hemangioma & Hemangiosarcoma (6,4,5,19)	(p=0.0001) (p=0.0003)	(p=0.0019) (p=0.0011)
	Liver/Adenoma (0,2,0,8)	(p=0.0005) (p=0.0017)	(p=0.0055) (p=0.0005)
	Liver/Combined Adenoma & Carcinoma (0,3,0,11)	(p=0.0001) (p=0.0001)	(p=0.0001) (p=0.0001)
	Skin, Other/Hemangiosarcoma (0,0,1,3)	(p=0.0130) (---) ^c	
	Ovary/Hemangiosarcoma (0,0,0,5)	(p=0.0003) (---)	(p=0.0199) (---)
	Vagina/Combined Hemangioma & Hemangiosarcoma (0,0,0,2)	(p=0.0485) (---)	
	Kidney/Adenoma (0,0,0,3)	(p=0.0187) (p=0.1146)	
	Kidney/Combined Adenoma & Adenocarcinoma (0,0,0,4)	(p=0.0037) (p=0.0194)	

- a. # Tumor counts are out of 60 animals in each group, except female high dose group(out of 58).
- b. The first p-value denotes the raw p-value per Peto test, the second p-value denotes the adjusted p-value.
- c. (---) denotes the adjusted p-value was not provided by the sponsor.

The statistical significance of the tests for both linear positive trend and pairwise comparison was evaluated using the adjusted p-values assessed at the 0.05 level of significance.

Under this criterion, tests did not show any statistically significant linear positive trend or increase in tumor incidence at high dose vs. control group in male mice. In female mice, the tumor types with statistically significant linear positive trends and increases at high dose vs. control group are : Multiple Organs /Hemangiosarcoma, Multiple Organs/Combined Hemangioma & Hemangiosarcoma, Liver/Adenoma, Liver/Combined Adenoma & Carcinoma. Moreover, a statistically significant linear trend was detected in Kidney/Combined Adenoma & Adenocarcinoma in female mice.

11c. Reviewer's analysis

The reviewer independently performed analyses on the survival and tumor data. In the survival data analysis the methods described in the papers of Cox (1972), and of Gehan (A generalized Wilcoxon test for comparing arbitrarily singly censored samples, Biometrika, 52 203-223, 1965) were used. The tumor data analyses were done using the methods described in the paper of Peto et al. (1980) and the method of exact permutation trend test, developed by the Division of Biometrics. The data used in the reviewer's analysis were provided by the sponsor on a floppy diskette.

Survival analysis: The intercurrent mortality data of the mouse study are given in Table 1. The plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for male and female mice are given in Figures 1a and 1b, respectively. The homogeneity of survival distributions of all four groups (Control, Low, Medium, and High) was tested separately for male and female mice using the Cox test and the Generalized Wilcoxon test. The tests did not show any statistically significant (at .05 level) linear positive trend or difference in the mortality in either sex.

The p-values of the linear positive trend and the pairwise tests are given in Tables 2A and 2B, respectively.

Tumor data analysis: The sponsor classified the tumor types as 'cause of death', 'not cause of death', and 'undetermined'. Following Peto et al.(1980), the reviewer applied the 'death rate method' to the first tumor type and the 'prevalence' method to the second and the third tumor types to test the linear positive trend in tumor rates. For tumor types occurring in both categories a combined test was performed. All tests were done using the method of exact permutation trend test, which is an extension of the Fisher exact test. The scores used in the reviewer's analyses were 0, 50, 150, and 450 for control, low, medium, and high dose groups, respectively. The time intervals used were 0 - 52, 53 - 78, 79-92, 93-104 weeks, and terminal sacrifice for both sexes. The incidence rates of tumor types with p-values less than 0.05 are listed below.

<u>TUMOR/ORGAN</u> <u>MALE MICE</u>	<u>TUMOR RATE</u>				<u>P-VALUE</u>
	<u>C</u> 60	<u>L</u> 60	<u>M</u> 60	<u>H</u> 60 ^a	
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	0	1	2	0.0276* ✓ ~~0.0276~~
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	14	10	9	21	0.0077* ✓ ~~0.0077~~
<u>FEMALE MICE</u>					
KIDNEY/TUBULAR ADENOMA	0	0	0	3	0.0143 ^a *
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	0	0	4	0.0029 ^a *
LIVER/HEPATOCELLULAR CARCINOMA	0	1	0	3	0.0224 ^a *
LIVER/HEPATOCELLULAR ADENOMA	0	2	0	8	0.000076 ^a *
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	0	3	0	11	0.0000 ^a *
MULTIPLE ORGANS/HEMANGIOSARCOMA	5	1	2	16	0.0000 ^a *
MULTIPLE ORGANS/HEMANGIOSARCOMA AND HEMANGIOMA	6	4	5	19	0.0000 ^a *
OVARY/HEMANGIOSARCOMA	0	0	0	5	0.000371 ^a *
SKIN OTHER/HEMANGIOSARCOMA	0	0	1	3	0.015013 ^a *

a: There are only 58 animals in female high dose group.

Multiple testing adjustment: The rule proposed by Haseman was used to adjust the effect of multiple testings. Haseman's rule states that in order to keep the overall false-positive rate at the nominal level of approximately five percent, tumor types with a spontaneous tumor rate of no more than one percent should be tested at .05 level, otherwise the level should be set at .01 (Haseman, (1983), A re-examination of false-positive rates for carcinogenesis studies, Fundamental and Applied Toxicology, 3: 334-339).

On the basis of Haseman's rule, the P-values marked with asterisks are considered to be statistically significant.

The incidence rates and p-values of all tumor types tested for linear trend are given in Table 3.

Pairwise comparisons of the controls with the high dose

In the following we will compare the incidence rates of the control groups with those of the high dose groups using the age adjusted Fisher exact test for tumor types whose linear trends were found to be statistically significant.

<u>TUMOR/ORGAN</u> <u>MALE MICE</u>	<u>TUMOR RATE</u>		<u>P-VALUE</u>
	<u>C</u> 60	<u>H</u> 60	
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	2	0.1694

<u>TUMOR/ORGAN</u> <u>MALE MICE</u>	<u>TUMOR RATE</u>		<u>P-VALUE</u>
	<u>C</u> 60	<u>H</u> 60	
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	14	21	0.0833
<u>FEMALE MICE</u>			
KIDNEY/TUBULAR ADENOMA	0	3	0.358*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	4	0.048*
LIVER/HEPATOCELLULAR CARCINOMA	0	3	0.105*
LIVER/HEPATOCELLULAR ADENOMA	0	8	0.0013*
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	0	11	0.00005*
MULTIPLE ORGANS/HEMANGIOSARCOMA	5	16	0.0034
MULTIPLE ORGANS/HEMANGIOSARCOMA AND HEMANGIOMA	6	19	0.0015*
<u>OVARY/HEMANGIOSARCOMA</u>	0	5	0.0214*
<u>SKIN OTHER/HEMANGIOSARCOMA</u>	0	3	0.1122

a: There are only 58 animals in female high dose group.

P-values marked with asterisks are considered to be statistically significant after adjusting for the effect of multiple tests.

Note: The results of statistical analysis of tumor data provided by the sponsor are based on the adjusted p-values by the method of Westfall and Young. In general, this technique provides more conservative adjusted p-values if number of testing variables is large. For this reason the conclusions of the sponsor and that of the reviewer may some times differ.

III. The rat study

IIIa. Design: Two separate experiments, one in male and one in female rats, were conducted. In each of these experiments there were three treated groups known as low, medium, and high, and one control group. For each sex, two hundred and forty CD(R)BR rats were randomly divided into equal groups of 60 animals each to form the treatment groups. The dose levels for the treated groups were 40, 80, and 170 mg/kg/day for the low, medium, and high dose groups, respectively. The control group received meal only.

The animals were checked twice daily for mortality and moribundity. A detailed physical examination was performed on each animal once a week for palpable masses, and gross signs of toxic and pharmacological effects. A complete histopathological examination was conducted on all animals found dead, killed moribund during the experiment or sacrificed at the end of the experiment.

11b. Sponsor's analysis

Survival analysis: Tables for survival rates of male and female rats were constructed. Percentages of animals survived were plotted against time separately for male and female rats.

The survival data were analyzed using the National Cancer Institute Package, which includes the test of homogeneity of survival distributions of treatment groups and the linear positive trend in mortality using the methods described in the paper of Cox (Regression models and life tables, Journal of the Royal Statistical Society, B, 34, 187-220, 1972), and of Tarone RE. (Tests for trends in life tables analysis. Biometrika 1975), respectively.

No significant differences in the survival rate were found among the treatment groups for either sex. A significant linear positive trend in the survival rate was detected in the female rats ($p=0.0438$).

Tumor data analysis: The sponsor used the prevalence method described in the paper of Peto et al. (Guidelines for sample sensitive significance test for carcinogenic effects in long-term animal experiments, Long term and short term screening assays for carcinogens: A critical appraisal, International agency for research against cancer monographs, Annex to supplement, World Health Organization, Geneva, 311-426, 1980) to analyze the tumor incidence data. The "ad hoc runs" procedure also described in the paper was used to generate the time intervals in the prevalence analysis. Tumors found at terminal necropsy were treated as occurring in a separate time interval. For each individual tumor site, two analyses were performed: the pairwise comparison for the high dose vs. control group and the linear positive trend test among four treatment groups. For the pairwise comparison, the asymptotic normal Z with the continuity correction was conducted; for the trend test, the ordinal scale of dose 0,1,2, and 3 was used as the independent variable for the control, low, median, and high dose groups, respectively. If the tumor incidence across treatment groups was fewer than 10, the exact permutation trend test was used to compute p-value, otherwise the normal approximation was employed.

Moreover, the sponsor also conducted the resampling-based multiple testing technique developed by Westfall and Young to compute the adjusted p-values. The adjusted p-values were obtained by using the bootstrap procedure of Westfall and Young in conjunction with the Cochran-Armitage test stratified over five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, weeks 93-104, and terminal necropsy (proposed by National Toxicology Program).

The tumor types having the p-values computed by Peto's method less than 0.05 along with their adjusted p-values are listed below.

<u>Sex</u>	<u>Tumor Type</u> <u>Tumor Count #^a</u>	<u>Trend</u>	<u>Pairwise</u> <u>(H. Vs. C.)</u>
Male	Testis/Interstitial Cell Tumor (3, 12, 12, 24)	(p=0.0001) ^b (p=0.0001)	(p=0.0001) (p=0.0001)
	Kidney/Combined Adenoma & Adenocarcinoma (3, 2, 1, 9)	(p=0.0301) (p=0.1690)	
Female	Kidney/Adenoma (0, 0, 2, 5)	(p=0.0036) (p=0.036)	
	Kidney/Combined Adenoma & Adenocarcinoma (0, 1, 2, 5)	(p=0.0036) ^b (p=0.1263)	

- a. # Tumor counts are out of 60 animals in each group, except female high dose group(out of 58).
- b. The first p-value denotes the raw p-value per Peto test, the second p-value denotes the adjusted p-value.

The statistical significance of the tests for both linear positive trend and pairwise comparison was evaluated using the adjusted p-values assessed at the 0.05 level of significance.

Under this criterion, a statistically significant linear positive trend and a statistically significant increase in high dose group over the control group were detected in the incidence of testicular interstitial cell tumors in male rats. In female rats, the tests showed only a statistically significant linear positive trend in the incidence of kidney tubular adenoma.

IIc. Reviewer's analysis

The reviewer independently performed analyses on the survival and tumor data. In the survival data analysis the methods described in the papers of Cox (1972), and of Gehan (1965) were used. The tumor data analysis were done using the methods described in the paper of Peto et al. (1980) and the method of exact permutation trend test, developed by the Division of Biometrics. The data used in the reviewer's analysis were provided by the sponsor on a floppy diskette.

Survival analysis: The intercurrent mortality data of the mouse study are given in Table 4. The plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for male and female mice are given in Figures 2a and 2b, respectively. The homogeneity of survival distributions of all four groups (Control, Low, Medium, and High) was tested separately for male and female mice using the Cox test and the Generalized Wilcoxon test.

No significant differences in survival distribution were found among the treatment groups for either sex. A significant linear positive trend in the survival rate was detected in the female rats.

The p-values of the linear positive trend and the pairwise tests are given in Tables 5A and 5B, respectively.

Tumor data analysis: The sponsor classified the tumor types as 'cause of death', 'not cause of death', and 'undetermined'. Following Peto et al. (1980), the reviewer applied the 'death rate method' to the first tumor type and the 'prevalence' method to the second and the third tumor types to test the linear positive trend in tumor rates. For tumor types occurring in both categories a combined test was performed. All tests except for those tumor types with total numbers of tumor bearing animals greater than 20 were done using the method of exact permutation trend test, which is an extension of the Fisher exact test. The scores used in the reviewer's analyses were 0, 40, 80, and 170 for the control, low, medium, and high dose groups, respectively. The time intervals used were 0 - 52, 53 - 78, 79-92, 93-104 weeks, and terminal sacrifice for both sexes.

The incidence rates of tumor types with p-values less than 0.05 are listed below.

<u>TUMOR/ORGAN</u>	<u>TUMOR RATE</u>				<u>P-VALUE</u>
	<u>C</u>	<u>L</u>	<u>M</u>	<u>H</u>	
<u>MALE RATS</u>	60	60	60	60	
→ KIDNEY/TUBULAR ADENOMA	3	1	1	7	0.0017*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	3	2	1	9	0.0062*
TESTIS/INTERSTITIAL CELL TUMOR	3	12	12	24	0.000007*
<u>FEMALE RATS</u>					
KIDNEY/TUBULAR ADENOMA	0	0	2	5	0.00399*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	1	2	5	0.011324*

Multiple testing adjustment: The rule proposed by Haseman was used to adjust the effect of multiple testings. Haseman's rule states that in order to keep the false-positive rate at the nominal level of approximately five percent, tumor types with a spontaneous tumor rate of no more than one percent should be tested at .05 level, otherwise the level should be set at .01 (Haseman, (1983), A re-examination of false-positive rates for carcinogenesis studies, Fundamental and Applied Toxicology, 3: 334-339).

On the basis of Haseman's rule, the P-values marked with asterisks are considered to be statistically significant after adjusting for the effect of multiple testings.

The incidence rates and p-values of all tumor types tested for linear trend are given in Table 6.

Pairwise comparisons of the controls with the high dose

In the following we will compare the incidence rates of the control groups with that of the high dose group using the age adjusted Fisher exact test for tumor types whose linear trends were found to be statistically significant.

<u>TUMOR/ORGAN</u>	<u>TUMOR RATE</u>		<u>P-VALUE</u>
	<u>C</u>	<u>H</u>	
<u>MALE RATS</u>	60	60	
~~KIDNEY/TUBULAR ADENOMA~~	~~3~~	~~7~~	~~0.0414~~
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	3	9	0.0494*
TESTIS/INTERSTITIAL CELL TUMOR	3	24	0.0000

FEMALE RATS

KIDNEY/TUBULAR ADENOMA	0	5	0.045*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	5	0.045*

P-values marked with asterisks are considered to be statistically significant.

Note: The results of statistical analysis of tumor data provided by the sponsor are based on the adjusted p-values by the method of Westfall and Young. In general, this technique provides more conservative adjusted p-values if number of testing variables is large. For this reason the conclusions of the sponsor and that of the reviewer may some times differ.

IV. Summary

The mouse study: The tests did not show any statistically significant (at 0.05 level) linear positive trends in mortality or significantly higher mortality in high dose group than any other dose groups in the pairwise comparisons for both sexes.

However, for tumor type analysis, Kidney/Tubular Adenoma & Adenocarcinoma, and Liver/Hepatocellular Adenoma & Carcinoma in male mice and Kidney/Tubular Adenoma, Kidney/Tubular Adenoma & Adenocarcinoma, Liver/Hepatocellular Carcinoma, Liver/Hepatocellular Adenoma, Liver/Hepatocellular Adenoma & Carcinoma, Multiple Organs/Hemangiosarcoma, Multiple Organs/Hemangiosarcoma & Hemangioma, Ovary/Hemangiosarcoma, and Skin other/Hemangiosarcoma in female mice showed statistically significant linear positive

trends.

Moreover, for pairwise comparisons of high dose vs. control groups, tumor types Kidney/Tubular Adenoma & Adenocarcinoma, Liver/Hepatocellular Adenoma, Liver /Hepatocellular Adenoma & Carcinoma, Multiple Organs /Hemangiosarcoma, Multiple Organs /Hemangiosarcoma & Hemangioma, and Ovary/Hemangiosarcoma in female mice showed statistically significant differences.

The rat study: A statistically significant (at .05 level) positive linear trend in mortality was found in female rats only. Pairwise comparisons did not show statistically significantly higher mortality in high dose group than any other dose groups or control group.

For tumor type analysis, Kidney/Tubular Adenoma, Kidney/Tubular Adenoma & Adenocarcinoma, and Testis/Interstitial Cell Tumor in male rats and Kidney/Tubular Adenoma, Kidney/Tubular Adenoma & Adenocarcinoma in female rats showed statistically significant liner positive trends.

Moreover, for pairwise comparisons of high dose vs. control groups, tumor types Testis/Interstitial Cell Tumor in male rats and Kidney/Tubular Adenoma, Kidney/Tubular Adenoma & Adenocarcinoma in female rats showed statistically significant differences.

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HFD-150/Dr. Joseph Sun

HFD-710/Chron

HFD-715/Dr. K. Lin

HFL-715/Dr. Chen

HFD-715/DRU 2.1.1 IND : Zileuton Mouse and Rat
carcinogenicity studies.

HFD-401/Dr. Contrera

HFD-502/Dr. Osterberg or Dr. Taylor

HFD-150/ Bunker

Table 1

Intercurrent mortality rates in the mouse study

Sex	Time(wks)	Control	Low	Medium	High
MALE					
	0 - 52	1/ 60 (1.67)	1/ 60 (1.67)	3/ 60 (5.00)	3/ 60 (5.00)
	53- 78	13/ 59 (23.33)	11/ 59 (20.00)	16/ 57 (31.67)	14/ 57 (28.33)
	79- 92	13/ 46 (45.00)	14/ 48 (43.33)	11/ 41 (50.00)	12/ 43 (48.33)
	93(ND)	8/ 33 (58.33)	7/ 34 (55.00)	5/ 30 (58.33)	13/ 31 (70.00)
	TERM. SACR	25/ 60 (41.67)	27/ 60 (45.00)	25/ 60 (41.67)	18/ 60 (30.00)
FEMALE					
	0 - 52	3/ 60 (5.00)	3/ 60 (5.00)	2/ 60 (3.33)	4/ 58 (6.90)
	53- 78	9/ 57 (20.00)	6/ 57 (15.00)	16/ 58 (30.00)	15/ 54 (32.76)
	79- 92	20/ 48 (53.33)	17/ 51 (43.33)	12/ 42 (50.00)	13/ 39 (55.17)
	93(ND)	8/ 28 (66.67)	12/ 34 (63.33)	4/ 30 (56.67)	9/ 26 (70.69)
	TERM. SACR	20/ 60 (33.33)	22/ 60 (36.67)	26/ 60 (43.33)	17/ 58 (29.31)

Note: Except the TERM. SACR. row, an entry of this table = number of animals dying or sacrificed in the time interval/number of animals entering the time interval. An entry in parenthesis = cumulative mortality rate; i.e. cumulative percent of animals dying up to the end of the time interval. An entry in the TERM. SACR. row = number of animals surviving to terminal sacrifice / initial number of animals. An entry in parenthesis in this row = percent of animals (of the initial number) surviving to terminal sacrifice.
93(ND) = Natural death between weeks 93 and 104.

Table 2A

P-values of tests for positive linear trend in mortality
in the mouse study

Test of homogeneity

<u>Sex</u>	<u>Test</u>	<u>P-value</u> (one tail chi-sqr.)
Male	Cox	.5906
	Wilcoxon	.6516
Female	Cox	.5247
	Wilcoxon	.4452

Test of Positive linear trend

<u>Sex</u>	<u>Test</u>	<u>P-value</u> (one tail Normal)
Male	Cox	.09975
	Wilcoxon	.11375
Female	Cox	.1371
	Wilcoxon	.10625

Table 2B

P-values of pairwise test for the differences in mortality between treated groups in mouse study

Male mouse

GROUP	EXACT ONE TAIL TEST	2X2 CHI- SQUARE TESTS N IN DEN	DIRECTION OF 2X2 CHI-SQ	CHI'S TEST		GENERALIZED L/P ANALYSIS	
				EXACT	INVERSE CONSERVATIVE	EXACT	INVERSE CONSERVATIVE
0 VS. 1	CHI SQ	.1332	NEG	.0779	.0779	.0537	.0537
	PROB	.3567		.7001	.7002	.8167	.8168
0 VS. 2	CHI SQ	.6339	NEG	.0039	.0039	.2300	.2307
	PROB	.4270		.9300	.9300	.6291	.6292
0 VS. 3	CHI SQ	.5609	POS	.6931	.6943	.9204	.9279
	PROB	.2234		.0044	.0046	.3333	.3334
1 VS. 2	CHI SQ	.0000	POS	.0732	.0751	.3343	.3341
	PROB	.3000		.7040	.7040	.9631	.9633
1 VS. 3	CHI SQ	1.7014	POS	1.0040	1.0022	1.4403	1.4391
	PROB	.0939		.2231	.2234	.2301	.2303
2 VS. 3	CHI SQ	1.2991	POS	.9409	.9403	.2132	.2130
	PROB	.1309		.6620	.6623	.6427	.6429

Female mouse

GROUP	EXACT ONE TAIL TEST	2X2 CHI- SQUARE TESTS N IN DEN	DIRECTION OF 2X2 CHI-SQ	CHI'S TEST		GENERALIZED L/P ANALYSIS	
				EXACT	INVERSE CONSERVATIVE	EXACT	INVERSE CONSERVATIVE
0 VS. 1	CHI SQ	.0000	NEG	.2607	.2603	.9443	.9430
	PROB	.3000		.6097	.6099	.3312	.3315
0 VS. 2	CHI SQ	.3596	NEG	.1819	.1817	.0324	.0323
	PROB	.2273		.6697	.6699	.0372	.0373
0 VS. 3	CHI SQ	.2133	POS	.2817	.2811	.3983	.3977
	PROB	.3215		.9936	.9940	.5200	.5203
1 VS. 2	CHI SQ	.3123	NEG	.0000	.0000	.6629	.6620
	PROB	.2082		.9968	.9968	.4963	.4967
1 VS. 3	CHI SQ	.4271	POS	1.4134	1.4103	2.6392	2.6329
	PROB	.2369		.2343	.2330	.1043	.1047
2 VS. 3	CHI SQ	1.9330	POS	1.2341	1.2300	.0001	.0006
	PROB	.0019		.2630	.2636	.3460	.3464

Table 3

Tumor rates and p-values of the tested tumor types for positive linear trend in mouse study

<u>TUMOR/ORGAN</u> <u>MALE MICE</u>	<u>TUMOR RATE</u>				<u>P-VALUE</u>
	<u>C</u> 60	<u>L</u> 60	<u>M</u> 60	<u>H</u> 60	
KIDNEY/TUBULAR ADENOMA	0	0	1	1	0.1351
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	0	1	2	0.0276*
LIVER/HEPATOCELLULAR ADENOMA	8	9	7	13	0.03526
LIVER/HEPATOCELLULAR CARCINOMA	6	1	2	8	0.0252
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	14	10	9	21	0.0077*
LIVER/HEMANGIOMA	1	0	1	2	0.1194
LIVER/HEMANGIOMA & HEMANGIOSARCOMA	2	3	3	3	0.28313
MULTIPLE ORGANS/HEMANGIOSARCOMA	1	4	2	6	0.0206
MULTIPLE ORGANS/HEMANGIOMA & HEMANGIOSARCOMA	3	6	3	8	0.0293
MULTIPLE ORGANS/MALIGNANT LYMPHOCYTIC LYMPHOMA	1	3	2	3	0.237
SKIN OTHER/HEMANGIOSARCOMA	0	0	0	2	0.052
FEMALE MICE					
KIDNEY/TUBULAR ADENOMA	0	0	0	3	0.0143*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	0	0	4	0.0029*
LIVER/HEPATOCELLULAR CARCINOMA	0	1	0	3	0.0224*
LIVER/HEPATOCELLULAR ADENOMA	0	2	0	8	0.000076*
LIVER/HEPATOCELLULAR ADENOMA AND CARCINOMA	0	3	0	11	0.0000*
MULTIPLE ORGANS/HEMANGIOMA	1	3	3	3	0.22
MULTIPLE ORGANS/HEMANGIOSARCOMA	5	1	2	16	0.0000*
MULTIPLE ORGANS/HEMANGIOSARCOMA AND HEMANGIOMA	6	4	5	19	0.0000*
OVARY/HEMANGIOSARCOMA	0	0	0	5	0.000371*
SKIN OTHER/HEMANGIOSARCOMA	0	0	1	3	0.015013*
VAGINA/HEMANGIOMA & HEMANGIOSARCOMA	0	0	0	2	0.065

a: There are only 58 animals in male high dose group.

* indicates statistically significant after Haseman's adjustment for the effect of multiple tests.

Table 4

Intercurrent mortality rates in the rat study

Sex	Time(wks)	Control	Low	Medium	High
MALE					
	0 - 52	1/ 60 (1.67)	2/ 60 (3.33)	4/ 60 (6.67)	3/ 60 (5.00)
	53- 78	8/ 59 (15.00)	7/ 58 (15.00)	10/ 56 (23.33)	7/ 57 (12.67)
	79- 92	10/ 51 (31.67)	10/ 51 (31.67)	9/ 46 (38.33)	6/ 50 (26.67)
	93(ND)	11/ 41 (50.00)	18/ 41 (61.67)	8/ 37 (51.67)	8/ 44 (40.00)
	TERM. SACR	30/ 60 (50.00)	23/ 60 (38.33)	29/ 60 (48.33)	36/ 60 (60.00)
FEMALE					
	0 - 52	1/ 60 (1.67)	1/ 60 (1.67)	1/ 60 (1.67)	1/ 60 (1.67)
	53- 78	13/ 59 (23.33)	10/ 59 (18.33)	5/ 59 (10.00)	4/ 59 (8.33)
	79- 92	9/ 46 (38.33)	9/ 49 (33.33)	11/ 54 (28.33)	10/ 55 (25.00)
	93(ND)	8/ 37 (51.67)	8/ 40 (46.67)	13/ 43 (50.00)	7/ 45 (36.67)
	TERM. SACR	29/ 60 (48.33)	32/ 60 (53.33)	30/ 60 (50.00)	38/ 60 (63.33)

Note: Except the TERM. SACR. row, an entry of this table = number of animals dying or sacrificed in the time interval/number of animals entering the time interval. An entry in parenthesis = cumulative mortality rate; i.e. cumulative percent of animals dying up to the end of the time interval. An entry in the TERM. SACR. row = number of animals surviving to terminal sacrifice / initial number of animals. An entry in parenthesis in this row = percent of animals (of the initial number) surviving to terminal sacrifice.
93(ND) = Natural death between weeks 93 and 104.

Table 5A

P-values of tests for positive linear trend in mortality
in the rat study

Test of homogeneity

<u>Sex</u>	<u>Test</u>	<u>P-value</u> (one tail chi-sqr.)
Male	Cox	.2196
	Wilcoxon	.3240
Female	Cox	.3193
	Wilcoxon	.2529

Test of Positive linear trend

<u>Sex</u>	<u>Test</u>	<u>P-value</u> (one tail Normal)
Male	Cox	.078
	Wilcoxon	.109
Female	Cox	.0345
	Wilcoxon	.0222

Table 5B

P-values of pairwise test for the differences in mortality between treatment groups in rat study

Male rat

GROUP	EXACT ONE TAIL TEST	2x2 CHI- SQUARE USING N IN DEN	DIRECTION ^a OF 2x2 CHI-SQ	CHI'S TEST		GENERALIZED L/M ANALYSIS	
				EXACT	INVERSE CONSERVATIVE	EXACT	INVERSE CONSERVATIVE
0 VS. 1	CHI SQ	.5371	POS	.3049	.3044	.5365	.5362
	PROB	.2319		.4773	.4776	.4639	.4640
0 VS. 2	CHI SQ	.0000	POS	.0422	.0422	.4030	.4036
	PROB	.5724		.8372	.8373	.5232	.5232
0 VS. 3	CHI SQ	1.6629	NEG	1.2719	1.2701	1.0634	1.0623
	PROB	.0985		.2394	.2398	.3024	.3027
1 VS. 2	CHI SQ	.5371	NEG	.1073	.1071	.0014	.0014
	PROB	.2319		.7433	.7435	.9703	.9703
1 VS. 3	CHI SQ	4.8120	NEG	3.7397	3.7461	2.9047	2.9776
	PROB	.01391		.0325	.0329	.0041	.0044
2 VS. 3	CHI SQ	1.6629	NEG	1.0639	1.0615	2.2310	2.2204
	PROB	.0985		.1722	.1724	.1355	.1355

Female rat

GROUP	EXACT ONE TAIL TEST	2x2 CHI- SQUARE USING N IN DEN	DIRECTION OF 2x2 CHI-SQ	CHI'S TEST		GENERALIZED L/M ANALYSIS	
				EXACT	INVERSE CONSERVATIVE	EXACT	INVERSE CONSERVATIVE
0 VS. 1	CHI SQ	.1334	NEG	.1304	.1303	.2116	.2115
	PROB	.3576		.7099	.7099	.6455	.6456
0 VS. 2	CHI SQ	.0000	NEG	.1363	.1362	.5595	.5590
	PROB	.5000		.7120	.7121	.4545	.4546
0 VS. 3	CHI SQ	2.1620	NEG	2.0323	2.0407	3.0091	3.0037
	PROB	.0705		.0912	.0914	.04064	.04064
1 VS. 2	CHI SQ	.0334	POS	.0009	.0009	.0625	.0625
	PROB	.4276		.9766	.9766	.0025	.0026
1 VS. 3	CHI SQ	.0571	NEG	1.3416	1.3406	2.1657	2.1630
	PROB	.1773		.2467	.2469	.1411	.1413
2 VS. 3	CHI SQ	1.6629	NEG	1.5200	1.5199	1.6702	1.6693
	PROB	.0985		.2175	.2176	.1962	.1963

Table 6

Tumor rates and p-values of the tested tumor types for positive linear trend in rat study

<u>TUMOR/ORGAN</u>	<u>TUMOR RATE</u>				<u>P-VALUE</u>
	<u>C</u>	<u>L</u>	<u>M</u>	<u>H</u>	
<u>MALE RAT</u>	60	60	60	60	
KIDNEY/TUBULAR ADENOMA	3	1	1	7	~~0.0017*~~ 0.026
KIDNEY/TUBULAR ADENOCARCINOMA	0	1	0	2	0.14
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	3	2	1	9	0.0062*
PITUITARY/NOS ADENOMA	25	29	29	21	0.8845
SKIN/PAPILLOMA	1	0	0	2	0.137
TESTIS/INTERSTITIAL CELL TUMOR	3	12	12	24	0.000007*
THYROID/FOLLICULAR ADENOMA	1	2	1	4	0.443
<u>FEMALE RATS</u>					
ADRENAL/ADENOMA	1	3	3	0	0.872
ADRENAL/PHEOCHROMOCYTOMA	1	2	2	2	0.452
KIDNEY/TUBULAR ADENOMA	0	0	2	5	0.00399*
KIDNEY/TUBULAR ADENOMA AND ADENOCARCINOMA	0	1	2	5	0.011324*
LIVER/HEPATOCELLULAR ADENOMA	0	2	0	3	0.0954
LIVER/HEPATOCELLULAR CARCINOMA	1	0	0	2	0.167
LIVER/HEPATOCELLULAR ADENOMA & CARCINOMA	1	2	0	5	0.0383

* indicates statistically significant after Haseman's adjustment for the effect of multiple tests.

Figure 1a

Kaplan-Mier Estimates of the survival distributions
(Male mice)

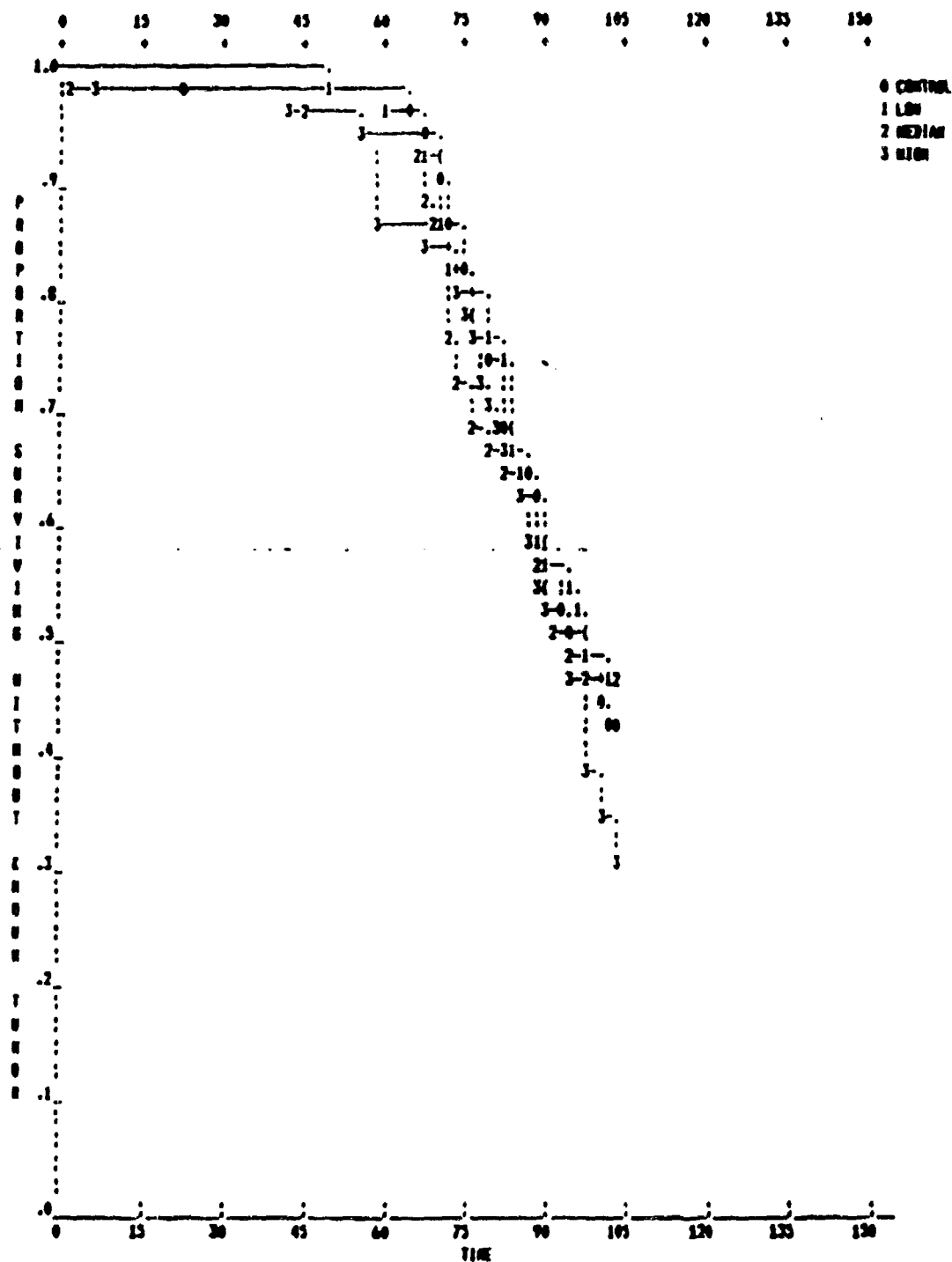


Figure 1b

Kaplan-Mier Estimates of the survival distributions
(Female mice)

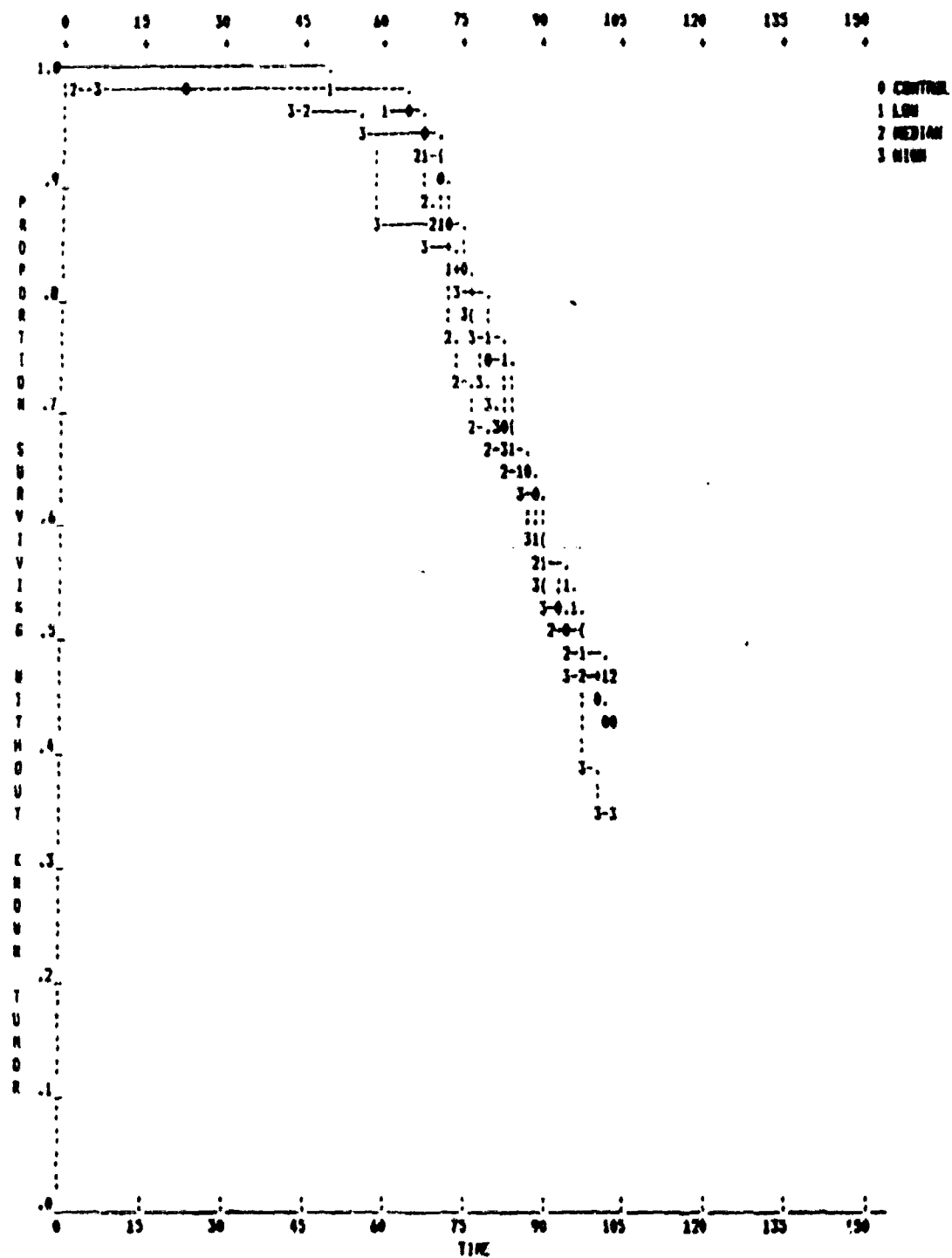


Figure 2a

Kaplan-Mier Estimates of the survival distributions
(Male rats)

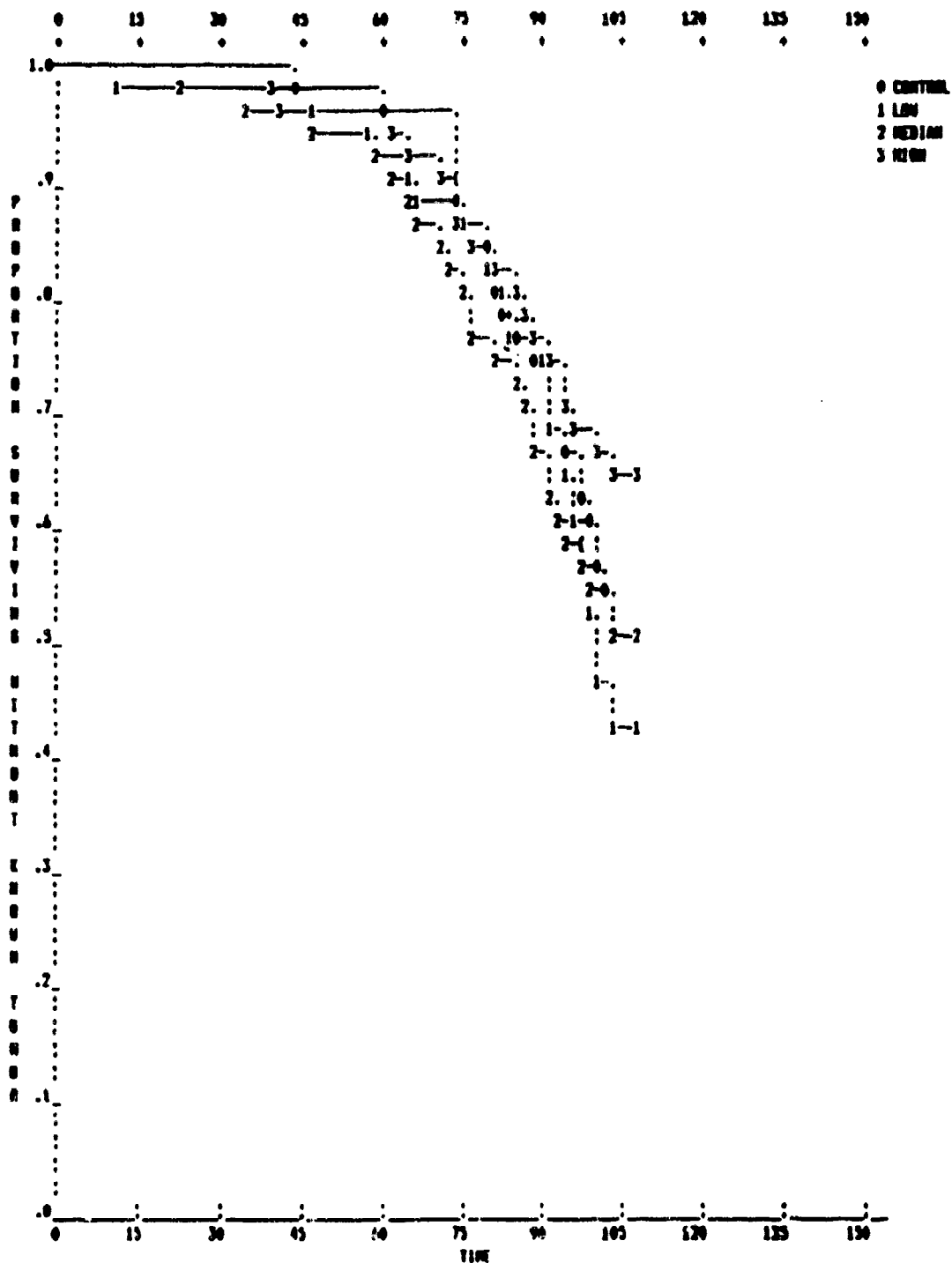
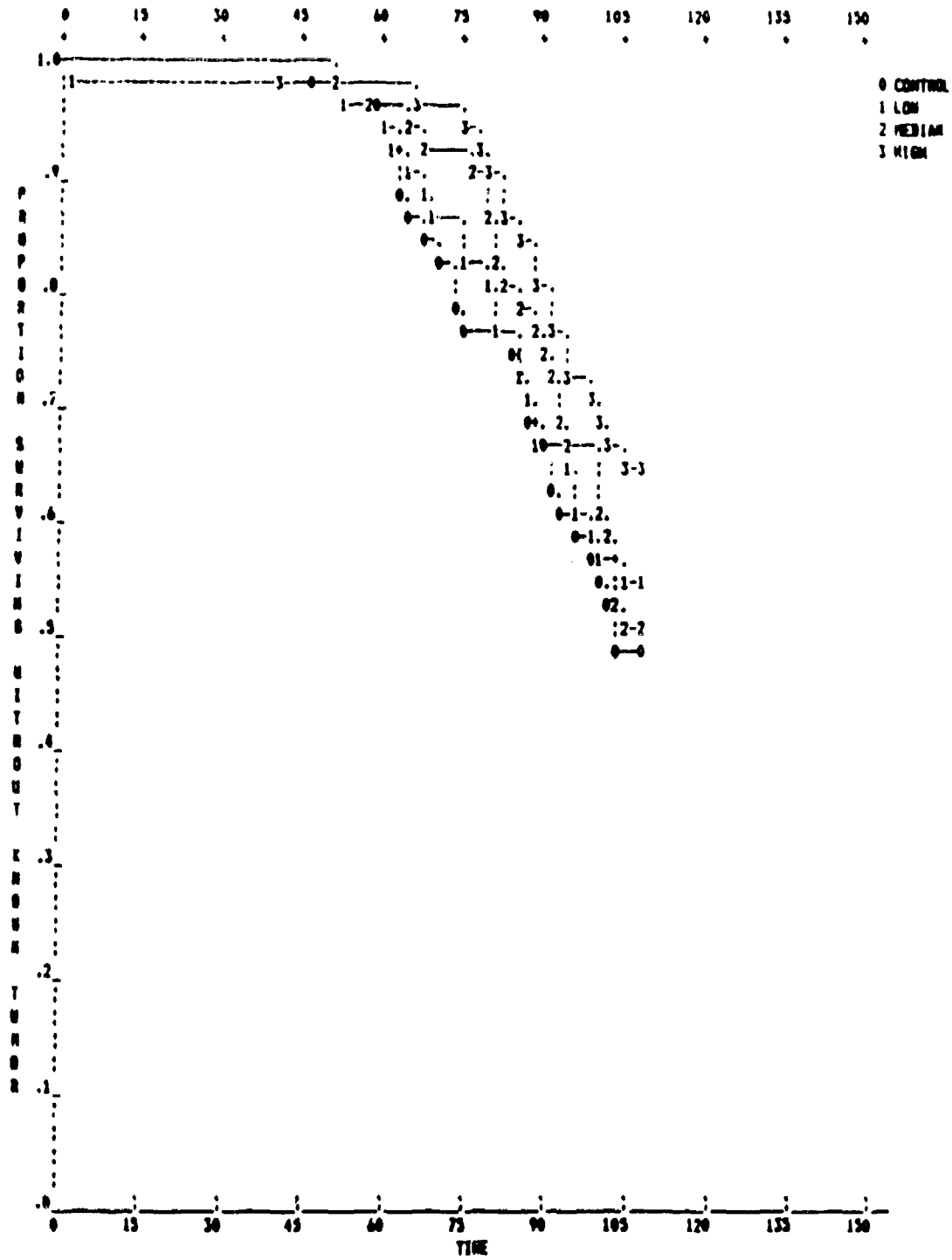


Figure 2b

Kaplan-Mier Estimates of the survival distributions
(Female rats)



Chemist Review

DIVISION OF PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-471 CHEM.REVIEW #: 5 REVIEW DATE: 11/15/96

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL	7/18/94	7/19/94	7/21/94
AMENDMENT	8/19/94	8/22/94	9/1/94
AMENDMENT	3/28/95	3/29/95	3/31/95
AMENDMENT	6/16/95	6/19/95	6/23/95
AMENDMENT	3/22/96	3/25/96	3/27/96
→ This Review			
AMENDMENT (AZ)	6/7/96	6/10/96	6/10/96
AMENDMENT (BZ)	7/23/96	7/24/96	7/26/96
AMENDMENT (BC)	9/11/96	9/12/96	9/12/96
AMENDMENT (BZ)	10/23/96	10/24/96	10/25/96
AMENDMENT (BZ)	11/7/96	11/8/96	11/13/96
AMENDMENT (BC)	11/7/96	11/8/96	11/13/96

NAME & ADDRESS OF APPLICANT: Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064-3500

DRUG PRODUCT NAME
Proprietary: Zyflo Tablets
Nonproprietary/USAN: Zileuton tablets
Code Name/#: A-64077, ABT-077, Code 74975
Chem.Type/Ther.Class: 1 S

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL. CATEGORY/INDICATION: A 5-lipoxygenase inhibitor for prevention and control of bronchospasm and for maintenance therapy of asthma patients.

DOSAGE FORM: immediate release tablets

STRENGTHS: 300,600 mg of zileuton

ROUTE OF ADMINISTRATION: oral, 600 mg QID

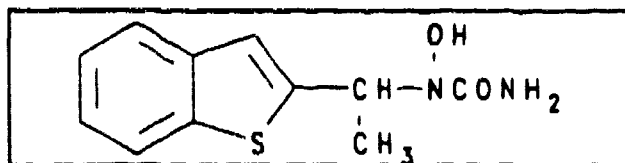
DISPENSED: Rx OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

N-(1-Benzo(b)thien-2-ylethyl)-
N-hydroxyurea

Molecular Formula: C₁₁H₁₂N₂O₂S

Molecular Weight: 236.29



CONSULTS:

- 1. Environmental assessment, submitted 8/22/94, reviewed 2/8/95 by FDA Environmental Officer, deficient, deficiencies sent to applicant by CSO (4/20/95). Response to the deficiency letter was reviewed and found adequate. Finding of no significant impact (FONSI) was issued on July 25, 1995.
- 2. Trademark name, ZYFLO, submitted in the 11/6/96 draft package insert after several unacceptable proposed names. The labeling committee accepted this name 10/18/96 in response to our request for a consult. Several name were proposed, but were rejected.
- 3. Biometrics requested 1/6/95, review dated 4/14/95 found all strengths packaged in 40 and 100 tablet bottles and push through blisters satisfactory for 24 month expiry. Additional data on peelable blister package and the 500 tablet bottle was submitted in the 6/16/95 amendment and found satisfactory in the 8/25/95 chemist's review.
- 4. Biopharmaceutics consult request regarding dissolution data and specifications was sent on August 25, 1995. The Biopharmaceutics reviewer performed a separate review and the deficiencies sent to applicant in the 10/19/95 NA letter. The response was reviewed by the Biopharmaceutics reviewer and resolved in the 9/9/96 biopharm review of the 3/22/96 amendment.
- 5. Establishment evaluation, submitted 9/21/94, acceptable 4/12/95. FUR was submitted 8/15/95, found acceptable 9/18/95, but an electronic message from HFD-324 on 1/24/96 sent an OAI notification that "YOU SHOULD NOT APPROVE THIS NDA". The OAI from San Juan includes items such as: unvalidated reworks, significant batch variability, high failure rate at release, and lack of particle specifications. HFD-324 requested an update from the DOs 8/2/96 and the facilities were found satisfactory on 8/9/96.
- 6. Methods validation, submitted 10/7/94 were completed at Detroit Lab. (8/8/95) and the St. Louis Lab (12/19/95) and found satisfactory for regulatory purposes.
- 7. The draft package insert submitted 11/6/96 has a satisfactory DESCRIPTION and HOW SUPPLIED section.

SUPPORTING DOCUMENTS:

<u>Support</u> <u>Doc#</u>	<u>Holder/</u> <u>Applicant</u>	<u>Content/</u> <u>Item</u>	<u>Status</u>	<u>Review</u> <u>Date</u>	<u>Letter</u> <u>Date</u>

REMARKS/COMMENTS:

Previous chemist's review (#4, JLeak, 4/24/96) of the 3/22/96 amendment found deficiencies which were listed in our FAX to the applicant dated 5/1/96. The submissions which followed satisfactorily address the deficiencies.

Enclosed is a list of supporting documents, a summary page, and a discussion of submissions and conversations with the applicant.

CONCLUSIONS & RECOMMENDATIONS:

The application is approvable from the standpoint of manufacturing and controls.

NOTE TO CSO:

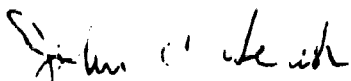
Please:

- 1) include in the approval letter the dosage sizes (300mg and 600mg)
- 2) inform applicant to use the USAN chemical name in the DESCRIPTION section of the package insert.
- 3) inform applicant to reinstate the statement "Blister pack with foil peelable backing" (or what else they plan on selling) in the description of the "Abbo-Pac" at the end of the HOW SUPPLIED section of the package insert.

cc:

Orig. NDA 20-471
HFD-150/Division File
HFD-150/JLeak/GPoochikian
HFD-151/CSO
HFD-150/medical officer

R/D Init by: GPU 11/19/96
filename: 20471C.NDA



John C. Leak, Ph.D., Review Chemist

APR 29 1996

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-471 CHEM.REVIEW #: 4 REVIEW DATE: 4/24/96

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL	7/18/94	7/19/94	7/21/94
AMENDMENT	8/19/94	8/22/94	9/1/94
AMENDMENT	3/28/95	3/29/95	3/31/95
AMENDMENT	6/16/95	6/19/95	6/23/95
AMENDMENT			
→ This Review	3/22/96	3/25/96	3/27/96

NAME & ADDRESS OF APPLICANT: Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064-3500

DRUG PRODUCT NAME

Proprietary: Leutrol Tablets
Nonproprietary/USAN: Zileuton tablets
Code Name/#: A-64077, ABT-077, Code 74975
Chem. Type/Ther. Class: 1 S

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL. CATEGORY/INDICATION: A 5-lipoxygenase inhibitor for prevention and control of bronchospasm and for maintenance therapy of asthma patients.

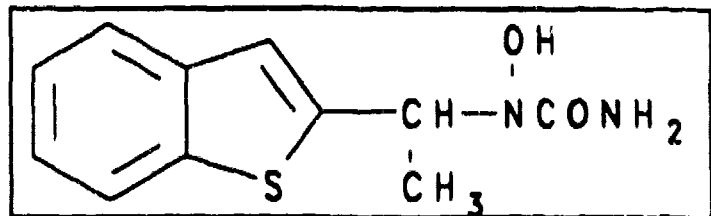
DOSAGE FORM: immediate release tablets
STRENGTHS: 300, 600 mg of zileuton
ROUTE OF ADMINISTRATION: oral, 600 mg QID
DISPENSED: Rx OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

N-(1-Benzo(b)thien-2-ylethyl)-
N-hydroxyurea

Molecular Formula: C₁₁H₁₂N₂O₂S

Molecular Weight: 236.29



CONSULTS:

- Environmental assessment, submitted 8/22/94, reviewed 2/8/95 by FDA Environmental Officer, deficient, deficiencies sent to applicant by CSO (4/20/95). Response to the deficiency letter was reviewed and found adequate. Finding of no significant impact (FONSI) was issued on July 25, 1995.
- Trademark name, submitted 8/22/94, acceptable 11/7/94. However, In the tradename, Abbott plans to develop a new name. as their
- Biometrics requested 1/6/95, review dated 4/14/95 found all strengths packaged in 40 and 100 tablet bottles and push through blisters satisfactory for 24 month expiry. Additional data on peelable blister package and the 500 tablet bottle was submitted in the 6/16/95 amendment and found satisfactory in the 8/25/95 chemist's review.
- Biopharmaceutics consult request regarding dissolution data and specifications was sent on August 25, 1995. The Biopharmaceutics reviewer performed a separate review and the deficiencies sent to applicant in the 10/19/95 NA letter are addressed in the last amendment and are being reviewed by the Biopharmaceutics reviewer.
- Establishment evaluation, submitted 9/21/94, acceptable 4/12/95. FUR was submitted 8/15/95, found acceptable 9/18/95, but an electronic message from HFD-324 on 1/24/96 sent an OAI notification that "YOU SHOULD NOT APPROVE THIS NDA". The OAI from San Juan includes items such as: unvalidated reworks, significant batch variability, high failure rate at release, and lack of particle specifications.
- Methods validation, submitted 10/7/94 were completed at Detroit Lab. (8/8/95) and the St. Louis Lab (12/19/95) and found satisfactory for regulatory purposes.

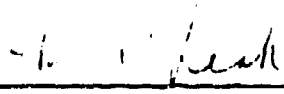
REMARKS/COMMENTS:

Previous chemist's review (CKim, 8/25/95) found deficiencies which were listed in our not approvable letter of 10/19/95. The 3/22/96 submission addresses the deficiencies.

CONCLUSIONS & RECOMMENDATIONS:

The deficiencies listed in the draft letter and any from the other disciplines should be sent to the applicant for correction. Since the drug product manufacturing facility is considered out of compliance, this will have to be corrected before the application can be approved.

cc:
Orig. NDA 20-471
HFD-150/Division File
HFD-150/JLeak/GPoochikian
HFD-151/CSO
HFD-150/medical officer



John C. Leak, Ph.D., Review Chemist

R/D Init by: *CUP* 4/29/96
filename: 20471B.NDA

Buccine

320

DIVISION OF PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-471 CHEM. REVIEW #: 3 REVIEW DATE: 8/25/95

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL	7/18/94	7/19/94	7/21/94
AMENDMENT	8/19/94	8/22/94	9/1/94
AMENDMENT	3/28/95	3/29/95	3/31/95
AMENDMENT (N-BC)*	6/16/95	6/19/95	6/23/95
AMENDMENT (N-BL)*	6/16/95	6/19/95	7/5/95

*Subject of this review

NAME & ADDRESS OF APPLICANT: Abbott Laboratories
One Abbott Park Road
Abbott Park, IL 60064-3500

DRUG PRODUCT NAME
Proprietary: Leutrol Tablets
Nonproprietary/USAN: Zileuton tablets
Code Name/#: A-64077, ABT-077, Code 74975
Chem. Type/Ther. Class: 1 8

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL. CATEGORY/INDICATION: A 5-lipoxygenase inhibitor for prevention and control of bronchospasm and for maintenance therapy of asthma patients.

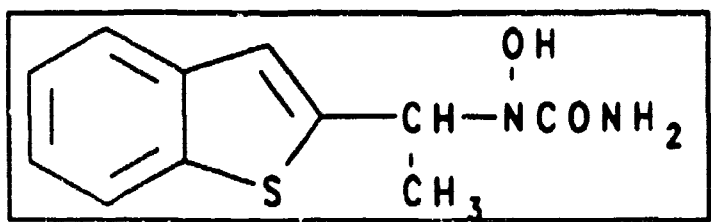
DOSAGE FORM: immediate release tablets
STRENGTHS: 300, 600 mg of zileuton
ROUTE OF ADMINISTRATION: oral, 600 mg QID
DISPENSED: X Rx OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea

Molecular Formula: C₁₁H₁₂N₂O₂S

Molecular Weight: 236.29



NDA #20-471

page 2

SUPPORTING DOCUMENTS:

<u>Support</u> <u>Doc#</u>	<u>Holder/</u> <u>Applicant</u>	<u>Content/</u> <u>Item</u>	<u>Status</u>	<u>Review</u> <u>Date</u>	<u>Letter</u> <u>Date</u>

CONSULTS:

- Environmental assessment: Submitted 8/22/94, reviewed 2/8/95 by FDA Environmental Officer found it deficient; deficiencies sent to applicant by CSO (4/20/95). Response to the deficiency letter was reviewed and found adequate. Finding of no significant impact (FONSI) was issued on July 25, 1995.
- Trademark name: Submitted 8/22/94, acceptable 11/7/94, however

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d

products in the U.S. marketplace. In the event Abbott

- Biometrics requested 1/6/95, review dated 4/14/95 found all strengths packaged in 40 and 100 tablet bottles and push through blisters satisfactory for 24 month expiry.
- Biopharmaceutics consult request regarding dissolution data and specifications was sent on August 25, 1995. The Biopharmaceutics reviewer should directly communicate with the applicant.
- Establishment evaluation, submitted 9/21/94, acceptable 4/12/95. FUR has been submitted (8/15/95).
- Methods validation, submitted 10/7/94, were completed at Detroit Lab., however, St. Louis Lab. is expected to complete their validation by the end of August, 1995 (see the telecon dated 8/16/95). Once St. Louis Lab. completes their validation, I will review the results from both laboratories.

REMARKS/COMMENTS:

Previous chemist's review #1 (JLeak, 12/21/94) found deficiencies which were listed in our letter of 1/12/95. Two telephone conversations discussing the deficiencies with the applicant were held 2/15/95 and 3/23/95. The 3/28/95 submission was reviewed (Review #2) by Dr. Leak and a deficiency letter was sent on May 17, 1995.

There are two amendments dated June 16, 1995. The N(BC) amendment responds to the CMC comments, while the N(BL) responds to package insert comments. The package insert is adequate from CMC viewpoint.

Data provided by the applicant indicate that there seems to be a difference in dissolution profile of batches manufactured in 1991-92 compared with those manufactured in 1993, e.g., the dissolution rate of zileuton is faster in batches manufactured in 1993. Based on this information, following questions are raised:

Is the proposed dissolution specification (dissolved in 45 minutes) based on data from drug product manufactured in 1992 appropriate in view of the faster dissolution rate of drug product manufactured in 1993? Is there any need for other specifications, e.g., dissolution profile, to ensure efficacy of the drug product? Is there any safety concern? A consult review request on these questions has been submitted to our Division of Biopharmaceutics as indicated above.

NDA #20-471

page 4.

CONCLUSIONS & RECOMMENDATIONS:

The additional deficiency listed in the draft letter should be sent to the applicant for correction.

Applicant is being advised of a potential problem with their proposed trade name Leutrol because of the recently approved NDA fo

cc:

Orig. NDA 20-471

HFD-155/Division File

HFD-155/CHKim/GPoochikian

HFD-155/JSun

HFD-155/JBuccine

HFD-155/RAnthracite

R/D Init by: *ACL for GP 8/25/95*
filename: n20-471a.616

Chong-Ho Kim 8/25/95
Chong-Ho Kim, Ph.D.
Review Chemist

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
 Review of Chemistry, Manufacturing, and Controls

NDA #: 20-471 **CHEM.REVIEW #:** 2 **REVIEW DATE:** 4/26/95

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL	7/18/94	7/19/94	7/21/94
AMENDMENT	8/19/94	8/22/94	9/1/94
→ This Review	3/28/95	3/29/95	3/31/95

NAME & ADDRESS OF APPLICANT: Abbott Laboratories
 One Abbott Park Road
 Abbott Park, IL 60064-3500

DRUG PRODUCT NAME

Proprietary: Leutrol Tablets
Nonproprietary/USAN: Zileuton tablets
Code Name/#: A-64077, ABT-077, Code 74975
Chem.Type/Ther.Class: 1 S

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL.CATEGORY/INDICATION: A 5-lipoxygenase inhibitor for prevention and control of bronchospasm and for maintenance therapy of asthma patients.

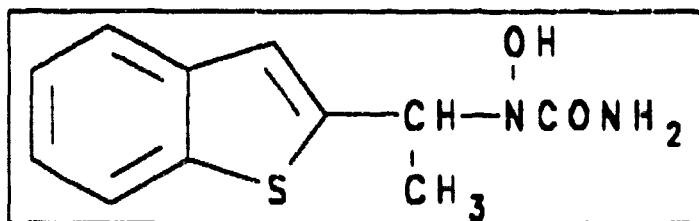
DOSAGE FORM: immediate release tablets
STRENGTHS: 300, 600 mg of zileuton
ROUTE OF ADMINISTRATION: oral, 600 mg QID
DISPENSED: X Rx OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL.WT:

N-(1-Benzo(b)thien-2-ylethyl)-
 N-hydroxyurea

Molecular Formula:
 $C_{11}H_{12}N_2O_2S$

Molecular Weight:
 236.29



CONSULTS:

Environmental assessment, submitted 8/22/94, reviewed 2/8/95 by FDA
Environmental Officer, deficient, deficiencies sent to applicant by CSO
and awaiting applicant's response.
Trademark name, submitted 8/22/94, acceptable 11/7/94
Biometrics requested 1/6/95, review dated 4/14/95 found all strengths packaged
in 40 and 100 tablet bottles and push through blisters satisfactory for
24 month expiry, but insufficient data on peelable blister package. A
deficiency concerning the peelable blister pack is included in the draft
letter. Data on the 120 and 500 tablet bottles were not analyzed by
Biometrics since only 6 months data on one lot of each size was
presented and there were not enough data points to provide for a
satisfactory analysis. See the comment under the discussion of item 12
below for further information on these two package sizes.
Biopharmaceutics, performing a separate review
Establishment evaluation, submitted 9/21/94, acceptable 4/12/95
Methods validation, submitted 10/7/94, pending

REMARKS/COMMENTS:

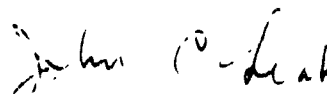
Previous chemist's review (JLeak, 12/21/94) found deficiencies
which were listed in our letter of 1/12/95. Two telephone
conversations discussing the deficiencies with the applicant were
held 2/15/95 and 3/23/95. The 3/28/95 submission addresses the
deficiencies.

CONCLUSIONS & RECOMMENDATIONS:

The additional deficiencies listed in the draft letter should
be sent to the applicant for correction.

cc:

Orig. NDA 20-471
HFD-150/Division File
HFD-150/JLeak/GPoochikian
HFD-151/CSO
HFD-150/medical officer



John C. Leak, Ph.D., Review Chemist

R/D Init by: *AP 5/4/95*
filename: 20471A.NDA

NDA 20-471 Abbott Laboratories Zileuton Tablets

1

DIVISION OF ONCOLOGY AND PULMONARY DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-471 CHEM. REVIEW #: 1 REVIEW DATE: 12/21/94

<u>SUBMISSION TYPE</u>	<u>DOCUMENT DATE</u>	<u>CDER DATE</u>	<u>ASSIGNED DATE</u>
ORIGINAL	7/18/94	7/19/94	7/21/94
AMENDMENT	8/19/94	8/22/94	9/1/94
<u>NAME & ADDRESS OF APPLICANT:</u>		Abbott Laboratories One Abbott Park Road Abbott Park, IL 60064-3500	

<u>DRUG PRODUCT NAME</u>	Leutrol Tablets
<u>Proprietary:</u>	Zileuton tablets
<u>Nonproprietary/USAN:</u>	A-64077, ABT-077, Code 74975
<u>Code Name/#:</u>	1 S
<u>Chem. Type/Ther. Class:</u>	

ANDA Suitability Petition/DESI/Patent Status: N/A

PHARMACOL. CATEGORY/INDICATION: A 5-lipoxygenase inhibitor for prevention and control of bronchospasm and for maintenance therapy of asthma patients.

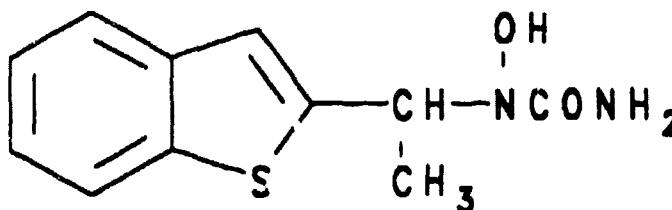
<u>DOSAGE FORM:</u>	immediate release tablets
<u>STRENGTHS:</u>	300, 600 mg of zileuton
<u>ROUTE OF ADMINISTRATION:</u>	oral, 600 mg QID
<u>DISPENSED:</u>	☒ Rx ☐ OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOL. WT:
N-(1-Benzo(b)thien-2-ylethyl)-
N-hydroxyurea

Molecular Formula: C₁₁H₁₂N₂O₂S

Molecular Weight: 236.29

SUPPORTING DOCUMENTS:



Support Doc#	Holder/ Applicant	Content/ Item	Status	Review Date	Letter Date

RELATED DOCUMENTS (if applicable):

CONSULTS:

Environmental assessment, submitted 8/22/94, pending
Trademark name, submitted 8/22/94, acceptable 11/7/94
Biometrics - ~~to be requested, see page 15 of review~~ requested 1/6/95
Biopharmaceutics, performing a separate review
Establishment evaluation, submitted 9/21/94, pending
Methods validation, submitted 10/7/94, pending

REMARKS/COMMENTS:

A number of package configurations are proposed, but significant stability data has been submitted for only some of the package configurations (bottles of 40 and 100 and push through blisters). Several consults are still outstanding and questions relating to both drug substance and drug product need to be addressed by the applicant.

CONCLUSIONS & RECOMMENDATIONS:

There are several deficiencies noted below in the draft letter which the applicant should be able to correct in a minimal amount of time.

cc:
Orig. NDA 20-471
HFD-150/Division File
HFD-150/JLeak/GPoochikian
HFD-151/CSO
HFD-150/medical officer
HFD-102/CKumkumian [#1 only]
R/D Init by:
filename: 20471FIL.NDA

gxl. f. 1/6/95

John C. Leak

John C. Leak, Ph.D., Review Chemist

BUCCEINE

JUL 25 1995

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

ZILEUTON TABLETS

300, 400 and 600 mg

NDA 20-471

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF PULMONARY DRUG PRODUCTS

(HFD-155)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-471

Zileuton Tablets

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 **Zileuton Tablets**, **Abbott Laboratories** has conducted a number of environmental studies and prepared an environmental assessment in accordance with 21 CFR 25.31a(a) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Zileuton is a synthetic drug which is administered as an oral tablet in the treatment of asthma and for the prevention and control of symptoms associated with reversible bronchospasm. The drug substance will be manufactured by Abbott Laboratories, North Chicago, Illinois and Abbott S.p.A., Aprilia, Italy. The drug product will be manufactured at Abbott Laboratories, Abbott Park, Illinois or Abbott Pharmaceuticals, Barceloneta, Puerto Rico. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Zileuton may enter the environment from excretion by patients or from emissions from manufacturing sites. Chemical and physical test results indicate that the majority of the drug substance will most likely be restricted to the aquatic environment. Data indicates that the substance is biodegradable by acclimated microflora and is susceptible to photolysis.

As zileuton is expected to persist in the aquatic environment for some time, the toxicity of the material to organisms was characterized. Acute static toxicity studies in water fleas (*Daphnia magna*) and rainbow trout (*Oncorhynchus mykiss*) and microbial inhibition studies indicate that the drug substance is generally not toxic to organisms at concentrations several orders of magnitude greater than the maximum expected environmental concentrations.

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Off-specification drug substance will be disposed of at a licensed incineration facility and unused or rejected drug product will be disposed of at a licensed landfill or incineration facility. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic procedures. 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, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

7/24/95
DATE

Nancy B. Sager

PREPARED BY
Nancy B. Sager
Environmental Scientist
Center for Drug Evaluation and Research

7/25/95
DATE

R. A. Jerussi

CONCURRED
Robert A. Jerussi, Ph.D.
Associate Director for Chemistry
Center for Drug Evaluation and Research

Attachment I: Environmental Assessment
Attachment II: Certifications (Abbott S.p.A)
Attachment III: Fate and Effects Summary
Attachment IV: Certifications
Attachment V: Material Safety Data Sheet

ATTACHMENT I

3.6 Environmental Assessment

NDA SUBSECTION 3.6
ENVIRONMENTAL ASSESSMENT
Zileuton Tablets
(300, and 600 mg)

Abbott Laboratories
100 Abbott Park Road
Abbott Park, Illinois 60064-3500

The National Environmental Policy Act requires Environmental Assessments (EAs) to be public documents. Subsections 3.6.1 through 3.6.14 (i.e., Items 1 through 14 of this EA) and accompanying Appendix A (from Subsection 3.6.15) are suitable for public disclosure. The latter nonconfidential subsections and appendix are complete with the exception of proprietary information. This proprietary information, which is contained in Appendices B (1 through 6), C (1 through 21), and D (1 and 2), could be beneficial to competitors and, therefore, must remain confidential. The text of the public document is based on the text of Appendix C. The latter appendix is intended for review as the confidential version of EA Items 1 through 15.

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3.6.1 DATE

May 30, 1995

3.6.2 NAME OF APPLICANT

Abbott Laboratories

3.6.3 ADDRESS

100 Abbott Park Road

Abbott Park, Illinois 60064-3500

3.6.4 DESCRIPTION OF THE PROPOSED ACTION

3.6.4.1 REQUESTED APPROVAL

Approval of NDA 20-471 is sought for the synthesis of zileuton bulk drug substance and the manufacture, packaging, distribution and use of the product designated in the Environmental Assessment (EA) as zileuton tablets--300, and 600 mg. Abbott Laboratories has filed an NDA pursuant to Section 505(b) of the Food, Drug, and Cosmetic Act for zileuton tablets packaged in high density polyethylene bottles and formed blister/foil packages. An EA has been submitted in accordance with 21 CFR § 25.31a(a).

The National Environmental Policy Act requires EAs to be public documents. Subsections 3.6.1 through 3.6.14 (i.e., Items 1 through 14 of this EA) and accompanying Appendix A (from Subsection 3.6.15) are suitable for public disclosure. The latter nonconfidential subsections and appendix are complete with the exception of proprietary information. This proprietary information, which is contained in Appendices B (1 through 7), C (1 through 21), and D (1 and 2), could be beneficial to competitors and, therefore, must remain confidential.

Appendix B is an assemblage of laboratory reports that are relevant to the environmental fate and effects of the drug substance. Appendix C contains all information presented in Subsections 3.6.1 through 3.6.14 with the addition of proprietary information discussed in appropriate places. Appendix D provides the Material Safety Data Sheets.

Appendix C is intended for review as the confidential version of Items 1 through 15. The text of the EA in Subsections 3.6.1 through 3.6.14 is based on the text in Appendix C. When Appendix C is cited in this EA, its enumerated subdivisions (i.e., C-1, C-2, etc.) are not indicated, because such citation could reveal confidential information. The source of specific information cited as Appendix C can be found in the appendix subsection (e.g., C.4.1) corresponding to the EA.

3.6.4.2 NEED FOR ACTION

Zileuton is a potent, specific inhibitor of 5-lipoxygenase, an enzyme involved in the synthesis of leukotrienes, a group of biochemicals implicated in the pathophysiology of asthma and other inflammatory disease states. These disease states have been shown to be associated with elevated levels of the leukotrienes.

The efficacy of zileuton in the treatment of patients afflicted with asthma is thought to be due to its suppression of leukotriene synthesis. The indication and usage of zileuton proposed in this EA is for the prevention and control of symptoms associated with reversible bronchospasm and as chronic therapy in symptomatic asthma patients requiring daily inhaled beta-agonists.

3.6.4.3 LOCATIONS OF MANUFACTURE

Four locations are involved in the manufacture of this product (Figure 3.6.4-1). Approval is sought to synthesize the bulk drug substance at two locations as follows: (1) Abbott Laboratories, 1401 Sheridan Road, North Chicago, Illinois 60064, and (2) Laboratory Abbott S.p.A., Via Pontina, Km 52, 04010 Campoverde, Aprilia, Italy.

Approval is also sought to manufacture, package, and distribute the tablets at two additional locations: (1) Abbott Laboratories, 100 Abbott Park Road, Abbott Park, Illinois 60064-3500, and (2) Abbott Pharmaceuticals, Inc., KM 58.0 Carretera 2, Cruce Davila, Barceloneta, Puerto Rico 00617. Tableting may occur at either or both of the latter two locations. All packaging operations are carried out at these two drug product manufacturing facilities.

3.6.4.4 LOCATIONS OF USE AND DISPOSAL

As medication prescribed to alleviate the symptoms of asthma, the tablets in which zileuton is present will be ingested by patients throughout the United States. The resulting substances that are eliminated (or excreted) will enter municipal treatment systems. With regard to disposal of material from manufacturing sources, off specification lots of bulk drug substance will be sent to one of a number of alternative contractors for incineration

1. Unused products (past the labeled expiration date) will be returned to Abbott Laboratories where they will be accumulated, classified (as appropriate), and sent offsite for landfilling as nonhazardous solid waste

Landfilling at this location is permitted by the

Rejected off

specification lots of drug product manufactured at the Abbott Park facility will be disposed of at the same approved landfill. Zileuton is not classified as a hazardous waste in accordance with US EPA regulations 40 CFR Part 261. Therefore, the material can be disposed of in a non-hazardous waste landfill or incinerator. Rejected off specification lots of drug product manufactured at the will be sent to an approved medical waste incinerator, which is currently

0021CF, and 1280-0021CG).

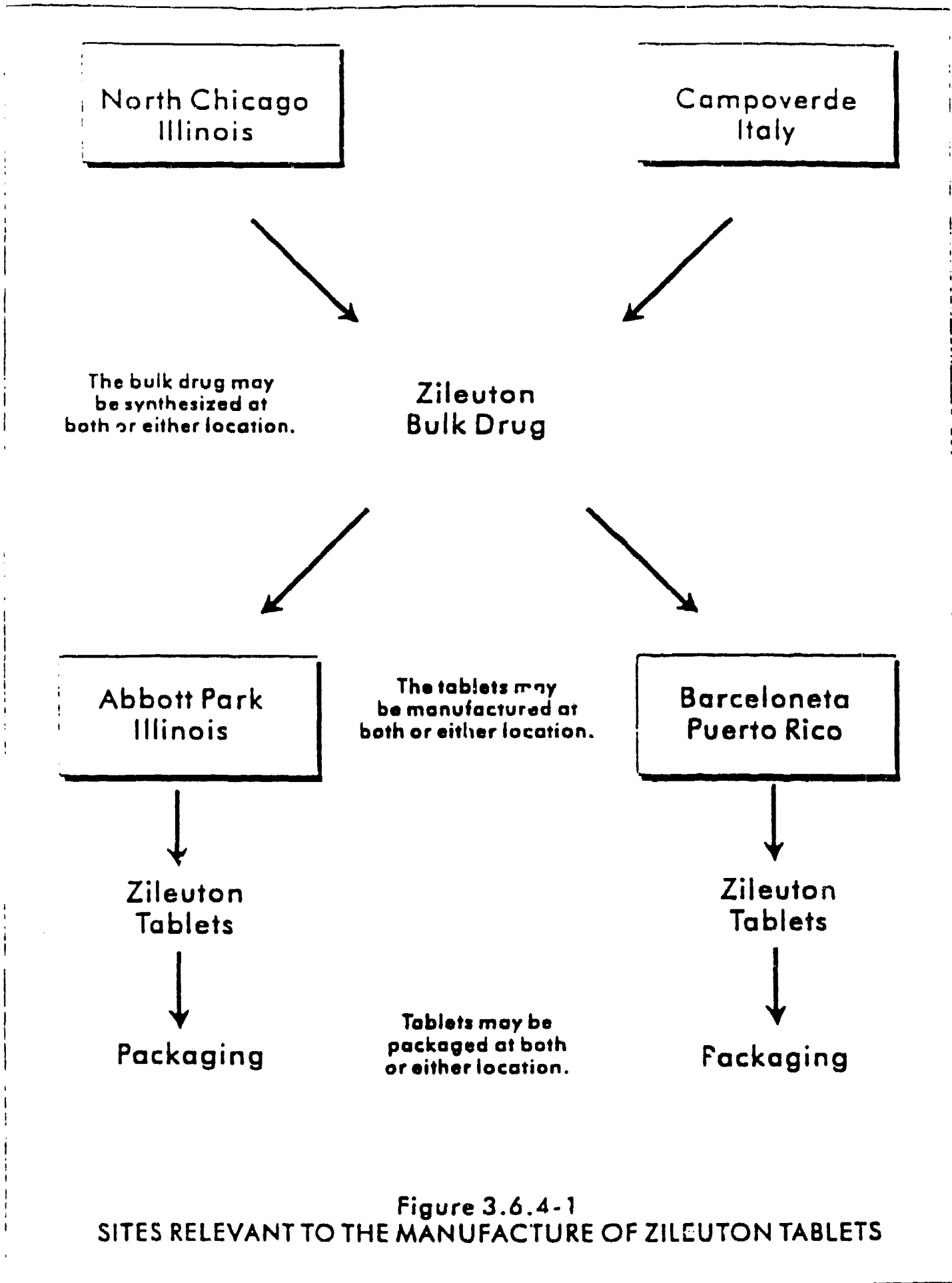


Figure 3.6.4-1
SITES RELEVANT TO THE MANUFACTURE OF ZILEUTON TABLETS

3.6.4.5 ENVIRONMENTAL SETTING OF FACILITIES

3.6.4.5.1 North Chicago/Abbott Park, Illinois

The properties of Abbott Laboratories are located within Lake County, Illinois. The North Chicago properties 600 to 1,000 feet west of Lake Michigan at an elevation ten to fifteen feet above the average 580 foot MSL (mean sea level) elevation of the lake. The Abbott Park property is located approximately five miles to the west. There are no other significant geographic features, such as mountains, lakes (aside from Lake Michigan) or rivers in close proximity to either manufacturing site. The area is topographically flat and slopes very gently to the east, toward Lake Michigan. Drainage is predominantly to the east-southeast, again toward the lake. Information on the geologic and hydrologic stratigraphy of the Chicago region is provided in Appendix C. The climate of northeastern Illinois is characterized by warm summers (74 to 94°F) and cold winters (20° to 32°F). The average annual rainfall is 32 inches; wind directions are highly variable.

Most industries and residences near the Abbott facilities are served by the City of North Chicago municipal water supply. The source of the municipal water supply is Lake Michigan. The Abbott Park facility currently uses both municipal water and groundwater from onsite wells. Wastewater is sewered to the treatment facility of the North Shore Sanitary District. Land use near the Abbott facilities is primarily residential and industrial. The portion of Lake County in which they are located is part of the Chicago metropolitan area. Land use (zoning) near the Abbott facilities is primarily residential and industrial.

3.6.4.5.2 Campoverde, Italy

The property of Laboratori Abbott S.p.A., Campoverde, Italy is located approximately 50 km south of Rome in a mainly agricultural area. The area is geographically flat, has an average elevation of 46 m above the mean sea level, and slopes gently to the west to the sea which is about 11 km distant. Drainage is predominately to the northeast toward the Spaccasassi Creek. The climate is typically Mediterranean characterized by moderate winter temperatures (0°C minimum) and by warm summers (32°C). The average annual rainfall is 930 mm. Wind directions are primarily southeast to northwest and west to east.

The Laboratori Abbott facility uses ground water from on-site wells. Wastewater is sewered to a wastewater treatment plant and then discharged to the Spaccassassi Creek. Land use near the Laboratori Abbott S.p.A. facility is primarily agricultural and residential.

Statements regarding environmental compliance are provided (Appendix C) from the appropriate governmental agency and a facility manager of Laboratori Abbott S.p.A.

3.6.4.5.3 Barceloneta, Puerto Rico

The manufacturing site of Abbott Pharmaceuticals, Inc., Barceloneta, Puerto Rico is situated in a mainly agricultural area. The flat land of this area has been devoted to pineapple growing and pasture for many decades. There are no significant geographic features, such as mountains, lakes, or rivers in proximity to the manufacturing facilities. During The 1970's several industrial plants were established nearby, most of which are engaged in pharmaceutical manufacturing. (Maps of the area are provided in Appendix C.) To date the agricultural and industrial uses (zoning) of land coexist in the region, though most of the land remains agricultural.

The climate of Puerto Rico, including the Barceloneta Region, is influenced by Puerto Rico's location in the Caribbean. At approximately 10 degrees north latitude, it is situated in the belt of prevailing, northeasterly tradewinds. The predominance of tradewinds results in little variability of wind direction. Puerto Rico's tropical climate also produces only a small range of temperature variation. Typically, the average summer and winter temperatures differ by less than 10 degrees Fahrenheit. The average annual temperature is approximately 78°F. Precipitation varies widely over the island due to local meteorological effects. On an annual basis, Barceloneta receives between 50 and 60 inches of rain. Water for operations at the Abbott facility is supplied by three shallow wells. An artisan well also exists at the facility, but is not currently used. Wastewater is treated onsite. Its effluent is piped to a facility of the Puerto Rico Aqueduct and Sewer Authority where it is treated further before being released 1.5 miles off the coast.

NDA 020471

FIRM: ABBOTT LABS

9 OF 9

TRADE NAME: ZYFLO FILMTAB 300/600MG

GENERIC NAME: ZILEUTON

3.6.5 IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

The drug substance--zileuton--will be prepared and administered in tablets. The molecular structure of zileuton is shown in Figure 3.6.5-1.

3.6.5.1 NOMENCLATURE

3.6.5.1.1 Chemical Abstracts

N-(1-Benzo(b)thien-2-ylethyl)-N-hydroxyurea

3.6.5.1.2 United States Adopted Name (USAN)

Zileuton

3.6.5.1.3 CAS Registry Number

111406-87-2

3.6.5.1.4 Laboratory Codes

Abbott-64077; ABT-077

3.6.5.1.5 Molecular Formula and Weight

C₁₁H₁₃N₂O₂S MW = 236.29

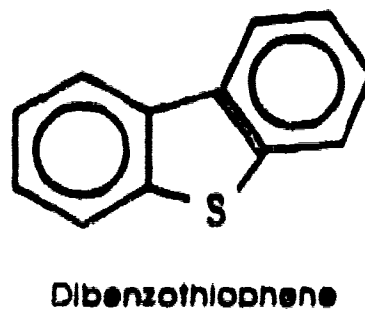
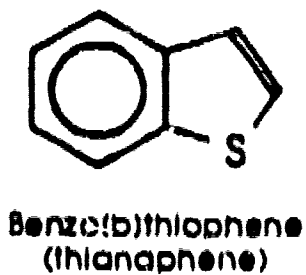
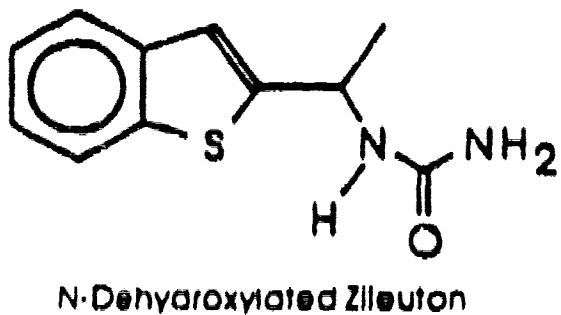
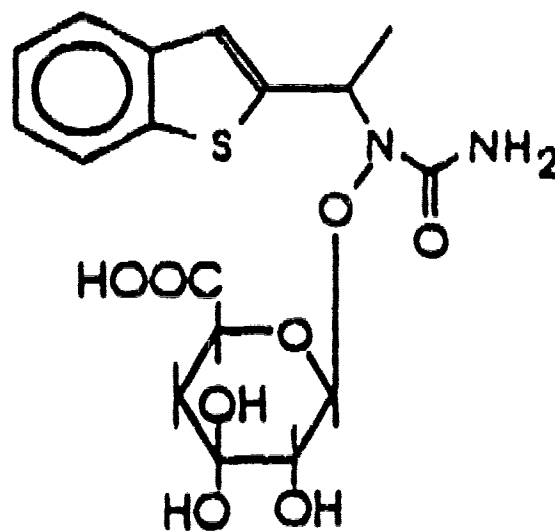
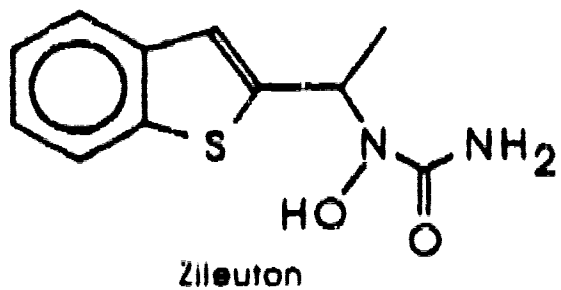


Figure 3.6.5-1
STRUCTURES OF ZILEUTON AND RELATED CHEMICALS

3.6.5.2 PHYSICAL DESCRIPTION

Zileuton is a substituted benzo(b)thiophene, as indicated in Figure 3.6.5-1. In appearance, zileuton is an off-white powder with no detectable odor. Its chemical and physical properties are listed in Table 3.6.5-1. Cited references to these properties are provided in the footnotes of the table. According to 21 CFR 58.3(d) and (i), determinations of physical or chemical characteristics are not included in the determinations that are to be carried out under conditions designated as Good Laboratory Practice.

Zileuton is a very weak acid ($pK_a = 10.3$) and would normally be a neutral substance in prepared aqueous solutions. However, the drug substance is complexed by the metal cations, Cu^{+2} and Fe^{+3} (Table 3.6.5-1), both of which are available in sewage systems and surface water. Thus, zileuton is expected to be bound in cationic complexes of these two metals when it is released to the ambient environment.

Table 3.6.5-1

Chemical and Physical Properties of Zileuton^(a)

Molecular formula	C ₁₁ H ₁₇ N ₂ O ₂ S
Molecular weight	236.29
Melting point	144.2 - 145.2°C
Solubility in water	100±20 mg/L
Density ^(b)	Not determined
Vapor pressure ^(a)	Not determined
Log octanol-water partition coefficient (log K _{ow}) ^(a)	2.1 (25°C)
Sediment-water distribution coefficient (K _d) ^(a)	
Surface Water Clay	114.9
Des Plaines River Sediment	5.63
Skokie Lagoons Sediment	5.62
Municipal Sewage Sludge	62.2
Acidic dissociation (proton loss) (pK _a)	10.3
Dissociation of metal complexes (pK _m) ^(b)	
Copper (+2)	6.05
Iron (+3)	5.52
Electromagnetic absorption (> 290 nm)	300 nm

(a) Data are taken from the NDA Section on Chemistry, Manufacturing and Controls, Report No. R&D/92/638, unless otherwise indicated. According to 21 CFR 312.3(d) and (i), determinations of physical or chemical characteristics are not included in the determinations that are to be carried out under conditions designated as Good Laboratory Practice.

(b) Not determined for zileuton because density is a colligative property rather than a molecular property. Colligative properties are those that are characteristic of a group of molecules, i.e., these properties depend on the spatial distribution of the molecules in that specific group. Thus, the density of zileuton (as a separate solid phase) is irrelevant to the behavior of the dissolved molecules, because the molecules will have become dissipated in the aqueous medium. When environmental releases of zileuton occur, they will always be in the dissolved state at concentrations well below the maximum solubility, precluding the formation of a separate solid phase.

- (c) Not determined for zileuton because the vapor pressure cannot be related to the volatility of the drug substance in the aquatic environment. The stability of its complexes with Cu^{+2} and Fe^{+3} indicate that zileuton will be bound to these metal cations when it is present in sewage or surface water. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant), however, assume that the dissolved chemical is not ionic or complexed and does not become adsorbed to suspended particulates or sediment (Mackay and Wolkoff, 1973; Lyman et al., 1982). If the chemical becomes part of an ionic complex--as would zileuton (Item 7, Subsection C.7.2.4)--its volatilization to the atmosphere cannot be regarded as an operative process and, therefore, can no longer be estimated. These restrictive conditions are often overlooked in the widespread application of this model.
- (d) The log K_{ow} is not indicative of the transport and fate of zileuton, because the drug substance will be complexed by environmental cations, such as Cu^{+2} and Fe^{+3} .
- (e) Appendix B-1 and Item 7 (Subsection C.7.2.5).
- (f) The dissociation of metal complexes is reported as pK_d in the NDA Section on Chemistry, Manufacturing and Controls, Report No. R&D/92/658. The pK_d (or pK_m) is defined as the negative log of the dissociation constant, K_d , in the relationship: $K_d = [\text{cation}][\text{zileuton}]/[\text{complex}]$, where brackets denote concentration. Dissociation of the complexes was studied at pH 9.44. At that pH, the ratio of neutral zileuton to its anion is 7.24.

Density is not determined for zileuton because this property is colligative rather than molecular. Colligative properties are those that are characteristic of a group of molecules, i.e., these properties depend on the spatial distribution of the molecules in that specific group. Thus, the density of zileuton (as a separate solid phase) is irrelevant to the behavior of the dissolved molecules, because the molecules will have become dissipated in the aqueous medium. When environmental releases of zileuton occur, the drug will always be in the dissolved state at concentrations well below the maximum solubility, precluding the formation of a separated solid phase.

Similarly, the vapor pressure of zileuton is not determined, because it cannot be related to the volatility of the drug substance in the aquatic environment. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic or complexed and does not become adsorbed to suspended particulates or sediment (Mackay and Wolkoff, 1973; Lyman et al., 1982). If the chemical becomes part of an ionic complex--as would zileuton--its volatilization to the atmosphere cannot be regarded as an operative process and therefore, can no longer be estimated. These restrictive conditions are often overlooked in the widespread application of this model.

3.6.5.3 IMPURITIES AND ADDITIVES

The impurities that have been identified in bulk lots of zileuton are discussed in the NDA Section on Chemistry, Manufacturing, and Controls. All impurities are present at a level of less than one percent. The impurities are not discussed individually in the present EA, because their structural similarity to zileuton will direct them to a similar environmental fate. The excipients that are present in the formulated tablets are listed in Appendix C.

3.6.6 INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

3.6.6.1 SYNTHESIS OF ZILEUTON (NORTH CHICAGO, ILLINOIS)

3.6.6.1.1 Substances Emitted During Manufacturing

Atmospheric Emissions

A summary of volatile emissions from the synthesis of zileuton is provided in Appendix C. The disposition of volatile organic materials in the atmosphere is also discussed in Appendix C.

Wastestreams

A material balance for the synthesis of zileuton is provided in Appendix C. The compositions of resulting wastestreams are tabulated. Several aqueous wastestreams will be sewerred to Abbott's North Chicago treatment plant which discharges to the North Shore Sanitary District (Russell Road, Gurnee, Illinois 60031). The wastestreams that are not sewerred will be sent offsite for incineration or reclamation by a waste contractor (Appendix C).

Solid Wastes

Wastes generated from the production of zileuton will be crushed (if feasible). Filter cartridges, fiber kegs, filter cakes, plastic bags, dust masks, gloves and other items of protective clothing will be collected in 20- or 30-yd³ rolloff boxes prior to transport and landfilling. This material is transported to the following transfer station prior to shipment to the non-hazardous landfill also shown below: Transfer Station: Waste Management - Northwest Transfer Facility, Wheeling, Illinois (Illinois EPA ID# ILD056657182); Non-hazardous Landfill: Greene Valley Landfill, Naperville, Illinois (Illinois EPA ID# 0438030001). Steel drums at North Chicago are cleaned in a drum washing unit and then shipped to a drum reconditioner.

3.6.6.1.2 Controls Exercised on Residuals and Emissions

Safety precautions for handling the chemicals listed in the material balance are described in Appendix C. The corresponding Material Safety Data Sheets are provided in Appendix D. Air emissions will be controlled as required by the Operating Permits from the Illinois Environmental Protection Agency (Appendix C). In the North Chicago facilities used for the production of zileuton bulk drug substance, the air emission controls utilized involve the use of condensers, vent condensers, water scrubbers, and dilute caustic scrubbers.

Records of emissions are maintained and inspected. Several wastestreams will be sewered while all other liquid wastestreams will be incinerated or reclaimed. The contractors for offsite waste disposal are listed in Appendix C. The sewered wastestreams are treated in Abbott's onsite facility before being released to the

3.6.6.1.3 Compliance of Proposed Action with Applicable Emissions Requirements

Equipment in which zileuton will be manufactured is all properly permitted for air emissions (Appendix C). Permit modifications will be requested (as needed) to correspond to changes in manufacturing. The permit for release of effluent from Abbott's wastewater treatment plant to the North Shore Sanitary District is also provided in Appendix C.

Certification of compliance with applicable emission requirements is provided in Appendix C.

3.6.6.1.4 Effect of Proposed Action on Compliance with Current Emission Requirements

Emissions and releases from the manufacture of zileuton bulk drug substance are not expected to exceed the limitations of current permits. If modifications of current air permits are required, they will be processed with sufficient time to allow production to proceed. Modifications can be prepared by Abbott within 30 days and would be reviewed by the Illinois Environmental Protection Agency within 90 days. The facility permit for release of treated effluent to the North Shore Sanitary District will not be exceeded by the synthesis of zileuton. Similarly, the generation of solid waste will not affect the permit for landfilling.

3.6.6.2 SYNTHESIS OF ZILEUTON (CAMPOVERDE, ITALY)

A declaration of responsibility has been issued by the Italian governmental agency that will regulate releases to the environment and safety of workers during the synthesis of zileuton (Appendix C). This agency monitors the Abbott facility at Campoverde to ensure its compliance with pertinent Italian laws. A copy of the letter from the Italian government agency, with certified English translation, is appended to this EA. Certification of compliance with regulations pertaining to the syntheses of zileuton has been provided by a facility manager of Laboratori Abbott S.p.A. (Appendix C).

3.6.6.3 PREPARATION OF THE TABLETS (ABBOTT PARK, ILLINOIS)

3.6.6.3.1 Substances Emitted During Manufacturing

Atmospheric Emissions

As indicated by the manufacturing instructions in Appendix C, water is the only volatile solvent that will be used to prepare the tablets. The water will evaporate before the tablets are packaged. Particulate emissions that are generated at this manufacturing facility cannot exceed three tons per year (Appendix C).

Aqueous Wastes

Equipment will be handscraped to remove residual solids before being washed with water. The washdown water--including room washdown--will be sewerd into process drains (Appendix C). The washdown water will be metered into the general wastestream for discharge into the sewer system of the . **Releases of zileuton** during tableting are discussed in Appendix C.

Solid Wastes

In addition to losses that will be sewerd, solid wastes from tableting (e.g., handscrapings) are expected. This material--as well as packaging rejects and protective clothing worn by employees--will be collected in drums and rolloff boxes for landfilling. These solid wastes will be transported t **Additional** information on solid wastes is provided in Appendix C.

3.6.6.3.2. Controls Exercised on Residuals and Emissions

Safety precautions for the chemicals introduced in the tableting formulation are provided in Material Safety Data Sheets (Appendix D). Volatile emissions are discussed in Appendix C. Particulates released from the tableting facility cannot exceed three tons per year and will be controlled by bag filters, HEPA filters, and Torit dust collectors (Appendix C). An equalization tank is employed to regulate the aqueous wastes that will be sewered into the general wastewater discharge. Solid wastes are landfill'

The proper handling and disposal of packaging wastes are carried out under a Basic Operating Procedure.

3.6.6.3.3 Compliance of Proposed Action with Applicable Emission Requirements

Handling procedures specified in the Material Safety Data Sheets will be followed throughout all operations. Particulate emissions from the tableting facility at Abbott Park is regulated under a permit issued by the Illinois Environmental Protection Agency. Wastewater from manufacturing must meet the General Pretreatment Standards in 40 CFR Part 403 and the Effluent Guidelines and Standards for Pharmaceutical Manufacturing in 40 CFR Part 439. The prohibitions and limitations for discharge into the sewer system of the North Shore Sanitary District are listed in Appendix C. Solid wastes will be landfilled by

Certification of compliance with applicable emission requirements is provided in Appendix C.

3.6.6.3.4. Effect of the Proposed Action on Compliance with Current Emission Requirements

Emissions and releases from the tableting of zileuton or packaging of zileuton tablets will not exceed the limitations of current permits. Manufacturing of this product will be scheduled to fit within the existing framework of activities for which current emission requirements are applicable.

3.6.6.4 PREPARATION OF THE TABLETS (BARCELONETA, PUERTO RICO)

3.6.6.4.1 Substances Emitted During Manufacturing

Atmospheric Emissions

Tablet manufacturing at Barceloneta will be carried out with the same manufacturing instructions and equipment as will be used in Abbott Park. Therefore, volatile emissions will also be the same. Particulate emissions will be collected in baghouses. Atmospheric emissions are considered negligible after treatment in high efficiency exhaust flow scrubbing equipment.

Aqueous Wastes

Water from equipment washdown, room cleaning, and wet scrubbers is sent to an onsite wastewater treatment plant. The estimated total percentage loss of tableting formulation to aqueous wastes is approximately the same as the percentage loss estimated for Abbott Park (Appendix C).

Solid Wastes

An estimation of the solid wastes produced during the manufacture of zileuton tablets at Barceloneta is provided in Appendix C.

3.6.6.4.2 Controls Exercised on Residuals and Emissions

Processing of chemicals will be carried out as directed in the Material Safety Data Sheets. Volatile emissions are discussed in Appendix C. Release of particulates to the atmosphere will be controlled by baghouses. Solid waste--including the collected dust and culls from tableting--will be segregated and stored in dedicated areas before final disposal. All solid wastes from the manufacture of zileuton tablets will be incinerated by

Aqueous wastes will be treated in an onsite wastewater treatment system. The system includes an equalization and neutralization basin, two biological reactors and a secondary aerobic digester as part of the primary and secondary treatment scheme. The treatment system also includes sludge dewatering and tertiary treatment as final units of operation. Sludge is landfilled onsite. The effluent is discharged to the Barceloneta Regional Wastewater Treatment Plant, a publicly owned treatment works operated by the Puerto Rico Aqueduct and Sewer Authority (PRAISA). The Barceloneta municipal sewer permit number is CAG9001.

3.6.6.4.3 Compliance of Proposed Action with Applicable Emission Requirements

Operating personnel are required to read and follow the chemical handling procedures described in the Material Safety Data Sheets. The current permit for operating equipment to lessen air pollution is provided in Appendix C. Water that will be discharged from the onsite treatment system must comply with the prohibitions and limitations of a facility agreement issued by the Puerto Rico Aqueduct and Sewer Authority.

Certification of compliance with applicable emission requirements is provided in Appendix C.

The incinerator that Chambers Medical Technologies operates in Hampton, South Carolina is permitted by the South Carolina Department of Health and Environmental Control (Permit No.'s 1280-0021CD, 1280-0021CE, 1280-0021CF, and 1280-0021CG).

3.6.6.4.4 Effect of the Proposed Action on Compliance with Current Emission Requirements

Emissions and releases from the tableting or packaging of zileuton will not exceed the limitations of current permits. These permits are issued for the facility, and thus manufacturing of this product will be scheduled to fit within the activities that are permitted.

3.6.6.5 OCCUPATIONAL SAFETY

Chemicals used in manufacture of the drug product are regulated by the Occupational Safety and Health Administration under its responsibility for (1) permissible exposure limits, (2) safe handling of flammable liquids, (3) safe handling of corrosives, and (4) hazard communication. Employees are trained in the proper operation of equipment in order to minimize potential safety, health and environmental risks. Extensive safety training is mandated, and Material Safety Data Sheets are available to personnel for chemicals handled in the manufacturing area. Monitoring of employee exposure to hazardous materials is conducted and personnel participate in annual physical evaluations. Employee education on health is provided through lectures.

The safe transport of all drug-related materials is ensured by following protocols which include formal qualification of vendors, training of personnel, and rigid specification of containers and materials. Access to drug substances and products is restricted to authorized personnel.

3.6.6.6 AMOUNT OF SUBSTANCES ENTERING THE ENVIRONMENT

The two routes by which the drug substance of this product can enter the environment are: (1) elimination from patients who are using the product and (2) release of wastewater from manufacturing.

3.6.6.6.1 Human Elimination

The drug substance is introduced to sewage systems primarily as a glucuronide conjugate and, to a lesser extent, as an N-dehydroxylated metabolite produced by intestinal microflora (NDA Subsection 6.4.2.3, Metabolism). Sewage microflora will hydrolyze the glucuronide to zileuton and glucuronic acid, and the structure of the metabolite is very similar to zileuton. Therefore, releases via human elimination are estimated in this subsection as if zileuton were the only substance being released.

The estimated concentration, i.e., Maximum Expected Emitted Concentration (MEEC), of zileuton at a typical wastewater treatment plant (in the fifth year following approval) is less than 1 ppm. This concentration is, of course, a worst-case estimate, because environmental processes leading to the drug's dissipation are not being considered. The calculation for the estimated concentration is provided in Appendix C.

3.6.6.6.2 Wastewater from Manufacturing

North Chicago

Synthesis of zileuton can result in a loss of zileuton by-products to wastewater, but zileuton itself is not released. The calculated concentration of zileuton by-products at the facility of the _____ that could result from releases at _____ (under the proposed action) would be in the ppb range (Appendix C). This is, of course, a worst-case estimate, because degradation and adsorption to sludge at Abbott's onsite treatment plant were not considered in the calculation of this estimate.

Abbott Park

The three facilities of the _____ operate at a flow rate of 19 million gal/day (NSSD, 1992). The concentration of zileuton at the treatment plant that could result from releases at Abbott Park (under the proposed action) would be in the ppb range (Appendix C).

Barceloneta

Wastewater from manufacturing is treated onsite before it is released to the publicly owned treatment plant of the Puerto Rico Aqueduct and Sewer Authority (PRASA). The capacity of the latter facility is 5 million gal/day (PRASA, 1993). If the concentration of released substances is considered to be unabated by onsite treatment, a worst-case concentration of zileuton at the PRASA facility can be estimated. The worst-case concentration of zileuton at the PRASA facility would be in the ppb range (Appendix C).

3.6.7 FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

In Item 7, information is presented that is relevant to the environmental transport and fate of zileuton. Assessment of its transport and fate is accomplished by an evaluation of processes affecting transport (between air, water, and soil) and processes affecting structural degradation. The methodology involved in this evaluation and its application to specific chemicals is discussed in Water-Related Environmental Fate of 129 Priority Pollutants (USEPA, 1979).

Zileuton is a substituted benzo(b)thiophene (Figure 3.6.5-1). The latter chemical--and dibenzothiophene as well--are constituents of coal-tar and crude oil (Boyles, 1984; Mueller et al., 1991). The use of coal-tar to preserve wood and the refining of crude oil have released these two sulfur-containing aromatic compounds to the natural environment for many decades. As instructed in the Environmental Technical Assistance Handbook (USFDA, 1987), available information on chemicals structurally similar to the drug substance--in this case, benzo(b)thiophene and dibenzothiophene--is utilized (wherever appropriate) to predict the environmental fate.

After zileuton is ingested, it is absorbed from the intestinal tract and eliminated in urine primarily as glucuronide conjugates (Figure 3.6.5-1). Human intestinal microflora are capable of dehydroxylating zileuton, and a small amount of this dehydroxylated degradate is also absorbed and eliminated. During sewage treatment, glucuronides are readily hydrolyzed by the sludge culture and, therefore, zileuton itself will be regarded as the substance released from patient usage.

3.6.7.1 AIR

No emission of zileuton to the atmosphere occurs during synthesis of the bulk drug, preparation of the product, or use of the product by patients. Zileuton is a solid (or solute in solution) at ambient temperatures. Its volatility from water is impeded by its tendency to form complexes with environmental cations, such as Cu^{+2} and Fe^{+3} . Dust from the manufacturing facilities will be trapped by vent filters that are collected for landfilling or incineration. If any drug substance should escape the dust collection system, it will either precipitate with rain or become photolyzed in the condensing moisture.

The principal fate of volatile materials released during manufacturing (Appendix C) is reaction with the oxidizing radical species of the atmosphere. (Hazardous Substances Databank, 1992; Howard et al., 1990).

3.6.7.2 WATER

Zileuton will be introduced to the aqueous environment via elimination by patients and via releases from manufacturing. Adsorption to sewage sludge and photolysis are expected to decrease its concentration before the sewage effluent is released to surface water (Subsections 3.6.7.2.1 and 3.6.7.2.5). However, if processes leading to abatement are not considered, worst-case concentrations for surface water can be estimated.

From Item 6 (Subsection 3.6.6.6.1), the concentration of zileuton at a typical wastewater treatment plant (in 1999) that would be due to patient usage is estimated to be less than 1 ppm. Because of variations in plant capacity and in rates of surface water flow, dilution factors for effluent can vary (depending on geographic location) from about 10^7 to essentially no dilution (i.e., settling ponds or intermittently dry drainage channels) (Metcalf & Eddy, Inc., 1979; Linsley et al., 1975). A typical dilution factor for many rivers of the United States is 10^3 and, thus, the corresponding typical concentration in surface waters that receive sewage plant effluent is estimated to be less than 1 ppb. It should be noted, however, that the concentration would be much less downstream from the effluent outfall.

The worst-case effect of tableting releases from Abbott Park on the concentration of zileuton in the Des Plaines River--the surface water that receives effluent from the North Shore Sanitary District--can be estimated with a similar calculation. From Subsection 3.6.6.6.2, the concentration of zileuton (at NSSD) that would be due to 1999 tableting releases under the proposed action would be in the ppb range. The effluent from this wastewater facility is discharged to the Des Plaines River which (near NSSD in Gurnee, Illinois) has a mean annual flow rate of 189 ft³/sec or 122 million gal/day (ISWS, 1992). Therefore, the dilution factor from the NSSD facility (19 million gal/day) to the river is 1.6×10^4 , and the concentration of zileuton (at NSSD) due to tableting at Abbott Park would be decreased by that factor.

A similar estimate for Barceloneta cannot be provided, because the effluent from the publicly owned treatment plant is piped 1.5 miles off the coast of Puerto Rico where it is discharged (PRASA, 1993).

At North Chicago, the sewered wastestreams from synthesis of the bulk drug do not contain zileuton (Appendix C). However, some by-products that have the benzo(b)thiophene ring structure could be sewered. Their worst-case concentration (without abatement) at the North Shore Sanitary District is calculated to be in the ppb range (Appendix C). The previous dilution factor for discharge to the Des Plaines River would decrease that concentration accordingly.

In the water of a sewage treatment facility, or in the surface water that dilutes the effluent, zileuton (or its by-products) would be affected by environmental processes that include photolysis, oxidation, hydrolysis, volatilization, adsorption, bioaccumulation, and biodegradation. These processes are evaluated individually (in this subsection) before a concluding statement is made on the probable fate and concentration of released substances in the aqueous environment.

3.6.7.2.1 Photolysis

The electromagnetic absorption spectrum of zileuton exhibits maximum absorbance at 300 nm (Table 3.6.5-1). This extension of the spectrum into the wavelength range of terrestrial sunlight (>290 nm) signifies that zileuton could be susceptible to photodegradation, which was, in fact, demonstrated when aqueous solutions were subjected to the irradiation of sunlight (Appendix B-2). The concentration of the solutions was approximately 4 ppm, and irradiation was carried out over several days in September at 40° north latitude (Savage, Maryland). Under these conditions, the half-life for photodegradation was 2.4 days. The estimated half-life for summer is 0.46 days, and for winter, 10 days (Appendix B-2).

The efficiency of photolysis as a degradative pathway in the environment, however, will be affected by the adsorption of zileuton to natural adsorbents (Subsection 3.6.7.2.5). Thus, adsorption of zileuton (after its release to the environment) could lessen the effect of photodegradation, if the adsorption removes zileuton from the shallow depths of a

sewage facility or surface water. The degradation of zileuton by this pathway, therefore, will depend not only on the availability of sunlight but also on (1) desorption of the drug from sludge or sediment and (2) the mixing of drug-containing water from lower depths with shallower water.

The zileuton by-products should be similarly photolabile, because their molecular structure also contains the benzo(b)thiophene ring. The photolysis of benzo(b)thiophene and dibenzothiophene (Figure 3.6.5-1) has been studied in samples of natural waters (Mill et al., 1981). At 40° north latitude (Palo Alto, California), the respective half-lives (in sunlight) were 28 and 94 hours.

3.6.7.2.2 Oxidation

Exposure of zileuton for one week to 5 percent hydrogen peroxide in water results in less than 1 percent degradation (NDA Subsection 3.3.1.5, Report No. R&D/92/674). Because zileuton does not degrade significantly under these conditions, it is unlikely that oxidation in surface water would proceed at a rate sufficient to serve as a mechanism for environmental dissipation.

3.6.7.2.3 Hydrolysis

Zileuton is stable to hydrolysis when dissolved in distilled water at ambient room temperature (Appendix B-3). After 3 weeks, 98.9 percent of the drug remains; the corresponding half-life for hydrolysis is 3.6 years. When zileuton is eliminated by human subjects, it will enter sewage treatment systems as its glucuronide conjugate. Zileuton will be regenerated from this conjugate by the action of hydrolytic enzymes, some of which are secreted into the aqueous medium of the sludge culture (USEPA, 1987; Janssen, 1979).

3.6.7.2.4 Volatilization

Transport from surface water to the atmosphere via volatilization is not an operative process, because zileuton forms highly stable complexes with Cu^{+2} and Fe^{+3} , both of which are available in sewage systems and surface water. Complexing of this type means that zileuton will exhibit ionic transport characteristics when it is present in the environment.

The glucuronide conjugate of zileuton can also be expected to form similar complexes prior to its decomposition. Estimations of volatility based on vapor pressure are regarded as invalid, if a chemical becomes part of an ionic complex. (Mackay and Wolkoff, 1973; Lyman et al., 1982).

3.6.7.2.5 Sorption/Desorption

The procedures outlined in USFDA (1987) were followed to obtain the distribution adsorption coefficient, K_d , for zileuton with bentonite (a typical surface water clay), Lake Michigan beach sand, Des Plaines River sediment, Skokie Lagoons sediment, and Milorganite (a mixture of sand and dried municipal sewage sludge) (Appendix B-1). After an equilibration period of 16 hours, the K_d values of Table 3.6.7-1 were calculated from the following relationship:

Table 3.6.7.1
Sorption of Zileuton to Natural Adsorbents^(a)

	<u>Bentonite (Surface Water Clay)</u>	<u>Lake Michigan Beach Sand</u>	<u>Des Plaines River Sediment</u>	<u>Skokie Lagoons Sediment</u>	<u>Municipal Sewage Sludge (Milorganite)</u>
K_d	114.9	0.96	5.63	5.62	62.2
σ	± 45.3	± 0.13	± 0.46	± 0.25	± 3.4
Irreversibly Bound ^(b)	86.5%	6.20%	14.4%	14.2%	16.4%
σ	$\pm 4.9\%$	$\pm 0.79\%$	$\pm 1.7\%$	$\pm 1.3\%$	1.0%

(a) Test mixture: 200 µg drug/1.0 g adsorbent.
Solution volume: 15 mL.
Solution: 0.01 M CaCl₂.
Testing details are given in Appendix B-1.

(b) The percentage of adsorbed zileuton retained by the tested adsorbent after the spent adsorbent was leached with acetonitrile, a solvent selected for its capacity to dissolve the drug substance. In a natural setting, the drug substance that is leached from a sediment would conceivably become adsorbed to the adjacent mass of sediment that had not yet been exposed to it.

$$K_d = \frac{\text{chemical sorbed } (\mu\text{g/gram})}{\text{chemical in solution } (\mu\text{g/mL})}$$

Attempted extraction of zileuton from the adsorbents with a polar solvent in which the drug is very soluble, i.e., acetonitrile, demonstrated that the drug remained adsorbed to the surface water clay to the extent of 86.5 percent. The Lake Michigan beach sand, the two sediments, and the sewage sludge retained about 6, 14 and 16 percent of the drug, respectively, after being leached by the acetonitrile.

Sorption/desorption, as studied under the test conditions, measures the result of a closed-system process that has reached equilibrium, i.e., the measurements are conducted on batches of material at the end of a prescribed time period. These test conditions are similar to the conditions for adsorption of zileuton to sludge during sewage treatment. In a natural setting, however, sorption/desorption is a dynamic, open-system process that does not reach a comparable equilibrium, i.e., leaching--and re-adsorption from the leachate--continuously involve fresh unaffected adsorbent. Clearly, the drug this is the only substance that is leached from a portion of sediment could concertededly become adsorbed to the adjacent mass of sediment that has not yet been exposed to it.

Given the conditions of the test and the aqueous systems to which the drug is released, it is reasonable to conclude from the data presented in Table 3.6.7-1 that the concentration of zileuton in a sewage system will be decreased by a factor of about 1/62 (or 1.6×10^{-2}) as the drug becomes adsorbed to the sludge. After the effluent has been discharged, contact with sediments--especially those that contain clay--will result in further adsorption of the drug.

3.6.7.2.6 Bioaccumulation/Bioconcentration

Bioaccumulation of chemicals generally refers to their introduction into animals by ingestion, while the bioconcentration of chemicals refers to their absorption from water by aquatic organisms. Bioaccumulation of zileuton in aquatic animals is unlikely, because its solubility (Table 3.6.5-1) should be sufficient to facilitate fecal elimination or transport via gills to the surrounding water.

A surface water bioconcentration factor (BCF) can be calculated for zileuton from the following equation (Lyman et al., 1982):

$$\log BCF = 0.76 \log K_{ow} - 0.23$$
$$(r^2 = 0.823; n = 84)$$

where:

- K_{ow} = octanol-water partition coefficient.
- r^2 = the coefficient of determination (proportional reduction in error).
- n = the number of chemicals from which the regression was developed.

Using the log octanol-water partition coefficient (2.1), from Table 3.6.5-1, the BCF calculated by this method is 23.2. This value indicates little or no tendency for bioconcentration of zileuton in aquatic life.

If terrestrial organisms come into contact with sediments, surface water, or landfilled sludge in which zileuton is present, bioaccumulation (via ingestion) is also not expected. Trabalka and Garten (1982) have reported that chemicals with log K_{ow} values of less than 3.5--or solubilities greater than 10 mg/L--do not accumulate in mammals or birds. These cutoffs values for log K_{ow} and solubility were based respectively on a frequency analysis of 123 paired observations for 68 chemicals and 90 paired observations for 47 chemicals. The log K_{ow} and aqueous solubility of zileuton are 2.1 and approximately 100 mg/L, respectively, precluding its bioaccumulation in these animal receptors.

3.6.7.2.7 Biodegradation

The aerobic biodegradation of zileuton in water was studied in accordance with the guidelines in USFDA (1987). As measured by carbon dioxide production, the drug substance degraded to the extent of 2.6 percent during the 66-day test period (Appendix B-4). Based on these data, it can be concluded that zileuton is resistant to biodegradation under the conditions of the test. The negative test results, however, do not exclude the possibility that the drug substance could be (1) biodegradable by a more acclimated microbial culture or (2) biodegradable under environmental conditions that the test does not mimic.

Two studies have been published on the biodegradation of benzo[b]thiophene, the aromatic rings, structure of zileuton (Figure 3.6.5-1) (Boyles, 1984; Mueller et al., 1991). Benzo[b]thiophene and dibenzothiophene occur in crude oil and coal-tar as a mixture with many other chemical components. The degradation of individual components was determined when the crude oil and coal-tar (as creosote) were incubated, respectively, with an adapted microbial culture and an indigenous soil culture. Three-week incubation of the crude oil resulted in 22 percent degradation of the benzo[b]thiophene but no degradation of the dibenzothiophene. In the creosote mixture, greater than 98 percent of the benzo[b]thiophene was converted to carbon dioxide and sulfate while only 68 percent of the dibenzothiophene was destroyed.

In an acclimated sewage culture, or in the natural environment, zileuton may be degradable by extant microflora via cometabolism with other organic materials. As pointed out at the beginning of Item 7, human intestinal microorganisms can apparently dehydroxylate zileuton (Figure 3.6.5-1). This dehydroxylation should certainly continue in the sewage systems. The resulting degradate, as a substituted urea, could then be broken down further by the hydrolytic enzymes of the sludge culture (Janssen, 1979). Also, the fungal extracellular enzymes responsible for catalyzing the oxidation of aromatic rings may be capable of catalyzing the breakdown of zileuton as well (Bumpus and Aust, 1987; USEPA, 1987).

3.6.7.2.8 Probable Fate of Zileuton in Aquatic Systems

The processes that affect the environmental fate of zileuton are summarized in Table 3.6.7-2. Zileuton is photolabile, and its destruction is expected in the shallow depths of aquatic systems. As zileuton enters a sewage treatment facility, most of it will become adsorbed to the sludge culture. However, whenever this microbial mass and its associated water is exposed to sunlight or to the irradiation of fluorescent fixtures, zileuton will decompose photolytically. A small amount of zileuton will be released in effluent but it should also undergo photodestruction, as mixing of the river water exposes the drug substance to sunlight. Similar destruction of the zileuton by-products (released from synthesis of the drug) can be expected, because of their structural similarity to zileuton.

The concentrations of zileuton that were discussed at the Subsection 3.6.7.2 did not consider losses that would occur from degradation or adsorption. However, adsorption to sludge and photodegradation will diminish whatever amount of the drug substance that is released. Therefore, the actual concentrations that will be present near an effluent outfall can be expected to be several orders of magnitude smaller and would continue to diminish with the passage of time. Downstream from the effluent outfall, the expected environmental concentration (EEC) (PMA, 1991) of zileuton or its by-products will be essentially zero.

3.6.7.3 SOIL

Zileuton is not released to soil but it could be landfilled with sludge to which it had become adsorbed. If this situation should occur, the zileuton would be photodegraded as it becomes exposed at the surface of the sludge. Biodegradation would also be expected. Leaching, however, would not be expected, because the clay liner of landfills would adsorb the drug (Table 3.6.7-1).

Table 3.6.7-2

Summary of Environmental Transport and Fate of Zileuton

Environmental Process	Summary Statement	Confidence of Data ^(a)
Photolysis	Direct photolysis of zileuton by terrestrial sunlight is the process that will account for most of its destruction in environmental surface waters.	High
Oxidation	Oxidation of zileuton will not proceed at a significant rate in the environment.	High
Hydrolysis	Nonenzymatic hydrolysis will be very slow under ambient conditions.	High
Volatilization	This is not an operative process, because zileuton will be present in the environment as part of the ionic complexes that it forms with copper and iron.	High
Sorption	Zileuton will be adsorbed by both sludge and sediments. This is the principal process that determines the transport of this drug in the environment.	High
Bioaccumulation/ Bioconcentration	There is little or no tendency for bioaccumulation in terrestrial animals or bioconcentration in aquatic organisms.	Low
Biodegradation	Zileuton will be degraded if the microflora become acclimated to it.	Medium

^(a) Levels of confidence are based on criteria discussed in USEPA (1979). High confidence requires that the data are quantitative; rate constants and half-lives are either explicitly described or can be calculated from the results. Medium confidence is assigned to quantitative data reported for a different but structurally related compound. A low confidence ranking is given to theoretical estimates or to speculative statements.

3.6.8 ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

Three environmental effects studies have been conducted with zileuton: (1) *Daphnia* acute toxicity, (2) freshwater fish acute toxicity, and (3) microbial growth inhibition (Appendices B-5, B-6, and B-7). These studies were conducted in accordance with the guidelines in USFDA (1987). The results are presented in Table 3.6.8-1.

The lowest no-observed-effect concentration (NOEC) is ≥ 19.4 mg/L for the 96-hour toxicity to rainbow trout. The next higher NOEC is 23.1 mg/L for water fleas. For the seven microbial organisms tested, a minimum inhibitory concentration (MIC) could not be determined, because no inhibition of growth was observed at 90 mg/L, the approximate limit of solubility of the drug substance. For comparison, the Maximum Expected Emitted Concentration (MEEC) for zileuton is less than 1 mg/L (Subsection 3.6.6.6.1).

In Subsection 3.6.7.2, the worst-case (no abatement) concentration of zileuton in the river that would receive effluent from the North Shore Sanitary District is much less (< 1 ppb) than the toxic effect levels. Moreover, the sewage plant itself will not be affected, because the minimum inhibitory concentration for microorganisms is greater than any expected concentration. Thus, the concentrations of zileuton in the environment that could potentially result from approval of the proposed action will not have an adverse effect on living organisms.

Similarly, the worst-case (no abatement) concentration (ppb range) of the zileuton by-products at the North Shore Sanitary District (Subsection 3.6.6.6.2) are also much lower than the lowest toxic effect concentration of zileuton. Although the structures of the by-products are slightly different from the structure of zileuton, their concentrations indicate that their effects (if any) on living organisms should also not be adverse. Moreover, it must be pointed out that these worst-case concentrations were calculated as if environmental processes (e.g., adsorption and photodegradation) were not rapidly diminishing the concentrations of released substances. In fact, the expected environmental concentration (EEC) (PMA, 1991) of zileuton (or its by-products) is essentially zero.

Table 3.6.8-1
Toxicity Testing of Zileuton with Representative Environmental Organisms

Test Organism	Conditions	Results	Study Report
Water Flea (<i>Daphnia magna</i>)	Acute toxicity (TAD No. 4.08) (USFDA, 1987)	NOEC 23.1 mg/l. (48 hr) EC ₅₀ 55.7 mg/l. (48 hr)	Battelle C00015DA
Rainbow Trout (<i>Oncorhynchus mykiss</i>)	Acute toxicity (TAD No. 4.11) (USFDA, 1987)	NOEC ≥ 19.4 mg/l. (96 hr) EC ₅₀ >19.4 mg/l. (96 hr)	Battelle C00016FB
Nitrogen-fixing Bacteria (<i>Azotobacter chroococcum</i>)	Microbial growth inhibition (TAD No. 4.02) (USFDA, 1987)	MIC >90 mg/l.	Battelle SC920184
Blue-green algae (<i>Nostoc</i>)	Microbial growth inhibition (TAD No. 4.02) (USFDA, 1987)	MIC >90 mg/l.	Battelle SC920184
Soil Bacteria (<i>Pseudomonas fluorescens</i>) (<i>Bacillus megaterium</i>)	Microbial growth inhibition (TAD No. 4.02) (USFDA, 1987)	MIC >90 mg/l.	Battelle SC920184
Ascomycetes (<i>Chaetomium globosum</i>)	Microbial growth inhibition (TAD No. 4.02) (USFDA, 1987)	MIC >90 mg/L	Battelle SC920184
Mold (<i>Aspergillus niger</i>) (<i>Penicillium notatum</i>)	Microbial growth inhibition (TAD No. 4.02) (USFDA, 1987)	MIC >90 mg/L	Battelle SC920184

TAD = Technical Assistance Document

NOEC = the concentration at which no effects are observed.

EC₅₀ = the concentration that produces an effect (e.g. immobilization) in 50 percent of the population being tested.

MIC = the minimum inhibitory concentration for growth of the test organism.

3.6.9 USE OF RESOURCES AND ENERGY

The proposed action requires a moderate commitment of company resources. However, chemicals that will be used are common commodities of commerce. Moreover, no irreversible or irretrievable commitment of limited national resources will be involved.

As discussed in Item 8 (Subsection 3.6-8), the environmental impact of releases from manufacturing and use of the product is negligible. Therefore, it is unlikely that threatened or endangered species could be affected.

The State of Illinois does not regard property in the vicinity of the Abbott Park or North Chicago facilities to have historical or archaeological importance (Appendix C). Documents from the government of Puerto Rico (Appendix C) indicate that there is one archaeologically important site near Barceloneta, but no historically important sites. The address of this archaeological site is not available for public disclosure. However, it is unlikely to be located near Abbott's facility because the land of the surrounding industrial park has been extensively excavated and filled.

3.6.10 MITIGATION MEASURES

Controls on emissions at North Chicago, Abbott Park, and Puerto Rico are described in Appendix C. Compliance of the proposed action with applicable emission requirements is also provided in Appendix C.

Occupational safe handling procedures are described in Appendices C and D. Unused drugs (past the labeled expiration date) are returned to Abbott Laboratories for disposal by landfilling (Subsection 3.6.4.4.).

Waste minimization studies are an ongoing activity at Abbott facilities. As their results become available, practical measures to increase control of wastes are incorporated into manufacturing procedures.

3.6.11 ALTERNATIVES TO THE PROPOSED ACTION

No potential adverse environmental impacts have been identified for the proposed action. Little release of zileuton to the environment is expected and, after entering the environment, it would be removed from potential exposure pathways by adsorption to natural adsorbents or photodegradation in surface water. Because no adverse environmental impact is expected, alternatives to the proposed action are not being considered. If this product were not manufactured (as a no-action alternative), zileuton would not become available as medication to alleviate the symptoms associated with asthma.

3.6.12 PREPARER

Norman W. Gabel

Senior Scientist

N.W. Gabel & Assoc.

8201 Corporate Drive, Suite 620

Landover, Maryland 20785

3.6.13 CERTIFICATION

The undersigned certifies that the information presented herein and provided to N.W. Gabel & Assoc. by Abbott Laboratories (applicant) is true, accurate, and complete (with exception of proprietary information) to the best of our knowledge.

Signature James D. Stock Date May 30, 1995
Title Associate Director, Regulatory Affairs

The undersigned certifies that the information presented herein is true, accurate, and as complete as provided (with the exception only of proprietary material) to N.W. Gabel & Assoc. for preparation in accordance with 21 CFR 25.31(a).

*Signature _____ Date _____
Title _____

* This document was prepared but not signed by Norman W. Gabel who is now deceased. Abbott Laboratories certifies that the information presented herein is true, accurate, and complete, as prepared, to the best of our knowledge.

3.6.14 REFERENCES

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APPENDICES

- A. Fate and Effects Studies for Environmental Assessment of Zileuton.**
- B-1 Binding Characteristics of Zileuton on Various Soils and Stability in Sodium Hypochlorite Solutions.**
- B-2 Sunlight-Induced Photolytic Degradation of Zileuton in Aqueous Solution.**
- B-3 Hydrolytic Degradation of Zileuton.**
- B-4 Aerobic Biodegradation of Zileuton in Water.**
- B-5 A Study on the Daphnia Acute Toxicity of Zileuton.**
- B-6 A Study on the Freshwater Fish (Rainbow Trout) Acute Toxicity of Zileuton.**
- B-7 Microbial Growth Inhibition by Zileuton.**
- C Proprietary Information for the Environmental Assessment of Zileuton.**
- D-1 Material Safety Data Sheets for the Synthesis of Zileuton.**
- D-2 Material Safety Data Sheets for the Preparation of Zileuton Tablets.**

ATTACHMENT II

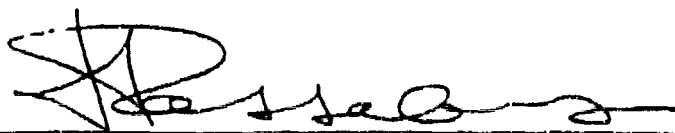
**Certified Translation of Italian
Government Agency Letter**

BURG TRANSLATIONS, INC.

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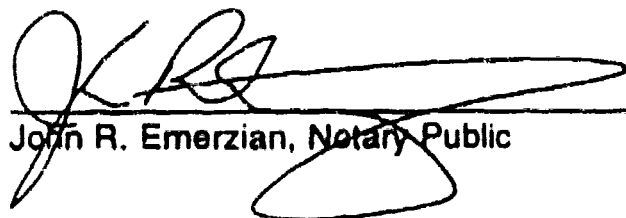
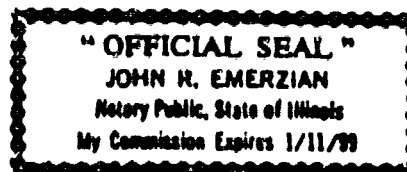
May 24, 1995

I, Lodovico Passalacqua, having been duly sworn, depose and say that the foregoing translation from Italian into English of the LETTER DATED APRILIA, 05/ 21/1993 FROM THE LOCAL SANITATION UNIT LT/1 - APRILIA TO LABORATORI ABBOTT S.P.A. has been translated by Burg Translations, Inc., and that it represents, according to the best of my knowledge and belief, a true and accurate rendering of the original text.



Manager

Subscribed and sworn before me on
May 24, 1995.


John R. Emerzian, Notary Public

a complete language service

Local Sanitation Unit LT/1 - Aprilia
Via Degli Oleandri 94 - Tel. (06) 92.47.44 - 92.07.022
Tax code: 91001320596 - V.A.T. Ref. 01021400591

Aprilia, on: 05/21/1993

File no.: 827/93 Ref.:
Service: PUBLIC HEALTH Office:

To LABORATORI ABBOTT S.p.A.
S.S. 148 - PONTINA - KM 52.00
APRILIA (LT) - ITALY

RE: ZILEUTON PRODUCTION

Pursuant to your request about the production of ZILEUTON and all other products manufactured by the firm Laboratori Abbott S.p.A., of Aprilia (LT), Italy, we state as follows: on the basis of Article 14 of the Law number 833/78, as well as all of the other laws which are listed hereinunder, the Public Health Service of the Local Sanitation Unit LT/1 in Aprilia (LT) is the agency which is responsible for monitoring all environmental emissions into the air, water and soil, as well as for the hygiene and safety at the work place.

- LAW 833/78: ESTABLISHMENT OF THE NATIONAL HEALTH SERVICE.
- D.P.R. 203/88 - D.M. 07/12/1990: POLLUTION EMISSIONS INTO THE ATMOSPHERE.
- LAW 319/76 - LAW 650/79 - D.L. 132/92 - D.L. 133/92: PROTECTION OF THE WATERS FROM POLLUTION.
- D.P.R. 915/82 - LAW 475/88: SPECIAL AND TOXIC WASTE DISPOSAL.
- D.P.C.M. 03/01/1991: DIFFUSION OF NOISE INTO THE OUTSIDE ENVIRONMENT.
- D.P.R. 175/88: RISKS OF SIGNIFICANT ACCIDENTS.
- D.P.R. 577/82: PREVENTION OF FIRES.
- D.P.R. 524/82: SAFETY SIGNALS.
- D.P.R. 547/55 - D.P.R. 303/56 - D.P.R. 277/91: HYGIENE AND SAFETY AT THE WORK PLACE.

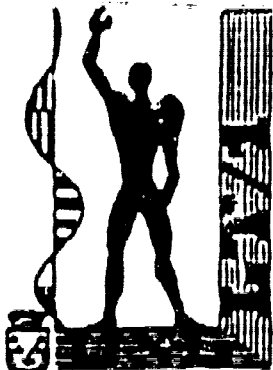
Sincerely yours,

/Signature/

(Dr. A. ... /illegible/)
Director of the Public Health Service

/Official stamp/

1564



Unità Sanitaria Locale LT/1 - Aprilia

VIA DEGLI OLEANDRI, 84 - TEL. (06) 82.47.44 - 82.07.022

Cod. Fisc. 81001320988 - Part. IVA 01021400881

Aprilia, 21/05/1993

Prot. N. 827/93 RIV.
Servizio IGIENE PUBBLICA Ufficio

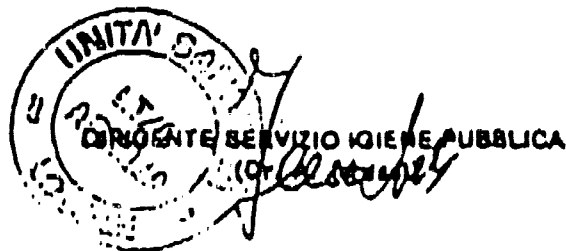
Alla Soc. LABORATORI ABBOTT S.p.A
S.S 148 PONTINA Km. 52,00
APRILIA (LT)

OGGETTO: FABBRICAZIONE ZILEUTON

In merito alla Vs richiesta relativa alla fabbricazione di ZILEUTON e di tutti gli altri prodotti fabbricati dalla Soc. Laboratori Abbott S.p.A. di Aprilia (LT), Italia, si dichiara che : in base all'art. 14 della legge 833/78 ed a tutte le altre leggi sottoelencate, il servizio di igiene pubblica dell'Unità Sanitaria Locale LT/1 di Aprilia (LT) è l'organo preposto al controllo di tutte le emissioni inquinanti in aria, acqua e suolo e dell'igiene e sicurezza nei luoghi di lavoro.

- . LEGGE 833/78 : ISTITUZIONE DEL SERVIZIO SANITARIO NAZIONALE
- . D.P.R. 203/88 - D.M. 12/07/90 : EMISSIONI INQUINANTI IN ATMOSFERA.
- . LEGGE 319/76 - LEGGE 650/79 - D.L. 132/92 - D.L. 133/92 : TUTELA DELLE ACQUE DALL'INQUINAMENTO.
- . D.P.R. 915/82 - LEGGE 475/88 : SMALTIMENTO RIFIUTI
- . D.P.C.M. 01/03/91 : DIFFUSIONE DI RUMORE NELL'AMBIENTE ESTERNO
- . D.P.R. 175/88 : RISCHI DI INCIDENTI RILEVANTI
- . D.P.R. 577/82 : PREVENZIONE INCENDI
- . D.P.R. 524/82 : SEGNALETICA DI SICUREZZA
- . D.P.R. 547/55 - D.P.R. 303/56 - D.P.R. 277/91 : IGIENE E SICUREZZA NEI LUOGHI DI LAVORO

In fede



 UNITA' SANITARIA LOCALE LT/1
 DIRIGENTE SERVIZIO IGIENE PUBBLICA

ATTACHMENT III

APPENDIX A

Fate and Effects Studies for Environmental Assessment of Zileuton

APPENDIX A

Fate and Effects Studies For Environmental Assessment of Zileuton

FDA Guideline	Type of Study	Result	Significance
3.01	Water Solubility	100±20 mg/L	Baseline data for other studies.
3.02	Log Octanol-Water Partition Coefficient	2.1	Estimation of partitioning into aquatic biota from surface water. The log K_{ow} is not indicative of the transport or fate, however, because the drug substance will form complexes with Cu^{+2} and Fe^{+3} giving it cationic characteristics.
3.03	Vapor Pressure	ND	Vapor pressure of zileuton cannot be related to its environmental volatility. Estimations of volatility from surface water that are based on vapor pressure (and a calculated Henry's constant) assume that the dissolved chemical is not ionic and does not become part of an ionic complex (Mackay and Wolkoff, 1973; Lyman et al. (1982). (This restrictive condition is often overlooked in the widespread application of this model.) If the chemical becomes strongly complexed by environmental cations such as Cu^{+2} and Fe^{+3} --as does zileuton (Item 7, Subsection C.7.2.4--its volatilization to the atmosphere cannot be regarded as an operative process and, therefore, can no longer be estimated.
3.04	Dissociation Constant (pK _a)	10.3	Zileuton would be present as a neutral, uncharged molecule, if cations such as Cu^{+2} and Fe^{+3} were not also in the aquatic environment.

FDA Guideline	Type of Study	Result	Significance
3.05	UV-Visible Spectrum	Peak maximum at 300 nm	Photolysis may occur in shallow surface water.
3.06	Melting Temperature	144.2 - 145.2°C	Bulk zileuton is solid at ambient conditions.
3.07	Density	ND	The density of the solid phase of a substance cannot be related to the behavior of its dissolved molecules.
3.08	Sorption and Desorption	Zileuton is strongly adsorbed to sludge and clay.	This is the process that determines the environmental transport of zileuton.
3.09	Hydrolysis	At pH 7, the half-life was 3.6 years.	Hydrolysis may be too slow to contribute to the environmental fate of zileuton.
3.10	Photodegradation in Water	The half-life of zileuton measured in September at 40° N latitude was 2.4 days.	Photodegradation of zileuton will account for most of its destruction in environmental surface waters.
3.11	Biodegradation in Water	Under the test conditions, degradation was very slow. However, acclimated cultures have been demonstrated to degrade benzo[b]thiophene.	Breakdown of zileuton may require an acclimated microbial culture.
3.12	Biodegradation in Soil	ND	Soil is not an environment of concern.

FDA Guideline	Type of Study	Result	Significance
4.01	Algal Toxicity	ND	Photodegradation of zilcuton in shallow surface water will prevent the exposure of algae to the drug substance. Moreover, the expected environmental concentration (EEC) of zilcuton is essentially zero.
4.02	Microbial Growth Inhibition	No inhibition of growth was observed at 90 mg/L, the limit of solubility.	The microorganisms of sewage and surface water would not be affected by the worst-case concentrations of zilcuton.
4.06	Seed Germination	ND	Soil is not an environment of concern.
4.07	Seedling Growth	ND	Soil is not an environment of concern.
4.08	Daphnia Acute Toxicity	The no-observed-effect concentration (NOEC) is 23.1 mg/L.	The worst-case concentrations are much lower than the toxic concentration.
4.09	Daphnia Chronic Toxicity	ND	Zilcuton will not be persistent in surface water and, therefore, will not present itself as a chronic problem.
4.10	Hyalella Toxicity	ND	The adsorbed zilcuton is not available to this organism; the dissolved zilcuton will not be persistent due to its photodegradation.
4.11	Freshwater Fish Acute Toxicity	The no-observed-effect concentration (NOEC) is greater than or equal to 19.4 mg/L.	The worst-case concentrations are much lower than the toxic concentration.
4.12	Earthworm Subacute Toxicity	ND	Soil is not an environment of concern.

ATTACHMENT IV

In a separate communication, Abbott Laboratories authorized release of pages 220-222 (Volume 017) and pages 134-137 (Volume 018) which were originally classified as confidential.

ABBOTT LABORATORIES
CHEMICAL AND AGRICULTURAL PRODUCTS DIVISION
NORTH CHICAGO

GENERAL ENVIRONMENTAL STATEMENT OF COMPLIANCE

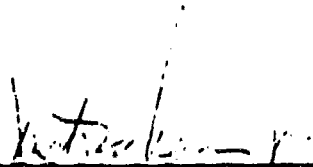
ABBOTT LABORATORIES states that it is in material compliance with, or on an enforceable schedule to be in compliance with, applicable emission requirements set forth in permits, consent decrees and administrative orders relating to the production of Zileuton bulk drug at its facilities in North Chicago, Illinois, as well as applicable emission requirements set forth in federal, state and local statutes and regulations relating to the production of Zileuton bulk drug.



Jim McClain
CAPD Environmental Manager

ABBOTT LABORATORIES
PHARMACEUTICAL PRODUCTS DIVISION
A B B O T T P A R K
GENERAL ENVIRONMENTAL COMPLIANCE STATEMENT

ABBOTT LABORATORIES states that it is in material compliance with, or on an enforceable schedule to be in compliance with, applicable emission requirements set forth in permits, consent decrees and administrative orders relating to the production of Zileuton at its facilities in Abbott Park, Illinois, as well as applicable emission requirements set forth in federal, state and local statutes and regulations relating to the production Zileuton.



JOSEPH M. NEMMERS, JR.
PLANT MANAGER



ABBOTT

PUERTO RICO OPERATIONS
P. O. BOX 278
BARCELONETA, P. R. 00617

Subsidiary of
Abbott Laboratories

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GENERAL ENVIRONMENTAL COMPLIANCE STATEMENT

ABBOTT LABORATORIES states that it is in material compliance with, or on an enforceable schedule to be in compliance with, applicable emission requirements set forth in permits, consent decrees and administrative orders relating to the production of Zileuton at its facilities in Barceloneta, Puerto Rico, as well as applicable emission requirements set forth in federal, state and local statutes and regulations relating to the production of Zileuton.

Luis A. Rivera

LUIS A. RIVERA FIGUEROA
MANAGER/ENVIRONMENTAL
ENGINEER

LARF/mc
Statement

ATTACHMENT V

In a separate communication, Abbott Laboratories authorized release of pages 220-222 (Volume 017) and pages 134-137 (Volume 018) which were originally classified as confidential.

MATERIAL SAFETY DATA SHEET

ABBOTT LABORATORIES
CHEMICAL & AGRICULTURAL PRODUCTS DIVISION
NORTH CHICAGO, ILLINOIS 60064
EMERGENCY TELEPHONE 1-708-937-6100
ABBOTT CHEMTREC 1-800-424-9300

ISSUE DATE: 03/17/92 TSCA STATUS: Exempt APPROVAL: _____

LIST/CODE: N/D/N/D

PRODUCT NAME: Zileuton; A-64077
CHEMICAL NAME: N-(1-benzo(b)thien-2-ylethyl)-N-hydroxyurea.
DOT CLASSIFICATION: Not regulated

HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

NAME (CAS NO.)	OSHA PEL	ACGIH TLV	ABBOTT LIMIT
A-64077* (111406-87-2)	NL	NL	**
**Hazardous per OSHA criteria			
***Internal Guideline = 1.0 mg/m ³ (8-hour TWA)			

PHYSICAL PROPERTIES

Appearance: White crystalline powder with no detectable odor
Solubility: Insoluble in water and methanol
Boiling Point: N/A Melting Point: 158-160C
pH: N/D Vapor Pressure: N/A
Vapor Density: N/A Density: N/D
Viscosity: N/A

FIRE AND EXPLOSION DATA

Flash Point: N/A
Extinguishing Media: Use appropriate media for underlying cause of fire
Special Fire Fighting Procedures: Wear protective clothing and if necessary self-contained breathing apparatus.
Unusual Fire and Explosion Hazards: Bulk drug ignition sensitivity is "strong"; explosion severity is "severe" and explosibility index is "severe"

PRODUCT NAME: Zileuton; A-64077

REACTIVITY

Incompatibility: Iron containing solutions

Hazardous Decomposition or By-products: Oxides of nitrogen, sulfur, and carbon.

Conditions to Avoid: N/D

HEALTH HAZARD DATA

Routes of Entry: Inhalation - YES Skin - N/D Ingestion - ?

Oral Toxicity: LD50=410 to >4000mg/kg in mice and rats. TDLo = 500 mg/kg in dogs

Dermal Toxicity: N/D

Inhalation Toxicity: N/D. In rats and guinea pigs, intratracheal dosages of 2.5 mg/kg or more produced blood concentrations consistent with pharmacologic efficacy

Corrosiveness: No

Dermal Irritation: Non-irritating in a skin irritation test in rabbits

Ocular Irritation: Practically non-irritating in an eye irritation test in rabbits

Dermal Sensitization: N/D

Special Target Organ Effects: A-64077 produced injury to the liver, kidneys, testes and prostate gland, as well as, alterations in estrus cycling accompanied by focal uterine metaplasia in preclinical studies in adult animals. In reproduction studies in animals, A-64077 produced a prolongation of gestation, increased estrus cycling, fetotoxicity, delayed ossification, some fetal abnormalities and maternal toxicity

Carcinogenicity: NTP - NL IARC - NL OSHA - NL ACGIH - N

Signs and Symptoms of Exposure: None known. In clinical trials, the data suggested only nausea, and GI upset. Workplace experience suggests the possibility of tightness in the chest and dizziness

Medical Conditions Aggravated by Exposure: None known. Animal data suggest liver, renal, testes or prostate injury or impairment. Clinical information suggest GI lesions

PRODUCT NAME: Zileuton; A-64077

HEALTH HAZARD DATA (cont)

Emergency and First Aid Procedures: Remove from source of exposure.
If skin or eye contact occur, flush with copious amounts of water.
If irritation persists or signs of toxicity occur, seek medical attention. No known antidote. Provide symptomatic/supportive care necessary

SPECIAL PROTECTION INFORMATION

Ventilation: Use local ventilation to control dust

Respirator: Approved respirator with high efficiency particulate filter or supplied-air respirator.

Gloves: Rubber

Eye Protection: Safety glasses/goggles

Other Protection: N/D

SPECIAL HANDLING AND STORAGE

Special Precautions: N/D

Spill or Release Procedures: Wear approved respirator and chemically compatible gloves. Sweep up spillage. Avoid dust. Place in appropriate container for disposal. Ventilate and wash spill site

Waste Disposal: Dispose of material in accordance with federal, state, and local regulations

Other Handling: N/D

PRODUCT NAME: Zileuton; A-64077

Legend

N/A = NOT APPLICABLE
N/D = NOT DETERMINED
NL = Not Listed
L = Listed
C = Ceiling
S = Short Term
(R) = A registered trademark of Abbott Laboratories
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