

Walsh
HFD-180

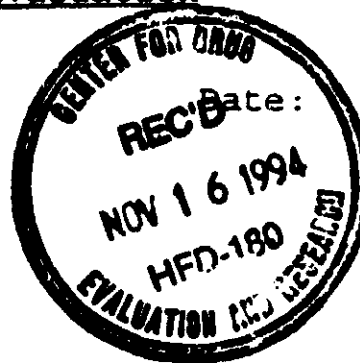
Statistical Review and Evaluation

NDA #: 20-406

Applicant: TAP Pharmaceuticals

Name of Drug: Prevacid (Lansoprazole)

Documents Reviewed:



1. "Statistical Analysis of Mortality and Tumor Incidence Data of a Two Year Oral Oncogenicity Study in Mice of Lansoprazole.", International Research and Development Corp. Study No. 126-052, TD91-025, Report No. R&D/93/514, Sep. 9, 1993, Submitted Nov. 12, 1993.
2. "Statistical Analysis of Mortality and Tumor Incidence Data of a Two Year Oral Oncogenicity Study in Mice of Lansoprazole.", International Research and Development Corp. Study No. 126-057, TD91-276, Report No. R&D/93/731, Dec. 1. 1993. Submitted Dec. 22, 1993.
3. NDA submission volumes 5.3 to 5.5, Special NDA submission, Date of Document, Feb. 18, 1994, Data Diskettes for six animal tumorigenicity studies.

I. Background

Six animal carcinogenicity studies (study numbers TA91-024, 1148/CA, TD91-025, TD91-276, 87-3158 and 86-3028) included in this NDA submission have been reviewed by the Division of Biometrics. In the previous reviews, statistical analyses were performed on each study independently. Dr. Chopra, HFD-180, who is the reviewing pharmacologist of this NDA has requested the Division of Biometrics to test the positive linear trend in some incidental tumor rates based on the combined data of studies TD91-025 and TD91-276. The following tumors: hepatocellular adenoma, hepatocellular carcinoma, and combined hepatocellular adenoma/carcinoma in both sexes, and rete testis adenoma in males are included in the following analyses.

II. Reviewer's Analyses

In TD91-025 mouse study, Lansoprazole was administered by gavage once daily to 70 Crl:CD-1 BR mice/sex/group at dosage levels of 0, 0, 15, 75, 150, and 300 mg/kg/day for two years. The data of second control group (vehicle control B) in this study were not submitted by the sponsor. In TD91-276 mouse study, Lansoprazole was administered by gavage once daily to 70 Crl:CD-1 BR mice/sex at dosage level of 0 and 600 mg/kg/day for two years. Dr. Chopra indicated that studies TD91-025 and TD91-276 were conducted at the same laboratories by the same group of scientists and were evaluated by the same pathologists during the similar time frames. The following analyses were based on

the data of both studies. They include dose groups of 0, 15, 75, 150, and 300 mg/kg/day from TD91-025 study and dose groups of 0 and 600 mg/kg/day from TD91-276 study.

The intercurrent mortality rates for both male and female mice (see Tables 1 and 2) were tested for linear trend according to the death rate method described in the paper of Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) using the time intervals 0-50, 51-80, and 81-104 weeks. Since there are two control groups, three separate sets of analyses were applied to these data sets. In the first set of analyses (called C1), the data of first control (from TD91-025 study), low, medium-1, medium-2, high-1, and high-2 dose groups were used. In the second set of analyses (called C2), the data of second control (from TD91-276 study), low, medium-1, medium-2, high-1, and high-2 dose groups were used. In the third set of analyses (called C1+C2), the combined data of both controls, low, medium-1, medium-2, high-1, and high-2 dose groups were used. The actual dose levels 0, 0, 15, 75, 150, 300, and 600 mg/kg/day were the scores assigned to the first control, the second control, low, medium-1, medium-2, high-1, and high-2 dose groups, respectively. The results of the analyses showed that there were significant (at 0.05 level) linear trends in the intercurrent mortality rate in both male (C1: $p < 0.0001$, C2: $p < 0.0001$, C1+C2: $p < 0.0001$) and female mice (C1: $p < 0.0001$, C2: $p < 0.0001$, C1+C2: $p < 0.0001$).

The method of age-adjusted exact permutation trend test was used to test the linear trend in hepatocellular adenoma, hepatocellular carcinoma, and combined hepatocellular adenoma/carcinoma in both sexes, and rete testis adenoma in males. The results of the above analyses showed that there was a significant (at 0.05 level) positive linear trend in the following neoplastic lesions:

(1) Liver hepatocellular adenoma (C1: $p = 0.0002$, C2: $p = 0.0001$, C1+C2: $p = 0.0001$), and combined hepatocellular adenoma and carcinoma (C1: $p = 0.0001$, C2: $p = 0.0001$, C1+C2: $p < 0.0001$) in females.

(2) Liver hepatocellular adenoma (C1: $p = 0.0001$, C2: $p < 0.0001$, C1+C2: $p < 0.0001$), liver hepatocellular carcinoma (C1: $p = 0.0224$, C2: $p = 0.0149$, C1+C2: $p = 0.0109$), combined hepatocellular adenoma and carcinoma (C1: $p = 0.0002$, C2: $p = 0.0001$, C1+C2: $p = 0.0001$), and rete testes adenoma (C1: $p = 0.0035$, C2: $p = 0.0036$, C1+C2: $p = 0.0014$) in males.

However, the prevalence rates of liver hepatocellular carcinoma in male mice in the concurrent control group is greater than one percent. It is considered as a common tumor in this strain of mice. For a common tumor, we consider a positive linear trend not to occur by chance of variation only if the p-value is smaller than 0.01.

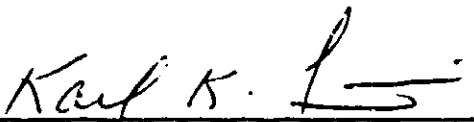
Therefore, we do not regard the positive linear trend in liver hepatocellular carcinoma in male mice as statistically significant. The incidence rates of the tumor types with significant linear trends are given in Tables 3 to 8.

The pairwise comparisons between the high dose and control groups also showed that there are significantly higher incidence rates in the high dose group in the liver hepatocellular adenoma (C1: $p = 0.0004$, C2: $p = 0.0002$, C1+C2: $p = 0.0001$), combined hepatocellular adenoma and carcinoma (C1: $p = 0.0005$, C2: $p = 0.0003$, C1+C2: $p = 0.0001$), and rete testes adenoma (C1: $p = 0.0411$, C2: $p = 0.0451$, C1+C2: $p = 0.0099$) in male mice. For female mice, there are significantly higher incidence rates in the high dose group in the liver hepatocellular adenoma (C1: $p = 0.0226$, C2: $p = 0.0184$, C1+C2: $p = 0.0078$), and combined hepatocellular adenoma and carcinoma (C1: $p = 0.0226$, C2: $p = 0.0184$, C1+C2: $p = 0.0078$) only when both control groups are combined.



Daphne Lin, Ph.D.
Mathematical Statistician

Concur:

 11/15/84
Karl K. Lin, Ph.D., Group Leader, SARB

cc: Original NDA 20-406

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HFD-715/DRU 2.1.1, Prevacid, Tap Pharmaceuticals

Table 1
Intercurrent Mortality Rates
Male Mice

<u>Weeks</u>	<u>Control-1</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High-1</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	70	1	1.4	70	0	0	70	7	10	70	6	8.6	70	6	8.6
51-80	69	6	8.7	70	10	14.3	63	20	31.7	64	23	35.9	64	17	26.6
81-104	63	25	39.7	60	19	31.7	43	13	30.2	41	19	46.3	47	18	38.3
Term.	38			41			30			22			29		

<u>Weeks</u>	<u>Control-2</u>			<u>High-2</u>		
	S	D	%	S	D	%
0-50	70	2	2.85	70	6	8.57
51-80	68	10	14.7	64	24	37.5
81-104	58	19	32.75	40	24	60.0
Term.	39			16		

Peto Test: C1: $p < 0.0001$
 C2: $p < 0.0001$
 C1+C2: $p < 0.0001$

Notes: S: Number of animals starting during the period
 D: Deaths
 %: Percent of death during the period

Table 2
Intercurrent Mortality Rates
Female Mice.

<u>Weeks</u>	<u>Control-1</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High-1</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	70	1	1.4	70	0	0	70	1	1.4	70	1	1.4	70	7	10
51-80	69	11	15.9	70	19	27.1	69	22	31.8	69	19	27.5	63	25	39.7
81-104	58	26	44.8	51	21	41.2	47	22	46.8	50	16	32	38	14	36.8
Term.	32			30			25			34			24		

<u>Weeks</u>	<u>Control-2</u>			<u>High-2</u>		
	S	D	%	S	D	%
0-50	70	4	5.71	70	13	18.57
51-80	66	11	16.6	57	30	52.6
81-100	55	25	45.45	27	15	55.5
Term.	30			12		

Peto Test: C1: $p < 0.0001$
 C2: $p < 0.0001$
 C1+C2: $p < 0.0001$

Notes: S: Number of animals starting during the period
 D: Deaths
 %: Percent of death during the period

Table 3
Tumor Incidence Rates
Female Mice, Hepatocellular adenoma

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	1	0	7
51-80	0	11	0	19	0	22	0	19	0	25
81-104	1	26	0	21	0	22	0	16	0	14
Terminal	0	32	0	30	1	25	4	34	6	24
<u>Total</u>	<u>1</u>	<u>70</u>	<u>0</u>	<u>70</u>	<u>1</u>	<u>70</u>	<u>4</u>	<u>70</u>	<u>6</u>	<u>70</u>

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	4	0	13
51-80	0	11	0	30
81-100	0	25	0	15
Terminal	1	30	4	12
<u>Total</u>	<u>1</u>	<u>70</u>	<u>4</u>	<u>70</u>

Peto Test: C1: p = 0.0002
C2: p = 0.0001
C1+C2: p = 0.0001

Pairwise Comparison: C1: p = 0.0226
C2: p = 0.0184
C1+C2: p = 0.0078

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 4
Tumor Incidence Rates
Female Mice, Hepatocellular adenoma/carcinoma

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	1	0	1	0	7
51-80	0	11	0	19	0	22	0	19	0	25
81-104	1	26	0	21	0	22	0	16	0	14
Terminal	0	32	0	30	1	25	4	34	7	24
<u>Total</u>	<u>1</u>	<u>70</u>	<u>0</u>	<u>70</u>	<u>1</u>	<u>70</u>	<u>4</u>	<u>70</u>	<u>7</u>	<u>70</u>

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	4	0	13
51-80	0	11	0	30
81-100	0	25	0	15
Terminal	1	30	4	12
<u>Total</u>	<u>1</u>	<u>70</u>	<u>4</u>	<u>70</u>

Peto Test: C1: $p = 0.0001$
C2: $p = 0.0001$
C1+C2: $p < 0.0001$

Pairwise Comparison: C1: $p = 0.0226$
C2: $p = 0.0184$
C1+C2: $p = 0.0078$

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 5
Tumor Incidence Rates
Male Mice, Hepatocellular adenoma

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	7	0	6	0	6
51-80	0	6	0	10	0	20	1	23	0	17
81-104	0	25	3	19	1	13	1	19	4	18
Terminal	10	38	8	41	4	30	2	22	9	29
<u>Total</u>	<u>10</u>	<u>70</u>	<u>11</u>	<u>70</u>	<u>5</u>	<u>70</u>	<u>4</u>	<u>70</u>	<u>13</u>	<u>70</u>

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	2	0	6
51-80	1	10	0	24
81-104	2	19	4	24
Terminal	2	39	11	16
<u>Total</u>	<u>5</u>	<u>70</u>	<u>15</u>	<u>70</u>

Peto Test: C1: $p = 0.0001$
C2: $p < 0.0001$
C1+C2: $p < 0.0001$

Pairwise Comparison: C1: $p = 0.0004$
C2: $p = 0.0002$
C1+C2: $p = 0.0001$

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 6
Tumor Incidence Rates
Male Mice, Hepatocellular carcinoma

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	7	0	6	0	6
51-80	0	6	0	10	0	20	0	23	0	17
81-104	2	25	1	19	0	13	5	19	3	18
Terminal	3	38	5	41	4	30	0	22	1	29
Total	5	70	6	70	4	70	5	70	4	70

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	2	0	6
51-80	0	10	0	24
81-104	2	19	3	24
Terminal	1	39	6	16
Total	3	70	9	70

Peto Test: C1: p = 0.0224
C2: p = 0.0149
C1+C2: p = 0.0109

Pairwise Comparison: C1: p = 0.021
C2: p = 0.0087
C1+C2: p = 0.0065

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 7
Tumor Incidence Rates
Male Mice, Hepatocellular adenoma/Carcinoma

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	7	0	6	0	6
51-80	0	6	0	10	0	20	1	23	0	17
81-104	2	25	4	19	1	13	5	19	6	18
Terminal	12	38	11	41	6	30	2	22	10	29
<u>Total</u>	<u>14</u>	<u>70</u>	<u>15</u>	<u>70</u>	<u>7</u>	<u>70</u>	<u>8</u>	<u>70</u>	<u>16</u>	<u>70</u>

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	2	0	6
51-80	1	10	0	24
81-104	4	19	7	24
Terminal	3	39	12	16
<u>Total</u>	<u>8</u>	<u>70</u>	<u>19</u>	<u>70</u>

Peto Test: C1: p = 0.0002
C2: p = 0.0001
C1+C2: p = 0.0001

Pairwise Comparison: C1: p = 0.0005
C2: p = 0.0003
C1+C2: p = 0.0001

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 8
Tumor Incidence Rates
Male Mice, Testis, Adenoma, Rete Testis

<u>Weeks</u>	<u>Control-1</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High-1</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	1	0	0	0	7	0	6	0	6
51-80	0	6	0	10	0	20	0	23	0	17
81-104	0	25	0	19	0	13	0	19	0	18
Terminal	0	38	0	41	1	30	1	22	1	29
Total	0	70	0	70	1	70	1	70	1	70

<u>Weeks</u>	<u>Control-2</u>		<u>High-2</u>	
	T	N	T	N
0-50	0	2	0	6
51-80	0	10	0	24
81-104	0	19	1	24
Terminal	0	39	2	16
Total	0	70	3	70

Peto Test: C1: p = 0.0035
C2: p = 0.0036
C1+C2: p = 0.0014

Pairwise Comparison: C1: p = 0.0411
C2: p = 0.0451
C1+C2: p = 0.0099

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.



walsh

ADDENDUM TO STATISTICAL REVIEW

Date: NOV 28 1994

NDA #: 20-406/Drug Class 1s

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: acute healing in severe Reflux Esophagitis

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.483, dated November 12, 1993, responses dated April 7 and May 2, 1994.

This review is an addendum to the review dated June 10, 1994 for acute RE. The medical officer Gallo-Torres, M.D., Ph.D. raised the issue that the number of severe RE patients in the trials submitted was small, and hence the generalization of safety and efficacy is problematic. With this in mind, this statistical reviewer requested additional, unplanned subset analyses of the sponsor.

The results in the severe disease subsets are consistently in favor of lansoprazole, but not consistently statistically significant.

Although these subset analyses were not planned in the original protocols, nor were the trial sizes intended to support efficacy, some trials provide statistically significant support for efficacy in severe Reflux Esophagitis.

ACUTE SEVERE REFLUX ESOPHAGITIS

We did not ask the sponsor for study M87-092, since none of its grades were found Severe. The sponsor added M90-422 which we do not remember

seeing before, and M92-721, a new study, both using a definition of Grade 4 which is acceptable as Severe RE. The results are summarized in Table A, following.

We see in Table A, statistically significant results in two trials. One is the Ranitidine controlled trial, M89-349, and the other is the new placebo controlled trial, M92-721. Trial M92-721 has not been submitted and reviewed in detail. If Trial M92-721 is acceptable, then we have two trials supporting the efficacy of lansoprazole for acute treatment of severe RE, separately from support for mild to moderate RE.

Table A				
8 Week Healing Rates for Acute Treatment of Severe RE				
Studies	Placebo	Ranitidine 150mg BID	Lansoprazole	
			15mg	30mg
M88-269	————	4/11 (36%)	————	5/ 8 (63%)
M89-349	————	0/11 (0%)	————	13/23 (57%)
M89-393	————	0/ 3 (0%)	————	5/ 8 (63%)
M90-422	————	2/ 7 (29%)	————	8/14 (57%)
M92-721	1/12 (8%)	————	10/17 (59%)	24/37 (65%)
bold	indicates statistical significance versus control at the 0.01 level.			

CONCLUSION

For acute treatment of severe RE, if trial M92-721 is found well-controlled and otherwise acceptable, we have two trials in support of efficacy.

Furthermore, in 3 additional trials, including 2 substantially reviewed trials, lansoprazole has a numerically better result than the control group (placebo or ranitidine.)

There is an overall result for lansoprazole per the review dated June 10, 1994 for acute RE. If approved for acute RE treatment, this reviewer finds no reason to exclude Severe RE from the labeling.

Ferrin Harrison, Ph.D.
Mathematical Statistician

Ferrin Harrison 11/28/94

This addendum to a review consists of 3 pages of text and 4 pages of proposed transparency.

Concur: Dr. Huque, Ph.D.

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11/28/94

Dr. Dubey, Ph.D.

62
11-28-94

cc: orig. NDA No. 20-406

HFD-180

HFD-180/ Dr. Fredd

HFD-180/ Dr. Gallo-Torres

HFD-180/ Ms. Maria Walsh

HFD-713/ Dr. Dubey [File DRU 1.3.2]

HFD-713/ Dr. Huque

HFD-713/ Dr. Ferrin Harrison

Chron.

HFD-713/HARRISONF/11-28-1994/wp51/lanrese2.doc

Acute Healing in Severe Reflux Esophagitis by Prevacid (Lansoprazole)

- Medical officer raised issue: number of severe RE patients in the trials submitted was small, hence the generalization of safety and efficacy is problematic?
- Statistical reviewer requested additional, unplanned subset analyses of the sponsor to address.
- 5 trials with Severe patients, 3 previously reviewed, 2 new
- Although analysis not planned, powered or stratified for, 5 differences favor lansoprazole and 2 differences are statistically significant

ACUTE SEVERE REFLUX ESOPHAGITIS

- o did not ask for M87-092, since none of its grades were found Severe.
- o sponsor added M90-422 which we do not remember seeing before
- o sponsor added M92-721, a new study, the second supporting efficacy in the Severe subset.

8 Week Heal Rates Acute Treatment, Severe RE

Studies Reviewed:

Studies	Placebo	Ranitidine 150mg BID	15mg	Lansoprazole 30mg
M88-269	_____	4/11 (36%)	_____	5/ 8 (63%)
M89-349	_____	0/11 (0%)	_____	13/23 (57%)
M89-393	_____	0/ 3 (0%)	_____	5/ 8 (63%)

Studies Not Reviewed:

Studies	Placebo	Ranitidine 150mg BID	15mg	Lansoprazole 30mg
M90-422	_____	2/ 7 (29%)	_____	8/14 (57%)
M92-721	1/12 (8%)	_____	10/17 (59%)	24/37 (65%)

bold indicates statistical significance versus control at the 0.01 level.

CONCLUSION for ACUTE REFLUX ESOPHAGITIS

- if trial M92-721 is found well-controlled and otherwise acceptable, we have 2 trials in support of efficacy.
- furthermore, in 3 additional trials, including 2 substantially reviewed trials, lansoprazole has a numerically better result than the control group (placebo or ranitidine.)
- overall result for lansoprazole for Reflux Esophagitis
- if approved, this reviewer finds no reason to exclude Severe RE from the labeling.

STATISTICAL REVIEW

Date: OCT 27 1994

NDA #: 20-406/Drug Class 1s

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: maintenance of healing in Reflux Esophagitis

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.483, dated November 12, 1993, responses dated April 7 and May 2, 1994.

Ia. INTRODUCTION

This review is for the RE (Reflux Esophagitis) maintenance or intermittent treatment indication. Separate reviews address the indications: Reflux Esophagitis, and Duodenal Ulcer.

The pivotal studies are M88-271 and M89-350, with more severe patients in M89-350. Their purpose is to show the safety and efficacy of lansoprazole in delaying recurrence of reflux esophagitis.

Ib. FEATURES OF THE DESIGN OF STUDIES M88-271, M89-350

The esophagitis grading scale used was TAP's modification of the Savary-Miller scale (see Table A on page 2.) There is a further modification to evaluate patients with RE in the presence of Barrett's epithelium (see Table B, on page 2.)

Study M89-350 enrolled only patients from the acute studies M89-349 and M89-393.

To enter the double-blind maintenance treatment phase, patients were required to have endoscopically documented healing (Grade 0 or 1) $\leq$ 7 days prior to the start receiving double-blind medication. The patient entered from one of three paths:

1. Patient completed a short-term study with endoscopically documented healing (Grade 0 or 1).
2. Patient completed a short-term study without endoscopically documented healing. Next, the patient received open-label lansoprazole at 30mg for eight weeks, and was healed (Grade 0 or 1) at the end of eight weeks, or was dropped.
3. This path is for Study M88-271 only. The patient had never

been in a lansoprazole study and had documented erosive RE (grade 2-4). The patient received open-label lansoprazole at 30mg for eight weeks, and was healed (Grade 0 or 1) at the end of eight weeks, or was dropped.

Table A
TAP Grading Scale for Principal RE Maintenance
Studies M88-272 and M89-350 without Barrett's

Grade	Description
0	Normal appearing mucosa by endoscopy
1	Mucosal edema, hyperemia or friability of mucosa
2	One or more erosions or ulcerations in the squamocolumnar junction
3	Erosions or ulcerations involving $\leq 50\%$ of the distal 5cm of the esophagus proximal to the squamocolumnar junction
4	Multiple erosions or ulcerations involving $>50\%$ of the distal 5cm of the esophagus or a single large ulcer $> 5\text{mm}$ in diameter proximal to the squamocolumnar junction

Table B
TAP Grading Scale for Principal RE Maintenance
Studies M88-272 and M89-350 with Barrett's Epithelium

Grade	Description
2	One or more erosions or ulcerations involving $<10\%$ of the distal 5cm of the esophagus proximal to the anatomical gastroesophageal junction
3	Erosions or ulcerations involving $10\%-50\%$ of the distal 5cm of the esophagus proximal to the anatomical gastroesophageal junction or an ulcer measuring 3mm-5mm in diameter
4	Multiple erosions or ulcerations involving $>50\%$ of the distal 5cm of the esophagus (as defined for Barrett's patients) or a single large ulcer $>5\text{mm}$ in diameter

Efficacy evaluations during blinded treatment were scheduled at Months 1,2,3,6,9 and 12, with endoscopy at each visit. In

addition, patients were asked to return for a visit at any time if signs or symptoms of erosive RE returned. At unscheduled visits, patients could undergo endoscopy. If possible, all endoscopies were to be performed by the same endoscopist throughout the study.

The primary efficacy evaluation was the time to first recurrence of erosive RE as determined by endoscopy, of Grade ≥ 2 . Probabilities of remaining healed after Months 1, 2, 3, 6, 9 or 12 months of therapy were estimated using the life-table methodology for the evaluable and intent-to-treat populations. Life-table methodology assumes that patients who are censored have the same rates of recurrence of RE as those who did not withdraw. This and two other analyses were used:

- 1) life-table analysis adjusting for censoring
- 2) assume all censored patients remained healed after censoring
- 3) assume all censored patients had recurrence after censoring

In addition to the per-protocol analysis, the following analyses were requested at the pre-NDA meeting to further test and substantiate the findings:

- 1) scheduled visits analysis
- 2) healing was considered Grade 0 instead of Grade 0 or 1
- 3) analysis of patients of Grades 3-4 prior to acute treatment.

Additionally, Gelusil use (average tablets per day) from baseline to first recurrence or the end of the study, whichever came first, was recorded. Gelusil use was analyzed by comparison for each pair of treatment groups using the Wilcoxon two-sample test.

II.a DESCRIPTION OF TRIAL M88-271

Study M88-271 was a blinded, randomized, parallel group, dose-response study that compared the efficacy of two doses of lansoprazole (15 mg, and 30 mg) to that of placebo. The principal efficacy endpoint was time to first recurrence.

Patients with documented healed esophagitis entered into a one-year maintenance treatment phase and were assigned to a fixed dose of study medication. However, if at any time during the 12 months the RE recurred, or duodenal or gastric ulcer was noted on endoscopy, the patient was treated with open-label lansoprazole 30mg QD for eight weeks. If healing was again documented by endoscopy, the patient returned to the originally assigned double-blind study medication. If the patient failed to heal on

this oper soproazole treatment, as demonstrated
endoscop patient was dropped from the study, as a
treatmer

Lansoprazole was administered orally as a once daily dose in the morning before breakfast. No other anti-ulcer medications were allowed except the antacid Gelusil taken on an as-needed basis. No chronic use of ulcerogenic drugs, including NSAIDs or aspirin was allowed. However, the occasional use of NSAIDs (≤ 2 doses per week) or treatment with ≤ 325 mg of aspirin per day was acceptable.

II.b RESULTS AND REVIEWER'S COMMENTS ON TRIAL M88-271

A total of 186 patients were enrolled by 25 investigators. Of the 186, 128 came from Study M88-269, and 58 had never been previously enrolled into a lansoprazole study. Out of the 186, 173 were eligible for entry into the double-blind maintenance phase, and were randomized.

Of the 173 patients who were randomized into the maintenance treatment phase, 170 were included in at least one primary efficacy analysis, and all 173 were included in the intent-to-treat analysis.

Forty-one (41) patients withdrew prematurely from the study during the maintenance phase:

21/57 (37%) placebo patients,
11/60 (18%) lanso 15mg, and
9/56 (15%) lanso 30mg.

Of these 41 premature withdrawals, 16 discontinued due to treatment failure, 12 due to relapse of erosive RE (10 placebo, 2 lansoprazole 30mg), and 4 experienced

symptoms of RE sufficiently severe to request withdrawal from the study (2 placebo, 2 lansoprazole 15mg.)

On its face, the effect size appears substantive, as shown in Table C and the preceding Figure 1 by differences in healing

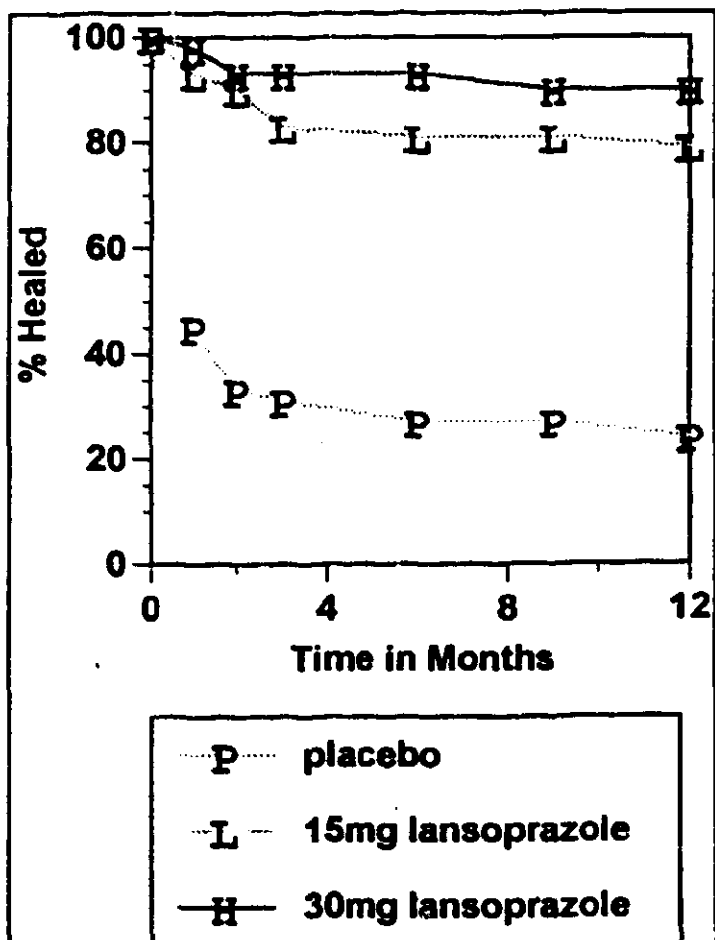


Figure 1 Trial M88-271
Primary Per-Protocol
Efficacy Analysis
treating dropouts as having
same rate as completers

rates of around 50% between lansoprazole and placebo. Details beyond Table C are given in Table 7a, in the appendix. The difference is developed after a month or two and is retained for the duration, noting differences of 10% are not statistically significant for this trial. The sponsor provided intent-to-treat rates, and they are comparable to the efficacy subset analysis. Additional analyses are given in the appendix, in Tables 2, 3 and 4, showing the robustness of the result. However, dose-response is not shown.

Table C PRIMARY ANALYSIS Percentage of Evaluable Patients Remaining Healed in Principal RE Maintenance Study M88-271 Life Table Analysis				
<u>Treat</u>	<u>0-1 months</u>	<u>1-2 month</u>	<u>2-3 month</u>	<u>3-6 month</u>
Pla. Ulcer/Total (%)	30/55 (55%)	7/25 (28%)	1/16 (6%)	2/15 (13%)
Drop/Total (%)	0/55 (0%)	0/25 (0%)	2/16 (13%)	0/15 (0%)
Product-Limit	45%	33%	31%	27%
15mg Ulcer/Total (%)	4/59 (7%)	2/54 (4%)	4/50 (8%)	1/45 (2%)
Drop/Total (%)	0/59 (0%)	1/54 (2%)	2/50 (4%)	1/45 (2%)
Product-Limit	93%	90%	83%	81%
30mg Ulcer/Total (%)	1/56 (2%)	3/53 (6%)	0/49 (0%)	0/43 (0%)
Drop/Total (%)	0/56 (0%)	2/53 (4%)	1/49 (2%)	6/43 (14%)
Product-Limit	98%	93%	93%	93%
Either lansoprazole versus placebo at 12 months: $p < .001$				

The Product-Limit healing rate estimator shown in Tables C, D and appendix 7a, 7b are computed as follows. The Product-Limit for the end of the first interval on placebo is $1 - 30/55 = 45\%$. The Product-Limit for the second interval on placebo is $45\%(1 - 7/25)$. For the third interval, $45\%(1 - 7/25)(1 - 1/16)$. For the fourth and additional intervals, keep multiplying by $(1 - \text{ulcers}/\text{total})$ for all the data up to the time limit, hence the name Product-Limit estimator. Thus patients who are censored are included in the denominator until they are censored, which is more informative than completely excluding them from analysis.

III.a DESCRIPTION OF TRIAL M89-350

Study M89-350 was a blinded, randomized, parallel group, dose-response study that compared the efficacy of two doses of lansoprazole (15 mg, and 30 mg) to that of placebo. The

principal efficacy endpoint was time to first recurrence. The patients had a recent history of erosive RE which had proven resistant to treatment with at least 12 weeks of therapy with one or more histamine H₂-receptor antagonists, given in standard (ranitidine 150 BID) or higher doses.

Patients with documented healed esophagitis entered into a one-year treatment phase and were assigned to a fixed dose of study medication. However, if at any time during the 12 months the RE recurred, or duodenal or gastric ulcer was noted on endoscopy, the patient was treated with open-label lansoprazole 30mg QD for eight weeks. If healing was again documented by endoscopy, the patient returned to the originally assigned double-blind study medication. If the patient failed to heal on open-label lansoprazole, as demonstrated endoscopically, the patient was dropped from the study, regarded as treatment failure.

III.b RESULTS AND REVIEWER'S COMMENTS ON TRIAL M89-350

A total of 180 patients were enrolled by 16 investigators. Out of the 180, 163 were eligible for entry into the double-blind maintenance phase. Of the 17 dropped between enrollment and double-blind maintenance, 15 were unhealed after the 8-week open label healing period, 1 discontinued with an adverse event and 1 was lost to follow up.

Of the 163 patients who entered the maintenance phase, 146 (90%) were included in at least one primary efficacy analysis, and all 163 were included in the intent-to-treat analysis. There were 71 premature withdrawals from the study:

39/56 (55%) placebo patients
15/53 (28%) lanso 15mg
17/54 (31%) lanso 30mg

Of the 71 withdrawals, there were 37 treatment failures:
27 relapse of RE
10 withdrawal on RE symptoms

Of the 37 treatment failures,

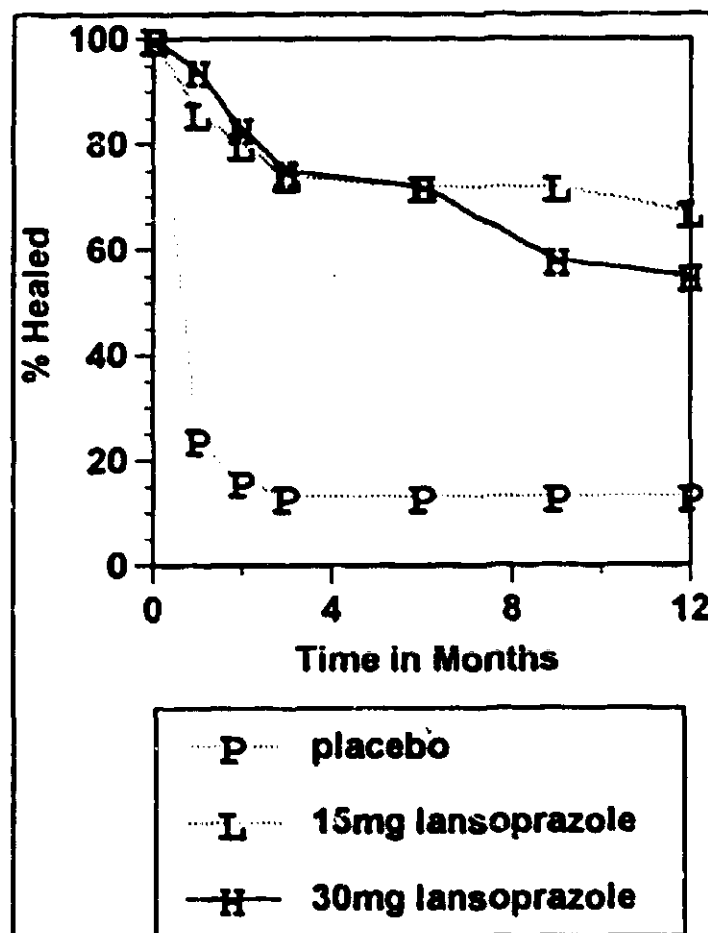


Figure 2 Trial M89-350
Primary Per-Protocol
Efficacy Analysis
treating dropouts as having
same rate as completers

21 were on placebo.

Baseline imbalance was not found with respect to gender, race, esophagitis grade, hiatal hernia, tobacco use, alcohol use, caffeine use, or previous treatment (lanso 8 week, 16 week, ranitidine.) Nor was baseline imbalance found with respect to age or weight by gender.

As shown in Table D and the preceding Figure 2, the rates diverge strongly and early. Details beyond Table D are given in Table 7b, in the appendix. After the second month, the 6/47 placebo patients remain healed, while the lansoprazole healing rates drop gradually. Given the large early difference and early censoring in placebo, this reviewer is not concerned by the estimated decrease in the difference in healing rates between lansoprazole and placebo, and similarly for lansoprazole 15mg versus 30mg. The results of the intent-to-treat analysis are similar, except the placebo healing rates decline gradually so the estimated difference is more stable over time. Alternate analyses are in the appendix, Tables 5, 6 and 4

Table D
PRIMARY ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Study M89-350
Life Table Analysis

<u>Treat</u>		<u>0-1 months</u>	<u>1-2 month</u>	<u>2-3 month</u>	<u>3-6 month</u>
Pla.	Ulcer/Total (%)	35/46 (76%)	3/ 9 (33%)	0/ 6 (0%)	1/ 5 (20%)
	Drop/Total (%)	1/46 (2%)	2/ 9 (22%)	0/ 6 (0%)	1/ 5 (20%)
	Product-Limit	24%	16%	16%	13%
15mg	Ulcer/Total (%)	7/50 (14%)	3/43 (7%)	3/39 (8%)	1/36 (3%)
	Drop/Total (%)	0/50 (0%)	3/43 (7%)	1/39 (3%)	0/36 (0%)
	Product-Limit	86%	80%	74%	72%
30mg	Ulcer/Total (%)	3/48 (6%)	5/45 (11%)	4/39 (10%)	1/29 (3%)
	Drop/Total (%)	1/48 (2%)	0/45 (0%)	1/39 (3%)	6/29 (21%)
	Product-Limit	94%	83%	75%	72%

Either lansoprazole versus placebo at 12 months: $p < .001$

IIII. REVIEWER'S COMMENTS ON SAFETY IN TRIALS M88-271 & M89-350

Since the indication is for chronic treatment, safety is of particular interest. This reviewer cannot fully address safety, but looked at the more consistent changes in blood of

lansoprazole patients with corroboration of change between trials, with the results summarized in appendix Table 8.

To summarize the results in words, hematocrit, hemoglobin and basophils drop during treatment in their respective orders, with no discernible change in the first month, but the decrease is always apparent in the sixth month.

Potassium is sometimes increased in M88-271, but generally decreased in M89-350.

Alkaline phosphatase is generally but not consistently (not consistent over time) increased in both trials.

GGT is generally increased in M88-271, but shows only one period of change in M89-350 and it is a decrease. However, the insignificant changes in M89-350 are increases.

Some patients were withdrawn due to severe changes in some of these and other parameters.

The metabolic chemistry parameters might be construed to favor the drug:

Serum albumin is somewhat more consistent than GGT, dropping in the 15th month (3 months after treatment concluded) in both trials; with a significant decrease in the 3rd month in M88-271.

Cholesterol decreases by a small amount, achieving significance only in the 12th month in M88-271 but generally significant in M88-350.

V. REVIEWER'S COMMENTS AND CONCLUSIONS
(that may be conveyed to the sponsor)

In all analyses, the data indicate the drug is superior to placebo.

Since the drug is shown superior to placebo in 3 per-protocol analyses, 3 pre-NDA analyses, and 4-8 post-NDA analyses, this reviewer finds two trials supporting the efficacy of the drug relative to placebo.

Dose response is not supported. There are many insignificant results from tests for drug response, with p-values from 0.062 to 0.763, none below 0.05.

A safety concern is that there is a mean decrease in hematocrit, hemoglobin and basophils during sustained treatment.

OVERALL CONCLUSION

Two trials support efficacy relative to placebo.

Dose response (30mg vs. 15mg) is not supported.

Sustained treatment leads to a mean decrease in hematocrit, hemoglobin and basophils.

Ferrin Harrison 10/27/94

Ferrin Harrison, Ph.D.
Mathematical Statistician

This review consists of 9 pages of text and 8 pages of tables.

Concur: Dr. Huque

Huque 10/27/94

Dr. Dubey

6 10-27-94.

cc: orig. NDA No. 20-406

HFD-180

HFD-180/ Dr. Fredd

HFD-180/ Dr. Gallo-Torres

HFD-180/ Ms. Maria Walsh

HFD-713/ Dr. Dubey [File DRU 1.3.2]

HFD-713/ Dr. Huque

HFD-713/ Dr. Ferrin Harrison

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HFD-713/HARRISONF/10-27-1994/wp51/lanmaint.doc

TABLE 1
Pivotal Reflux Esophagitis Maintenance or Intermittent Treatment Studies
Duration of Drug Treatment: 12 Months

Study Number	Study Design	Treatment Doses Studied (Randomization)	Evaluable Endoscoped Patients Effectiveness Outcome
M88-271 (U.S.)	Double-Blind, randomized fixed dose, multicenter, parallel-group, 3 month posttreatment follow up.	Lansoprazole 15 mg QD 30 mg QD Placebo randomization (1:1:1)	Time to first recurrence of RE. Estimated likelihood of remaining healed at 12 months was 24% placebo, 79% lanso 15mg, 90% lanso 30mg.
M89-350 (U.S.)	Double-Blind, randomized fixed dose, multicenter, parallel group, 3 month posttreatment follow up.	Lansoprazole 15 mg QD 30 mg QD Placebo randomization (2:1:1)	Time to first recurrence of RE. Estimated likelihood of remaining healed at 12 months was 13% placebo, 67% lanso 15mg, 55% lanso 30mg.

Table 2
PER-PROTOCOL ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Study M88-271

treating withdrawals as having the rates of completers

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=55)	45%	33%	31%	27%	27%	24%
Lanso 15mg (N=59)	93%	90%	83%	81%	81%	79%
Lanso 30mg (N=56)	98%	93%	93%	93%	90%	90%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .098$

treating withdrawals as ulcer recurrences

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=55)	45%	33%	27%	24%	22%	18%
Lanso 15mg (N=59)	93%	88%	78%	75%	71%	66%
Lanso 30mg (N=56)	98%	89%	88%	77%	71%	68%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .763$

treating withdrawals as non-recurrences

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=55)	45%	33%	31%	27%	27%	25%
Lanso 15mg (N=59)	93%	90%	83%	81%	81%	80%
Lanso 30mg (N=56)	98%	93%	93%	93%	91%	91%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .088$

Table 3
PRE-NDA ALTERNATE ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Study M88-271 -

with baseline esophagitis Grades 3 or 4

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=35)	43%	29%	26%	23%	23%	23%
Lanso 15mg (N=33)	97%	94%	80%	77%	77%	73%
Lanso 30mg (N=32)	97%	90%	90%	90%	86%	86%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .266$

baseline grade 2-4, defining healing as grade 0

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=41)	17%	15%	15%	12%	8%	4%
Lanso 15mg (N=41)	80%	73%	66%	63%	58%	55%
Lanso 30mg (N=43)	100%	90%	86%	80%	78%	72%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .062$

baseline grade 2-4, healing grade 1-2,
excluding unscheduled visits

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=55)	50%	38%	35%	30%	30%	28%
Lanso 15mg (N=59)	95%	91%	86%	84%	84%	82%
Lanso 30mg (N=56)	98%	93%	93%	93%	90%	90%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .210$

Table 4
POST-NDA ALTERNATE ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Studies M88-271 and M89-350

visits used	Trial	Method	variable	-2log(L)	p-value
all visits	M88-271	Logistic	symptmat	39.12	p<.0001
			severity	29.498	p<.0001
		CoxReg	symptmat	45.24	p<.0001
			severity	37.152	p<.0001
	M89-350	Logistic	symptmat	11.886	p=.0026
			severity	7.463	p=.0240
		CoxReg	symptmat	41.713	p<.0001
			severity	33.254	p<.0001
visits used	Trial	Method	variable	-2log(L)	p-value
scheduled only	M88-271	Logistic	symptmat	40.757	p<.0001
			severity	29.929	p<.0001
		CoxReg	symptmat	43.392	p<.0001
			severity	35.392	p<.0001
	M89-350	Logistic	symptmat	15.306	p=.0005
			severity	9.61	p=.0082
		CoxReg	symptmat	26.361	p<.0001
			severity	17.862	p=.0001

CoxReg: Cox Regression; Logistic: Logistic Regression
 In Logistic Regression, patients with ulcers at unscheduled visits are non-informatively censored.

	— covariables —	
heartburn	symptmat	severity
none	0	0
mild	0	1
moderate	1	2
severe	1	3

For logistic regression, the two models are

Ho: Intercpt Symptmat/Severity	Ha: Intercpt Symptmat/Severity Lanso (lansoprazole vs placebo) Lansodos (15mg vs 30mg lansoprazole)

For Cox regression, the nonparametric life curve absorbs the intercept term, so the models simplify. For the model
 Ho: Intercept versus Ha: Intercpt, Symptmat/Severity, the result is statistically significant for all cases in this table.

Table 5
PER-PROTOCOL ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Study M89-350

treating withdrawals as having the rates of completers

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=47)	24%	16%	13%	13%	13%	13%
Lanso 15mg (N=50)	86%	80%	74%	72%	72%	67%
Lanso 30mg (N=49)	94%	83%	75%	72%	58%	55%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .435$

treating withdrawals as ulcer recurrences

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=47)	23%	13%	13%	9%	6%	4%
Lanso 15mg (N=50)	86%	80%	72%	70%	66%	56%
Lanso 30mg (N=49)	92%	82%	71%	57%	43%	39%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .119$

treating withdrawals as non-recurrences

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=47)	26%	19%	19%	17%	17%	17%
Lanso 15mg (N=50)	86%	80%	74%	72%	72%	68%
Lanso 30mg (N=49)	94%	84%	76%	73%	63%	61%

15mg vs Pla $p < .001$ 30mg vs Pla $p < .001$ 30mg vs 15mg $p = .602$

Table 6
PRE-NDA ALTERNATE ANALYSIS
Percentage of Evaluable Patients Remaining Healed
in Principal RE Maintenance Study M89-350 -

with baseline esophagitis Grades 3 or 4

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=35)	0*	0%	0%	0%	0%	0%
Lanso 15mg (N=33)	79%	75%	63%	58%	58%	52%
Lanso 30mg (N=32)	100%	86%	77%	77%	59%	53%

15mg vs Pla p<.001 30mg vs Pla p<.001 30mg vs 15mg p=.586

baseline grade 2-4, defining healing as grade 0

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=44)	16%	10%	6%	0*	0%	0%
Lanso 15mg (N=44)	80%	73%	63%	61%	55%	49%
Lanso 30mg (N=45)	78%	68%	68%	59%	42%	42%

15mg vs Pla p<.001 30mg vs Pla p<.001 30mg vs 15mg p=.626

baseline grade 2-4, healing grade 1-2,
excluding unscheduled visits

Treatment	Interval in Months					
	0-1	1-2	2-3	3-6	6-9	9-12
Placebo (N=47)	31%	21%	21%	17%	17%	17%
Lanso 15mg (N=50)	86%	80%	77%	75%	75%	68%
Lanso 30mg (N=49)	94%	83%	78%	75%	62%	58%

15mg vs Pla p<.001 30mg vs Pla p<.001 30mg vs 15mg p=.570

* All placebo patients withdrew or recurred during this interval.

Table 7a
PRIMARY PER-PROTOCOL ANALYSIS
Outcomes in Principal RE Maintenance Study M88-271

Treatment	(Contents of Table)	0-1 months	1-2 month	2-3 month	3-6 month	6-9 month	9-12 month
Placebo	Recurrence/Total (%)	30/55 (55%)	7/25 (28%)	1/16 (6%)	2/15 (13%)	0/12 (0%)	1/11 (9%)
	Withdrawal/Total (%)	0/55 (0%)	0/25 (0%)	2/16 (13%)	0/15 (0%)	1/12 (8%)	1/11 (9%)
	Product-Limit Healed	45%	33%	31%	27%	27%	24%
Lanso 15mg QD	Recurrence/Total (%)	4/59 (7%)	2/54 (4%)	4/50 (8%)	1/45 (2%)	0/42 (0%)	1/40 (3%)
	Withdrawal/Total (%)	0/59 (0%)	1/54 (2%)	2/50 (4%)	1/45 (2%)	2/42 (5%)	2/40 (5%)
	Product-Limit Healed	93%	90%	83%	81%	81%	79%
Lanso 30mg QD	Recurrence/Total (%)	1/56 (2%)	3/53 (6%)	0/49 (0%)	0/43 (0%)	1/41 (2%)	0/38 (0%)
	Withdrawal/Total (%)	0/56 (0%)	2/53 (4%)	1/49 (2%)	6/43 (14%)	2/41 (5%)	2/38 (5%)
	Product-Limit Healed	98%	93%	93%	93%	90%	90%

Table 7b
PRIMARY PER-PROTOCOL ANALYSIS
Outcomes in Principal RE Maintenance Study M89-350

Treatment	(Contents of Table)	0-1 months	1-2 month	2-3 month	3-6 month	6-9 month	9-12 month
Placebo	Recurrence/Total (%)	35/46 (76%)	3/9 (33%)	0/6 (0%)	1/5 (20%)	0/3 (0%)	0/2 (0%)
	Withdrawal/Total (%)	1/46 (2%)	2/9 (22%)	0/6 (0%)	1/5 (20%)	1/3 (33%)	1/2 (50%)
	Product-Limit Healed	24%	16%	16%	13%	13%	13%
Lanso 15mg QD	Recurrence/Total (%)	7/50 (14%)	3/43 (7%)	3/39 (8%)	1/36 (3%)	0/33 (0%)	2/30 (7%)
	Withdrawal/Total (%)	0/50 (0%)	3/43 (7%)	1/39 (3%)	0/36 (0%)	2/33 (7%)	3/30 (10%)
	Product-Limit Healed	86%	80%	74%	72%	72%	67%
Lanso 30mg QD	Recurrence/Total (%)	3/48 (6%)	5/45 (11%)	4/39 (10%)	1/29 (3%)	5/26 (19%)	1/20 (5%)
	Withdrawal/Total (%)	1/48 (2%)	0/45 (0%)	1/39 (3%)	6/29 (21%)	2/26 (8%)	1/20 (5%)
	Product-Limit Healed	94%	83%	75%	72%	58%	55%

Table 8
**Replicated Statistically Significant and Corroborated Changes
 in blood of Lansoprazole patients from Baseline
 in Principal RE Maintenance Studies M88-271 and M89-350**

TRIAL M88-271		— Mean Change from Lansoprazole Therapy Baseline (Standard Deviation) by Visit —									
		1 month	2 months	3 months	6 months	9 months	12 months	15 months			
Hemoglobin (g/dL)		-0.08 (0.70)	-0.05 (-0.73)	-0.08 (0.73)	-0.18 (0.76)	-0.17 (0.83)	-0.22 (0.84)	-0.28 (0.76)			
Hematocrit (%)		0.1 (2.87)	-0.3 (3.13)	-1.3 (3.63)	-2.1 (3.23)	-2.4 (3.43)	-1.8 (3.43)	-1.9 (3.19)			
Basophils (%)		0.05 (0.40)	-0.05 (0.50)	-0.04 (0.38)	-0.11 (0.41)	-0.19 (0.47)	-0.25 (0.44)	-0.14 (0.56)			
Potassium (mEq/L)		0.07 (0.39)	0.02 (0.40)	0.11 (0.40)	0.07 (0.43)	0.11 (0.39)	0.05 (0.41)	0.04 (0.33)			
Alkaline Phosphatase (U/L)		0.7 (9.6)	1.9 (8.9)	1.7 (10.1)	1.5 (11.3)	3.1 (10.8)	3.3 (11.9)	4.0 (13.6)			
GGT (U/L)		-0.1 (9.7)	1.6 (9.3)	3.1 (14.9)	2.6 (9.7)	3.3 (10.1)	3.9 (13.4)	3.6 (16.8)			
Albumin (g/dL)		-0.05 (0.33)	-0.05 (0.27)	-0.03 (0.25)	-0.01 (0.26)	-0.05 (0.27)	-0.01 (0.24)	-0.11 (0.21)			
Cholesterol (mg/dL)		-2.1 (29.9)	-0.2 (24.1)	-0.2 (30.1)	2.5 (29.3)	-2.2 (25.8)	-7.5 (30.4)	-0.5 (49.2)			
TRIAL M89-350		— Mean Change from Lansoprazole Therapy Baseline (Standard Deviation) by Visit —									
		1 month	2 months	3 months	6 months	9 months	12 months	15 months			
Hemoglobin (g/dL)		-0.11 (0.89)	-0.11 (0.97)	-0.20 (0.98)	-0.34 (1.07)	-0.35 (1.34)	-0.17 (1.15)	-0.12 (1.32)			
Hematocrit (%)		-0.5 (3.27)	-0.8 (3.73)	-1.1 (3.71)	-1.5 (4.14)	-1.3 (4.29)	-1.1 (3.93)	-1.2 (4.76)			
Basophils (%)		0.04 (0.47)	0.02 (0.54)	-0.06 (0.47)	-0.14 (0.38)	-0.28 (0.46)	-0.27 (0.42)	-0.33 (0.44)			
Potassium (mEq/L)		-0.05 (0.33)	-0.06 (0.35)	-0.08 (0.36)	-0.12 (0.33)	-0.15 (0.35)	-0.17 (0.34)	-0.14 (0.39)			
Alkaline Phosphatase (U/L)		1.1 (9.6)	2.6 (11.5)	2.2 (14.2)	3.0 (13.3)	4.5 (12.3)	4.2 (10.6)	5.4 (14.2)			
GGT (U/L)		0.9 (25.7)	3.4 (26.5)	-5.4 (29.1)	1.9 (40.4)	3.3 (34.2)	3.3 (39.2)	4.1 (39.4)			
Albumin (g/dL)		-0.02 (0.28)	-0.02 (0.24)	-0.02 (0.29)	-0.04 (0.28)	-0.06 (0.29)	-0.06 (0.28)	-0.09 (0.27)			
Cholesterol (mg/dL)		-1.0 (23.7)	-5.3 (27.6)	-6.5 (29.8)	-13. (28.6)	-7.8 (30.4)	-3.0 (32.9)	-6.9 (33.9)			

bold indicates p<.05 versus baseline.



STATISTICAL REVIEW

Date: **SEP 23 1994**

NDA #: 20-406/Drug Class 1s

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: acute Duodenal Ulcer

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.486, 2.636-2.639,
especially 2.409, 2.415, 2.474, dated
November 12, 1993

I. INTRODUCTION

This review is for the DU (Duodenal Ulcer) indication. Separate reviews address the indications: Reflux Esophagitis (completed); Gastric Ulcer; and maintenance of healing in Reflux Esophagitis.

The pivotal studies are M87-090 and M88-268, both of four weeks. Their purpose is to show the safety and efficacy of lansoprazole in treating duodenal ulcers of normal responsiveness.

II.a DESCRIPTION OF TRIAL M87-090 (U.S.)

Study M87-090 was a parallel-group, dose-response study that compared four weeks of lansoprazole 15mg, 30mg and 60mg, to placebo. Patients were to be endoscoped at screening (within 7 days prior to receiving study medication) and after two and four weeks of treatment, to show efficacy. During the posttreatment period, endoscopies were performed at one, three and six months or upon presentation with symptoms. Recurrence of ulcer disease was defined as a return of an ulcer at any location in the duodenum or stomach after documented healing.

Patients entered had an endoscopically documented, acute DU with a crater size of at least 3mm diameter.

One dose of nonsteroidal anti-inflammatory drug (NSAID) per week or one aspirin per day was allowed prior to screening and could be continued during the study. Gelusil tablets, provided by TAP, were to be taken for relief of discomfort as needed.

II.b REVIEWER'S AND SPONSOR'S ANALYSES AND RESULTS: TRIAL M87-090

The enrollment was 296 patients by 28 investigators in the U.S.

Of these patients, 284/296(96%) were included in at least one of the primary efficacy analyses. All 296 were included in the intent-to-treat analysis. The number of premature withdrawals was 17/75(23%) placebo, and 10/221(5%) lansoprazole. Out of these 27/296(9%) withdrawals, 5 were attributed to treatment failure, and all 5 were on placebo.

Combined across investigators, no statistically significant differences were detected at baseline for weight, race, gender, tobacco use, alcohol use, caffeine use, ulcer size, or night abdominal pain. However, there was a difference in the mean age of patients among treatment groups, as shown in Table A, following. The p-value is not adjusted for multiple comparisons.

Table A
Efficacy Subset
Duodenal Ulcer Study M87-090
Baseline Patient Ages in Years
Cochran-Mantel-Haenszel Analysis adjusted for Investigator

	Placebo	Lansoprazole			
		15mg	30mg	60mg	
Number of Patients	72	68	74	70	
Mean±SD age in years	45±13	48±15	41±13	44±14	
Minimum-Maximum age	20-72	19-74	21-72	21-75	p=.023

There was also a difference in day abdominal pain, shown in Table B, following. The p-values are not adjusted for multiple comparisons.

Table B
Intent-to-Treat
Duodenal Ulcer Study M87-090
Day abdominal pain at Baseline
Cochran-Mantel-Haenszel Analysis adjusted for Investigator

		Placebo	Lansoprazole		
			15mg	30mg	60mg
None	(0)	13	16	11	9
Mild	(1)	32	23	18	18
Moderate	(2)	20	27	34	33
Severe	(3)	10	7	12	13
Mean Score		1.36	1.34	1.63	1.68

Intent-To-Treat p=.045; Efficacy Subset p=.080

Intent-to-treat results are shown in Table C, following. The efficacy subset results are similar to the intent-to-treat

results. A distinction between doses (15mg, 30mg, and 60mg) of lansoprazole is not shown statistically. A distinction between each dose of lansoprazole and placebo is shown at four weeks.

The Cochran-Mantel-Haenszel test is usually applied and has a valid statistical interpretation under homogeneity of treatment effects across centers. At two weeks, the p-values for lansoprazole versus placebo are small, but there is significant lack of homogeneity between investigators at two weeks. However, a logit analysis by this reviewer does not uncover any relationship between investigators and treatment effect. As shown in Figures 1 and 3, in the appendix, the centers have dispersed results. There is a strong relationship between center and healing regardless of treatment at 2 weeks. The summary of healing rates by investigator are given in Table 2, in the appendix.

Table C
Intent-to-Treat Healing Rates
Duodenal Ulcer Study M87-090
Cochran-Mantel-Haenszel analysis adjusted for investigator

Week	Placebo	Lansoprazole		
		15mg	30mg	60mg
2	8 (11%)	29 (40%)	27 (36%)	27 (37%)
4	31 (41%)	60 (82%)	67 (89%)	64 (88%)
Total at risk	75	73	73	73

Week	Drug versus placebo			Drug vs. 15mg		vs. 30mg
	15mg	30mg	60mg	30mg	60mg	60mg
2	p<.001	p<.001	p<.001	p=.618	p=.994	p=.746
4	p<.001	p<.001	p<.001	p=.268	p=.338	p=.835

Breslow-Day Test of Homogeneity

Week	Drug versus placebo			Drug vs. 15mg		vs. 30mg
	15mg	30mg	60mg	30mg	60mg	60mg
2	p=.014	p=.071	p=.004	p=.132	p=.400	p=.062
4	p=.368	p=.764	p=.181	p=.293	p=.147	p=.261

Drug versus placebo consistently has p<.001 for efficacy.

III.a DESCRIPTION OF TRIAL M88-268

Study M88-268 was a blinded, parallel-group dose-response study. This study compared two doses of lansoprazole, 15mg and 30mg QD, versus placebo and ranitidine 300mg HS.

The eligibility criteria included endoscopically documented acute

Duodenal Ulcers with crater sizes at least 3mm in diameter.

III.b SPONSOR'S ANALYSES AND RESULTS IN TRIAL M88-268

The enrollment was 301 patients under 30 investigators in the U.S. Out of 301, 280 were included in at least one primary efficacy analysis, and 300 were included in the intent-to-treat analysis. The patient excluded from the intent-to-treat analysis had esophagitis Grade 2 at screening and was discontinued from the study after less than seven days of therapy.

Table D
Evaluable subset Analysis of trial M88-268
Slight Baseline difference in Caffeine Usage, p=.03

	Placebo	Ranitidine 300mgHS	Lansoprazole 15mg	30mg	Total
Did not Use	8 (20%)	20 (24%)	6 (8%)	16 (21%)	50 (18%)
Used Caffeine	33	62	74	61	230
Total Patients	41	82	80	77	280

Table E
Intent-to-Treat Analysis of trial M88-268
Cochran-Mantel-Haenszel analysis adjusted for Investigator

Week	Placebo	Ranitidine 300mgHS	Lansoprazole 15mg	30mg
2	14 (32%)	26 (30%)	30 (35%)	35 (42%)
4	19 (43%)	57 (66%)	76 (88%)	64 (76%)
Total at Risk	44	86	86	84
Drug vs. Placebo		Ran. vs	Drug versus Ranitidine	
Week 15mg	30mg	Placebo	15mg	30mg
2	p=.843*	p=.282*	p=.571	p=.501
4	p<.001	p<.001	p=.024	p=.093
			p=.001	p=.108

* - p=.073, .075 for homogeneity across investigators; no p<.05 for homogeneity across investigators.

Combined across investigators, no statistically significant differences were detected at baseline for age, race, gender, tobacco use, alcohol use, body weight, or ulcer size. There was a slight difference in caffeine use, found only in the evaluable subset, as shown in Table D. If adjusted for multiple comparisons, the p-value is not significant. This reviewer notes the slight difference appears to be mainly in the lansoprazole 15mg group, toward more caffeine users. A parallel analysis

adjusting for caffeine use was provided by the sponsor.

Tables E and F show the sponsor's efficacy results for intent-to-treat and efficacy subsets respectively, adjusted for investigator effects. There are some hints of lack of homogeneity. At 4 weeks, the apparent lack of homogeneity is strongest when the p-value for efficacy is strongest, so the suspicion that the assumption of homogeneity is violated has little impact. The healing rates on drug by center are shown in Figures 2 and 4 in the appendix, showing high dispersion. The healing rates by center are shown in Table 3 in the appendix.

Table F
Efficacy Subset Analysis of trial M88-268
Cochran-Mantel-Haenszel analysis adjusted for investigator

Week	Placebo	Ranitidine 300mgHS	Lansoprazole 15mg	Lansoprazole 30mg	
2	14/41 (34%)	25/82 (31%)	28/80 (35%)	34/77 (44%)	
4	19/40 (48%)	55/78 (71%)	72/78 (92%)	61/76 (80%)	
Week	Drug vs. Placebo		Ran. vs Placebo		Drug
	15mg	30mg	15mg	30mg	15-30mg
2	p=.953*	p=.392	p=.512	p=.534	p=.404
4	p<.001**	p<.001	p=.020*	p=.002	p=.076

* - p=.058, .090 for homogeneity across investigators

** - p=.020 for homogeneity across investigators.

Table G
Intent-To-Treat Analyses of trial M88-268
CMH analysis adjusted for investigator and Caffeine Use

Week, Caffeine	Placebo	Ranitidine 300mgHS	Lansoprazole 15mg	Lansoprazole 30mg	
2, No	3/ 8 (38%)	6/21 (29%)	5/ 9 (56%)	8/17 (47%)	
2, Yes	11/36 (31%)	20/65 (31%)	25/77 (33%)	27/67 (40%)	
4, No	5/ 8 (63%)	15/21 (71%)	8/ 9 (89%)	13/17 (76%)	
4, Yes	14/36 (39%)	42/65 (65%)	68/77 (88%)	51/67 (76%)	
Week	Drug vs. Placebo		Ran. vs Placebo		Drug
	15mg	30mg	15mg	30mg	15-30mg
4	p<.001	p<.001	p=.015	p<.001	p=.150

Next, the sponsor provided parallel analyses which are adjusted for caffeine use, in Tables G and H. The results of the alternate analysis at 4 weeks are comparable to not adjusting for caffeine use, with only one non-significant result, for 30mg

lansoprazole versus ranitidine. For the assumption of homogeneity, no p-values go below 0.15 for lack of homogeneity. This analysis addresses this reviewer's concerns regarding the effects of caffeine use and differences between investigators; it appears both are acceptable at four weeks.

Table H						
Efficacy Subset Analyses of trial M88-268						
CMH analysis adjusted for investigator and Caffeine Use						
Week,		Placebo	Ranitidine	Lansoprazole		
Caffeine			300mgHS	15mg	30mg	
2,	No	3/ 8 (38%)	6/20 (30%)	3/ 6 (50%)	7/16 (44%)	
2,	Yes	11/33 (33%)	19/62 (31%)	25/74 (34%)	27/61 (44%)	
4,	No	5/ 8 (63%)	14/19 (74%)	6/ 6 (all)	13/16 (81%)	
4,	Yes	14/31 (44%)	41/59 (70%)	66/72 (92%)	48/60 (80%)	
Drug vs. Placebo		Ran. vs		Drug versus Ran.		Drug
Week	15mg	30mg	Placebo	15mg	30mg	15-30mg
4	p<.001	p<.001	p=.017	p<.001	p=.160	p=.027

IV. REVIEWER'S COMMENTS AND CONCLUSIONS (that may be conveyed to the sponsor)

The sponsor has submitted two pivotal trials for Duodenal Ulcer considered by this reviewer. Trial M87-090 is controlled by placebo, with three doses of drug, and trial M88-268 is controlled by placebo and ranitidine, with two doses of drug. Both appear well controlled.

Both studies M87-090 and M88-268 show evidence of effectiveness of lansoprazole (versus to placebo) after 4 weeks of treatment. This effectiveness was observed consistently for all three doses (15mg, 30mg, 60mg) in study M87-090 and for both (15mg, 30mg) in study M88-268, each at 4 weeks. In addition, study M88-268 shows lansoprazole is at least as good as ranitidine 300mg HS.

The healing rate data did not provide substantial evidence for 2 weeks treatment for two reasons:

1. In study M87-090 an overall effect is suggested at 2 weeks, but the result is not homogeneous across centers when tested by the Breslow-Day test of homogeneity (see also Figure 1, appendix.)
2. The overall result of M88-268 was not significant at 2 weeks. Furthermore, there is a trend toward lack of consistency across investigators (see also Figure 2, appendix.)

A possible topic for the sponsor is the apparent lack of homogeneity between centers at 2 weeks, which is absent at 4 weeks. The presence and absence of homogeneity is corroborated between trials by Breslow-Day tests at the 0.15 level customarily used to detect lack of homogeneity between centers. If a covariate were found to explain the lack of homogeneity, and confirmed by phase IV clinical trials, there might be a patient population for which the drug could be labeled for 2 weeks of treatment. Another possible explanation is between center variation in the evaluation of patients at 2 weeks, which the sponsor may wish to reduce in future clinical trials.

Two minor issues in trial M88-268 include imbalance in caffeine use, and dose response in favor of 15mg over 30mg. The analysis adjusting for caffeine use corroborates the unadjusted results. The dose response for 15mg over 30mg is not supported by M87-090.

V. CONCLUSION

Two trials support the effectiveness of lansoprazole, against Duodenal Ulcer at 15mg or 30mg for 4 weeks.

A difference in efficacy between 30mg and 15mg is not supported, so safety can be considered without expecting a loss of efficacy.

The efficacy of 2 weeks treatment is not supported.

Ferrin Harrison 9/23/94
Ferrin Harrison, Ph.D.
Mathematical Statistician

This review consists of 7 pages of text, 3 pages of tables, and 1 page of figures.

Concur: Dr. Huque *Huque* 9/26/94

Dr. Dubey *Dubey* 9-26-94

cc: orig. NDA No. 20-406

HFD-180
HFD-180/ Dr. Fredd
HFD-180/ Dr. Gallo-Torres
HFD-180/ Ms. Maria Walsh
HFD-713/ Dr. Dubey [File DRU 1.3.2]
HFD-713/ Dr. Huque
HFD-713/ Dr. Ferrin Harrison
Chron.

HFD-713/HARRISONF/9-23-1994/wp51/landul.doc

TABLE 1
Pivotal Duodenal Ulcer Studies
Duration of Drug Treatment: 4 Weeks

Study Number	Study Design	Treatment Doses Studied (Randomization)	Evaluable Endoscoped Patients Effectiveness Outcome
M87-090 (U.S.)	Double-Blind, randomized, fixed dose (3 doses), placebo-controlled, multicenter, parallel-group, 6 month posttreatment follow up	Placebo Lansoprazole 15 mg QD 30 mg QD 60 mg QD (1:1:1:1)	Healing rates for evaluable: 2week 4week 11% 46% placebo, 42% 89% lansoprazole 15mg, 36% 92% lansoprazole 30mg, 39% 90% lansoprazole 60mg
M88-268 (U.S.)	Double-Blind, randomized, fixed dose (2 doses), active&placebo control, parallel-group, multicenter	Placebo Ranitidine 300 mg HS Lansoprazole 15 mg QD 30 mg QD	Healing rates for evaluable: 2week 4week 34% 48% placebo, 30% 70% ranitidine 300mg 35% 92% lansoprazole 15mg 44% 80% lansoprazole 30mg

Table 2
Duodenal Ulcer Study M87-090
Summary of 2 Week Intent-to-Treat Healing Rates by Investigator

	2 week Healed/Total					4 week Healed/Total	
	lansoprazole					lansoprazole	
	placebo	15mg	30mg	60mg	15&30&60	placebo	15&30&60mg
Aaronson	0/7	0/8	0/7	1/8	1/23	5/ 7	19/23
Avner	0/5	3/5	3/5	3/5	9/15	0/ 5	8/15
Berry	0/3	2/3	2/2	2/2	6/ 7	0/ 3	3/ 7
Bruns	0/2	1/2	0/2	1/2	2/ 6	1/ 2	5/ 6
Dorsch	0/6	1/5	2/6	0/6	3/17	3/ 6	9/17
Erfling	1/3	1/3	2/3	0/3	3/ 9	1/ 3	4/ 9
Fisher	1/1	0/1	1/1	0/1	1/ 3	1/ 1	2/ 3
Fitch	0/5	0/5	0/5	0/5	0/15	4/ 5	13/15
Houston	1/1	0/1	0/0	0/1	0/ 2	1/ 1	1/ 2
Jones	1/3	2/2	1/3	1/2	4/ 7	1/ 3	7/ 7
Koval	1/1	0/1	1/1	1/1	2/ 3	0/ 1	2/ 3
Mertesdorf	0/1	1/1	1/1	1/1	3/ 3	0/ 1	2/ 3
Movva	0/4	0/3	1/4	1/4	2/11	1/ 4	7/11
Nano	0/1	1/1	0/1	0/1	1/ 3	0/ 1	2/ 3
Nelson J	0/0	1/1	1/1	0/0	2/ 2	0/ 0	2/ 2
Nelson K	1/5	3/4	3/5	4/4	10/13	3/ 5	6/13
Neuman	0/2	0/2	0/2	0/2	0/ 6	2/ 2	5/ 6
Oliver	0/3	3/4	3/3	2/4	8/11	0/ 3	6/11
Rafoth	0/1	0/0	0/1	0/1	0/ 2	0/ 1	0/ 2
Rowekamp	0/2	1/3	2/2	1/2	4/ 7	0/ 2	2/ 7
Sahba	0/5	2/5	0/4	4/5	6/14	0/ 5	7/14
Schwartz	0/3	2/2	2/3	3/3	7/ 8	1/ 3	5/ 8
Scowcroft	0/3	1/3	1/4	1/3	3/10	0/ 3	7/10
Shivakumar	2/2	0/2	0/2	0/2	0/ 6	2/ 2	2/ 6
Simmons	0/3	2/3	0/3	1/3	3/ 9	0/ 3	3/ 9
Thune	0/0	1/1	0/1	0/1	1/ 3	0/ 0	0/ 3
Whitaker	0/1	1/1	0/1	0/0	1/ 2	0/ 1	1/ 2
Wintroub	0/2	0/1	1/2	0/1	1/ 4	0/ 2	1/ 4

Table 3
Duodenal Ulcer Study M88-268
Summary of 2 Week Intent-to-Treat Healing Rates by Investigator

	2 week Healed/Total				4 week Healed/Total	
	placebo	lansoprazole			placebo	lansoprazole
		15mg	30mg	15&30mg		15&30mg
Aaronson	0/3	0/6	0/6	0/18	2/3	16/18
Avner	1/2	2/5	4/4	11/14	2/2	14/14
Berry	0/1	2/2	2/2	4/ 7	0/1	7/ 7
Brady	0/0	1/1	1/2	2/ 3	0/0	3/ 3
Bruns	1/2	1/2	1/3	3/ 7	2/2	6/ 7
Demicco	1/1	1/2	2/2	4/ 6	1/1	5/ 6
Dorsch	1/1	0/2	1/2	2/ 6	1/1	5/ 6
Erfling	0/2	2/2	2/2	4/ 6	1/2	5/ 6
Fumo	0/1	1/2	0/0	2/ 4	0/1	3/ 4
Godiwala	0/0	0/1	0/0	0/ 1	0/0	1/ 1
Goff	0/1	1/2	2/2	5/ 6	0/1	6/ 6
Herb	2/2	3/5	2/4	7/14	2/2	12/14
Jones	1/1	2/2	2/3	8/ 9	1/1	9/ 9
Keller	0/1	0/1	0/0	0/ 1	1/1	1/ 1
Kogut	0/3	5/6	4/6	12/17	0/3	16/17
Koval	0/1	0/2	2/3	3/ 7	0/1	4/ 7
Lanza	1/3	3/6	3/7	9/19	1/3	17/19
Levine	0/1	1/3	3/3	7/10	0/1	10/10
Movva	0/2	2/7	1/6	3/17	1/2	11/17
Nelson K	2/2	2/4	3/4	7/12	1/2	8/12
Nichols	1/1	0/0	0/0	0/ 1	1/1	0/ 1
Oliver	1/1	1/2	1/2	2/ 6	1/1	5/ 6
Rowekamp	0/0	1/1	0/0	2/ 2	0/0	2/ 2
Sahba	1/2	3/5	4/5	11/16	1/2	13/16
Schwartz	1/1	1/2	2/2	4/ 6	0/1	6/ 6
Scowcroft	0/4	4/6	2/6	7/19	1/4	16/19
Shivakumar	0/2	0/2	1/3	2/ 8	1/2	6/ 8
Sigmund	0/1	0/0	0/0	0/ 0	0/1	0/ 0
Silvers	1/2	3/6	2/4	6/14	2/2	14/14
Sperling	0/0	0/0	0/1	0/ 1	0/0	0/ 1

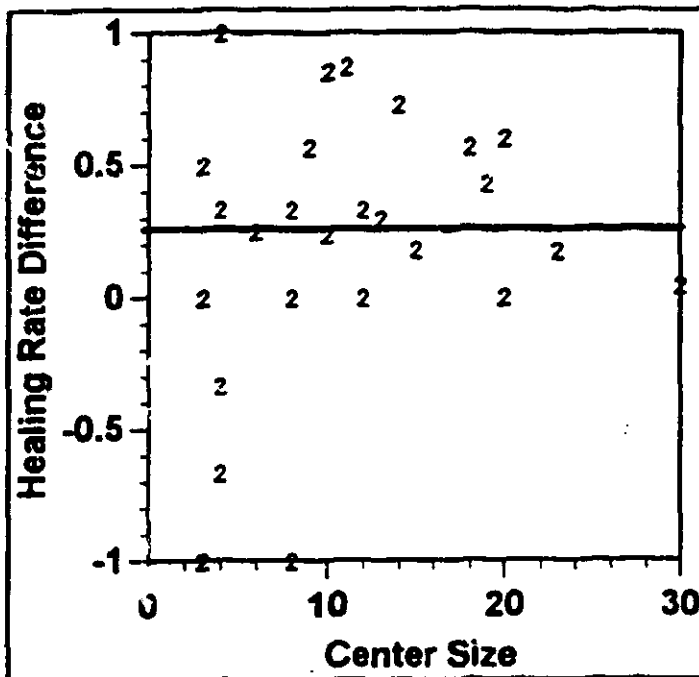


Figure 1 Trial M87-090, 2 weeks
Intent-to-Treat Healing Rate
Differences by Center Size
Not Homogeneous
pooled mean is 0.269

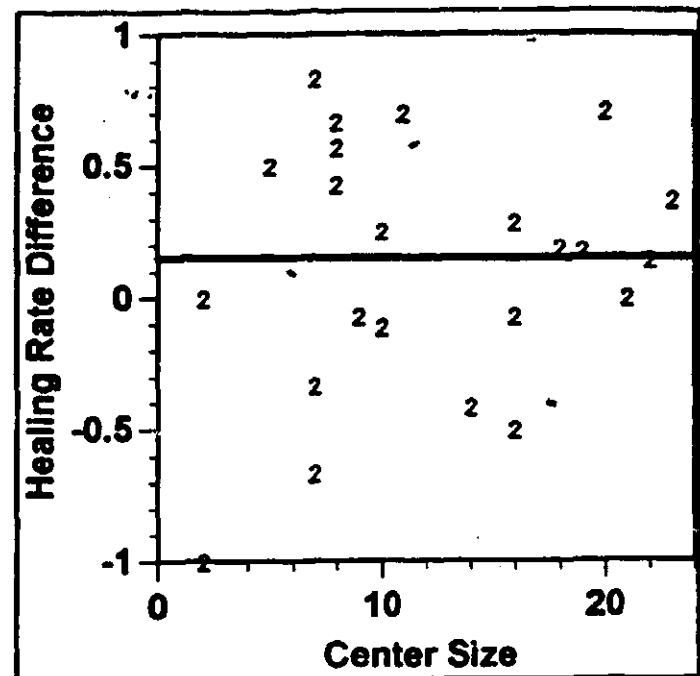


Figure 2 Trial M88-268, 2 weeks
Intent-to-Treat Healing Rate
Differences by Center Size
possibly not homogeneous
pooled mean is 0.153

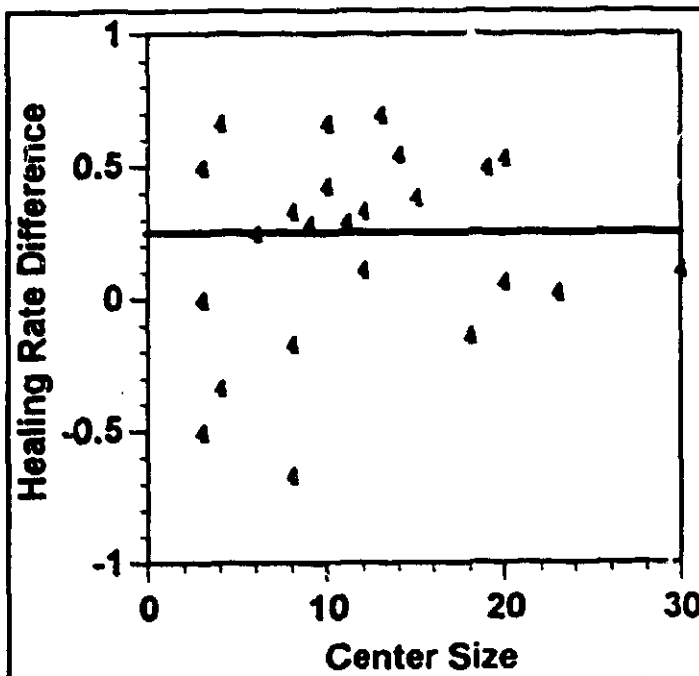


Figure 3 Trial M87-090, 4 weeks
Intent-to-Treat Healing Rate
Differences by Center Size
pooled mean is 0.246

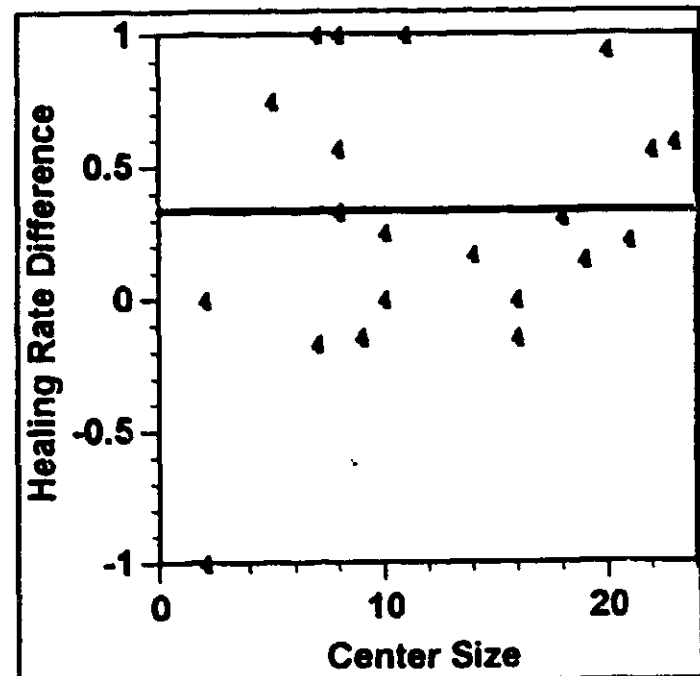


Figure 4 Trial M88-268, 4 weeks
Intent-to-Treat Healing Rate
Differences by Center Size
pooled Mean is 0.337

STATISTICAL REVIEW

Date: JUN 10 1994

NDA #: 20-406

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: Reflux Esophagitis

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.486, 2.636-2.639,
especially 2.409, 2.415, 2.474, dated
November 12, 1993

I. INTRODUCTION

This review is for the RE (Reflux Esophagitis) indication. Separate reviews address the indications: resistant or poorly responsive Reflux Esophagitis; maintenance of healing in Reflux Esophagitis; Duodenal Ulcer; and Gastric Ulcer.

The pivotal studies are M87-092 and M88-269. Their purpose is to show the safety and efficacy of lansoprazole in treating reflux esophagitis of normal responsiveness.

II.a DESCRIPTION OF TRIAL M87-092

Study M87-092 was a blinded, randomized, parallel group, dose-response study that compared the efficacy of three doses of lansoprazole (15 mg, 30 mg, and 60 mg) to that of placebo. Efficacy evaluations were scheduled at weeks 4, 6 and 8. Endoscopies were mandatory at weeks 4 and 8, and at 6 if not healed at week 4.

Patients with completely healed lesions at the end of eight weeks of therapy were evaluated during a six-month posttreatment (untreated) period with the patient off all anti-ulcer therapy except Gelusil. If possible, all endoscopies for a particular patient were to be performed by the same endoscopist.

The eligibility criteria were acute esophagitis, endoscopically documented: mucosa with frank ulceration, erosions, neovascularization, or white plaques, Grade 2 or higher; no Barrett's epithelium. Ulcers were graded by Kaul's modification of the Savary-Miller scale, shown in Table A as follows (next page.)

Table A
Reflux Esophagitis Study M87-092
Kaul's modified Savary-Miller Scale

Grade	Description
0	No abnormalities
1	Mucosal edema and hyperemia
2	Friability of mucosa with bleeding under instrumentation
3	Isolated ulcerations
4	Confluent ulcerations

II.b SPONSOR'S ANALYSES AND RESULTS IN TRIAL M87-092

The enrollment was 292 patients under 34 investigators in the U.S. Out of 292, 269 were included in at least one efficacy analysis and 288 in the intent-to-treat analysis, excluding 4 who entered inappropriately and terminated after less than ten days therapy.

Combined across investigators, no statistically significant differences were detected at baseline for age, race, gender, tobacco use, alcohol use, caffeine use, esophagitis grade, or female body weight. There were statistically significant differences in male body weight, as shown in Table B, following.

Table B
Reflux Esophagitis Study M87-092
Baseline Body Weight of Males

	Placebo	<u>Lansoprazole</u>			Pooled
		15mg	30mg	60mg	
Number of Patients	41	46	47	47	181
Mean±SD in lbs	182±35	189±27	191±34	204±36	192±34
Minimum-Maximum	129-297	120-263	132-280	131-295	120-297

unadjusted p=.018 with 9 endpoints

The sponsor regarded the difference as clinically insignificant. With respect to the randomization, this reviewer notes that adjustment for 9 endpoints, even with correlation taken into account, will obviously take the adjusted p-value above 0.05, so the baseline imbalance does not cast doubt on the randomization. Clinically, since the direction of the imbalance is increasing in the dose of drug, we would not expect the imbalance to bias the trial results toward exaggerating the efficacy of lansoprazole. The imbalance in body weight does not appear to merit further attention.

There were not many patients of Grade 4, as shown in Table C, following.

Table C Reflux Esophagitis Study M87-094 Baseline Intent-to-Treat Grades					
Grade	Placebo	Lansoprazole			Total
		15mg	30mg	60mg	
2	27	23	24	32	106
3	36	41	39	39	155
4	5	9	9	8	31
Total	68	73	72	79	292

The efficacy subset analysis gives similar results to the intent-to-treat analysis shown in the following Table D. The efficacy subset analysis finds a significant difference between 15mg and 30mg lansoprazole at four weeks and the 0.05 significance level. For the subset with grade 3 or 4 esophagitis at baseline, the sponsor obtains the same results for ITT and efficacy subsets (statistical significance between drug and placebo, and at 4 weeks, statistical significance for the difference between 30mg:15mg and 60mg:15mg.)

Table D Reflux Esophagitis Study M87-094 Cochran-Mantel-Haenszel, stratified by investigator Intent-to-Treat Healing Rates						
Week	Placebo	Lansoprazole				
		15mg	30mg	60mg		
4	20 (30%)	46 (64%)	52 (73%)	60 (76%)		
6	32 (49%)	57 (79%)	62 (87%)	68 (86%)		
8	31 (47%)	60 (83%)	62 (87%)	70 (89%)		
Total at risk	66	72	71	79		
Week	Drug versus placebo			Drug vs. 15mg		vs. 30mg
	15mg	30mg	60mg	30mg	60mg	60mg
4	p<.001	p<.001	p<.001	p=.105	p=.046	p=.735
6	p<.001	p<.001	p<.001	p=.108	p=.128	p=.880
8	p<.001	p<.001	p<.001	p=.415	p=.250	p=.705
Drug is consistently statistically significantly better than placebo at the 0.05 significance level.						
At 4 weeks, 60mg is better than 15mg at the 0.05 level.						

To address possible bias at baseline, the sponsor adjusted

healing rates for concomitant factors of gender, age, esophagitis grade at baseline, alcohol use, tobacco use, caffeine use, and found all lansoprazole doses significantly superior to placebo at the Week 8 analysis.

This reviewer is aware of the statistically significant difference between investigator Sahba and the remaining investigators, in favor of placebo versus 60mg. Given the direction and the strong p-values versus placebo, this reviewer does not wish to raise heterogeneity as an issue. If the results were weaker, this reviewer would ask for an alternative statistical analysis using the DL, Dersimonian-Laird statistical methodology instead of CMH, Cochran-Mantel-Haenszel. Briefly, the difference in methodologies is that DL uses a random effects model, while CMH uses a fixed effects model, so DL is more appropriate when heterogeneity is present. If additional interest is expressed, this reviewer will ask for Dersimonian-Laird analysis.

With respect to effect size and dose, perusal of the preceding Table D shows a clear effect against placebo, with inconclusive support of effects between doses. If rapid healing is desired, one might argue for more than 15mg, but the p-values for 60mg versus 30mg are nowhere close to significant. The evidence is so weak that the numeric direction of the effect for 60mg versus 30mg is reversed at week 6.

III.a DESCRIPTION OF TRIAL M88-269

Study M88-269 was a blinded, randomized, parallel group study. This study compared lansoprazole 30mg QD to ranitidine 300mg (150 mg BID) for an eight week trial. Efficacy evaluations were scheduled at weeks 2, 4, 6 and 8. Endoscopies were mandatory at weeks 2 and 8, and at weeks 4 and 6 if not healed at the prior visit. If possible, all endoscopies for a particular patient were to be performed by the same endoscopist.

The eligibility criteria included endoscopically documented acute erosive reflux esophagitis of at least Grade 2. Patients had discontinued chronic use of ulcerogenic drugs at least one month prior to screening. One dose of NSAID per week, one aspirin per day, or up to 10 mg prednisone or equivalent per day was allowed prior to screening and could be continued during the study. During the treatment period, no antiulcer medication was allowed except Gelusil tablets, which were provided by TAP for relief as needed, and the amount consumed was tracked and analyzed.

There is yet another modification of the esophagitis grading scale, as shown in Table E, following (next page.)

Table E
Reflux Esophagitis Study M88-269
TAP's modification of Kaul's modification of
the Savary-Miller grading scale

Grade	Description
0	Normal-appearing mucosa by endoscopy
1	Mucosal edema, hyperemia, and/or friability
2	at least 1 erosions/ulcerations in squamocolumnar junction
3	erosions/ulcerations not exceeding 50% of distal 5 cm of esophagus, proximal to squamocolumnar junction
4	multiple erosions/ulcerations exceeding 50% of distal 5 cm of esophagus or single large ulcer at least 5 mm in diameter proximal to squamocolumnar junction

III.b SPONSOR'S ANALYSES AND RESULTS IN TRIAL M88-269

The enrollment was 247 patients by 29 investigators. Of these patients, 242 (98%) were included in at least one primary efficacy analysis, and all 247 were included in the intent-to-treat analysis. Ten lansoprazole patients withdrew prematurely, and 8 ranitidine patients withdrew prematurely. Only one premature withdrawal was attributed to treatment failure, and it was in lansoprazole.

There were a fair number of patients (136 out of 247) of Grade 3 and 4, as shown in Table F, following.

Table F
Reflux Esophagitis Study M88-269
Baseline Intent-to-Treat Grades

Grade	Ranitidine 150 mg BID	Lansoprazole 30 mg QD	Total
1	0	1	1
2	56	54	110
3	61	56	117
4	11	8	19
Total	128	119	247

Combined across investigators, no statistically significant differences were detected at baseline for age, race, gender, tobacco use, alcohol use, caffeine use, esophagitis grade, or body weight. Three patients in each group had Barrett's epithelium (involving ≤ 3 cm from gastroesophageal junction) noted on the endoscopic examination.

Table G
Reflux Esophagitis Study M88-269
Cochran-Mantel-Haenszel, stratified by investigator
Intent-to-Treat Healing Rates

Week	Ranitidine 150 mg BID	Lansoprazole 30 mg QD	Treatment Difference	Investigator Homogeneity
2	49/128 (38%)	77/119 (65%)	p<.001	p=.592
4	64/128 (50%)	95/119 (80%)	p<.001	p=.908
6	80/128 (63%)	107/119 (90%)	p<.001	p=.943
8	86/128 (67%)	106/119 (89%)	p<.001	p=.990

For these dose regimens, lansoprazole has a consistently statistically significantly greater healing rate, $p \leq .001$, than ranitidine.

The intent-to-treat analysis is given in Table G, preceding on this page. The efficacy subset analysis gives the same statistical results as the intent-to-treat analysis, statistical significance for each comparison of lansoprazole to ranitidine (with or without adjustment for multiple tests of hypothesis.) The statistical significance holds for weeks 2 and 8 when healing is defined as reaching Grade 0.

IV. REVIEWER'S COMMENTS AND CONCLUSIONS (that may be conveyed to the sponsor)

The sponsor has submitted two pivotal trials for Reflux Esophagitis considered by this reviewer. Trial M87-092 is controlled by placebo, and trial M88-269 is controlled by ranitidine. Both appear well controlled.

The sponsor's efficacy result versus placebo is supported by a highly statistically significant result in trial M87-092 for 15mg, 30mg, and 60mg. The sponsor's efficacy result is supported by a highly statistically significant result in trial M88-269 for lansoprazole 30mg QD versus ranitidine 150mg BID.

With respect to the effect size or choice of dose, there appears to be little reason to prefer 60mg over 30mg. The statistical result for 15mg versus placebo is very strong in trial M87-092. The healing rate at 4 weeks is lower for 15mg than for 30mg or 60mg, with statistical significance for 60mg; at 8 weeks, no difference was detected.

V. CONCLUSION

The sponsor has submitted two apparently well controlled and pivotal trials for mild to moderate Reflux Esophagitis supporting the efficacy of a dose of 30mg. The evidence of trial M87-092 supports a dose of 15mg.

Ferrin Harrison 6/7/94

Ferrin Harrison, Ph.D.
Mathematical Statistician

This review consists of 7 pages of text and 1 page of table.

Concur:

Dr. Huque

Huque 6/7/94

Dr. Dubey

6/10/94

cc: orig. NDA No. 20-406

HFD-180

✓ HFD-180/ Dr. Fredd

HFD-180/ Dr. Gallo-Torres

HFD-180/ Ms. Maria Walsh

HFD-713/ Dr. Dubey [File DRU 1.3.2]

HFD-713/ Dr. Huque

HFD-713/ Dr. Ferrin Harrison

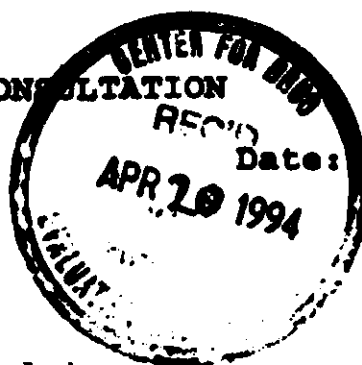
Chron.

HFD-713/HARRISONF/6-7-1994/wp51/lanrel.doc

TABLE 1
Pivotal Reflux Esophagitis Studies
Duration of Drug Treatment: 8 Weeks

Study Number	Study Design	Treatment Doses Studied (Randomization)	Evaluable Endoscoped Patients Effectiveness Outcome
M87-092 (U.S.)	Double-Blind, randomized fixed dose, placebo- controlled, multicenter, parallel-group, posttreatment follow-up of 6 months	Lansoprazole Placebo 15 mg QD 30 mg QD 60 mg QD (4 groups, 1:1:1:1)	Healing Rates at 4 weeks were 68% for lansoprazole 15 mg, 81% for 30 and 60mg doses and 33% for placebo; at 8 weeks rates were 91%, 95%, 94% and placebo 53%, respectively.
M88-269 (U.S.)	Double-Blind, randomized fixed dose, d active-controlled, multicenter, parallel-group	Lansoprazole 30 mg QD 60 mg QD Ranitidine 150 mg BID (2 groups, 1:1)	Healing rates at 4 weeks were 82% for lansoprazole 30 mg, versus 52% for ranitidine 150 mg BID; at 8 weeks rates were 92% and 70% respectively.

STATISTICAL CONSULTATION



APR 20 1994

NDA #: 20-406

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: maintenance of healing in Reflux Esophagitis; -
Duodenal Ulcer; Reflux Esophagitis; Gastric Ulcer;
Pathological hypersecretory conditions including
Zollinger-Ellison syndrome

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.483, especially
2.420, dated November 12, 1993

The questions submitted to the sponsor were incomplete, submitted without context, and are as follows for resubmission.

ADDITIONAL ANALYSES REQUESTED

These additional analyses are for the intent-to-treat and efficacy subsets. The endpoint is first erosion (if any.)

For some analyses, a covariate score for symptomatology is to be developed (as clinically and statistically meaningful as possible.) For example, there are daytime and nighttime heartburn incidences, which can be added up over the period prior to an endpoint or censoring to make a score. The two purposes of such a covariate are to attempt to address:

- 1) ascertainment bias issue: frequent symptom driven endoscopies lead to bias against the placebo group, and
- 2) correlation between the surrogate endpoint (erosions) and clinical benefit.

Please provide the following analyses:

- 2a Logistic regression analysis, using all endoscopies. To address possible ascertainment bias, test symptomatology or total incidence of erosions or both for each patient as a covariate.
- 2b Logistic regression analysis, using only scheduled endoscopies. To address erosions as surrogates for clinical benefit, test symptomatology for each patient as a covariate.

- 4a Cox regression, using all endoscopies. Develop a symptomatology as a covariate and test for treatment by covariate interaction, and significance of treatment when covariate is in the model. This analysis addresses possible ascertainment bias.
- 4b Cox regression, using only scheduled endoscopies. Develop a symptomatology as a covariate and test for treatment by covariate interaction, and significance of treatment when covariate is in the model. Please break ties by the actual time of endoscopy, rather than treating all endoscopies at a 'visit' as simultaneous. This analysis addresses erosions as surrogates for clinical benefit.

Please send your response on a floppy disk in addition to volume(s). Files of size up to one megabyte are acceptable, preferably with an index if numerous. Acceptable formats include ASCII, or DOS WordPerfect 5.1, preferably with the base font, line width and page size coded in each file and the intended type of printer identified. Please note that 132 column output can be accommodated in either format.

Please also send the SAS datasets used to prepare these analyses, with endpoints and covariates used clearly identified and sample SAS codes. The SAS datasets should be in either PC SAS format, or SAS 5 transport files with codes to convert them into PC SAS 6 datasets.

Ferrin Harrison 4/20/94

Ferrin Harrison, Ph.D.
Mathematical Statistician

This letter of request for information consists of 2 pages of text.

Concur: Dr. Huque *Huque 4/19/94*

cc: orig. NDA No. 20-406

HFD-180
HFD-180/ Dr. Fredd
HFD-180/ Dr. Gallo-Torres
HFD-180/ Ms. Maria Walsh
HFD-713/ Dr. Dubey [File DRU 1.3.2]
HFD-713/ Dr. Huque
HFD-713/ Dr. Ferrin Harrison
Chron.

HFD-713/HARRISONF/4-20-1994/wp51/lanmain2.doc

STATISTICAL CONSULTATION

Date: MAR 16 1994

NDA #: 20-406

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: maintenance of healing in Reflux Esophagitis;
Duodenal Ulcer; Reflux Esophagitis; Gastric Ulcer;
Pathological hypersecretory conditions including
Zollinger-Ellison syndrome

Documents Reviewed: Volumes 2.1-2.3, 2.393-2.483, especially
2.420, dated November 12, 1993

The extent of the sponsor's analyses is insufficient to complete statistical review of trials M88-271 and M89-350.

WHAT THE SPONSOR HAS DONE

The sponsor has provided the following statistical analyses:

- 1 Cochran-Mantel-Haenszel life-table analysis (tables with ordered responses), intent-to-treat and efficacy, including all endoscopies. In this analysis the endpoint is the time of the first recurrence of erosions, with no use of data gathered after the first erosion.
- 2 The same analysis excluding unscheduled endoscopies. To be considered scheduled, the endoscopy has to be within 1 week of the target time for months 1, 2, 3, and within 2 weeks of the target time for months 6, 9 and 12.
- 3 The same analyses defining healing as achieving Grade 0 only, rather than Grade 1 or 0.
- 4 Secondary (point prevalence) analysis of all observations of erosions during each 90 day period, analyzed by the two-sample Wilcoxon rank-sum test.

ADDITIONAL ANALYSES REQUESTED

These additional analyses are for two endpoints, and for the intent-to-treat and efficacy subsets. The first endpoint is first erosion (if any.) The second endpoint is treatment failure

as defined in the protocol (failed rescue.)

For some analyses, a covariate score for symptomatology is to be developed (as clinically and statistically meaningful as possible.) For example, there are daytime and nighttime heartburn incidences, which can be added up over the period prior to an endpoint or censoring to make a score. The two purposes of such a covariate are to attempt to address:

- 1) ascertainment bias issue: frequent symptom driven endoscopies lead to bias against the placebo group, and
- 2) correlation between the surrogate endpoint (erosions) and clinical benefit.

The crude rate is the number of events divided by the total number of patients at baseline (randomized or efficacy subset.)

Please provide the following analyses:

- 1a Crude rate analysis, using all endoscopies.
- 1b Crude rate analysis, using only scheduled endoscopies.
- 2a Logistic regression analysis, using all endoscopies. To address possible ascertainment bias, test symptomatology or total incidence of erosions or both for each patient as a covariate.
- 2b Logistic regression analysis, using only scheduled endoscopies. To address erosions as surrogates for clinical benefit, test symptomatology for each patient as a covariate.
- 3 For pre-trial treatment versus randomized treatment: tabulate frequency distribution, and incidence of each outcome, analyzing for possible imbalance. If this or other analyses are present in the submission, please give references.
- 4a Cox regression, using all endoscopies. Develop a symptomatology as a covariate and test for treatment by covariate interaction, and significance of treatment when covariate is in the model. This analysis addresses possible ascertainment bias.
- 4b Cox regression, using only scheduled endoscopies. Develop a symptomatology as a covariate and test for treatment by covariate interaction, and significance of treatment when covariate is in the model. Please break ties by the actual time of endoscopy, rather than treating all endoscopies at a

'visit' as simultaneous. This analysis addresses erosions as surrogates for clinical benefit.

Please send your response on a floppy disk in addition to volume(s). Files of size up to one megabyte are acceptable, preferably with an index if numerous. Acceptable formats include ASCII, or DOS WordPerfect 5.1, preferably with the base font, line width and page size coded in each file and the intended type of printer identified. Please note that 132 column output can be accommodated in either format.

Please also send the SAS datasets used to prepare these analyses, with endpoints and covariates used clearly identified and sample SAS codes. The SAS datasets should be in either PC SAS format, or SAS 5 transport files with codes to convert them into PC SAS 6 datasets.

Ferrin Harrison 3/16/94

Ferrin Harrison, Ph.D.
Mathematical Statistician

This letter of request for information consists of 3 pages of text.

Concur: Dr. Huque

Huque 3/16/94

cc: orig. NDA No. 20-406

HFD-180
HFD-180/ Dr. Fredd
HFD-180/ Dr. Gallo-Torres
HFD-180/ Ms. Maria Walsh
HFD-713/ Dr. Dubey [File DRU 1.3.2]
HFD-713/ Dr. Huque
HFD-713/ Dr. Ferrin Harrison
Chron.

HFD-713/HARRISONF/3-16-1994/wp51/lanmaint.doc

STATISTICAL CONSULTATION

Date: FEB 10 1994

NDA #: 20-406

Applicant: TAP Pharmaceuticals

Drug Name: Prevacid (Lansoprazole)

Pharmacologic Category of the Drug: Hydrogen pump inhibitor

Indications: Duodenal Ulcer; Reflux Esophagitis; maintenance of healing in Reflux Esophagitis; Gastric Ulcer; Pathological hypersecretory conditions including Zollinger-Ellison syndrome

Documents Reviewed: datasets on disk, computer dated 12/7/93 and 12/9/93

This statistical consult was requested by Dr. Hugo Gallo-Torres, to provide the investigative branch with a starting point in the key DU (duodenal ulcer) maintenance trials of lansoprazole currently submitted.

Dr. Gallo-Torres' question regarding DU trials was, "are there centers where all treated patients were 100% healed?"

There are two such centers in M87-090, one of which looks statistically suspicious, Jones #2940 with 7 patients. These are shown in Table 1 in the appendix.

There are 21 out of 28 such centers in M88-268, none of which look suspicious given the overall result. However, the number of patients per center is small, so any test for inhomogeneity will have low power. These are shown in Table 2 in the appendix.

An additional caveat is that sponsors often label the variables in their datasets in a manner which is unclear to the reviewer, or include errors in their datasets, so the data in this consult should be regarded as a starting point and not as confirmed or certain.

A small number of treated patients in M87-090 did not have the disease state at the start (EVFLAG=0), indicated in Table A, following.

Table A
Patients without Duodenal Ulcer at Baseline in trial M87-090

<u>Investigator</u>		<u>Patient Prescription</u>		<u>Lansoprazole</u>	<u>SUCCESS</u>
<u>Number</u>	<u>Name</u>	<u>Number</u>	<u>Day</u>	<u>Dose</u>	
INVNO	INVNAME	PTNO	ENRXDY	TREAT	
2927	BERRY		28	15 mg	1
2941	MERTESDORF		124	30 mg	0
2948	OLIVER		-3	60 mg	1
2948	OLIVER		-3	15 mg	1
2951	SAHBA		64	60 mg	0
2955	SHIVAKUMAR		-8	15 mg	1
3094	AVNER		-5	15 mg	1
3094	AVNER		-5	15 mg	1
3096	WINTROUB		57	60 mg	0

For trial M88-268, treated patients with no disease at baseline (treated as non-successes in preceding table, regardless of their classification by the sponsor) are in Table B, as follows.

Table B
Patients without Duodenal Ulcer at Baseline in trial M88-268

<u>Investigator</u>		<u>Patient Prescription</u>		<u>Lansoprazole</u>	<u>Sponsor</u>
<u>Number</u>	<u>Name</u>	<u>Number</u>	<u>Day</u>	<u>Dose</u>	
INVNO	INVNAME	PTNO	ENRXDY	TREAT	SUCCESS
2925	AARONSON		29	30 mg	1
2925	AARONSON		31	30 mg	1
2927	BERRY		30	15 mg	1
2928	BRUNS		29	15 mg	1
2928	BRUNS		27	30 mg	1
2928	BRUNS		29	30 mg	1
2951	SAHBA		-3	30 mg	1
2951	SAHBA		29	30 mg	1
2965	KOVAL		-3	15 mg	1
2965	KOVAL		-5	15 mg	1
4257	KOGUT		32	15 mg	1
4261	SILVERS		15	15 mg	1
4261	SILVERS		29	15 mg	1
4262	SPERLING		-4	30 mg	1

OVERALL CONCLUSION

There are two centers in trial M87-090 in which all patients receiving drug are successes. One of these centers looks statistically suspicious, Jones #2940 with 7 patients.

There are 21 out of 28 centers in trial M88-268 in which all patient receiving drug are successes. None of the centers look statistically suspicious given the overall result. However, the number of patients per center is small, so any statistical test for inhomogeneity has low power.

Ferrin Harrison Feb 10, 1994

Ferrin Harrison, Ph.D.
Mathematical Statistician

This consult consists of 3 pages of text, and 2 pages of tables.

Concur: Dr. Huque *Huque*
2/10/94

cc: orig. NDA No.

HFD-180
HFD-180/ Dr. Fredd
HFD-180/ Dr. Gallo-Torres
HFD-180/ Ms. Maria Walsh
HFD-713/ Dr. Dubey [File DRU 1.3.2]
HFD-713/ Dr. Huque
HFD-713/ Dr. Ferrin Harrison
Chron.

HFD-713/HARRISONF/2-10-1994/wp51/lansinv.doc

Table 1
Trial M87-090 (Duodenal Ulcer)

Investigator #	Failure	Success	Total
AARONSON 2925	4	19	23
BERRY 2927	4	3	7
BRUNS 2928	1	5	6
ERFLING 2934	5	4	9
FISHER 2935	1	2	3
FITCH 2936	2	13	15
HOUSTON 2939	1	1	2
JONES 2940	0	7	7
MERTESDO 2941	1	2	3
MOVVA 2942	4	7	11
NANO 2943	1	2	3
NEUMAN 2944	1	5	6
NELSON 2945	0	2	2
NELSON 2947	7	6	13
OLIVER 2948	3	8	11
RAFOTH 2949	2	0	2
ROWEKAMP 2950	5	2	7
SAHBA 2951	7	7	14
SCHWARTZ 2953	3	5	8
SCOWCROF 2954	3	7	10
SHIVAKUM 2955	3	3	6
SIMMONS 2956	6	3	9
THUNE 2957	3	0	3
WHITAKER 2959	1	1	2
KOVAL 2965	1	2	3
AVNER 3094	5	10	15
DORSCH 3095	8	9	17
WINTROUB 3096	3	1	4
Total	85	136	221
	38%	62%	

Table 2
Trial M88-268 (Duodenal Ulcer)
treating no disease as a non-success

Investigator #	Failure	Success	Total
AARONSON 2925	1	11	12
BERRY 2927	0	4	4
BRUNS 2928	1	4	5
ERFLING 2934	0	4	4
JONES 2940	0	5	5
MOVVA 2942	4	9	13
NELSON 2947	1	7	8
OLIVER 2948	0	4	4
ROWEKAMP 2950	0	1	1
SAHBA 2951	0	10	10
SCHWARTZ 2953	0	4	4
SCOWCROF 2954	1	11	12
SHIVAKUM 2955	0	5	5
KOVAL 2965	0	5	5
AVNER 3094	0	9	9
DORSCH 3095	0	4	4
BRADY 4249	0	3	3
DEMICCO 4251	0	4	4
FUMO 4252	0	2	2
GOFF 4253	0	4	4
HERB 4254	1	8	9
KELLER 4256	0	1	1
KOGUT 4257	0	12	12
SILVERS 4261	0	10	10
SPERLING 4262	0	1	1
LANZA 4265	1	12	13
LEVINE 4267	0	6	6
GODIWALA 4759	0	1	1
Total	10 6%	161 94%	171

HFD-180-180

Statistical Review and Evaluation

Part II

NDA #: 20-406

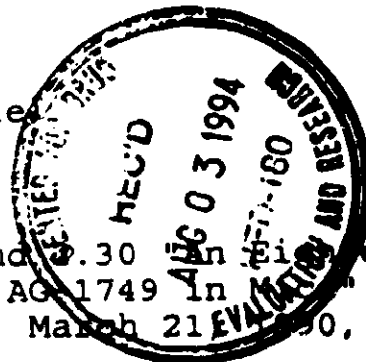
Date: JUL 25 1991

Applicant: TAP Pharmaceuticals

Name of Drug: Prevacid (Lansoprazole)

Documents Reviewed:

1. NDA submission volumes 2.22 and 2.30 "An Eighteen Month Oral Gavage Oncogenicity Study of AG-1749 in Mice", Project No. 87-3158, Report No. A-29-680, March 21, 1990, Submitted Nov. 12, 1993.
2. NDA submission volumes 2.43 and 2.53 "A Two-year Oral Gavage Oncogenicity Study of AG-1749 in Rats.", Project No. 86-3028, Report No. A-29-681, Jan. 25, 1990, Submitted Nov. 12, 1993.
3. Special NDA submission, Date of Document, June 17, 1994, Data Diskettes for two animal tumorigenicity studies.



I. Background

Six animal carcinogenicity studies (three in rats and three in mice) were included in this NDA submission. The reviewer has reviewed four of them (TA91-024, 1148/CA, TD91-025, and TD91-276), and a statistical review and evaluation report on those four studies was issued on June 17, 1994. This report contains reviews of studies 87-3158 and 86-3028. The purpose of these studies was to assess the potential tumorigenicity of the test material, Prevacid (Lansoprazole), when given by gavage to rats and mice for 24 and 18 months, respectively. Dr. Chopra, HFD-180, who is the reviewing pharmacologist of this NDA has requested the Division of Biometrics to perform the statistical review and evaluation of these two studies. The data submitted on computer floppy diskettes were used in the reviewer's independent analyses.

II. The Rat Study - 86-3028 (Report A-29-681)

II. a. Design

In this study, six hundred Charles River Sprague-Dawley CD rats (300 males, 300 females) were randomly distributed into five groups (60 per sex/group). Rats in groups II, III, IV, and V received AG-1749 via oral gavage at dose levels of 1.5, 5.0, 15.0 and 50.0 mg/kg/day, respectively, five days per week for two years. Animals in group I, the control, received the vehicle (5% gum arabic solution) at the same dose volume as administered to the treated animals. Physical observations, body weight and food consumption measurements were

performed on all animals pretest and at selected intervals during the treatment period. After at least 24 months of treatment, all survivors were sacrificed. Complete gross postmortem examinations were conducted on all animals. Histopathological evaluation of selected tissues were conducted on all animals in control and high dose groups. Sections of stomach and testes of animals in all groups were evaluated. Treatment of this study began on November 13, 1986 and ended on November 21, 1988.

II. b. Sponsor's Analyses

The Cox test ("Regression models and life tables (with discussion)", J. Royal Stat. Soc., Series B, 34: 187-220, 1972) was used to compare the survival distributions among groups for homogeneity. The Tarone test ("Tests for trends in life tables analysis, Biometrika, 62: 679-682, 1975) was used to test for linear trends. In addition, the Kaplan-Meier estimate of survival curve ("Nonparametric estimation from incomplete observations", Journal of the American Statistical Association, 53, 457-481, 1958) was calculated for each sex/treatment group and displayed graphically over time. The above analyses showed that no significant differences in survival distributions were detected for either sex, nor were the tests for linear trends statistically significant. There is no effect of treatment with AG-1749. Survivorship analysis did not indicate any effect of treatment. Median survival times by the Kaplan-Meier method of estimation were 650 days for males and 683 for females. The percents of animals surviving to terminal necropsy were 33%, 32%, 30%, 28%, and 27%, respectively, for 0, 1.5, 5, 15, and 50 mg/kg groups for males, and 32%, 43%, 47%, 47%, and 40% for females. The Kaplan-Meier survival curves are shown in Figures 1 and 2 for males and females, respectively.

In the first stage of sponsor's tumor data analysis, the chi-square test, Armitage's test for monotonic trend in the doses, and the Fisher Exact tests of treated groups vs control were performed on the numbers of animals with all neoplasms, all benign neoplasms, and all malignant neoplasms. In the second stage, the prevalence method given in the paper of Peto et al. ("Guidelines for Simple, Sensitive Significance Tests for Carcinogenic Effects in Long-Term Animal Experiments", In Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal, International Agency for Research on Cancer Monographs, Annex to Supplement 2, World Health Organization, 311-426, 1980) was used to analyze the data of the testes and thyroid neoplastic and hyperplastic lesions. Two time intervals - one before the terminal sacrifice and one at the terminal sacrifice were used in the analyses. The sponsor indicated that the incidences of bilateral or unilateral interstitial cell tumors were combined. The same thing was done for the incidence of bilateral or unilateral hyperplasia. The incidences of c-cell adenoma and carcinoma (c-cell tumors) were also combined for analysis. In addition to the trend test, pairwise comparisons were also made comparing the incidence in each dose group with the control group for

testicular tumors and hyperplasia.

The results of the above analyses revealed that the number of treated males and females from groups II through V with one or more primary, benign or malignant neoplasms, seen microscopically, was considered to be similar to that seen in their respective controls, except for those seen in the testes. Table 1 listed the total numbers of animals with all neoplasms, all benign neoplasms, and all malignant neoplasms for both sexes. Table 2 summarized the incidences of neoplastic and hyperplastic lesions found in the testes and thyroid in rats. The percents of male rats affected by testicular interstitial cell tumor were 1.7%, 3.3%, 3.4%, 10%, and 20%, respectively, for 0, 1.5, 5, 15, and 50 mg/kg groups. The test for a positively linear trend was highly significant ($p < 0.0001$). Pairwise comparisons of the 50 mg/kg and 15 mg/kg against controls showed significant increases in the tumor incidences at the 0.05 level of significance. The percents of male rats affected by testicular interstitial cell hyperplasia were 3.3%, 5.0%, 1.7%, 13.3%, and 33%, respectively, for 0, 1.5, 5, 15, and 50 mg/kg groups. The test for a positively linear trend was highly significant ($p < 0.0001$). Pairwise comparisons of the 50 mg/kg and 15 mg/kg against controls showed significant increases of testicular hyperplasia at the 0.05 level of significance. No significant linear trend was detected in thyroid c-cell tumor and hyperplasia for either sex.

Based on the above analyses, the sponsor concluded that "the results of this study have demonstrated that with the possible exception of interstitial cell tumors seen in the testes and a carcinoid tumor in the stomach of one female in group V, AG-1749 did not exhibit an oncogenic effect."

II.c. Reviewer's analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas, Breslow, and Gart ("Trend and Homogeneity Analyses of Proportions and Life Table Data", Computers and Biomedical Research, 10, 373-391, 1977) were used to test for heterogeneity in survival distribution. The p-values of the Cox test were 0.9701 and 0.3053 for males and females, respectively. Hence, there was no statistically significant difference (at 0.05 level) in survival distribution in both sexes. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values were 0.9815 and 0.3430 for males and females, respectively. In addition, the high dose group survival was compared with that of the control group for both sexes. The test results did not reveal any significant difference in survival between high dose and control groups for both sexes.

The intercurrent mortality rates for both male and female rats (see Table 3) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-80, 81-104 weeks. The actual dose levels 0, 1.5, 5, 15, and 50 mg/kg/day were the scores assigned to the control, low,

medium-1, medium-2, and high dose groups, respectively. The results of the analyses showed that there was no significant (at 0.05 level) linear trend in the intercurrent mortality rate in male ($p = 0.4492$) and female ($p = 0.2748$) rats.

The methods described in Peto et al. (1980) and the methods of age-adjusted exact permutation trend test were used to test the linear trend in the tumor data. The results of the above analyses showed that there was a significant (at 0.05 level) positive linear trend in the following neoplastic lesions:

(1) Testes U/interstitial tumor ($p = 0.0123$), testes B/interstitial tumor ($p = 0.0004$), combined testes U/interstitial and B/interstitial tumors ($p < 0.00001$), combined testes U/interstitial, B/interstitial, and B/mesothelioma tumors ($p < 0.00001$) and pancreas acinar cell adenoma ($p = 0.0411$) in males.

(2) Thyroid gland c-cell adenoma ($p = 0.0418$) in females.

However, the prevalence rates of thyroid gland c-cell adenoma in female rats in the concurrent control group is greater than one percent. It is considered as a common tumor in this strain of rats. For a common tumor, we consider a positive linear trend not to occur by chance of variation only if the p-value is smaller than 0.01. Therefore, we do not regard the positive linear trend in thyroid gland c-cell adenoma in female rats as statistically significant. The incidence rates of the tumor types with significant linear trends are given in Tables 4 to 8.

The statistical test results also showed that there are marginally significant (at 0.05 level) positive linear trends in liver hepatocellular carcinoma ($p = 0.0663$), skin fibroma ($p = 0.0526$), uterus endometrial polyp ($p = 0.0838$) in female rats, and in lymphoretic tiss histiocytic sarcoma ($p = 0.0679$) in male rats. The incidence rates of these tumor types are given in Tables 9 to 12.

Comparing the incidence rates of testes interstitial cell tumor (U/interstitial tumor and B/interstitial tumor) of high dose group (20%) in this study with those of historical control data base in Table 13, we found that the incidences of testes interstitial cell tumor of this study are at the high end of the historical control ranges (3.4% to 23.5%).

III. The Mouse Study - 87-3158 (Report # A-29-680)

III. a. Design

In this study, AG-1749 was administered via gastric intubation to 560 Charles River CD-1 mice (70/sex/group) at dosage levels of 1.5, 5.0, 15 and 50 mg/kg/day 5 day/week, for eighteen months. Control animals (70/sex) received the vehicle, 5% gum arabic solution, at the same dose volume as administered to the treated animals. Physical

observations, body weight and food consumption measurements were performed on all animals pretest and at selected intervals during the treatment period. After at least 18 months of treatment, all survivors were sacrificed. Selected organs were weighed and organ/body weight ratios calculated during the first day of the terminal sacrifice. Complete gross postmortem examinations were conducted on all animals including the control group. Histopathological evaluations of selected tissues were conducted on all animals in control and high dose groups and on all unscheduled deaths. In addition, histopathological evaluation of all gross lesions was also conducted on all low- and mid-dose groups (groups II, III, and IV). Treatment of this study began on May 1, 1987 and ended on November 13, 1988.

III. b. Sponsor's Analyses

Similar to the rat study, the Cox test, the Tarone test and the Kaplan-Meier method were used to analyze the survival data. The above analyses showed that there was no statistically significant difference in survival among the treated and control groups for females. Median survival time for females determined by the Kaplan-Meier method of estimation was 537 days. There were statistically significant differences in survivorship for the males. Both the Cox and Gehan-Breslow tests indicated statistically significant differences in survivorship ($p = 0.002$), and decreasing trend in survivorship with dose ($p = 0.001$). Individual test of treated groups vs control indicated that the 15.0 and 50.0 dose groups were different from control. The Kaplan-Meier survival curves are shown in Figures 3 and 4 for males and females, respectively.

The chi-square test, Armitage's test for monotonic trend in the doses, and the Fisher Exact tests of treated groups vs control were performed on the total numbers of animals with all primary neoplasms, all benign neoplasms, and all malignant neoplasms. For these particular tumor types, the sponsor indicated that there is not an effect on the number of tumor animals from the test material used in AG-1749. Table 14 listed the total numbers of animals with different kinds of neoplasms for both sexes. Table 15 listed the numbers of animals examined and the tumor incidences for each organ/tumor types for both sexes.

Based on the above test results, the sponsor concluded that "although the total neoplasms were increased in the high-dose males (malignant) and females (benign), the increase was slight and was attributed to a low incidence of neoplasms in a number of tissues. Therefore administration of AG-1749 to mice for 18 months was not associated with an oncogenic effect." Treatment related microscopic findings were noted in animals receiving 5, 15, and 50 mg/kg/day of AG-1749. The findings included hyperplasia of the glandular mucosa which occurred with an increased incidence and severity in group V followed by group IV and vesiculation/vacuolation of parietal cells was also noted in group IV and V. The no effect level based on microscopic

stomach findings was 1.5 mg/kg/day."

III.c. Reviewer's analyses and Comments

The Cox test and the generalized Wilcoxon test described in the paper of Thomas et al. (1977) were used to test for heterogeneity in survival distribution. The p-values of the Cox test were 0.0073 and 0.7034 for males and females, respectively. Hence, there was no statistically significant difference (at 0.05 level) in survival distribution in female mice. However, there was a statistically significant difference in survival distribution in males. A similar conclusion was obtained in the generalized Wilcoxon test. The p-values of the generalized Wilcoxon test were 0.0150 and 0.6791 for males and females, respectively.

The intercurrent mortality rates for both male and female mice (see Table 16) were tested for linear trend according to the death rate method described in the paper of Peto et al. (1980) using the time intervals 0-50, 51-70, and 71-78 weeks. The actual dose levels 0, 1.5, 5, 15, and 50 mg/kg/day were the scores assigned to the control, low, medium-1, medium-2, and high dose groups, respectively. The results of the analyses showed that no statistical significant (at 0.05 level) linear trend in the intercurrent mortality rate was detected in females ($p = 0.1383$). However, there was a significant (at 0.05 level) linear trend in the intercurrent mortality rate in male mice ($p = 0.0032$).

Noted that the sponsor did not identify "death or sacrifice status" in their submitted data diskettes. The above data analyses assumed that animals died after weeks 78 are terminal sacrificed which caused some differences in numbers of terminally sacrificed animals between sponsor's and the reviewer's analyses. The numbers of terminally sacrificed animals in reviewer's analyses are 33, 30, 36, 33, and 38 for 0, 1.5, 5, 15, and 50 mg/kg groups in females, respectively, and 40, 34, 30, 21, and 23 in males, respectively. However, the numbers of terminally sacrificed animals in sponsor's analyses are 34, 28, 32, 34, and 39 for 0, 1.5, 5, 15, and 50 mg/kg groups in females, respectively, and 42, 35, 31, 21, and 23 in males, respectively.

Table 15 listed the numbers of animals examined and tumor incidences for each organ/tumor type. They are consistent with the tumor incidences generated from the sponsor's submitted data diskettes. Due to the limitation of the sponsor's submitted data, it is impossible to use the age-adjusted method in testing linear trends in tumor data. In the following analyses, the age-unadjusted exact permutation trend test was used to test the linear trend in the tumor data. Since there is no significant linear trend in the intercurrent mortality rate in female mice, the age-unadjusted method is appropriate to analyze the tumor data of female mice. However, it is not true for the tumor data of male mice. The results of the above analyses showed that no significant (at 0.05 level) dose related trend in the tumor types tested was detected in both sexes.

The reviewer also evaluated the validity of the experiment. The following two issues are important in determining the validity of an experiment: (1) The numbers of animals alive over the course of the study to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors. (2) If the doses are high enough to present a reasonable tumor challenge to the animals.

With regard to the first issue, the following criteria or rules of thumb have been proposed by some experts in the field:

(A) Haseman proposes (through personal communication with Dr. Karl Lin) that a 50% survival rate of the 50 initial animals in the high dose group between weeks 80-90 will be considered as a sufficient number and an adequate exposure. However, the percentage can be lower or higher if the number of animals used in each treatment/sex is larger or smaller than 50 as long as there will be between 20 to 30 animals still alive during these weeks.

(B) Chu, Ceuto, and Ward ("Factors in the Evaluation of 200 National Cancer Institute Carcinogen Bioassays", Journal of Toxicology and Environmental Health, 8, 1981, pp. 251-280) propose that an experiment that has not shown a chemical to be carcinogenic should have (high dose) groups of animals with greater than 50% survival at one year (52 weeks).

In this study, the survival rates of the high dose mice at one-year were 87.14% and 97.14% for males and females, respectively. These one-year survival rates satisfied the criterion of Chu et al. (1981). The survival rates of the control, low, medium-1, medium-2, and high dose mice in the terminal sacrifice were 57.14%, 48.57%, 42.85%, 30%, and 32.85%, respectively, for males, and 47.14%, 42.85%, 51.42%, 47.14%, and 54.28%, respectively, for females. There were not sufficient male mice in the medium-2 and high dose groups living long enough to get an adequate exposure to the chemical and to be at risk of forming late-developing tumors based on Haseman's proposition.

With regard to the second issue, in the paper of Chu, Ceuto and Ward (1981), the following criteria for dose adequacy are mentioned.

(A) "A dose is considered adequate if there is a detectable loss in weight gain of up to 10% in a dosed group relative to the controls."

(B) "The administered dose is also considered an MTD (Maximum Tolerated Dose) if dosed animals exhibit clinical signs or severe histopathologic toxic effects attributed to the chemical."

(C) "In addition, doses are considered adequate if the dosed animals show a slightly increased mortality compared to the controls."

Figures 5-6 are the mean body weight curves of control and treated groups in male and female mice. In Tables 17-18, summaries of mean body weight data of the male and female mice are given. All treated

male mice gained more body weight than the control group. The weight gains in the male low, medium-1, medium-2, and high dose groups are 40.6%, 46.87%, 18.75%, and 34.37%, respectively, more than that of the male control group. The weight gain in the female low dose group is 1.94% less than that of the female control group. However, the weight gains of the female medium-1, medium-2, and high dose groups are 11.65%, 2.91%, and 7.76% more than that of the control group. It seems that the high dose is below MTD from the above mentioned criteria. However, before drawing the final conclusion that the high dose is below MTD, the clinical and toxic signs of the treated animals still need to be checked.

IV. Summary

IV.a. The Rat Study

The test results revealed that there was no statistically significant difference in survival distribution, and linear trend in intercurrent mortality rate in both sexes.

Results of tumor data analyses showed a significant dose-related trend in testes U/interstitial tumor ($p = 0.0123$), testes B/interstitial tumor ($p = 0.0004$), combined testes U/interstitial and B/interstitial tumors ($p < 0.00001$), combined testes U/interstitial, B/interstitial, and B/mesothelioma tumors ($p < 0.00001$) and pancreas acinar cell adenoma ($p = 0.0411$) in male rats.

Comparing the incidence rates of testes interstitial cell tumor (U/interstitial tumor and B/interstitial tumor) of high dose group (20%) in this study with those of historical control data base in Table 13, we found that the incidences of testes interstitial cell tumor of this study are at the high end of the historical control ranges (3.4% to 23.5%).

IV.b. The Mouse Study

The test results revealed that there was no statistically significant difference (at 0.05 level) in survival distribution in female mice. However, there was a statistically significant difference in the survival distribution in males.

Applying Peto's method to test the positive linear trend in intercurrent mortality rates, the results of the analyses showed that no statistical significant (at 0.05 level) linear trend in the intercurrent mortality rate was detected in females. However, there was a significant linear trend in the intercurrent mortality rate in male mice ($p = 0.0032$).

Due to the limitation of the sponsor's submitted data, the age-unadjusted exact permutation trend test was used to test the linear trend in the tumor data. The results of the above analyses showed that no significant (at 0.05 level) dose related trend was detected

in both sexes. However, before drawing the final conclusion that the chemical is not carcinogenic, the validity of the experiment has been evaluated. As mentioned previously, the numbers of animals alive over the course of the study and the adequacy of dose levels are the two important factors considered in the evaluation.

The analysis of survival data show that there were not sufficient male mice in the medium-2 and high dose groups living long enough to get an adequate exposure to the chemical. In addition, the analysis of weight gain data show that the high dose is below MTD. It is important to check the clinical and toxic signs of the animals in the high dose groups in the final determination of the adequacy of the high dose.

Daphne Lin

Daphne Lin, Ph.D.
Mathematical Statistician

Concur:

Karl K. Lin 7/20/94

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Figure 1

FIGURE 1
KAPLAN-MEIER SURVIVAL CURVES FOR MALE RATS
TWO YEAR ORAL GAVAGE ONCOGENICITY STUDY OF AG-1749

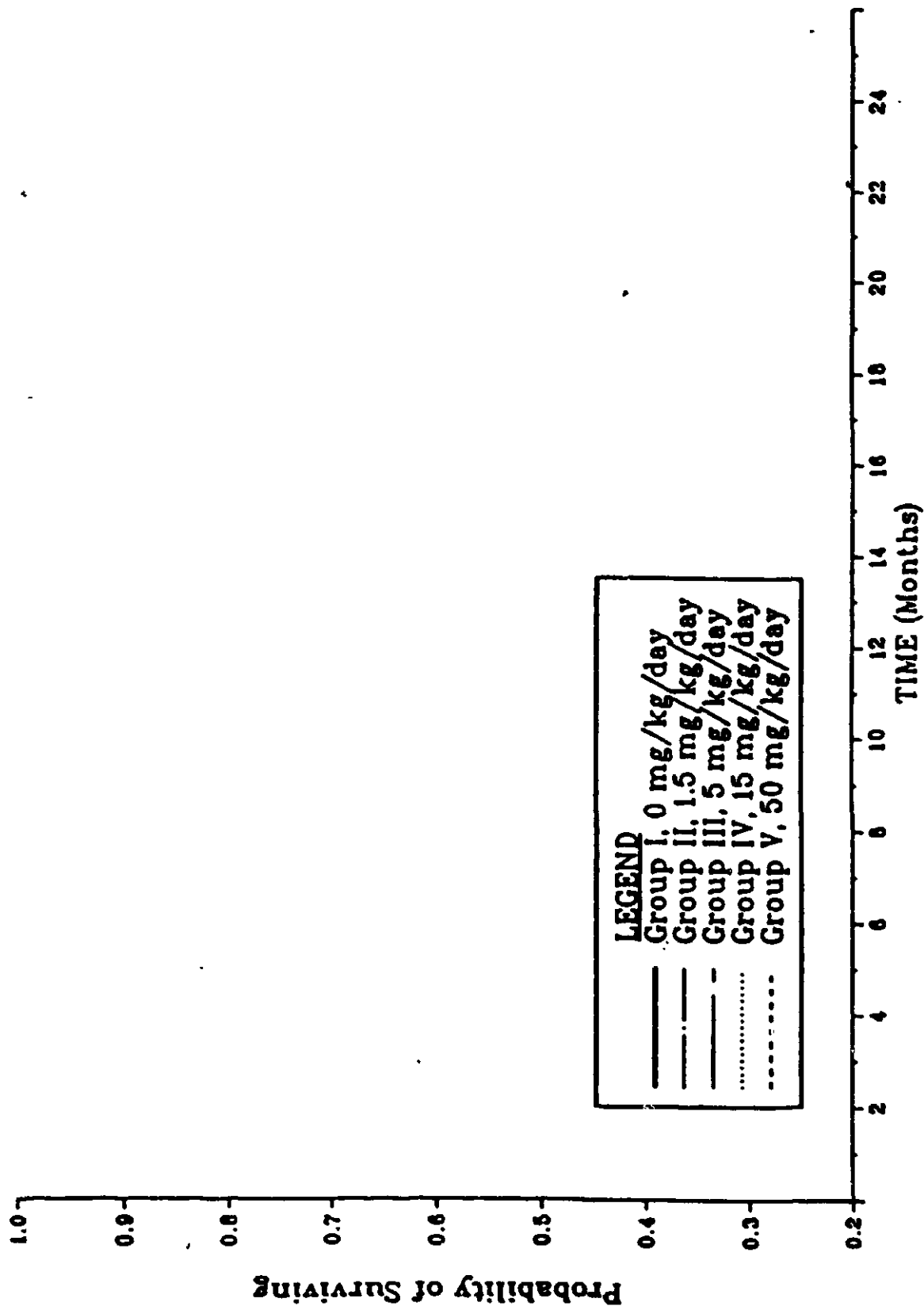


Figure 2

FIGURE 2

KAPLAN-MEIER SURVIVAL CURVES FOR FEMALE RATS

TWO YEAR ORAL GAVAGE ONCOGENICITY STUDY OF AG-1749

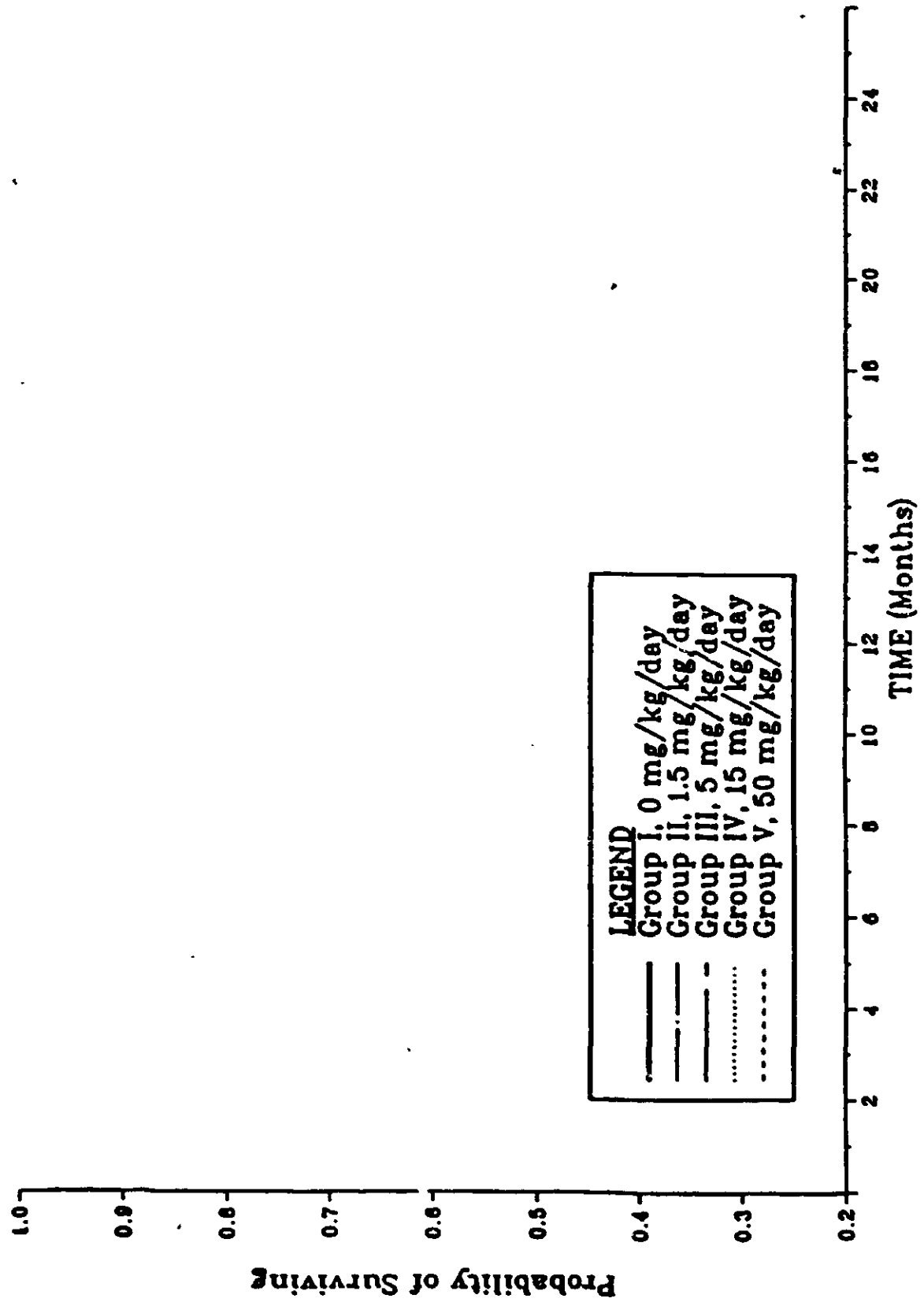


Figure 3

87-3158

F 1-1
Figure 1
An Eighteen Month Oral Gavage Oncogenicity Study
of AG-1749 in Mice

Survival Curve in Male Mice

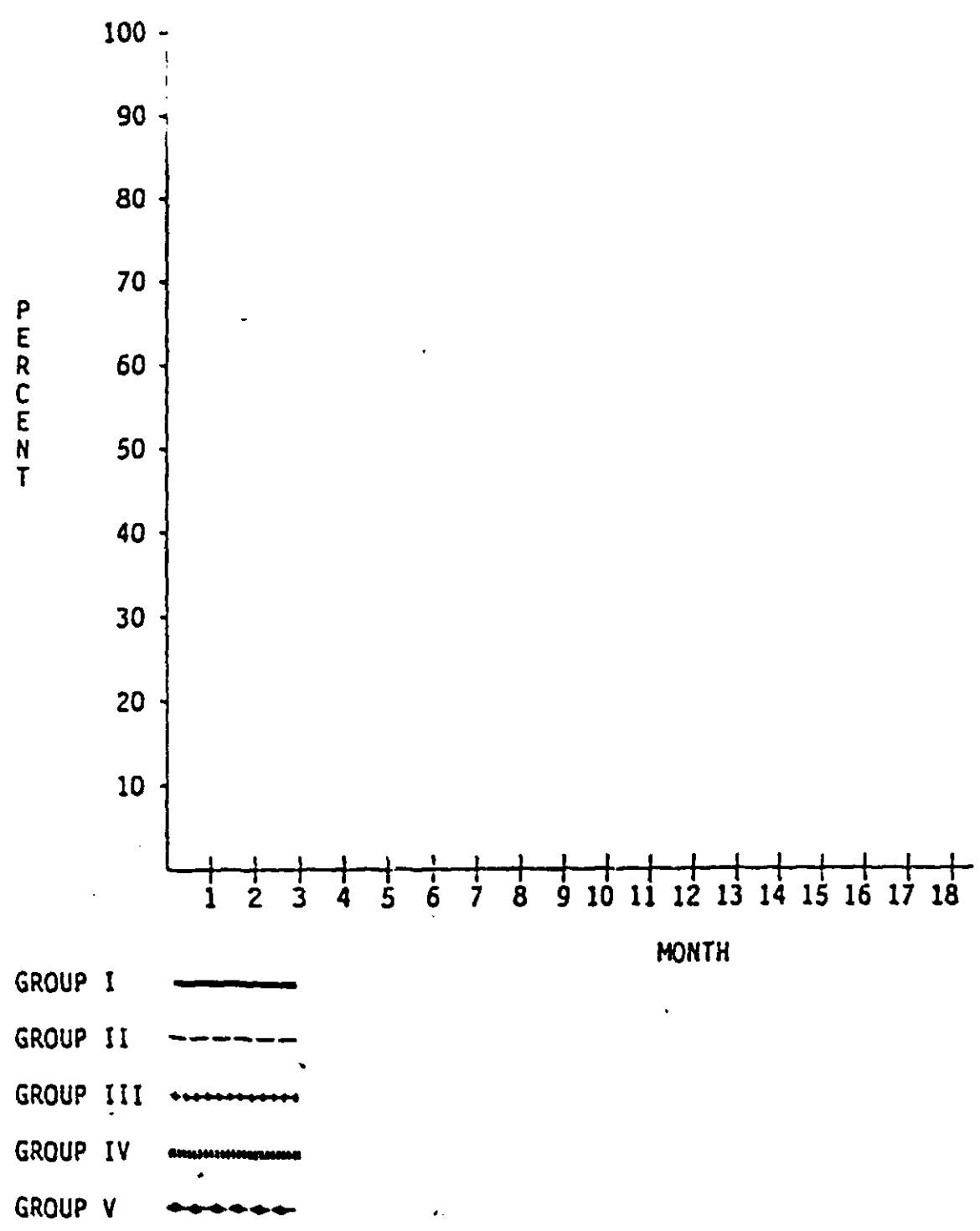


Figure 4

F 2-1
Figure 2
An Eighteen Month Oral Gavage Oncogenicity Study
of AG-1749 in Mice

87-3158

Survival Curve in Female Mice

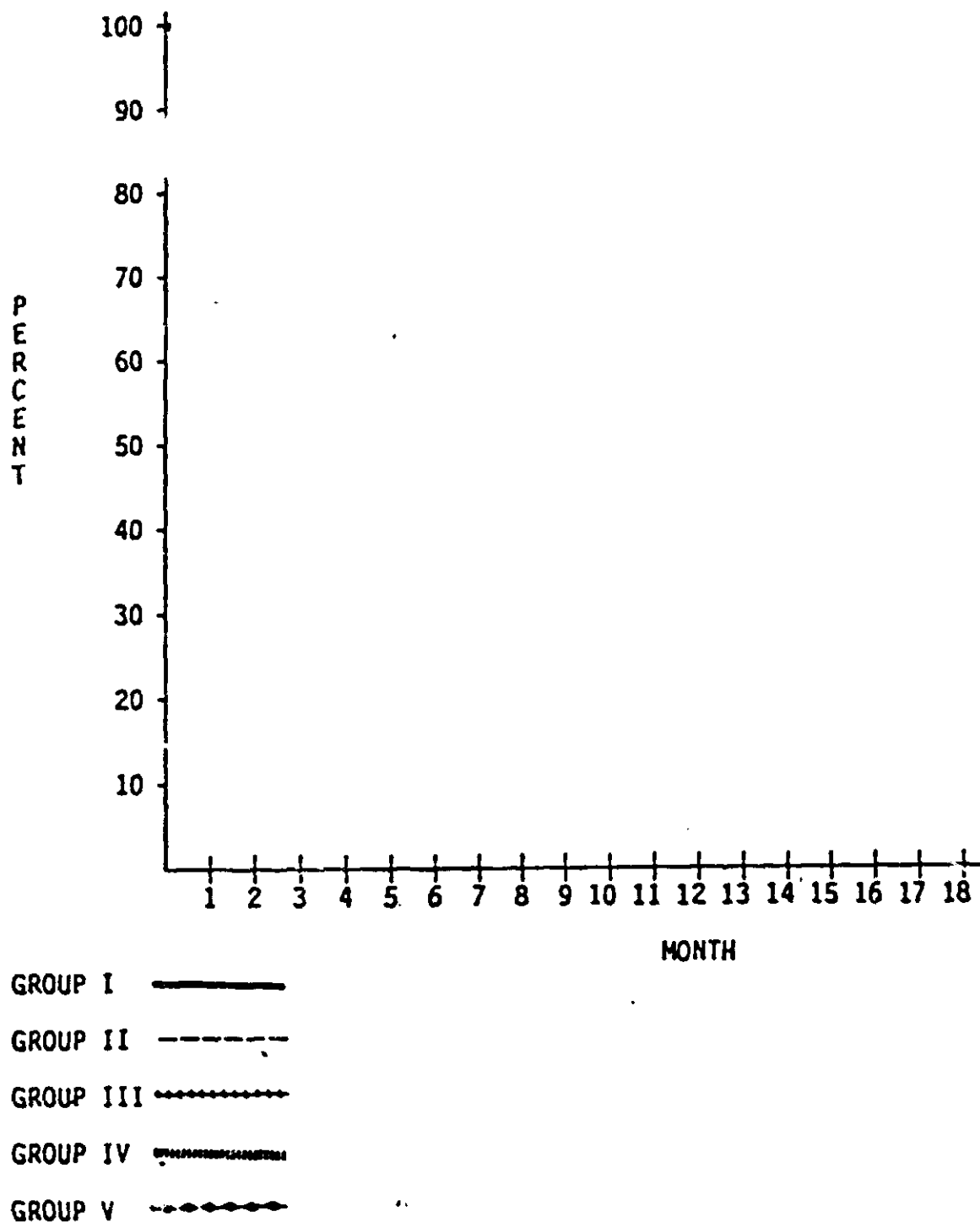


Figure 5

87-3158

Figure 3
An Eighteen Month Oral Gavage Oncogenicity Study
of AG-1749 in Mice

Group Mean Body Weights in Male Mice

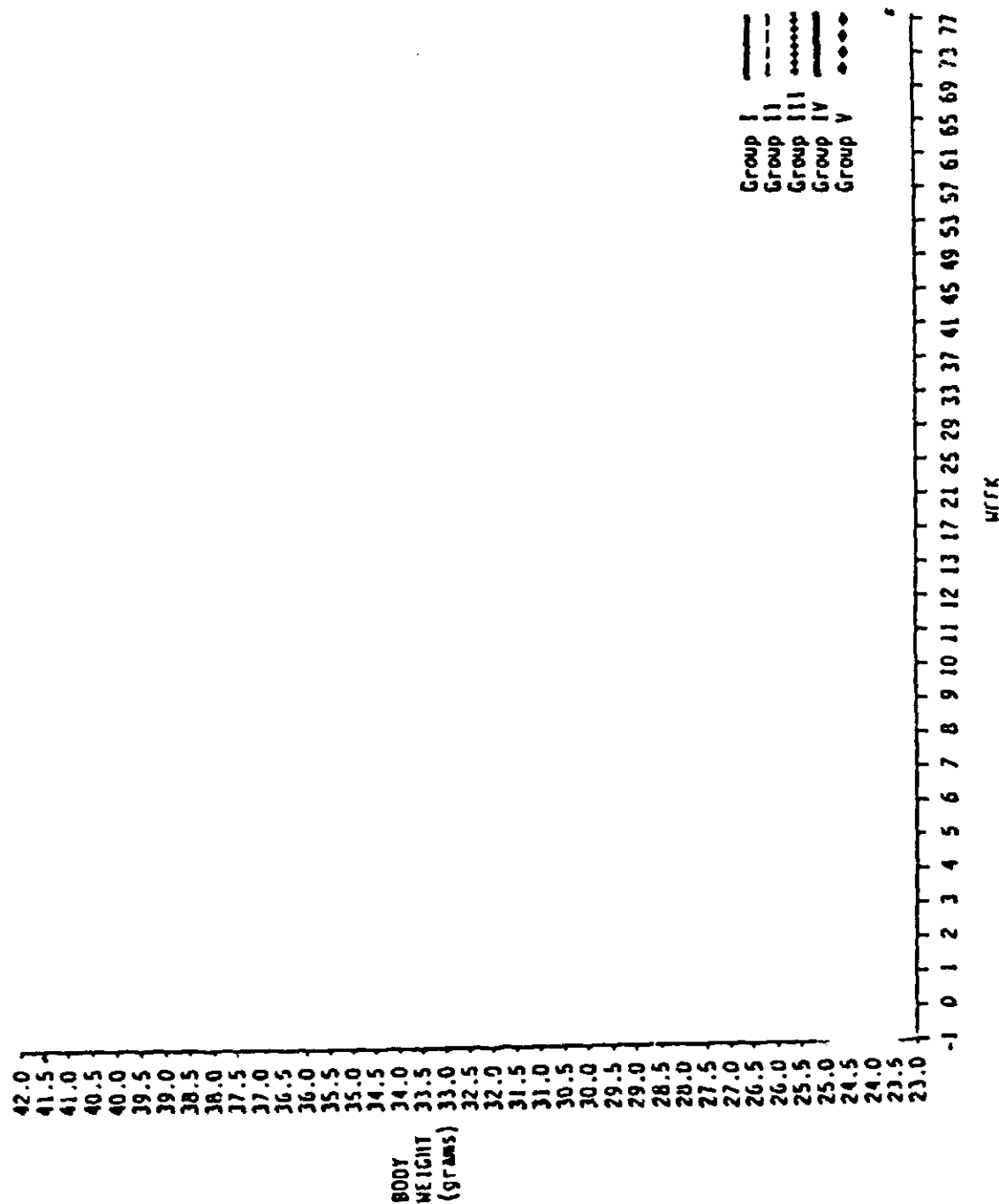
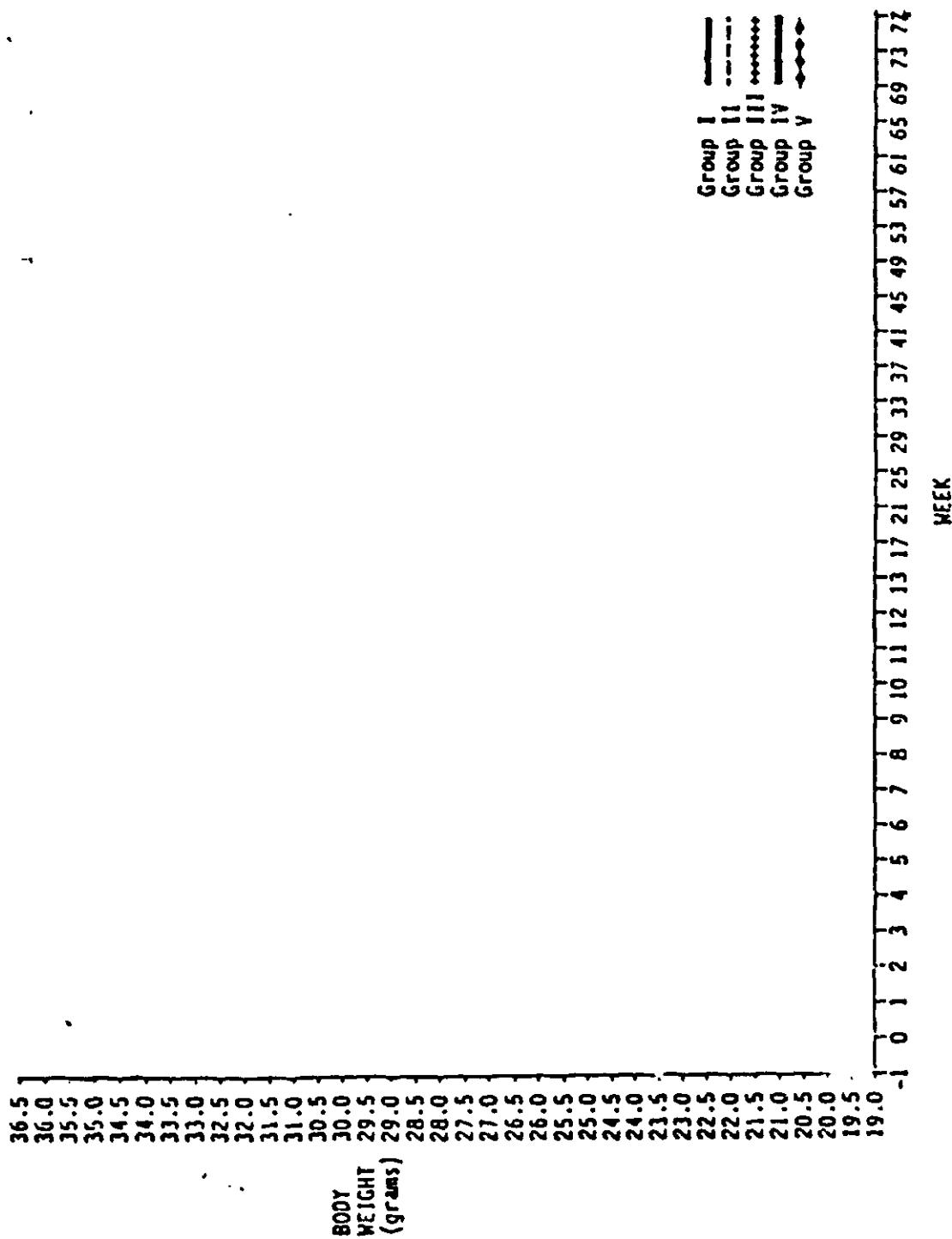


Figure 6

87-3158

Figure 4
An Eighteen Month Oral Gavage Oncogenicity Study
of AG-1749 in Mice

Group Mean Body Weights in Female Mice



F 4-1

Table 1

5. Pathology Report (cont.):

-46-

86-3028

BIO/PHARMICS, INC.
 DEPARTMENT OF PATHOLOGY
 EAST HILLSTONE, NJ 08873

*** PATH/TOX SYSTEM OUTPUT ***
 A TWO YEAR ORAL GAVAGE ONCOGENICITY STUDY
 OF AG-1729 IN RATS
 INCIDENCE SUMMARY

PRINTED: 23-JAN-90
 STUDY NUMBER: 863028

... NUMBER OF ANIMALS AFFECTED ...

TABLE INCLUDES:	SEX:	MALE:
SCREEN-ALL; WEEKS-ALL	GROUP:	1 2 3 4 5
DEATH-ALL; FIMO-ALL; SUBSET-ALL	NUMBER:	60 60 60 60 60
NEOPLASM CLASSIFICATION SUMMARY		
TOTAL PRIMARY NEOPLASMS	71 62 56 67 72	
ANIMALS WITH ONE OR MORE	43 37 30 43 47	
PERCENT WITH ONE OR MORE	72% 62% 52% 72% 78%	
TOTAL BENIGN NEOPLASMS	60 50 47 53 42	
ANIMALS WITH ONE OR MORE	38 32 37 36 42	
PERCENT WITH ONE OR MORE	63% 53% 62% 60% 70%	
TOTAL MALIGNANT NEOPLASMS	11 12 9 14 10	
ANIMALS WITH ONE OR MORE	11 10 4 13 10	
PERCENT WITH ONE OR MORE	18% 17% 10% 22% 17%	

5. Pathology Report (cont.):

Table 1 (continued)

-47-

86-3028

BIO/DYNAMICS, INC.		PRINTED: 23-JAN-90	
DEPARTMENT OF PATHOLOGY		STUDY NUMBER: 863028	
EAST HILLSTONE, NJ 08273		... NUMBER OF ANIMALS AFFECTED ...	
...		...	
TABLE INCLUDES:		SEX: FEMALE.....	
SEX: GROUP=ALL; SCREEN=ALL; YEARS=ALL		GROUP: 1. 2. 3. 4. 5.	
DEATH=ALL; FIND=ALL; SUBSET=ALL		NUMBER: 60 60 60 60 60	
NEOPLASM CLASSIFICATION SUMMARY			
.....			
TOTAL PRIMARY NEOPLASMS		102 106 97 92 105	
ANIMALS WITH ONE OR MORE		56 54 55 51 50	
PERCENT WITH ONE OR MORE		93% 90% 92% 85% 83%	
.....			
TOTAL BENIGN NEOPLASMS		79 80 81 68 82	
ANIMALS WITH ONE OR MORE		52 50 53 44 48	
PERCENT WITH ONE OR MORE		87% 83% 88% 77% 80%	
.....			
TOTAL MALIGNANT NEOPLASMS		23 26 16 24 23	
ANIMALS WITH ONE OR MORE		20 20 15 22 20	
PERCENT WITH ONE OR MORE		33% 33% 25% 37% 33%	

Table 2

Table 1
Incidence of Neoplastic and Hyperplastic Lesions in Testes and Thyroid Glands

SEX	ORGAN	LESION	AQ-1749						Test for Trend					
			0	1.5	3	15	30		0	1.5	3	15	30	P-VALUE
			mg/kg/day DOS SAC ^b	mg/kg/day DOS SAC	mg/kg/day DOS SAC	mg/kg/day DOS SAC	mg/kg/day DOS SAC		mg/kg/day DOS SAC	mg/kg/day DOS SAC	mg/kg/day DOS SAC	mg/kg/day DOS SAC	mg/kg/day DOS SAC	
M	TESTES	INTERSTITIAL CELL TUMOR	(0/40) ^a (1/30) (0/41) (2/19) (1/42) (1/17) (1/43) (5/17) ^a (0/44) (4/16) ^a											0.0001
M	TESTES	INTERSTITIAL CELL HYPERPLASIA	(1/40) (1/30) (1/41) (2/19) (0/42) (1/17) (3/43) (5/17) ^a (10/44) (10/16) ^a											0.0001
M	THYROID	C-CELL TUMOR	(1/30) (4/30) (3/41) (1/1) (1/41) (0/0) (3/41) (0/1) (3/43) (2/15)											0.3703
M	THYROID	C-CELL HYPERPLASIA	(19/30) (16/30) (14/41) (1/1) (14/41) (0/0) (16/41) (0/1) (12/43) (14/15)											0.1134
F	THYROID	C-CELL TUMOR	(2/30) (0/17) (7/31) (0/0) (5/32) (0/0) (2/30) (0/2) (4/33) (4/24)											0.0916
F	THYROID	C-CELL HYPERPLASIA	(26/30) (16/17) (31/31) (0/0) (20/32) (0/0) (19/30) (1/2) (23/33) (23/24)											0.5830

^a DOS = Natural death or moribund kill

^b SAC = Terminal sacrifice

^c (a/b) = IMO. with Tumor/No. Examined

* Significant increase in the total incidence from the control group ($p = 0.0139$ for testicular tumor and $p = 0.0172$ for hyperplasia). The p values almost reach significance at the Bonferroni adjusted level 0.0125 (= 0.05/4).

** Highly significant increase in the total incidence from the control group ($p < 0.0005$).

Table 3
Intercurrent Mortality Rates
Male Rats

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	60	3	5.0	60	3	5.0	60	2	3.3	60	4	6.7	60	3	5.0
51-80	57	20	35.1	57	16	28.1	58	14	24.1	56	11	19.6	57	14	24.6
81-104	37	17	45.9	41	19	46.3	44	26	59.1	45	27	60	43	25	58.1
Term.	20			22			18			18			18		

Peto Test: p-value = 0.4492

Female Rats

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	60	0	0	60	1	1.7	60	1	1.7	60	2	3.3	60	3	5.0
51-80	60	20	33.3	59	13	22.0	59	15	25.4	58	11	18.9	57	9	15.8
81-104	40	22	55	46	20	43.5	44	16	36.4	47	20	42.6	48	23	47.9
Term.	18			26			28			27			25		

Peto Test: p-value = 0.2748

Notes: S: Number of animals starting during the period
D: Deaths
%: Percent of death during the period

1 - 4

Table 4
Tumor Incidence Rates
Male Rats, Testes U/Interstitial Tumor

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
C-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	0	14	0	11	1	14
81-104	0	17	0	19	1	26	0	27	2	25
Terminal	1	20	1	22	0	18	3	18	2	18
<u>Total</u>	<u>1</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>3</u>	<u>60</u>	<u>5</u>	<u>60</u>

Peto Test: p-value = 0.0123

Pairwise Comparison: High vs. Control: P-value = 0.1054 (one-sided)

Table 5
Tumor Incidence Rates
Male Rats, Testes B/Interstitial Tumor

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	0	14	0	11	0	14
81-104	0	17	0	19	0	26	1	27	3	25
Terminal	0	20	1	22	1	18	2	18	4	18
<u>Total</u>	<u>0</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>3</u>	<u>60</u>	<u>7</u>	<u>60</u>

Peto Test: p-value = 0.0004

Pairwise Comparison: High vs. Control: P-value = 0.0083 (one-sided)

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 6
Tumor Incidence Rates
Male Rats, Testes U/Interstitial & B/Interstitial Tumors

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	0	14	0	11	1	14
81-104	0	17	0	19	1	26	1	27	5	25
Terminal	1	20	2	22	1	18	5	18	6	18
Total	1	60	2	60	2	60	6	60	12	60

Peto Test: p-value < 0.00001

Pairwise Comparison: High vs. Control: P-value = 0.0012 (one-sided)

Table 7
Tumor Incidence Rates
Male Rats, Testes U/Interstitial, B/Interstitial Tumors
and B/Mesothelioma

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	0	14	0	11	1	14
81-104	0	17	0	19	1	26	2	27	5	25
Terminal	1	20	2	22	1	18	5	18	6	18
Total	1	60	2	60	2	60	7	60	12	60

Peto Test: p-value < 0.00001

Pairwise Comparison: High vs. Control: P-value = 0.0012 (one-sided)

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 8
Tumor Incidence Rates
Male Rats, Pancreas Acinar Cell Adenoma

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	0	14	0	11	0	14
81-104	0	17	0	19	0	26	0	27	1	25
Terminal	0	20	0	22	0	18	0	18	1	18
<u>Total</u>	0	60	0	60	0	60	0	60	2	60

Peto Test: p-value = 0.0411

Pairwise Comparison: High vs. Control: P-value = 0.2820 (one-sided)

Table 9
Tumor Incidence Rates
Female Rats, Liver Hepatocellular Carcinoma

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	0	0	1	0	1	0	2	0	3
51-80	0	20	0	13	0	15	0	11	0	9
81-104	0	22	0	20	0	16	1	20	1	23
Terminal	0	18	0	26	1	28	0	27	1	25
<u>Total</u>	0	60	0	60	1	60	1	60	2	60

Peto Test: p-value = 0.0663

Pairwise Comparison: High vs. Control: P-value = 0.2972 (one-sided)

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 10
Tumor Incidence Rates
Female Rats, Skin Fibroma

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	0	0	1	0	1	0	2	0	3
51-80	0	20	0	13	1	15	0	11	0	9
81-104	1	22	0	20	0	16	1	20	2	23
Terminal	0	18	0	26	1	28	4	27	2	25
<u>Total</u>	<u>1</u>	<u>60</u>	<u>0</u>	<u>60</u>	<u>2</u>	<u>60</u>	<u>5</u>	<u>60</u>	<u>4</u>	<u>60</u>

Peto Test: p-value = 0.0526

Pairwise Comparison: High vs. Control: P-value = 0.2340 (one-sided)

Table 11
Tumor Incidence Rates
Female Rats, Uterus Endometrial Polyp

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	0	0	1	0	1	0	2	0	3
51-80	0	20	0	13	1	15	1	11	0	9
81-104	0	22	1	20	2	16	1	20	2	23
Terminal	1	18	1	26	1	28	1	27	3	25
<u>Total</u>	<u>1</u>	<u>60</u>	<u>2</u>	<u>60</u>	<u>4</u>	<u>60</u>	<u>3</u>	<u>60</u>	<u>5</u>	<u>60</u>

Peto Test: p-value = 0.0838

Pairwise Comparison: High vs. Control: P-value = 0.1643 (one-sided)

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 12
Tumor Incidence Rates
Male Rats, Lymph-Retic Tiss Histiocytic Sarcoma

<u>Weeks</u>	<u>Control</u>		<u>Low</u>		<u>Medium-1</u>		<u>Medium-2</u>		<u>High</u>	
	T	N	T	N	T	N	T	N	T	N
0-50	0	3	0	3	0	2	0	4	0	3
51-80	0	20	0	16	1	14	1	11	0	14
81-104	0	17	0	19	0	26	2	27	3	25
Terminal	0	20	1	22	0	18	0	18	0	18
<u>Total</u>	<u>0</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>1</u>	<u>60</u>	<u>3</u>	<u>60</u>	<u>3</u>	<u>60</u>

Peto Test: p-value = 0.0679

Pairwise Comparison: High vs. Control: P-value = 0.2003 (one-sided)

Notes: T: Number of necropsies with the above tumor.
N: Number of necropsies.

Table 13

5. Pathology Report (cont.):

-54-

A Two Year Oral Gavage Oncogenicity Study
of AG-1749 in Rats
Pathology Report

86-3028

Figure 4

Historical Control Data (Testes)

AG-1749

Group	I	II	III	IV	V
Testes					
Interstitial Cell Tumor	1/60	2/60	2/59	6/60	12/60
%	(1.7)	(3.3)	(3.4)	(10.0)	(20.0)
Interstitial Cell Hyperplasia	2/60	3/60	1/59	8/60	20/60
%	(3.3)	(5.0)	(1.7)	(13.3)	(33.3)

Data Base

Testes	
Interstitial Cell Tumor	Overall Incidence 116/1185 (9.8%) Range - High 27/115 (23.5%) - Low 4/116 (3.4%)
Interstitial Cell Hyperplasia	Overall Incidence 17/1185 (1.4%) Range - High 3/42 (7.1%) - Low 0/118 (0.0%)

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*** PATH/TOX SYSTEM OUTPUT ***
AN EIGHTEEN MONTH ORAL GAVAGE ONCOGENICITY STUDY
OF AG-1769 IN MICE
INCIDENCE SUMMARY

STUDY NUMBER: 873158

--- NUMBER OF - ANIMALS - AFFECTED ---

TABLE INCLUDES:

SEX=M; GROUP=ALL; SCREEN=ALL; WEEKS=ALL
DEATH=ALL; FIND=B,M,X; SUBSET=1

SEX: MALE.....
GROUP: -1- -2- -3- -4- -5-

NUMBER: 70 70 70 70 70 70
.....-.-.-.-.-

NEOPLASM CLASSIFICATION SUMMARY

TOTAL PRIMARY NEOPLASMS
ANIMALS WITH ONE OR MORE 26 21 14 20 27
PERCENT WITH ONE OR MORE 20 17 12 17 25
..... 29% 24% 17% 24% 36%

TOTAL BENIGN NEOPLASMS
ANIMALS WITH ONE OR MORE 22 16 8 9 18
PERCENT WITH ONE OR MORE 17 16 7 9 18
..... 24% 23% 10% 13% 26%

TOTAL MALIGNANT NEOPLASMS
ANIMALS WITH ONE OR MORE 4 5 6 11 9
PERCENT WITH ONE OR MORE 4 5 5 10 9
..... 6% 7% 7% 14% 13%

TOTAL METASTATIC NEOPLASMS
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

TOTAL LOCALLY INVASIVE NEOPLASMS
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

TOTAL OTHER NEOPLASMS
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

Table 14 (continued)

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PAGE: 1

*** PATH/TOX SYSTEM OUTPUT ***
AN EIGHTEEN MONTH ORAL GAVAGE ONCOGENICITY STUDY
OF AG-1749 IN MICE
INCIDENCE SUMMARY

STUDY NUMBER: 873158

--- NUMBER OF - ANIMALS - AFFECTED ---

TABLE INCLUDES:

SEX=F; GROUP=ALL; SCREEN=ALL; WEEKS=ALL
DEATH=ALL; FIND=B,M,X; SUBSET=T

SEX: FEMALE
GROUP: 1. 2. 3. 4. 5.

NEOPLASM CLASSIFICATION SUMMARY

NUMBER: 70 70 70 70 70

TOTAL PRIMARY NEOPLASMS 27 17 18 17 25
ANIMALS WITH ONE OR MORE 24 14 17 16 19
PERCENT WITH ONE OR MORE 34% 20% 24% 23% 27%

TOTAL BENIGN NEOPLASMS 13 12 9 11 20
ANIMALS WITH ONE OR MORE 12 12 9 10 15
PERCENT WITH ONE OR MORE 17% 17% 13% 14% 21%

TOTAL MALIGNANT NEOPLASMS 14 5 9 6 5
ANIMALS WITH ONE OR MORE 13 4 9 6 5
PERCENT WITH ONE OR MORE 19% 6% 13% 9% 7%

TOTAL METASTATIC NEOPLASMS 0 0 0 0 0
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

TOTAL LOCALLY INVASIVE NEOPLASMS 0 0 0 0 0
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

TOTAL OTHER NEOPLASMS 0 0 0 0 0
ANIMALS WITH ONE OR MORE 0 0 0 0 0
PERCENT WITH ONE OR MORE 0% 0% 0% 0% 0%

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PAGE: 1

STUDY NUMBER: 873158

SEX: MALE.....

1. 2. 3. 4. 5.

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1970	0	0	0	0
1971	0	0	0	0
1972	0	0	0	0
1973	0	0	0	0
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2067	0	0	0	0
2068	0	0	0	0
2069	0	0	0	0
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2071	0	0	0	0
2072	0	0	0	0
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*** PATH/TOX SYSTEM OUTPUT ***									
AN EIGHTEEN MONTH ORAL GAVAGE ONCOGENICITY STUDY									
OF AG-1769 IN MICE									
INCIDENCE SUMMARY									
TABLE INCLUDES:									
SEX= M; GROUP= ALL; SCREEN= ALL; WEEKS= ALL									
DEATH= ALL; FIND= B, M, X; SUBSET= T									
STUDY NUMBER: 873158									
NUMBER OF ANIMALS AFFECTED ...									
ORGAN AND FINDING DESCRIPTION									
FROM PREVIOUS PAGE **									
SKIN (SK)									
--H-SARCOMA, NOT OTHERWISE SPECIFIED									
--B-PILOMATRIXOMA									
LYMPHO-BEYIC (LR)									
--H-MYELOSARCOMA/GRANULOCYTIC LEUKEMIA									
--H-LYMPHOMA/LEUKEMIA									
--H-HISTIOCYTIC SARCOMA									
** END OF LIST **									

SEX:	MALE	
GROUP:	1	2
NUMBER:	70	70
NUMBER EXAMINED:	69	47
	0	48
	0	58
	0	70
	0	0
	0	0
	0	0
NUMBER EXAMINED:	1	0
	0	1
	0	5
	1	0
	0	0
	0	5
	0	0
	0	0

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PAGE: 2

Table 15 (continued)

*** PATH/TOX SYSTEM OUTPUT ***									
AN EIGHTEEN MONTH ORAL GAVAGE ONCOGENICITY STUDY									
OF AG-1749 IN MICE									
INCIDENCE SUMMARY									
STUDY NUMBER: 873158									
NUMBER OF ANIMALS AFFECTED									
SEX: -1- -2- -3- -4- -5-									
GROUP: -1- -2- -3- -4- -5-									
NUMBER: 70 70 70 70 70									
ORGAN AND FINDING DESCRIPTION									
TOP OF LIST									
LUNGS (LU)									
--M-BRONCHIOLAR/ALVEOLAR ADENOCARCINOMA									
--B-BRONCHIOLAR/ALVEOLAR ADENOMA									
LIVER (LI)									
--M-HEMANGIOSARCOMA									
--M-HEPATOCELLULAR CARCINOMA									
--B-HEPATOCELLULAR ADENOMA									
--B-HEMANGIOMA									
SPLEEN (SP)									
--M-HEMANGIOSARCOMA									
--B-HEMANGIOMA									
URINARY BLADDER (UB)									
--B-LEIOMYOMA									
OVARIES (OV)									
--B-U/ NEANGIOMA									
UTERUS (UT)									
--M-ENDOMETRIAL SARCOMA									
--M-ADENOCARCINOMA									
--M-MISTICOTIC SARCOMA									
--M-NEANGIOSARCOMA									
--M-LEIOMYOSARCOMA									
--B-ENDOMETRIAL STROMAL POLYP									
--B-LEIOMYOMA									
--B-NEANGIOMA									
--B-NEURILEMOMA									
ADRENAL GLANDS (AD)									
--B-U/ CORTX: ADENOMA									
--B-U/ MEDULLA: ADENOMA									
THYROID GLAND (TH)									
--B-U/ FOLLICULAR ADENOMA									

Table 16
Intercurrent Mortality Rates
Male Mice

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	70	9	12.8	70	10	14.3	70	4	5.7	70	12	17.1	70	9	12.8
51-70	61	9	14.7	60	12	20.0	66	17	25.8	58	18	31.0	61	23	37.7
71-78	52	12	23.1	48	14	29.2	49	19	38.8	40	19	47.5	38	15	39.5
Term.	40			34			30			21			23		

Peto Test: p-value = 0.0032

Female Mice

<u>Weeks</u>	<u>Control</u>			<u>Low</u>			<u>Medium-1</u>			<u>Medium-2</u>			<u>High</u>		
	S	D	%	S	D	%	S	D	%	S	D	%	S	D	%
0-50	70	5	7.1	70	12	17.1	70	5	7.1	70	7	10	70	2	2.9
51-70	65	18	27.7	58	13	22.4	65	15	23.1	63	17	26.9	68	21	30.9
71-78	47	14	29.8	45	15	33.3	50	14	28	46	13	28.3	47	9	19.1
Term.	33			30			36			33			38		

Peto Test: p-value = 0.1383

Notes: S: Number of animals starting during the period
 D: Deaths
 %: Percent of death during the period

Summary of Mean Body Weight with (Standard Deviation)

The Male Mice Study

Dose	Week 0	Week 77	Gain in Wt.	Gain rel. to Control
Control	29.9 (1.8)	36.3 (3.2)	6.4	-
Low	29.2 (1.7)	38.2 (3.4)	9.0	40.6%
Med-1	29.4 (1.5)	38.8 (2.9)	9.4	46.87%
Med-2	29.9 (1.6)	37.5 (3.4)	7.6	18.75%
High	29.6 (1.9)	38.2 (4.4)	8.6	34.37%

Table 18

Summary of Mean Body Weight with (Standard Deviation)

The Female Mice Study

Dose	Week 0	Week 77	Gain in Wt.	Gain rel. to Control
Control	22.8 (1.9)	33.1 (2.7)	10.3	-
Low	22.6 (1.6)	32.7 (3.1)	10.1	- 1.94%
Med-1	22.8 (1.6)	34.3 (2.4)	11.5	11.65%
Med-2	22.7 (1.7)	33.3 (3.6)	10.6	2.91%
High	22.8 (1.6)	33.9 (3.2)	11.1	7.76%

Note: A negative sign stands for decrease in weight gain relative to control.

H11. 620
W1212

Statistical Review and Evaluation

DATE:

JUN 17 1994

NDA#: 20-406

APPLICANT: TAP Pharmaceuticals Inc.

NAME OF DRUG: Prevacid (Lansoprazole) 30 mg and 15 mg Capsules

DOCUMENTS REVIEWED: Unnumbered Volume Dated 5/26/94 Containing a Coverletter and Output from Sponsor's Statistical Analyses

I. Background

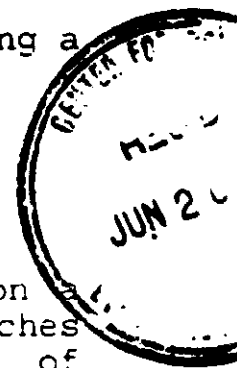
This volume contained the data listings (in hardcopy and on a diskette) and results of the statistical analyses of three batches each of 30 mg and of 15 mg capsules filled into bottles of 100, of 30, and into blister packs. Dr. A. Shaw (HFD-180) requested the Division of Biometrics to review and evaluate the sponsor's analyses.

Sponsor's Results

The sponsor used the PC-SAS stability program which is also used in the Division of Biometrics. He analyzed the data per strength and per package type.

The 30 mg capsules filled into bottles of 100 were studied for 30 months. The data regressed to individual lines. Two of the lines had negative slope estimates, one line had a positive slope estimate. The sponsor computed the lower 95 % confidence limit for each line and the intersection of this limit with the lower (90 %LC) specification limit. In this manner the data from each batch estimated an extrapolated expiration dating period of 84 months. The same three batches were also filled into bottles of 30. The stability data of this configuration regressed to parallel lines, each of which estimated the expiration dating period to be 84 months. The slope estimate was negative. The same product was studied for 24 months in blister packs. These data regressed to a common line and again estimated an extrapolated expiration dating period of 84 months.

Three batches of 15 mg capsules were packaged in the same way as the 30 mg capsules, namely into bottles of 100, into bottles of 30, and into blister packs. The 30 months data of the 15 mg capsules in bottles of 100 regressed to parallel lines. Each line estimated an extrapolated expiration dating period of 84 months. The 30 months data of the 15 mg capsules in bottles of 30 regressed to a common line and estimated the expiration dating period again to be 84



months. Similarly, this product in blister packs regressed to a common line with a 84 months extrapolated expiration dating period.

III. Reviewer's Results

There were two files on the diskette, Lansdata.wk4 and Lansdata.txt. The first file could not be read by this reviewer but the second file contained all stability data as listed in the volume. No further attempt was made to identify the Lansdata.wk4 file.

As the sponsor used the program which was provided by the Division of Biometrics this reviewer did not rerun all analyses but verified the results of two of the sponsor's analyses. She obtained identical results and therefore assumed that all analyses of the sponsor are numerically correct.

Each data set supported an extrapolated expiration dating period of 84 months. Though the sponsor had 24 and 30 months of actual data, extrapolation well beyond this point assumes that the product's degradation pattern beyond the actual data will remain identical to the one estimated by the actual data.

The sponsor apparently assumed that the product will only degrade over time and therefore used only the lower 95 % CL and the lower specification limit in the calculation of the expiration date. Actually, one batch had a positive slope estimate in one instance. In this case it is reasonable to check whether the upper 95 % CL and the upper specification limit give a shorter expiration date. This reviewer performed this calculation and no change in the estimated expiration dating period was found.

IV. Summary and Conclusion

The sponsor used the program provided by the Division of Biometrics in estimating the expiration date for this product. This reviewer therefore only verified the correctness of two analyses. Finding no discrepancies she assumed that the remaining analyses were also correct.

There were three batches of 30 mg capsules. These batches were studied for 30 months when filled into bottles of 100 or into bottles of 30. When packaged into blister packs the product was studied for 24 months. Each set of data (the same three batches in different packaging) estimated an extrapolated expiration dating period of 84 months.

There were also three batches of 15 mg capsules which were filled into the same packaging. Again each data set estimated an extrapolated expiration dating period of 84 months.

In summary, the product appears to remain within its specification limits well beyond the observed data. Extrapolation far beyond the observed data assumes that the degradation pattern does not change from the one estimated based on the actual data.

Roswitha E Kelly
Roswitha E Kelly

Concur:

Karl K. Lin 6/16/94
Karl K. Lin, Ph.D.

cc: HFD-180/NDA 20-406 Original
HFD-180/Dr. Shaw
HFD-710/Chron
HFD-715/Dr. Fairweather
HFD-715/Dr. Lin
HFD-715/R. Kelly
HFD-715/DRU 2.2.1 Prevacid Capsules, TAP Pharm., Stability
HFD-715/RKELLY/6/13/94/wp-prevacid.rev

Pharm

M E M O R A N D U M

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

DATE: January 25, 1995

FROM: Supervisory Pharmacologist
Division of Gastrointestinal and
Coagulation Drug Products, HFD-180

SUBJECT: NDA 20,406 (PREVACID/Lansoprazole Delayed-Release
Capsules) - Labeling - Revisions For Pre-clinical
Pharmacology Portion.

TO: NDA 20,406

A Supervisory Pharmacologist's addendum is under preparation to address certain aspects of Dr. Y.M. Chopra's pharmacology review (dated January 23, 1995) of this NDA. In this present memorandum, the preclinical portions of the Sponsor's version of the labeling are reproduced below. Our evaluation of the Sponsor's versions, our recommendations and the revised portions for the final labeling follow.

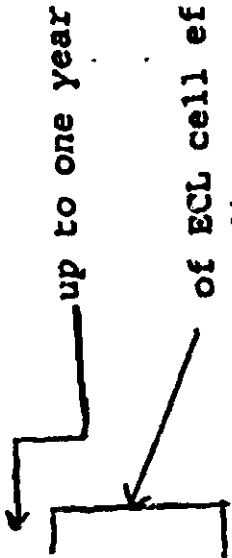
Pharmacodynamics

Enterochromaffin-like (ECL) cell effects

Sponsor's Version:

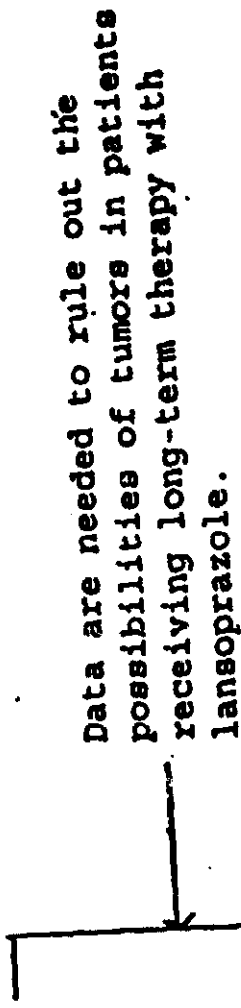
Gastric biopsy specimens from the body of the _____ approximately 150

stomach from _____ patients treated
continuously with lansoprazole for
_____ have not shown evidence



of ECL cell effects as noted in rat
studies.

During lifetime exposure of rats with up to 150
mg/kg/day of lansoprazole dosed seven days per
week, marked hypergastrinemia preceded ECL
cell proliferation and carcinoid formation,
especially in female rats.



Evaluation: In the second paragraph, Sponsor describes the ECL cell proliferative effects after life time exposure only in rats. The quoted dose was the maximum dose. Since the effects were noted at other doses also, quoting a dose range would be more representative of the data. The ECL proliferative effects were also elicited in mice in the carcinogenicity study. As indicated in the following table, the incidences of diffuse ECL cell hyperplasia and micronodular hyperplasia were increased in a dose related manner. Low incidences of carcinoid induction were also noted, though not in a dose related manner.

Incidences (Number of Animals)*

ECL Cell Proliferative Lesion	Treatment Groups (mg/kg/day)					
	0	15	75	150	300	600
Diffuse Hyperplasia						
Male	4	12	44	51	60	18
Female	6	13	42	53	54	34
Micronodular Hyperplasia						
Male	1	--	1	6	16	24
Female	1	--	4	6	7	23
Carcinoids						
Male	--	--	--	1	2	--
Female	--	1	--	--	1	--

* No. of animals treated = 70

Exclusion of the findings in mice gives the impression that the effect of lansoprazole is specific to a single species, i.e. rats.

Recommendation: Dose ranges used in rat and mouse studies should be included. A statement about the occurrence of ECL cell proliferative lesions in mice should also be included.

Division's Version:

Second Paragraph:

During lifetime exposure of rats with ⁵ up to 150 mg/kg/day of lansoprazole dosed seven days per week, marked hypergastrinemia preceded ECL cell proliferation and carcinoid formation, especially in female rats. ✓

ECL cell proliferative lesions were also seen in mice exposed to lansoprazole 15 to 600 mg/kg/day.

Data are needed to rule out the possibilities of tumors in patients receiving long-term therapy with lansoprazole.

Endocrine Effects**Sponsor's Version:*****Endocrine effects***

Human studies for up to eight weeks have not detected any clinically significant effects on the endocrine system. Hormones studied include testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEA-S), prolactin, cortisol, estradiol, insulin, aldosterone, parathormone, glucagon, thyroid stimulating hormone (TSH), triiodothyronine (T₃), thyroxine (T₄), and somatotrophic hormone (STH). Lansoprazole in oral doses of 15 to 60 mg for up to one year, had no clinically significant effect on sexual function. In addition, lansoprazole in oral doses of 15 to 60 mg for two to eight weeks had no clinically significant effect on thyroid function.

In 24-month carcinogenicity studies in rats with daily dosages up to 150 mg/kg, proliferative changes in the Leydig cells of the testes, including benign neoplasm, were increased over control incidence. No endocrine changes were observed in mice exposed for a lifetime at maximum tolerated dosages. Lansoprazole and especially a rat-specific metabolite (M-1) have been shown to slow testosterone synthesis and enhance testosterone elimination in the rat.

Evaluation:

The second paragraph above describes the results of 24-month carcinogenicity studies in rats with respect to the proliferative lesions in testes. The word "changes" was used instead of "lesions". It, also does not include the strain of the rat. It is important to quote the Sprague-Dawley strain of the rat, since the background incidence of testicular interstitial cell adenoma in this strain is very low (mean = 4.68%, range = 1.4 to 10.0%). Treatment related increased incidences of the lesions are meaningful when viewed in the context of background incidences in the strain. On the other hand, it is a very common tumor with high incidence of 80 to 98% in Fischer 344 rats (Goodman et al, Toxicology and Applied Pharmacology 48: 237-248, 1979 and Maekawa et al, Gann 74: 365-372, 1983) and 78% in Wistar rats (Teerds et al, J. Andrology, 12: 171-179, 1991) and treatment related increased incidence in these latter strains are not that meaningful. Many studies with pharmaceuticals employed these strains in the past. It is also not certain that the mechanisms involved are the same for each agent or the strain.

Sponsor did not include the observed treatment-related proliferative lesions in the Leydig cells of testes and the occurrence of interstitial cell tumors at a dose of 50 mg/kg/day in the 1-year toxicity study in Sprague-Dawley rats. No such tumors were observed in the concurrent control animals (NDA Volume 2.14).

It is also stated in the second paragraph that no endocrine changes were observed in mice exposed for a lifetime at maximum tolerated doses. This is an inaccurate statement. Treatment with lansoprazole (75, 150, 300 and 600 mg/kg/day) produced dose related significant ($p < 0.0014$) incidences (1.4 to 4.3%) of adenoma of rete testis, while the concurrent controls had no such incidence. This is a very rare tumor for animals and man.

The last sentence refers to a rat specific metabolite (M-I) and its presumed slowing effect on testosterone synthesis and enhancing effect on testosterone elimination in the rat. M-I metabolite is the reduction product of lansoprazole (a sulfide derivative). Designating this metabolite as rat specific is a misnomer. It is not a major circulating metabolite in rat. It was isolated in rat, dog and human samples (page 420, NDA volume 2.2). Its presumed slowing effect is based on an in vitro study of its effect on HCG stimulated testosterone synthesis in isolated rat Leydig cells. The metabolite was dissolved in 1N hydrochloric acid (10 µg/ml) due its low solubility. The report

(A-29-1733) does not provide the testosterone data in the placebo cultures, but expresses it as 100%. It is difficult to judge the data, in the absence of actual placebo data for the amount of testosterone when the solvent (1N hydrochloric acid) was added to the cultures as placebo. The recently provided submission (Amendment # 033, dated December 29, 1994) also did not provide such data.

Recommendation:

In the second paragraph, the strain of the rat should be identified as Sprague-Dawley with a low average background incidence of testicular interstitial cell adenoma. The findings of the proliferative lesions including the testicular interstitial cell adenoma in the 1-year toxicity study of rats should also be indicated. The statement of negative findings in mice should be replaced with the findings of rete testis adenoma in the carcinogenicity study. The last sentence referring to M-1 metabolite should be deleted.

Division's Version:

Endocrine effects

Human studies for up to eight weeks have not detected any clinically significant effects on the endocrine system. Hormones studied include testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEA-S), prolactin, cortisol, estradiol, insulin, aldosterone, parathormone, glucagon, thyroid stimulating hormone (TSH), triiodothyronine (T₃), thyroxine (T₄), and somatotrophic hormone (STH). Lansoprazole in oral doses of 15 to 60 mg for up to one year, had no clinically significant effect on sexual function. In addition, lansoprazole in oral doses of 15 to 60 mg for two to eight weeks had no clinically significant effect on thyroid function.

In 24-month carcinogenicity studies in rats with daily dosages up to 150 mg/kg, proliferative changes in the Leydig cells of the testes, including benign neoplasms, were increased over control incidence.

Sprague-Dawley

The incidence of such lesions are low in this strain of rat. Proliferative changes in the Leydig cells, including a benign neoplasm, was also seen after 1 year exposure to 50 mg/kg/day. In 24 month carcinogenicity studies in CD-1 mice, daily doses of 75 mg/kg/day or higher, produced a rare tumor-adenoma of rete testis.

Other Effects

Sponsor's Version:

Other effects

No systemic effects of lansoprazole on the central nervous system, lymphoid, hematopoietic, renal, hepatic, cardiovascular or respiratory systems have been found in humans. Other rat-specific findings after lifetime exposure included focal pancreatic atrophy, diffuse lymphoid hyperplasia in the thymus, and enhancement of the spontaneous retinal atrophy common to albino laboratory rats.

Based on _____ patients examined for visual disturbances, no visual toxicity has been determined as yet, but additional data are needed.

Evaluation:

Under "other effects", spontaneous retinal atrophy is depicted as common to albino laboratory rats with the implication that lifetime exposure produced increased incidence. There was a dose related increase due to lansoprazole treatment in both sexes. Focal retinopathy was detected in ophthalmoscopic examination in quite a few rats of both sexes at 6 months and 12 months in the 1-year chronic toxicity study, but it was considered unrelated to treatment. However, in the carcinogenicity study (IRDC), ophthalmoscopic examination was limited to lens only in the surviving animals during study week 95.

Recommendation:

It should be stated that no ophthalmoscopic examination of the retina was undertaken during the course of the carcinogenicity study.

Division's Version:

Other effects

No systemic effects of lansoprazole on the central nervous system, lymphoid, hematopoietic, renal, hepatic, cardiovascular or respiratory systems have been found in humans. Other rat-specific findings after lifetime exposure included focal pancreatic atrophy, diffuse lymphoid hyperplasia in the thymus, and spontaneous retinal atrophy. ←

Based on ___ patients examined for visual disturbances, no visual toxicity has been determined as yet, but additional data are needed.

However, no ophthalmoscopic examination of the retina was undertaken during the course of these studies.

Carcinogenesis, Mutagenesis, and Impairment of Fertility

Sponsor's Version

Rats and mice exposed continuously to 150 mg/kg/day or 300 mg/kg/day, respectively, for 24 months failed to provide evidence for a primary carcinogenic effect. Increased incidence of proliferative changes, leading to benign neoplasia in several tissues, were indicative of mechanisms secondary to hormonal perturbations within the animal model. They included gastric carcinoids secondary to sustained hypergastrinemia in female rats and Leydig cell tumors secondary to altered testosterone homeostasis in male rats. Gastric carcinoids have been shown to occur in female rats given proton pump inhibitors, albeit not in humans. The development of Leydig cell tumors in laboratory rats is enhanced by a wide variety of nongenotoxic pharmaceutical and dietary products and is confined to this species. A metabolite of lansoprazole (M-1) found in rats but not humans was shown to be the most potent inhibitor of testosterone synthesis in this species. Adverse hormonal effects from lansoprazole have been investigated but not shown to occur in humans.

Lansoprazole was not mutagenic in *in vitro* *Salmonella typhimurium* and *Escherichia coli* assays. A mouse micronucleus test at up to 5000 mg/kg (approximately 10,000 times the human dose) was negative for the induction of micronuclei. Results from a rat *in vivo/in vitro* unscheduled DNA synthesis assay in rat hepatocytes were negative. Also, a mammalian cell mutagenesis assay was negative.

In vitro cytogenetics studies showed increased levels of aberrations consisting mainly of chromatid breaks which occurred only at cytotoxic concentrations. These cytotoxic concentrations were at least 50 to 60 times expected clinical blood levels of parent drug. Therefore, such concentrations will not be reached in humans.

Lansoprazole had no effect on fertility in either male or female rats. In animals allowed to have a normal parturition, lansoprazole treatment produced a slight prolongation in gestation only at the highest dosage tested (150 mg/kg/day) which was also toxic to the dams.

Evaluation:

Sponsor's title for this subsection is missing the words "Impairment of" that should precede the word "Fertility". The description under the carcinogenicity portion of the label is nonspecific. It lacks specific information on the findings of the carcinogenicity studies in rats and mice. It doesn't provide any dose comparisons to recommended clinical dose.

Two acceptable twenty-four month rat (Sprague-Dawley) oral carcinogenicity studies of lansoprazole were conducted. In one study conducted at I.R.D.C. labs in U.S., lansoprazole was tested at doses of 5, 25, 75 and 150 mg/kg/day. One group of males was also treated for 18 months with 150 mg/kg/day followed by a drug free period of 6 months. In the other study conducted at Takeda in Japan, lansoprazole doses of 5, 15 and 50 mg/kg/day were tested. An additional group of male rats was treated with 50 mg/kg day for 18 months followed by a drug free period of 6 months.

Treatment with lansoprazole also produced statistically significant increased incidences of testicular interstitial cell adenoma in a dose related manner in IRDC study ($p < 0.0001$) and Takeda Study ($p < 0.0001$). In both studies the incidences of these tumors exceeded the incidences in the historical controls (1.88% in IRDC Study and 2.6% in Takeda Study). Unlike other strains of rats, the background incidence in Sprague-Dawley rats is very low (Mean 4.68%, Range 1.4 to 10%). In the animals in which the drug was withdrawn in the last 6 months of the study, it did not reduce the incidence in the Takeda Study, while in the IRDC Study it reduced only slightly (67.1% in animals treated for 24 months vs 51.4% in animals treated for 18 months, $p = 0.04$). Testicular interstitial cell adenoma also occurred in one of thirty male Sprague-Dawley rats (3.3%) in the 1 year toxicity study which was indicative of decreased latency. The sponsor tried to minimize the occurrence of the tumors by citing the occurrence with other pharmaceuticals and dietary products. This statement is not acceptable since other strains of rats with high background incidence of this tumor (Wistar rat - 78%, Fischer 344 rat - 98%) may have been employed with those products. The sponsor did not provide a convincing explanation for the mechanism involved for the occurrence of this tumor. Their efforts to imply altered testosterone secretion and LH secretion are unconvincing and at best highly equivocal and contradictory.

Lansoprazole treatment did not have any effect on serum testosterone and LH levels throughout the Takeda Study. Sponsor did not explore the possibility of any involvement of testicular paracrine/autocrine products such as Inhibin or Activin in the tumorigenesis. Both hormones are produced in the testicular interstitial cells and sertoli cells and they do influence the Leydig cell function in vitro (J.M. Saez, Endocrine Reviews 15:574-626, 1994). In the last but one sentence under carcinogenesis information, it was stated that a metabolite of lansoprazole (M-1) found in rats but not humans was the most potent inhibitor of testosterone in this species. This statement is not accurate and unacceptable due to several reasons. M-1 metabolite is the reduction product of lansoprazole (a sulfide derivative). Designating it as rat specific is a misnomer. It is not a major circulating metabolite in the rat. It was isolated in rat, dog and human materials (Page 420, NDA Volume 2.2). It was not tested in vivo. Its presumed inhibitory effect is based on an vitro study of its effect on HCG-stimulated testosterone synthesis in isolated rat Leydig cells. In that study, it was dissolved in 1 N hydrochloric acid (10 µg/ml) due to its low solubility. The report (A-29-1733) does

not provide the specific testosterone data for the placebo effect (1 N hydrochloric acid) except showing it as 100%. It is not possible to judge the effect of the metabolite in the absence of placebo data.

The labeling portion for carcinogenicity did not include any statement about the treatment related occurrence of retinal atrophy. Although a reference to this is made under "other effects" in "Pharmacodynamics" Section, it should be stated under carcinogenesis also. Its occurrence (IRDC Study) was dose related in females (5 to 150 mg/kg/day) and males (25 to 150 mg/kg/day). Focal retinopathy was also detected on ophthalmoscopic examination in a few rats of both sexes at 6 months and 12 months in the 1-year toxicity study, but it was considered unrelated to treatment. In the carcinogenicity studies, ophthalmoscopic examination of the retina was not undertaken. It was therefore not possible to determine the time of onset of this retinopathy. The sponsor did not provide a specific convincing mechanism involved for the lansoprazole induced retinal atrophy in rats. Although sponsor implied that the effect of lansoprazole is mediated through depletion of Taurine in serum and retina, no such effect was clearly demonstrated in the studies submitted so far. Therefore it is necessary to mention in the labeling that the mechanism involved is unknown and the risk to patients on long term treatment is also unknown.

One acceptable twenty-four month oral carcinogenicity study was conducted in CD-1 mice at IRDC labs. This study was initiated in February 1991 with doses of 15, 75, 150 and 300 mg/Kg/day doses. Another dose of 600 mg/Kg/day with concurrent controls was added in September, 1991. Treatment with lansoprazole produced a dose related increase in gastric ECL cell proliferative effects as indicated by the increased incidences of diffuse hyperplasia and micronodular hyperplasia in both sexes. It also produced gastric ECL cell carcinoid tumors, but the incidences were low and not dose related (see Table below).

Incidences (Number of Animals)*

ECL Cell Proliferative Lesion	Treatment Groups (mg/kg/day)					
	0	15	75	150	300	600
Diffuse Hyperplasia						
	4	12	44	51	60	18
	6	13	42	53	54	34
Micronodular Hyperplasia						
	1	--	1	6	16	24
	1	--	4	6	7	23
Carcinoids						
	--	--	--	1	2	--
	--	1	--	--	1	--

* No. of animals treated = 70

Lansoprazole treatment also increased the incidence of liver tumors (hepatocellular adenoma plus carcinoma) in male mice ($p < 0.0001$) and female mice ($p < 0.0001$). The tumor incidences in the male mice of 300 and 600 mg/kg/day groups (22.8 and 27.1%, respectively) exceeded the incidence range in the historical controls (1.8 to 21.8%) and the strain background incidence range (0-17.3%). The incidences in the female mice of 150, 300 and 600 mg/kg/day groups (5.7 to 10.0%) exceeded the strain background incidence range

(0-2.8%). Lansoprazole treatment produced adenoma of rete testis in the male mice of 75, 150, 300 and 600 mg/kg/day groups. This is a rare tumor for mice. Its incidence of 1.4 to 4.3% was statistically significant ($p=0.0014$).

The mutagenicity portion of the labeling describes that a mammalian cell mutagenesis assay was negative. This description is nonspecific. The conducted assay was mouse lymphoma (L5178Y) cell/HGPRT forward mutation assay. The HGPRT locus in this cell system is not suitable to evaluate the clastogenic component of a genotoxic compound (Moore et al, Mutagenesis 4: 394-403, 1989) and therefore reference to this test should be deleted. It is not necessary to quote the doses of the bioassays. Lansoprazole produced repeatable positive clastogenic effects in the in vitro human lymphocyte chromosomal aberration tests. It should be clearly stated.

In the fertility portion reference to length of gestation is not needed since parturition was not affected.

Recommendation:

The carcinogenicity portion of the labeling should incorporate the findings of the carcinogenicity studies in rats and mice. The dose relationships between tested doses to recommended clinical dose should be incorporated. The portion dealing with the mutagenicity tests needs to be rewritten. The fertility section should be revised : excluding the reference to prolongation of gestation.

Division's Version:

In two 24-month carcinogenicity studies, Sprague-Dawley rats were treated orally with doses of 5, 15, 25, 50, 75 and 150 mg/kg/day (30, 90, 150, 300, 450 and 900 mg/m²/day). For a 50 kg person of average height (1.46 m² body surface area), these doses represent 1.4, 4.1, 6.8, 13.5, 20.3 and 40.5 times the recommended human dose (22.2 mg/m²/day, based on a dose of 30 mg/day) on a body surface area basis. Lansoprazole produced gastric enterochromaffin like (ECL) cell hyperplasia and ECL cell carcinoids in a dose-related manner in both male and female rats. It increased the incidence of intestinal metaplasia

of the gastric epithelium in both sexes. A dose related increased incidence of retinal atrophy was seen in female rats receiving 5 to 150 mg/kg/day (30 to 900 mg/m²/day, 1.4 to 40.5 times the recommended human dose based on body surface area) and male rats receiving 25 to 150 mg/kg/day (150 to 900 mg/m²/day, 6.8 to 40.5 times the recommended human dose based on body surface area). The timing of the onset of retinopathy and the specific mechanism involved were not determined. In male rats, lansoprazole treatment produced increased incidence of testicular interstitial cell adenoma in a dose-related manner. The incidences in rats receiving doses of 15 to 150 mg/kg/day (90 to 900 mg/m²/day, 4.1 to 40.5 times the recommended human dose based on body surface area) exceeded the low background incidence (range = 1.4 to 10%) for this strain of rat. Testicular interstitial cell adenoma also occurred in one of thirty rats treated with 50 mg/kg/day dose (300 mg/m²/day, 13.5 times the recommended human dose based on body surface area) in the 1-year toxicity study. The mechanism involved was not clearly established.

In a 24-month carcinogenicity study, CD-1 mice were treated orally with doses of 15, 75, 150, 300 and 600 mg/kg/day (45, 225, 450, 900 and 1800 mg/m²/day). These doses represent 2.0, 10.1, 20.3, 40.5 and 81.0 times the recommended human dose based on body surface area. Lansoprazole produced a dose related increased incidence of gastric ECL cell hyperplasia. It produced an increased incidence of liver tumors (hepatocellular adenoma plus carcinoma). The tumor incidences in male mice treated with 300 and 600 mg/kg/day doses (900 and 1800 mg/m²/day, 40.5 and 81.0 times the recommended human dose based on body surface area) and female mice treated with 150, 300 and 600 mg/kg/day doses (450, 900 and 1800 mg/m²/day, 20.3, 40.5 and 81.0 times the recommended human dose based on body surface area) exceeded the ranges of background incidences in historical controls and this strain of mice. Lansoprazole treatment produced adenoma of rete testis in male mice receiving 75, 150, 300 and 600 mg/kg/day (225, 450, 900 and 1800 mg/m²/day, 10.1, 20.3, 40.5 and 81.0 times the recommended human dose based on body surface area. Adenoma of rete testis is a very rare tumor in mice.

Lansoprazole was not mutagenic in in vitro Ames test, ex vivo rat hepatocyte UDS test, and in vivo mouse micronucleus test and rat bone marrow cell chromosomal aberration test. It was, however, positive in in vitro human lymphocyte chromosomal aberration assays.

Lansoprazole at oral doses up to 150 mg/kg/day (900 mg/m²/day, 40.5 times the recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

Pregnancy

Teratogenic Effects. Pregnancy category ---

Sponsor's Version:

Pregnancy

Pregnancy category B

In two teratology studies, lansoprazole at dosages up to 300 mg/kg/day (approximately 600 times the human dose) was administered to rats on Days 6 to 17 of pregnancy. At higher dosages (150 to 300 mg/kg/day), only decreased fetal body weights were observed. Also at higher dosages, reduced ossification of vertebrae was indicative of dose-related fetal toxicity. These fetal effects were only found at doses which caused maternal toxicity.

In rabbits, doses of lansoprazole up to 30 mg/kg/day (approximately 60 times the human dose) were administered on Days 6 to 16 of pregnancy. A dose-related effect on fetal mortality at 30 mg/kg/day was noted, but there were no treatment-related external, skeletal, or visceral abnormalities. These fetal effects were only found at doses which caused maternal toxicity.

Lansoprazole is not considered to be teratogenic.

There are no adequate or well-controlled studies in pregnant women. Therefore, lansoprazole should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Evaluation:

The statement about the maternally toxic dose of 300 mg/kg/day in rat teratology study needs to be deleted. The description needs revisions. The description of the results in rabbit teratology study also needs revision. The decrease in the number of fetuses was erratic and had no dose relationship. The last paragraph referring to pregnant woman did not provide correct description for a pregnancy category B classification.

Recommendations: The entire description needs to be revised.

Division's Version

Pregnancy

Teratogenic Effects. Pregnancy Category B. Teratology studies have been performed in pregnant rats at oral doses up to 150 mg/kg/day (900 mg/m²/day, 40.5 times the recommended human dose based on body surface area) and pregnant rabbits at oral doses up to 30 mg/kg/day (354 mg/m²/day, 16.0 times the recommended human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to lansoprazole.

There are, however, no adequate or well-controlled studies in pregnant woman. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Nursing Mothers

Sponsor's Version:

Nursing Mothers

Lansoprazole or its metabolites are excreted in the milk of rats. It is not known whether lansoprazole is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when lansoprazole is administered to a nursing woman.

Evaluation:

The Sponsor's labeling accurately depicts that lansoprazole or its metabolites are excreted in the milk of rats. However, it does not address the tumorigenic potential of lansoprazole in rats and mice. Since lansoprazole was tumorigenic in rat and mouse carcinogenicity studies the labeling needs to be revised to reflect the potential.

Recommendation:

The labeling needs revision to reflect the tumorigenic potential.

Division's Version:

Nursing Mothers

Lansoprazole or its metabolites are excreted in the milk of rats. It is not known whether lansoprazole is excreted in human milk. Because many drugs are excreted in human milk,

and because of the potential for tumorigenicity shown for lansoprazole in rat and mouse carcinogenicity studies, the 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:

Sponsor's Version:

Overdosage

In rats and mice, oral administration of lansoprazole in doses up to 5000 mg/kg (30,750 and 27,800 mg/m², respectively; approximately 10,000 times the human dose) did not result in animal deaths but only affected the color of the urine in mice. Lansoprazole is not removed from the circulation by hemodialysis.

Evaluation:

The mg/m² dose for rats and mice are incorrect. It needs to be stated that no clinical signs of toxicity were elicited. The dose ratio to human dose also needs to be corrected.

Recommendation:

Division's Version:

Overdosage

Oral doses up to 5000 mg/kg in rats (30,000 mg/m², 1351.4 times the recommended human dose based on body surface area) and mice (15,000 mg/m², 675.7 times the recommended human dose based on body surface area) did not produce deaths or any clinical signs.

✓ Lansoprazole is not removed from
the circulation by hemodialysis.

Jasti B. Choudary 1/28/95
Jasti B. Choudary, Ph.D., B.V.Sc.

CC:
NDA 20,406
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd

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NDA 20-406

Review #1

SPONSOR & ADDRESS: TAP Pharmaceutical Inc.,
Deerfield, Il.

REVIEWER: Yash M. Chopra, M.D., Ph.D.,
Pharmacologist

DATE OF REVIEW: January 9, 1995

DATE OF SUBMISSION: Original - November 12, 1993
Amendment - December 22, 1993
Amendment - February 18, 1994
Amendment - April 5, 1994
Amendment - June 30, 1994

HFD-180 RECEIPT DATE: Original - November 17, 1993
Amendment - December 23, 1993
Amendment - February 22, 1994
Amendment - April 6, 1994
Amendment - July 1, 1994

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

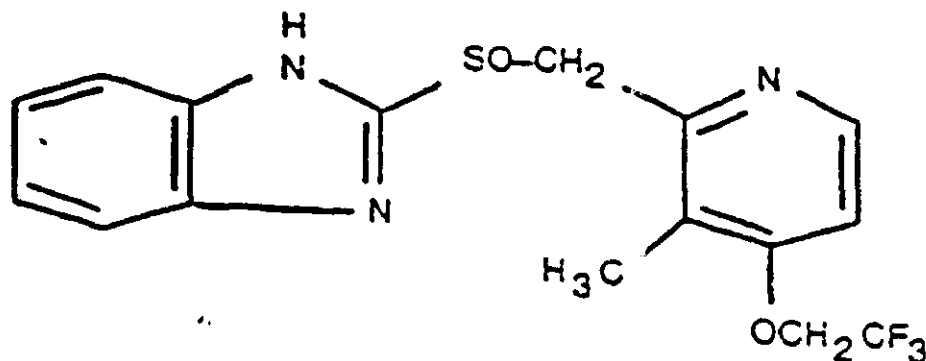
DRUG: Lansoprazole/PREVACID[®]/AG 1749/A-65006 15/30 mg Capsules

CHEMICAL NAME: 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]sulfinyl]benzimidazole.

MOLECULAR WEIGHT: 369.37

MOLECULAR FORMULA: C₁₈H₁₄F₃N₃O₂S

STRUCTURAL FORMULA:



FORMULATION: Each Prevacid delayed release capsule contains either 15 or 30 mg of lansoprazole in enteric coated granules. The formulation of the capsule is shown below:

	<u>15 mg</u>	<u>30 mg</u>
<u>CORE</u>		
\ lansoprazole		
\ magnesium carbonate, USP		
\ sugar spheres, NF		
\ sucrose, NF		
\ starch, NF		
\ low substituted, hydroxypropyl cellulose, NF		
\ hydroxypropyl cellulose, NF		
<u>ENTERIC LAYER</u>		
\ methacrylic acid copolymer		
\ polyethylene glycol, NF		
\ titanium dioxide, USP		
\ polysorbate 80, NF		
\ talc, USP		
\ colloidal silicon dioxide, NF		
<u>TOTAL</u>		

Each 30 mg capsule contains mg of methacrylic acid copolymer
 . Therefore, a person will be ingesting
 kg/day of during the treatment with lansoprazole.
 This amount of is within the previously approved quantity
 of 7.5 mg/kg/day in Erythromycin capsule formulation
 (NDA 62546). The contents of other inactive ingredients in the
 formulation were within the GRAS listing limits.

CATEGORY: Antisecretory Agent/Parietal cell (H^+-K^+)-ATP Pase
 Pump Inhibitor

RELATED INDs No: (1)
 , and (2)

MARKETING INDICATION: Lansoprazole is indicated in the short term treatment of reflux esophagitis, duodenal ulcers and long term treatment of pathological hypersecretory conditions including Zollinger-Ellison syndrome (ZES).

DOSE: Lansoprazole is recommended at an oral dose of 30 mg/day (or 0.6 mg/kg/day based on the assumption that an adult weighs 50 kg) before eating up to 8 weeks which is followed by a maintenance dose of 0.3 mg/kg/day for a long period. It is recommended at a dose of 15 mg/day (i.e., 0.3 mg/kg/day) orally up to 4 weeks in the treatment of duodenal ulcers. A starting daily single dose of 60 mg (i.e., 1200 ug/kg/day) has been recommended in pathological hypersecretory conditions including Zollinger-Ellison syndrome (ZES) which could be increased up to 3600 ug/kg/day for more than 3 years.

PRECLINICAL STUDIES AND TESTING LABORATORIES

Type of Study	Study/Report#	Drug Batch#	Lab Name	Page #

I. PHARMACOLOGY

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ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION:

Absorption and Pharmacokinetics:

1. Absorption of Orally Administered Lansoprazole and Omeprazole in 2 Vehicles in Rats	RED/91/663	..	Abbott	
2. Absorption of Plain Tablet, Enteric Coated Tablet or Enteric Coated Granules	A-29-578	Not Given	TK-CRD	
3. Effect of Dosage Forms of Lansoprazole on Bioavailability of Lansoprazole in Dogs and Man	A-29-1725	Not Given	TK-CRD	
4. Multiple Dose Pharmacokinetics in Female Cynomolgus Monkeys	A-29-1490	Not Given	TK-CRD	
5. Single Dose Pharmacokinetics in Rats: Dose Dependency	A-29-625	..	TK-CRD	
6. Single Dose Pharmacokinetics in Mice: Dose Dependency	A-29-704	Not Given	TK-CRD	
7. Single Dose Pharmacokinetics in Female Dogs	A-29-697	Not Given	TK-CRD	
8. Single Dose PK-In Vitro of Stereoisomers in Rats and Dogs	A-29-908	Not Given	TK-CRD	
9. Multiple Oral Dose Pharmacokinetics in Rats	A-29-529	..	TK-CRD	
10. Multiple Dose Pharmacokinetics in Dogs (Capsules)	A-29-628	Not Given	TK-CRD	
11. Multiple I.V. Dose Pharmacokinetic in Female Rats	A-29-1358	62-214AL	Abbott	
12. Multiple I.V. Dose Pharmacokinetics in Female Dogs	A-29-1359	--	TK-CRD	

13.Fate/Metabolism of p.o./i.v. Administered Lansoprazole	A-29-162	Not Given	TK-CRD
14.Pharmacokinetics/ Drug Metabolism Study in Rats and Dogs	A-29-876	Not Given	TK-CRD
15.Absorption of Orally/i.d. Administered Lansoprazole in Pylorus Ligated Rats	A-29-876	"	TK-CRD
16.Pharmacokinetics in Rats, Dogs and Man	A-29-1411	"	TK-CRD
17.10-Day Oral Toxicokinetics in Mice	A-29-696 R&D/90/380	"	TK-CRD

Distribution:

18.In vitro Protein Binding Study in the Rat, Dog and Human Plasma Samples	PPRD/88/201	A87-1027	Abbott
19.In vitro Erythro- cytic Binding in Rat, Dog and Human Plasma	A-29-876	Not Given	TK-CRD
20.Distribution of ¹⁴ C Lansoprazole in Rats and Dogs			
21.Placental Transfer of Lansoprazole after Oral dosing	A-29-702	"	TK-CRD
22.Autoradiographic Detection of Lansoprazole in Gastric Mucosa of Rats after P.O. dosing	A-29-1200	"	TK-CRD
23.Autoradiographic Detection of Lansoprazole in Gastric Mucosa of Rats after I.V. Dosing	A-29-1779	"	TK-CRD
24.Tissue Distribution in Mice after Repeated Oral Administration	A-29-936	"	TK-CRD
25.Tissue Distribution in Mice	A-29-1411	"	TK-CRD
26.Comparison of Distribution and Metabolism of Single I. V. and Oral Dose in Dogs	PPRD/88/344	06-677-A	Abbott

27. Metabolic Fate After I.V. Dosing in Male Rats and Dogs	A-29-1779	Not Given	TK-CRD

Metabolism:

28. In vitro Effects on Drug Metabolizing Enzymes	A-29-876	Not Given	
29. Hepatic Drug Metabolizing Activity in Mice and Rats After 14 Days Treatment with Lansoprazole	R&D/93/719	62-214	Abbott

Excretion:

30. Secretion in Milk of Lactating Rats	A-29-876	Not Given	TK-CRD
31. Excretion in Rats and Dogs	A-29-876	Not Given	TK-CRD
32. Biliary Excretion and Absorption in Rats	A-29-876	Not Given	TK-CRD

III. TOXICOLOGY

1. Acute Toxicity Studies:

a. Rat (p.o., s.c., i.p)	A-29-182	M610-002	TK-CRD
(i.v.)	A-29-898	233820	"
b. Mouse (p.o., s.c., i.p)	"	"	"
(i.v.)	"	"	"
c. Dog (p.o.)	A-29-1167	"	"

2. Acute Toxicity of Metabolites and Related Compounds in Rats and Mice	A-29-576	---	TK-CRD

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY STUDIES:

RATS

3. 2-Week I.V.	A-29-504	M610-016	TK-CRD
4. 4-Week I.V.	A-29-805	M610-016	TK-CRD
5. 4-Week Oral	A-29-167	M610-221	TK-CRD
6. 13-Week Oral	A-29-168	M610-002	TK-CRD
7. 13-Week Oral with 1 Month Recovery Period	A-90-151	M610-022	Abbott
8. 13-Week Oral with 4, 13 and 26 Weeks Recovery	A-29-452	M610-004	TK-CRD
9. 13-Week Oral	A-29-174	M610-	

10. 6-Month Oral	A-29-345	M610-005
11. 12-Month Oral	A-29-438	M610-005

MOUSE:

12. 13-Week Oral	A-126-049	38-006-AL
13. 3-Month Oral Dose Ranging	A-29-221	M610-003

DOG:

14. 2-Week I.V.	A-29-505	M-610-016	TK-CRD
15. 4-Week I.V.	A-29-806	M610-016	TK-CRD
16. 4-week Oral	A-29-169	M610-001	TK-CRD
17. 13-week Oral	A-29-170	M610-002	TK-CRD
18. 13-Week I.V.	A-29-1861	M610-050	
19. 6-Month Oral	A-29-348	M610-005	
20. 12-Month Oral	A-29-439	M610-005	

MONKEY:

21. 4-Week Oral Toxicity Study	A-29-1911	Z 528S24

CARCINOGENICITY STUDIES:

1. 78-Week in Mouse (5 days/week)	A-29-680	42-057-AL M610- 038, 0-49 and 050	
2. 104-Week in Mouse	R&D/93/547	,,	
3. 104-Week in Mouse	R&D/93/731	,,	
4. 104-Week in Rat	A-29-681	M610-004, 5 & 7	
5. 104-Week in Rat	R&D/93/546	M610-049	
6. 104-Week in Rat	A-29-1863	M610-038	TK-CRD

V. REPRODUCTIVE TOXICITY:

1. Oral Segment I. Toxicity in Rats	A-29-292	M610-004	
2. Oral Segment I. in Rats	TA-90-146	42-057-AL	
3. I. V. Segment I. in Rats (Males treated)	TA-29-1773	Z338201/ Z33802	TK-CRD

4. Oral Segment II. in Rats (Teratogenicity)	A-29-144	M610-001	TK-CRD
5. Oral Segment II (Modified Teratogenicity) Study in Rats	A-29-366	M610-004	
6. I.V. Segment II. (Teratogenicity) Study in Rats	A-29-1783	Z338201	TK-CRD
7. Oral Segment II. (Teratogenicity) Study in Rabbits	A-29-354	M610-004	
8. Oral Segment III. (peri & post natal) in rats	A-29-099	M610-004	
IV. MUTAGENICITY:			
1. Ames test	A-29-181	M610-001	TK-CRD
2. Mouse Micronucleus Test	A-29-251	M610-004	TK-CRD
3. Mouse Lymphoma Assay	A-29-710	M610-016	
4. In Vivo/In Vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay	A-29-718	M610-018	
5. In Vitro Chromosomal Aberration Test in Human Lymphocytes	A-29-650	M610-016	
6. In Vitro Chromosomal Aberration Test in Human Lymphocytes	A-29-699	M610-018	TK-CRD
7. In Vivo Bone Marrow Chromosomal Aberration Assay in Rats	TA-90-270	42-057-AL	
8. Micronucleus Test in ICR Mice	A-29-698	M610-018	TK-CRD
VI. SPECIAL TOXICITY STUDIES:			
1. In vitro Hemolytic Test in Rats	A29-902/ A29-1782	Z2338201	TK-CRD
2. In vitro Hemolytic Test in Rabbits	A29-901/ A29-1781	--	TK-CRD
3. I.V. Irritation Test in Rabbits	A29-900/ A29-1780	Z2338201	TK-CRD
4. Paravenous Irritation in Rabbits	A29-1818	Z2338201	TK-CRD

5. Antigenicity in Guinea Pigs and Mice	A29-1182	M610-016	TK-CRD
6. Immunotoxicity in Female Rats	TA-90-160	06-677AL	
7. In Vitro Effect on Leydig's Cell Testosterone Synthesis	PPRD/90/111	--	
8. Effect on Leydig's Cells Testosterone Synthesis in Presence of Testosterone Precursors in vitro	A-29-807	--	Abbott
9. Effect of Metabolites on hCG-stimulated Testosterone Production in Rat Leydig's Cells in Vitro	A-1548	--	TK-CRD
10. Effect of Acute and Chronic Treatment of Lansoprazole on Plasma Clearance and Metabolism of ³ H-Testosterone in Male Rats	R&D/92/493 V92-015	53-143-A1	Abbott
11. Effect on Testosterone Synthesis Pathway in Rat Leydig's Cells	A-29-1549	--	TK-CRD
12. Hormone Levels After Acute Dose of lansoprazole in rats	PPRD/90/045	--	
13. Effect of Metabolites on hCG stimulated Serum Testosterone in rats	A-29-1352	--	TK-CRD
14. Lansoprazole and its Metabolites on hCG stimulated testosterone in rats	A29-1733	--	TK-CRD
15. Serum Male Hormone in Male Rats Treated with Lansoprazole for 1 month	R&D92-024/ A91-310	--	Abbott
16. Effect on Steroidogenic Pathways Enzymes of Leydig's cells in Vitro	R&D/92/507	--	
17. Inhibition of Testosterone Synthesis in Mouse Leydig Cells	A-29-975	--	TK-CRD

18. Effect on Serum Testosterone and LH Levels in Mice	A-29-974	--	TK-CRD
19. On the possible Increased Incidences of Leydig's cell Tumor in 2-Year Rat Carcinogenicity Study	A-29-764	--	TK-CRD
20. Effect of Lansoprazole on Various Intervals of Mating and Gestation Periods in Rats: Preimplantation Losses and Female Sex Hormones	R&D93/539 A-93-021	--	TK-CRD
21. Serum Testosterone After Single Dose of Lansoprazole in Rats treated with Loading Doses of hCG	A-29-1532	--	TK-CRD
22. Testosterone Supplement Effect on Lansoprazole Induced Hyperplasia and Leydig Cells Tumors in Aged F33 Rats	A-29-1533	--	TK-CRD
23. Study on Adrenal Steroidogenesis After Loading Doses of ACTH in Rats	A-29-1547	--	TK-CRD
24. In Vitro Interaction of Lansoprazole and its Metabolites with Taurine	A-29-2036	M610-050	TK-CRD
25. Rat Electroretinography in 4-Week Toxicity Study	A-29-1990	M610-050	TK-CRD
26. Rat Electroretinography of 13 Week Toxicity Study	A-29-1991	M610-050	TK-CRD
27. Electroretinography of Rats included 13-Week Intravenous Tox. Study	A-29-1992	M610-050	TK-CRD
28. Taurine Levels of Liver, Retina and Serum in Young Rats of 4-Week Toxicity Study	A-29-2040	M610-050	TK-CRD
29. An Exploratory Report on Plasma Taurine Regulation of Female Rats in 3-Month Toxicity Study	TA-93-442	M610-050	TK-CRD

30. Distribution Tagged Lansoprazole and omeprazole in Ocular Tissues	A-29-2001	---	TK-CRD
31. Light Microscopic Autoradiography of I.V. Administered ¹⁴ C-Lansoprazole in Female Rats and Mice	CMR 93192	---	Abbott
32. Testosterone and LH of Rats after 19-week Subchronic Treatment in Aged Rats	A-29-1532	--	TK-CRD
33. In Vitro, Steroido- genic Enzymes Pathway Inhibition in Rat Liver	A-29-1547	--	TK-CRD

Abbrev Used: TK-CRD=Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan;

Abbott= Drug Metabolism & Toxicology Division, Abbott Laboratories, Abbott Park, Chicago.

The following studies submitted with initial submission of IND (capsules) and its various amendments dated 7/13/1990, 7/27/1990, 8/30/90, 5/1/91, 6/27/91, 7/29/91 and 3/12/92. These were reviewed earlier and have been incorporated in the present review: ADME Studies- (1) Localization of Lansoprazole in gastric mucosa: autoradiographic study- (Study # 29-1200) Tissue levels after repeated oral dosing in mice (Study # A-29-936), (2) Single dose kinetics & in-vitro metabolism of stereoisomers of Lansoprazole (Study # A-29-908), TOXICOLOGY (1) Acute toxicity studies in rats, mice and dogs (Study # A-29-182), (2) Acute toxicity of the partially degraded AG-1749, related compounds and its metabolites in mice (Study # A-29-576), (3) 4-week oral toxicity study in rats (Study # A-29-167), (4) 4-week i.v. toxicity in rats (Study # 805), (5) 4-week supplement i.v. toxicity in rats (Study # A-29-168) (6) 4-week i.v. toxicity in beagle dogs (Study # A-29-806), (7) 13-week oral toxicity study in rats (Study # A-29-168) (8) 13-week oral toxicity study in rats with a recovery group (Study # A-29-452), (9) 6-month oral toxicity study in rats (Study # A-29-345), (10) 12-month oral toxicity study in rats (Study # A-29-438), (10) 4-week oral toxicity study in dogs (Study # A-29-169), (11) 13-week oral toxicity study in dogs (Study # A-29-170), (12) 6-month oral toxicity study in dogs (Study # A-29-348) (13) 12-month oral toxicity study in dogs (Study # A-29-439). REPRODUCTIVE TOXICITY: (1) Segment I. Reproductive Toxicity in Rats (Study # A-29-292) (2) Segment I. Reproductive Toxicity Study in Rat (Study # RD90/249) (3) Segment II. (Teratogenicity)

toxicity in rats (Study # A-29-144), (4). Segment II. (Modified Teratogenicity) toxicity study in rats (Study # A-29-366), (5). Segment II (Teratogenicity) study in rabbits (Study # A-29-354) and (6) Segment III (Peri-and Postnatal) toxicity study in rats (Study # A-295-099). **MUTAGENICITY:** (1) Ames test (Study # A-29-181) (2) mouse micronucleus test (Study # A-29-251) (3) rat bone marrow cytogenetic assay (Study # R&D 90/387) (4) micronucleus test in ICR mice (Study # A-29-698) (5) mouse lymphoma assay (Study # A-29-710) (6) rat hepatocyte unschedule DNA synthesis assay (Study # A-29-718) (7) human lymphocyte chromosomal aberration assay (Study # A-29-718) (8) human lymphocyte chromosomal aberration assay (Study # A-29-699). **SPECIAL TOXICITY STUDIES:** (1) Antigenicity of Lansoprazole in guinea pigs and mice (Study # A-29-1182) (2) Immunotoxicity in rats (Study # R&D 90/278).

The following studies submitted with initial submission of IND (Injection) and were reviewed earlier (Pharmacology Review dated September 6, 1993). These reviews have been incorporated in the present review: (1) ADME studies in mice, rats and dogs (Study # A-29-177), (2) distribution study in rats, (3) i.v. metabolism studies in dogs (Study # PPRd 88/344, (4) Metabolic fate in mice, rats and dogs (Study # A-29-876) (5) Multiple dose studies in rats (Study # A-29-876). Toxicity Studies: (1) Acute toxicity in rats and mice (2) 2-week i.v. toxicity study in rats (Study # A-29-504) (3) 4-week (i.v.) study in rats (Study # A-29-805), (4) 2-week (i.v.) (Study # A-29-505), 4-week (i.v.) (Study # A-29-806) and 13-week (i.v.) toxicity studies in dogs (Study # A-29-1861). Reproductive Toxicity: (1) Fertility and general reproductive performance (Segment I) toxicity study in rats (i.v.) (Study # A-29-1783) (2) Teratology (Segment II) study in rats (i.v.) (Study # A-29-902 & A-29-1782). Special Toxicology Studies: (1) Hemolytic potential of lansoprazole (2) I.V. irritation Study in rabbits (Study # A-29-900) (3) Paravenous irritation Study (A-29-1818) (4) In vitro hemolysis study (A-29-1780) (5) Effect on hepatic drug metabolizing enzymes (A-29-876).

GOOD LABORATORY PRACTICE & QAU REGULATIONS:

Sponsor has included the statements that all preclinical studies were conducted in compliance with GLP and QAU regulations.

PHARMACOLOGY

The synthesis and secretion of gastric hydrochloric acid is initiated by an active transport of

Lansoprazole, a synthetic benzimidazole derivative, has been claimed to inhibit gastric acid secretion by inhibiting canalicular (H^+-K^+) -ATPase enzyme. It has been shown to cross into gastric parietal cells where it converts into metabolite that binds directly to the sulfhydryl group on the (H^+-K^+) -ATPase enzyme. The pharmacology of the compound is reviewed below:

Primary Pharmacology

A. In Vitro Studies:

1. Inhibition of Gastric Microsomal (H^+-K^+) -ATPase Activity:
 (H^+-K^+) -ATPase activity of gastric microsomes separated from fundic portion of dog and pig stomachs was measured in the absence/presence of lansoprazole. Lansoprazole (from uM concentration) inhibited (H^+-K^+) -ATPase activity of microsomes in a concentration dependent manner. IC_{50} values were 6.3 and 8.6 uM in the dog and pig microsomes, respectively.

2. Effect on Acid Formation in Histamine, carbachol or Dibutyryl Cyclic AMP Stimulated Isolated Canine Parietal Cells:
Canine gastric parietal cells were isolated by the method of Soll A.H. (J. Clin. Inves. 61: 370, 1978) and incubated at 37°C with 2 ml Earle's balanced salt solution containing 0.2 uCi of ^{14}C -aminopyrine. The accumulation of (^{14}C)-aminopyrine (an indicator for acid formation) in parietal cell was measured. 10^{-3} to $10^{-6}M$ concentrations of lansoprazole in the presence of 10 uM IBMX (a phosphodiesterase inhibitor), inhibited acid formation stimulated by 10 uM of histamine. Its IC_{50} value was 0.088 uM.

It inhibited the acid formation stimulated by uM of carbachol in a concentration dependent manner. Its IC_{50} value was 0.081 uM.

Dibutyryl c-AMP (1 mM) stimulated acid formation was also depressed by in a concentration dependent manner and IC_{50} value was uM.

PURGE

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B. (In Vivo Studies):

1. Effect of Lansoprazole on Surface pH of Gastric Mucosa: In 24 hr fasted 7 weeks old rats (JCL:SD strain), lansoprazole was administered at an oral dose of 1 to 30 mg/kg (in 5 % gum acacia, pH 4.5). Six rats/group were killed 0.25, 0.5, 2, 4 and 8 hr after the drug administration. Lansoprazole produced an increase in pH from 3 mg/kg dose significantly after 15 min of its administration. Its effect lasted for 1, 8 and 8 hr in animals included in 3, 10 and 30 mg/kg treatment groups. The pH of gastric mucosa of animals included in 3, 10 and 30 mg/kg treatment groups were: 3.7, 4.6 and 6.1 at 15 min. Therefore, lansoprazole increased gastric surface pH from an oral dose of 3 to 30 mg/kg.

2. Effect on Gastric Emptying in Rats: In 24 hr fasted rats, lansoprazole in 5 % gum acacia at an oral dose of 10 to 100 mg/kg, p.o. was given. Phenolsulfonphthalein (PSP) solution was administered 1 hr after the administration of the compound and the distance travelled by PSP in the drug treated and the control animals was determined. Lansoprazole showed no effect on gastric emptying of PSP.

3. Effect on Rat Gastric Mucosal Hemodynamics in hemorrhagic Shock Model: In anaesthetized rats, blood representing 2 % of the body weight (from carotid artery) was removed over 15 min beginning 30 min after the drug administration. The gastric mucosal blood volume was measured every 5 min by using organ

reflectance spectrophotometry before shock and 30 min after the drug administration. The gastric mucosal blood volume was measured after histamine (30 mg/kg, s.c.) or lansoprazole (10, 100 mg/kg, i.d.) during shock period. Lansoprazole suppressed the decrease in the gastric mucosal blood volume significantly ($p < 0.01$ vs control). It also caused a reduction in the decrease of gastric mucosal hemoglobin oxygen saturation (-40.8 vs -69.5 in control) in these animals during the experiment. Therefore, lansoprazole increases the gastric hemodynamics in rats.

4. Inhibitory Effect on Acid Secretion:

(i) In Rats:

a. Effect on Gastric Acid Secretion: In pylorus ligated rats, lansoprazole at an intraduodenal dose of 1 to 30 mg/kg, inhibited acid secretion in a dose dependent manner. The maximum effect was seen at 10 mg/kg dose and the effect at a dose of 30 mg/kg was almost similar to that of 10 mg/kg dose. ID_{50} was 3.6 mg/kg which was about 2.4 and 16.1 times greater than omeprazole and ranitidine.

b. Effect on Histamine Stimulated Acid Secretion: In unstimulated state, acid secretion was negligible and it was increased manifold by histamine (30 ug/kg, s.c.). Lansoprazole at intraduodenal doses of 0.3 to 10 mg/kg, inhibited histamine induced acid secretion in a dose dependent manner. The inhibitory effect of the compound was about 2.1 times more than omeprazole and 13.8 times of ranitidine.

In another study, the effect on histamine stimulated gastric acid secretion was studied after oral doses of lansoprazole, omeprazole and ranitidine. Lansoprazole (10 mg/kg, p.o.) inhibited gastric acid secretion by histamine (30 ug/kg, s.c.) and its activity was more than 100 mg/kg of ranitidine but almost similar to that omeprazole

An oral dose of 0.5 mg/kg/day of lansoprazole for 3 weeks exerted a relatively constant inhibitory effect on histamine induced gastric secretion in rats. A decrease of 90 % during week 1, 2 and 3 and about 65 % at 8 hr post-dosing during week 1 and 3 was observed. The decreases were not proportional to the drug serum levels as these were 68, 88 and 81 % at 1 hr; and 22, 29, and 28 % at 2 hr post-dosing, respectively during weeks 1, 2 and 3 of the study.

c. Effect on Bethanecol Stimulated Acid Secretion: In SD rats, bethanecol 93 mg/kg, s.c.) stimulated pepsin and acid secretion. Lansoprazole at intraduodenal doses from 3 to 30 mg/kg inhibited

acid secretion (ID_{50} =6.0 mg/kg) but did not affect the pepsin secretion. ID_{50} s for omeprazole and ranitidine were 10.5 and 15.6 mg/kg.

ii. In Heidenhain Pouch Dogs:

Single Dose:

a. Effect on Histamine Stimulated Acid Secretion: In 16-24 hr fasted male beagle dogs with Heidenhain pouch, the unstimulated acid secretion was negligible (values not given). But gastric secretion was increased in gastric secretion by histamine (30 ug/kg, s.c.). Lansoprazole at doses of 0.1, 0.3 and 1 mg/kg inhibited histamine stimulated acid secretion. The maximum effect was seen in 30 min of its administration and the effect lasted for 8 hr. ID_{50} for lansoprazole was 0.27 mg/kg. The inhibitory effect of omeprazole was almost equal to lansoprazole (ID_{50} of omeprazole=0.21 mg/kg). ID_{50} for ranitidine was 0.09 mg/kg. ID_{50} s for intravenously administered lansoprazole, omeprazole and ranitidine were almost similar and were 0.14, 0.15 and 0.11 mg/kg respectively.

b. Effect on Pentagastrin Stimulated Acid Secretion: In beagle dogs with Heidenhain pouch (as described in 'a'), lansoprazole at doses of 0.1, 0.3 and 1 mg/kg inhibited pentagastrin (3 ug/kg, s.c.) stimulated acid secretion. The maximum secretion was seen in 90 min and lansoprazole dose dependently inhibited the acid secretion and the maximum inhibition was seen in 30 min of its administration and the effect lasted for 24 hr. ID_{50} for lansoprazole was 0.22 mg/kg. The inhibitory effect of omeprazole was almost equal to lansoprazole (ID_{50} of omeprazole=0.26 mg/kg). ID_{50} for ranitidine was 0.09 mg/kg.

c. Effect on Bethanecol Stimulated Acid Secretion: In beagle dogs with Heidenhain pouch (as described in 'a'), lansoprazole at doses of 0.1, 0.3 and 1 mg/kg inhibited 100 ug/kg, s.c. administered bethanecol stimulated acid secretion. The maximum secretion was seen in 1.5 hr and lansoprazole dose dependently inhibited the acid secretion and the maximum inhibition was seen in 4 hr of its administration and the effect lasted for 8 hr. ID_{50} for lansoprazole was 1.61 mg/kg. The inhibitory effects of omeprazole and ranitidine were greater than lansoprazole as ID_{50} s were 0.67 and 0.24 mg/kg for omeprazole and ranitidine, respectively. Lansoprazole may also exert small effect by other mechanism.

d. Effect on Peptone Meal-Stimulated Acid Secretion: In beagle dogs with Heidenhain pouch (as described in 'a'), lansoprazole at doses of 0.1, 0.3 and 1 mg/kg inhibited peptone meal-stimulated (100 ml of a 20 % peptone meal) acid secretion in a

dose dependent manner. The inhibition was observed for 3 hr after the meal and a sustained release of acid was seen for this period of 3 hr. ID_{50} for lansoprazole was 0.36 mg/kg.

Multiple Dose:

a. Effect on Acid Secretion and Gastrin Secretion in Dogs:

Acid secretion in beagle dogs with Heidenhain pouch (as described in single dose 'a') was stimulated by histamine (30 ug/kg, s.c.) twice a day during the study. Orally administered lansoprazole at 0.5 mg/kg for 7 days inhibited stimulated acid secretion. After 30 min of the 1st dose of lansoprazole, 81 % of the secretion was inhibited. But this effect was transient as it reduced to 19 % by 2 hr on day 1. The maximum effect on the secretion was seen on day 3 and it was claimed to be present till the end of the study. The inhibitory effect disappeared after 48 hr of its administration.

4. Effect on Experimental Reflux Esophagitis and Experimental Stress Induced Ulcers in Rats: Both the pylorus and junction between the forestomach and corpus were ligated according to the procedure of Goto Y. and Kishi S. Jap. J. Pharmacol. 49 (suppl. 1, 193P, 1989) and drugs were administered intraduodenally immediately after ligation. Rats were sacrificed 4 hr after the drug administration and gastroesophageal portion was removed for evaluation of esophagitis index (% of lesions in esophagus). The esophagitis index in the control animals was 3.4 and perforation was reported in about 10% animals. Lansoprazole at 0.3 mg/kg inhibited the reflux esophagitis in a dose dependent manner and its ID_{50} was 0.7 mg/kg, i.d. Omeprazole inhibited reflux esophagitis and its ID_{50} was 13.7 mg/ug, i.d. Famotidine inhibited esophagitis and its ID_{50} was 12.0 mg/ug, i.d. Gastric secretion was inhibited by lansoprazole in a dose dependent manner and a significant effect was seen at a dose of 3 and 10 mg/kg. Famotidine from a dose of 3 to 10 mg/kg did not affect acid secretion.

5. Enantiomers Inhibitory Effects:

a. On (H^+-K^+) -ATPase in Canine Gastric Microsomes In Vitro:

Lansoprazole enantiomers (+, - or racemate) from 1 to 30 uM concentration inhibited (H^+-K^+) -ATPase activity of canine gastric microsomes in a concentration dependant manner. IC_{50} were 1.8 and 3.4 uM for d- and l- enantiomers respectively. Both of these compounds showed similar inhibition on these cells. In other study, IC_{50} were 2.1, 4.2 and 5.2 uM for lansoprazole, (+)- and the (-) enantiomers respectively.

b. On Acid Formation in Isolated Canine Parietal Cells In Vitro: Lansoprazole enantiomers (+, - or racemate) from 10 to 1000 nM concentration suppressed acid forming capacity of dibutyryl cyclic AMP stimulated isolated canine parietal cells in a concentration-dependent manner. IC_{50} of these enantiomers were 59, 59 and 82 nM for racemate, d- and l- isomers, respectively. The activity of the enantiomers were not different statistically and the activity was considered equal. This indicated that lansoprazole enantiomers exert similar inhibitory effect on acid production and antisecretory effect. The pharmacology of these 2 isomers has not been studied by sponsor.

6. Prophylaxis in Induced Gastroduodenal Ulcers In Rats:

i. Gastric Ulcers:

Lansoprazole (1-30 mg/kg; p.o.) when administered 30 min prior to stress in rats (in a stress cage and immersed vertically to the level of xiphoid process in a water bath at 23°C for 5 hr), prevented formation of gastric ulcer which appeared to be in a dose dependent manner. A dose of 10 mg/kg of lansoprazole prevented gastric ulcers in 100 % animals and ID_{50} value was 2.5 mg/kg). Ranitidine and omeprazole were about 11.4 and 2.8 times less active than lansoprazole. ID_{50} values for omeprazole and ranitidine were 7 and 28.4 mg/kg, p.o. respectively.

A pretreatment with 0.3 to 10 mg/kg, i.d. of lansoprazole in pylorus ligated rats, prevented aspirin induced ulcers. Its ID_{50} was 0.7 mg/kg. Omeprazole and ranitidine in rats treated in a similar manner also prevented aspirin induced ulcers and their ID_{50} values were 3.1 and 10.1 mg/kg, i.d., therefore, it was 4.4 and 14.4 times as potent as omeprazole and ranitidine, respectively.

Lansoprazole (1 to 30 mg/kg, p.o.) inhibited gastric lesions induced by 1 ml of orally administered absolute ethanol, in a dose dependent manner. Its ID_{50} was 8.5 mg/kg. Omeprazole (3 to 30 mg/kg, p.o.) also effectively inhibited these lesions in rats and its ID_{50} was 15.3 mg/kg. Ranitidine was effective at a dose of 100 mg/kg dose and in only 47 % rats.

ii. Duodenal Ulcers:

A pretreatment of 0.3 to 10 mg/kg, p.o. of lansoprazole prevented duodenal ulcers induced by a single dose of 400 mg/kg of cysteamine in a dose dependent manner. Its ID_{50} was 1.1 mg/kg. Omeprazole (3 to 30 mg/kg, p.o.) and ranitidine also effectively inhibited these lesions in rats and their ID_{50} s were 5.7 and 4.0 mg/kg.

A 30 min pretreatment with an oral dose of 0.1 to 3 mg/kg of lansoprazole, prevented duodenal ulcers induced by a single s.c. dose of 200 mg/kg of omeprazole in a dose dependent manner. Its ID_{50} was 0.3 mg/kg. ID_{50} s for omeprazole (1 to 10 mg/kg, p.o.) and ranitidine (3 to 30 mg/kg) were 3.0 and 10.3 mg/kg.

7. Curative Effect on Chronic Gastroduodenal Ulcers Induced by Acetic Acid in Rats: The test was done in 7 weeks JcL:SD rats according to the procedure of Takagi K. et al (Jap. J. Pharmacol. 18: 9, 1968). In this study, animals were anesthetized, laprotomized and their stomach exposed. A 30 μ l of 20 % acetic acid solution was injected into subserosal layer at the junction of the corpus and antrum of the anterior wall. The stomach was closed and lansoprazole (3-30 mg/kg; p.o.) was given for 14 days beginning two days after operation. Area (mm^2) of the ulcer was measured under the dissecting microscope and used as an ulcer index. Lansoprazole accelerating healing in a dose dependent manner as 9, 60 and 64 % of animals included in 3, 10 and 30 mg/kg/day treatment groups respectively were found to have decreased ulcer index. Omeprazole was effective only at a dose of 30 mg/kg/day and other 2 lower doses were ineffective. Ranitidine did not cure acetic acid induced ulcers.

8. Acetic Acid Induced Duodenal Ulcers: The test was done in 7 weeks JcL:SD rats according to the procedure of Okabe S. et al (Am. J. Dig. Dis. 16: 277, 1971). In this study, animals were anesthetized, laprotomized and a round metal mold, 6 mm in diameter was tightly placed on the serosal surface of the duodenal wall, about 7 mm distal to the pylorus. Glacial acetic acid (60 μ l) was poured into mold and allowed to remain for 20 sec and acetic acid removed and surface rinsed with 50 μ l of 0.02 N NaOH. The abdomen was closed and lansoprazole (3-30 mg/kg; p.o.) was given for 14 days beginning two days after operation. The animals killed on day 15 and the area (mm^2) of the ulcer was measured as described above. Lansoprazole was effective at a dose of 30 mg/kg/day.

9. In vitro Inhibitory Activity Against Viability of Helicobacter Pylori: Lansoprazole from μ g/ml concentration reduced the acid forming activity of Helicobacter pylori. Its activity was comparable with that of bismuth citrate. Mean inhibitory concentration (MICs) of 2 compounds were from 3.13 to 12.5 μ g/ml. These concentrations were about 4 times higher than those of omeprazole. IC_{50} s of various antiulcer agents for their gastric acid secretion inhibitory effect of H pylori are given in the following table (taken from sponsor's submission vol. 2.5, pp 069):

TABLE 1. Activities of various antiulcer agents against
17 strains of *H. pylori*

Agent	MIC ($\mu\text{g/ml}$) ^a		
	Range	50%	90%
Bismuth citrate	6.25-25	6.25	12.5
Cimetidine	800->800	>800	>800
Ranitidine	>800	>800	>800
Famotidine	>800	>800	>800
Omeprazole	12.5-50	25	25
Lansoprazole	3.13-12.5	6.25	6.25

^a MICs were determined by the agar dilution method on brucella agar with a bacterial suspension of about 3×10^7 CFU/ml. 50% and 90% MICs for 50 and 90% of the strains.

10. Comparison of Gastric Secretion Action of Plasma

Metabolites: Lansoprazole and its metabolites, M-VI and M-VII, identified in human blood and in other animals, were tested for their effects on $(\text{H}^+-\text{K}^+)\text{-ATPase}$ activity in canine gastric microsome, dibutyl (db) cyclic AMP-stimulated acid formation in isolated canine parietal cells, histamine stimulated acid secretion in conscious Heidenhain pouch dogs. Lansoprazole and inhibited the $(\text{H}^+-\text{K}^+)\text{-ATPase}$ activity in canine gastric microsome in a concentration dependent manner and IC_{50} s were 6.3 and 3.0 μM respectively. did not show any activity. were not shown to inhibit acid formation stimulated by db-cyclic AMP-stimulated acid formation in canine parietal cells up to 10^{-5}M concentration. Lansoprazole (0.1, 0.3 and 1 mg/kg, i.v.) inhibited histamine stimulated acid secretion dose dependently and ID_{50} was 0.14 mg/kg, i.v. inhibited 23 and 6% of the stimulated acid at a dose of 1 mg/kg, i.v.

Lansoprazole and its metabolites, were also tested for their effects on histamine stimulated gastric acid secretion in anesthetized rats and water immersion stress ulcer. Lansoprazole (0.3, 1, 3 and 10 mg/kg, i.p.) inhibited histamine stimulated acid secretion dose dependently and ID_{50} was 0.4 mg/kg, i.p. inhibited 40 and 30% of the stimulated acid secretion at a dose of 10 mg/kg, i.p. Lansoprazole inhibited the gastric lesions dose dependently and ID_{50} was 0.7 mg/kg. (10 mg/kg, i.p.) inhibited 32, and 24% of the lesions at a dose of 10 mg/kg, i.p. Therefore, these metabolites showed marginal and insignificant effects on these parameters.

SECONDARY PHARMACOLOGY

1. Intestinal Motility: Intestinal propulsion of charcoal was studied in fasted rat by administering 2 ml of 10 % charcoal (suspended in 5 % gum acacia) orally, 1 hr after the administration of lansoprazole. The animals killed and small intestines removed and the rate of propulsion of charcoal in lansoprazole treated animals was compared with control animals. Lansoprazole at these doses did not affect intestinal propulsion in the animals.

2. Bile Secretion in rats: It did not affect bile flow (no absolute figures for treated animals was given but bile flow in control animals was 406 ul/30 min in control animals) at an intraduodenal dose of 10 to 100 mg/kg. Therefore, lansoprazole exerted no significant effect on bile secretion.

3. Isolated Smooth Muscle of Duodenum and Stomach:

a. Rats: Lansoprazole at 10^{-6} , 10^{-5} and 10^{-4} M concentration decreased the amplitude of the contraction of stomach by 10, 19 and 69% respectively. The tone of contraction of stomach was decreased by 17, 17 and 32% respectively. Lansoprazole at 10^{-4} M concentrations decreased the amplitude and the tone of duodenal contraction by 42 and 32% respectively.

b. Rabbits and Guinea Pigs: Lansoprazole did not affect the amplitude of contraction of isolated rabbit ileum and guinea pig trachea from 1 to 100 μ M. It (at 10^{-6} and 10^{-5} M concentration) did not show any influence on the contraction induced by spasmogens like 10^{-6} M acetylcholine, 10^{-6} M histamine and 10^{-5} M serotonin or 10^{-3} M Barium chloride in these preparations. But, at 10^{-4} M, a reduction of 23, 34, 58 and 29 % amplitude of contraction induced by acetylcholine, histamine, serotonin or Barium chloride respectively was seen, respectively in these smooth muscle preparations.

4. General CNS and Behavioral Observations Effects: An oral dose of 30 or 100-300 mg/kg of lansoprazole produced a decrease in spontaneous motor activity in mice. The onset of the action was in 15 to 75 min after an oral dose of 100-300 mg/kg. It was delayed to 1.5 hr after a low dose of 30 mg/kg dose of the compound. The body tone was decreased from 300 and 1000 mg/kg oral dose of lansoprazole. Muscle coordination/activity in mice was not affected up to an oral dose of 300 mg/kg of lansoprazole. It (30 to 300 mg/kg, p.o.) did not affect electroshock-induced convulsion but a dose greater than 30 mg/kg prolonged pentobarbital induced sleeping time. This effect of the compound might be its effect on the hepatic enzyme system.

The frequency of writhing (induced by an 0.6 % acetic acid i.p. injection) was not affected up to a dose of 300 mg/kg/day of lansoprazole, therefore compound did not exert an analgesic effect. An i.p. dose of 100 mg/kg of lansoprazole was reported to exert no effect on EEG of mice (n=2). In rat phrenic nerve diaphragm preparation, lansoprazole up to a dose of $4 \times 10^{-4} M$ (n=3), did exert any effect in neuromuscular transmission.

In conscious unrestrained cats with chronically implanted electrodes (placed by stereotaxic instrument), lansoprazole at 100 mg/kg, i.p. did not exert any effect on the spontaneous EEG or behavior up to 7.5 hr after the administration. In a restrained cat (head and bilateral femurs fixed on a frame), the response to an electrical stimulation of 0.1 Hz with 1 msec in pulse duration was not also altered by 100 mg/kg, i.p. of lansoprazole. The flexure response of the anterior tibialis muscle to supramaximal electrical stimulation of the central cut end of the Achilles tendon was measured in these cats.

Lansoprazole in oral doses of 30, 100 and 300 mg/kg did not exert anti-inflammatory effect against carrageenin-induced edema in rat paw. Phenylbutazone (25 mg/kg, p.o.) inhibited edema by 31 % in these animals.

5. Cardiovascular Pharmacology:

i. Isolated Guinea Pig Atria: Lansoprazole from 10^{-6} M concentration did not effect contactile force of electrically paced left atria or the spontaneous beating rate of the atrium.

ii. In Cats: Lansoprazole (300 mg/kg, i.d.) did not exert any effect on blood pressure or heart rate and respiration of anesthetized cats. It (300 mg/kg, i.d.) did not alter bradycardiac response produced by the electrically stimulated cervical vagus nerve, the pressor response to carotid occlusion, the contraction of nictating membrane to electrical stimulation. It did not affect the pressor response of vasoactive agents.

iii. In Dogs: Lansoprazole at a dose of 150 mg/kg, i.d., exerted no effect on respiration, blood pressure, heart rate or EKG pattern. A continuous infusion of 1, 3 or 10 mg/kg (infusion rate of 0.067 ml/kg/min, i.e., 0.033, 0.010 and 0.333 mg/kg/min respectively) of lansoprazole produced an 14.4% increase in heart rate and a slight increase of 5.9 % in pulmonary arterial pressure in 10 min of its administration. A dose of 10 mg/kg resulted in the peak plasma levels of 11.2 ug/ml (about 9 times the levels produced by a single clinical dose of 1.2 mg/kg).

6. Effect on Urinary and Renal System in Rats: Lansoprazole (30-300 mg/kg, p.o.) did not affect urinary total volume, sodium and potassium excretion.

ABSORPTION, DISTRIBUTION, METABOLISM, PHARMACOKINETICS AND EXCRETION:

ADME data submitted with the present NDA were reviewed earlier in IND (Initial submission and its amendments) and IND (Initial submission). These reviews are acceptable, identified and reproduced below. Other ADME studies submitted for the first time are also reviewed below:

A. ABSORPTION:

1. Relative Degree of Absorption of Orally Administered Lansoprazole and Omeprazole in Rats: (Study # R&D/91/663)

Name of the Conducting Laboratory:

Methods: This study was performed to determine the relative absorption of lansoprazole from 2 vehicles, i.e., 5% gum Arabic (pH 7.0 with 1 N potassium hydroxide) or methocel (pH 9.0 adjusted with 10% sodium bicarbonate). Plasma concentration (AUC_{0-12}) were calculated. These suspensions were administered at an oral gavage dose of 50 mg/kg orally in 2 groups of overnight fasted rats (20/group) and the relative degree of absorption of lansoprazole was determined for these 2 vehicles. The blood samples (0.5 ml/time period) were drawn 5, 15, 30, 45, 85, 105, 150, 210 and 300 min after the administration of the compounds for the estimation of the concentration of the compound by a HPLC technique. During this study, the relative absorption of omeprazole was also calculated.

Results: A peak plasma levels (AUC_{0-12}) of 6679 and 8470 ng.hr/ml of lansoprazole were observed within 90 and 70 min after its administration in 5% gum Arabic and methocel vehicles respectively. These values were not different, therefore, the absorption of lansoprazole by these 2 vehicles was similar. The peak plasma concentrations of omeprazole were also similar, when administered in these 2 vehicles.

In the preclinical pharmacology, ADME, toxicology and carcinogenicity studies, lansoprazole suspension for oral use was made with 5% gum Arabic and at pH of 6.8 to 7.1. Therefore, the vehicle selection for the preparation of the suspension in the study was correct and acceptable.

with plain, enteric coated tablets and enteric coated granules respectively. Therefore lansoprazole was bioavailable in equal amounts after the administration of these preparations. This was attributed to the high gastric pH in dogs.

In humans, the absorption of lansoprazole (AUC_{0-4hr}) was low from the plain tablet. The plasma concentrations (AUC_{0-8hr}) values were 1159, 2437 and 3890 ng.hr/ml for plain, enteric coated tablets and enteric coated granules in capsules, respectively. Lansoprazole was available in higher amounts from the granules more than enteric coated tablets or the plain tablets. Therefore, enteric coated granules in capsules might provide a better preparation.

4. Multiple Dose Pharmacokinetics In Female Cynomolgus Monkeys: (Study # A-29-1490)

Methods: In this study, the plasma concentrations of lansoprazole after a single dose and multiple doses (once/day for 1 week) administered by gavage, were compared. Lansoprazole suspension (1 %) was made in aqueous solution containing 5 % gum arabic and was administered in a volume of 5 ml/kg. Blood samples were drawn at 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hr after the first and the last dose on 7th day and pharmacokinetics parameters were determined.

Results: In monkeys, a single or repeated doses of lansoprazole attained mean concentrations (AUC_{0-24hr}) of 4.75 ± 4.99 and 10.56 ± 9.21 ug.hr/ml respectively in about 4 hr and with a $t_{1/2}$ of 3.3 hr. Therefore, these doses attained concentrations which were not statistically different from each other. The amount of the compound absorbed in monkey did not varied after repeated dosing.

MICE**2. Multiple-Dosing Study in Mice:** (Also dose-dependency; A-29-630/IND Amendment dated 7/27/90/Pharmacology Review dated 1/2/91):

Groups of mice (n=3) were given single oral doses of C¹⁴-AG-1749 (diluted with non-labeled AG-1749) at 1.5, 5, 15 and 50 mg/kg. The C_{max}s ranged between ug/ml, the t_{1/2} between hr, and the AUCs between ug.h/ml at mg/kg, respectively. For multiple-dosing, another group of mice received po AG-1749 at 50 mg/kg/d once daily for 7 days. A group of mice were also injected a single iv dose of 1.5 mg/kg. The C_{max}, t_{1/2}, AUC and the bioavailability (the oral AUC/iv AUC x oral dose/iv dose x100) increased with dose but not dose-dependently.

With multiple dosing, the C_{max}, t_{1/2}, and the AUCs were 1.52 ug/kg, 1.0 hr, and 1.31 ug.h/ml, respectively. The bioavailability of AG-1749, was in the range of 4-14%. The kinetics (C_{max}, t_{1/2}, AUCs etc.) of AG-1749 tended to increase with dose, but were not linear, and were inconsistent at higher doses. The kinetics of its metabolites were similar after single oral doses of AG-1749. See comprehensive summary below.

Pharmacokinetics in Mice (n=3) After Single or Multiple
Doses of AG-1749

Dose, mg/kg	T _{max} min	C _{max} ug/ml	t _{1/2} hr	AUC _{0-24hr} ug.h/ml
<u>Multiple Dosing:</u>				
1.5				
5				
15				
50				
<u>Single Dosing:</u>				
500				
1000				
2500				
5000				

1. Pharmacokinetics of AG-1749 in Mice: Dose-dependency
(A-29-704/IND Amendment dated 7/27/90/Pharmacology Review dated 1/2/91):

Pharmacokinetics of C¹⁴-AG-1749 was studied in mice (n=84) at single oral doses of 500, 1000, 2500, and 5000 mg/kg dissolved in 1 gum arabic. Serial blood samples were collected at 15, 30 min, and 1, 2, 4, 8, and 24 hr after dosing. The C_{max} values at 15 min post-dosing were 251, 451, 839, and 1543 ug/kg, and the AUCs were 492, 1026, 2430, and 4582 ug.h/ml at 500, 1000, 2500, and 5000 mg/kg, respectively. The t_{1/2} of the initial phase was 0.8 hr and 5.5 to 8.0 hr for phase II. Data indicated plasma levels of AG-1749 increased with the dose and there was no suggestion of accumulation of the parent compound or its metabolites.

DOGS

5. Single Dose Pharmacokinetics of AG-1749 in the Female Beagle Dog (Takeda Report A-29-697/IND Amendment dated 7/27/90/
Pharmacology Review dated 1/2/91):

Groups of female beagle dogs, 3 each, were given AG-1749 in a gelatin capsule at single doses of 5, 15, and 50 mg/kg, and the plasma levels were determined at 15, 30 min, and 1, 2, 4, 6, and 8 hr after dosing. The C_{max} was ug/ml; $t_{1/2}$ was h; and the AUCs were ug.h/ml at mg/kg doses, respectively. In the dog, the pharmacokinetics of AG-1749 was not linear; the plasma concentrations increased with the dose, but not dose-dependently. The T_{max} was hr at mg/kg doses while at mg/kg, it took hr to achieve the C_{max} .

Single-Dose Pharmacokinetics and In-Vitro Metabolism of Stereoisomers of Lansoprazole in Rats and Dogs
(Report A-29-908/IND Amendment dated 8/30/91/
Pharmacology Review dated 10/18/91)

Groups of male Wistar rats and beagle dogs, 3 each, were dosed with ^{14}C -labeled Lansoprazole (2.mg/kg po or iv) and blood levels of the radioactivity were determined at various times to calculate the pharmacokinetic parameters (see below). The in-vitro metabolism of Lansoprazole by the rat and dog liver was determined in the presence of NADPH-generating enzyme system using S-9 fractions. The plasma levels of the stereoisomers were determined by HPLC and liquid scintillation spectrometry. The plasma C_{max} (see next page) at 15 min was higher for the the (+)-isomer than for the (-)-isomer in both rats and dogs. The disappearance of the (+)-isomer from plasma in the rat was biphasic, but $t_{1/2}$ s for both isomers were similar in both rats and dogs.

	<u>RAT</u>		<u>DOG</u>	
	(+)-isomer	(-)-isomer	(+)-isomer	(-)-isomer
	<u>ORAL, 2 mg/kg</u>			
T_{max} , h				
C_{max} , ug/ml				
$T_{1/2}$, h, alpha				
beta				
AUC, ug.h/ml				
	<u>Intravenous, 2 mg/kg</u>			
$T_{1/2}$, h,				
AUC, ug.h/ml				

ND=not determined; n=3 each.

The enzyme kinetics (see below) of the isomers in both rats and dogs show that the mechanism of metabolism for both isomers (affinities of the isomers toward the hepatic metabolizing enzymes) may be the same but with different rates as seen by similar K_m s and different V_{max} s.

Kinetics of Stereoisomers of Lansoprazole in Rat and Dog

	Substrate	V_{max} , nMol/ min/mg protein	K_m uM	V_{max}/K_m	(-)/(+) ratio
<u>Rat</u>	(+)-isomer				2.24
	(-)-isomer				
<u>Dog</u>	(+)-isomer				2.05
	(-)-isomer				

2. Multiple-Dose Pharmacokinetics of AG-1749 in Rats
(Takeda Report A-29-629/IND Amendment dated 7/27/90/
Pharmacology Review dated 12/16/90):

Male Wistar rats were given C¹⁴-AG-1749 (combined with non-labeled drug) in single or multiple oral doses of 15 mg/kg/d, once daily for 4 weeks. Blood samples collected at 5, 15, 30 min, and 1, 2, 3, 4, 6, 8, and 24 hr post-dosing from 5 rats/time point were analyzed for AG-1749 and its metabolites. With multiple dosing, there was no accumulation of the parent compound or its metabolites as seen with no substantial changes in either kinetic parameter: C_{max} , AUC, or $t_{1/2}$.

Pharmacokinetics in Rats After Multiple Dosing with AG-1749

No. Doses	T_{max} hr	C_{max} ug/ml	$t_{1/2}$ hr	AUC ₀₋₂₄ ug.h/ml
<u>AG-1749</u>				
1				
7				
14				
28				
<u>Metabolites</u>				
1				
7				
14				
28				

6. Multiple-dose Pharmacokinetics of AG-1749 in the Dog
(Takeda Report A-29-628/IND Amendment dated 7/27/90/
Pharmacology Review dated 1/2/91):

In experiment 1, 3 female dogs were given a single oral dose of 30 mg/kg/d unlabeled AG-1749 in a gelatin capsule, once daily for 14 days. Plasma levels were determined 8 hr after the last dose on days 1, 7, and 14. C_{max} and AUC on day 1 were ug/ml and ug.h/ml, respectively. These decreased to about 10 and 20% on days 7 and 14, possibly due to increased elimination or hepatic induction. However, no data exist to explain the latter explanation.

In experiment 2, in order to determine if the treatment with AG-1749 affects the plasma levels of AG-1749 and its metabolites, 3 female dogs each were given, a single 30 mg/kg oral dose of C^{14} -AG-1749 (in 5% gum arabic solution) on days 1 and 9, and non-labeled AG-1749 on days 2 through 8. After a 4-wk washout period, these dogs were given an iv injection of 2 mg/kg C^{14} -AG-1749 (in DMSO) on days 1 and 9 and oral dose of the non-labeled AG-1749 on days 2-8. AG-1749 and its metabolites were determined on days 1 and 9. On oral dosing, the C_{max} of the parent drug decreased to 10%, while AUC decreased to 30%, while those of the metabolites decreased to 50-60%. Following iv dosing, the $t_{1/2}$ was 0.13 hr compared to 0.39 hr before the treatment. The AUC decreased to 39%, all of which suggested that the disappearance of AG-1749 increased with repeated dosing. The bioavailability of oral drug was not significantly different before and after treatment (34 and 28%, respectively), which according to sponsor, could be due to increased plasma clearance of the parent drug from plasma.

At 0.5 and 5 mg/kg/d given po for 1 or 7 days (study A-29-703), the plasma levels of AG-1749 (see below) increased with increasing dose: the C_{max} s on day 1 were 72 and 378 ug/ml after 0.5 and 5 mg/kg doses, respectively; while on day 7 these were 41 and 45 ug/ml suggesting some hepatic microsomal induction. But there was significant variability between doses and within the animals. Further, the levels were determined at between 0-4 hr for the 0.5 mg/kg dose, and between 0-8 hr for the 5 mg/kg dose. Thus the comparison of plasma levels with different doses appeared to be inappropriate and valid conclusions cannot be drawn from this study.

Pharmacokinetic Parameters in the Dogs Treated with AG-1749

Dose, mg/kg	No. of Doses	T _{max} , hr.	t _{1/2} , hr.	C _{max} , ug/ml	AUC, ug.h/ml
0.5	1				
5	1				
0.5	7				
5	7				

Levels determined at 0-4 hr after 0.5 mg/kg, and 0-8 hr after 5 mg/kg/d dose.

In summary, plasma levels (C_{max}s, AUCs, t_{1/2}s) of AG-1749 in mice, rats, and dogs (summarized on the next page) tended to increase with dose, but were not linear, particularly at higher doses. The non-linearity observed could be due to a significant degree of variability between doses and species used.

Summary of Kinetics of AG-1749 in Mice, Rats and Dogs

Dose, mg/kg	C _{max} ug/ml	MICE		t _{1/2} h	C _{max} ug/ml	RATS		t _{1/2} h	C _{max} ug/ml	DOGS		t _{1/2} h
		AUC ug. h/ml				AUC ug. h/ml				AUC ug. h/ml		
SINGLE DOSING*												
1.5	0.03	0.01	0.02	ND	ND	ND	ND	ND	ND	ND	ND	
5	0.23	0.07	0.2	0.11	0.07	0.3	0.3	1.1	1.7			
15	0.45	0.26	1.1	0.34	0.32	0.7	0.7	1.4	1.3			
50	1.84	1.23	0.9	0.92	1.84	1.0	1.3	2.7	0.9			
150	ND	ND	ND	2.87	9.7	1.0	ND	ND	ND			
MULTIPLE DOSING**												
15 mg/kg												
1 dose	ND	ND	ND	0.96	10.3	8.1	ND	ND	ND	ND	ND	
7 doses	ND	ND	ND	1.43	9.4	11.0	ND	ND	ND	ND	ND	
30 mg/kg												
1 dose	ND	ND	ND	ND	ND	ND	0.56	1.42	ND			
7 doses	ND	ND	ND	ND	ND	ND	0.07	0.14	ND			
50 mg/kg												
1 dose	1.84	1.23	0.9	ND	ND	ND	ND	ND	ND	ND	ND	
7 doses	1.52	1.31	1.0	ND	ND	ND	ND	ND	ND	ND	ND	

ND=not determined. The bioavailability of AG-1749 with oral route was between % in mice and % in dogs. * = In the single dosing studies, blood sampling was as follows: mice- at 5, 10, 20, 30 min. and 1, 2, 4 hr after dosing; rats- 24 hr after dosing; dogs, at 15, 30 min., and 1, 2, 4, 6, 8 hr after dosing. ** = In multiple dosing studies, blood sampling was as follows: mice- at 5, 10, 20, 30 min. and 1, 2, 4 hr after dosing; rats- at 5, 10, 30 min. and 1, 2, 3, 4, 6, 8, and 24 hr after dosing; dogs- time of blood sampling was not clear.

Multiple-Dose Pharmacokinetics of AG-1749 in Female Rats
After Intravenous Administration
(Report A-29-1358/IND Initial Submission/
Pharmacology Review dated 9/2/93)

Methods: Female Wistar rats (n=6) were given Lansoprazole (10 mg/kg/day) intravenously for one day, 2 weeks or 4 weeks. Serial blood samples were collected from the jugular vein at 0, 5, 10, 15 and 30 min., 1, 2 and 4 hours after drug administration on day 1, week 2, and week 4 of the study. Plasma concentrations of AG-1749 were measured by HPLC methods.

Results: There was a slight increase in $AUC_{0-\infty}$ values after repeat dosing. However, $t_{1/2}$ (i.e. 0.4 hr) values remain same at all three sampling period. The pharmacokinetic profile of AG-1749 in female rats after multiple I.V. doses (4-weeks) were comparable to that found after a single I.V. dose.

Pharmacokinetic Parameters in Female Rats (n=6) After Repeat I.V. Doses (10 mg/kg/day)

<u>Parameters</u>	<u>Day 1</u>	<u>Week 2</u>	<u>Week 4</u>
Co (mcg/ml)			
$t_{1/2}$ (hr)			
AUC (mcg.h/ml)			

Co = plasma concentration extrapolated to time zero.

Multiple-Dose Pharmacokinetics of AG-1749 in Female Dogs
After Intravenous Administration
(Report A-29-1359/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Female beagle dogs (n=3) were given intravenously 3 mg/kg/day of Lansoprazole for 4 weeks. Blood samples were collected from cephalic vein at 0, 5, 10, 15 and 30 min. and 1, 2 and 4 hours after drug administration on day 1, week 2 and week 4 of the study. Lansoprazole levels in plasma were measured by HPLC methods.

Results: The pharmacokinetic profile after drug administration on day 1, week 2 and week 4 were comparable. The $t_{1/2}$ (beta) ranged from hours and no accumulation of the drug was evident in this study.

Pharmacokinetic Parameters in Female Dogs
After I.V. Dose (3 mg/kg/day)

Parameters	Day 1	Week 2	Week 4
Co (mcg/ml)			
$t_{1/2}$ (alpha)			
$t_{1/2}$ (beta)			
AUC ₀₋₂₄ (mcg.h/ml)			

Co = plasma concentration extrapolated to time zero.

Metabolic Fate of I.V./P.O. Administered Lansoprazole
in Rats and Dogs

(Study A-29-162/IND Initial Submission/
Pharmacology Review dated 8/12/90)

The study (metabolic fate of AG-1749 in rats and dogs, # A-29-162, p427, v2 dated 9/25/86) was conducted at the sponsor's labs in Japan.

Absorption: Based on the ratios of AUCs 24 or 72 hr after oral or intravenous administrations of 2 mg/kg dose of ¹⁴C-AG-1749, it appears that about 37 and 63% of the dosed compound is available to the rats and dogs, respectively.

Plasma levels: In rats, min after an oral dose of 2 mg/kg ¹⁴C-AG-1749, the plasma C_{max} of AG-1749 was ug/ml, and the levels decreased with an apparent t_{1/2} of hr. In the dog, after an oral dose of 2 mg/kg, the plasma C_{max}s were ug/ml at minutes, respectively. (page 436, and figure 4B, v2), followed by a biphasic decline with respective t_{1/2}s of hr. As calculated AUCs, % of the plasma radioactivity comprised of unchanged AG-1749, respectively in rats and dogs with remainder consisting of metabolites, suggesting rapid metabolism in rats and dogs.

Distribution: Tissue levels were determined at 0.5, 24, 72 hr after oral dosing of ¹⁴C-AG-1749 in rats. At 0.5 hr after dosing, the radioactivity was widely distributed in tissues with relatively high concentrations in the GI wall and liver. By 72 hr, the radioactivity in most tissues had decreased to very low levels except in the thyroid where it was still high: ug.eq/g compared to none in the plasma; ug.eq/g in the liver and ug.eq/g in the stomach/intestine combined. In terms of radioactivity entering the erythrocytes, in the rats, 30 min after an oral dose of ¹⁴C-AG-1749, 26% of the blood radioactivity entered erythrocytes, while in the dogs, 11-17% of the blood radioactivity entered the erythrocytes 0.25, 1, and 4 hr after dosing.

Accumulation: It was studied in rats and dogs after giving oral doses for 7 or 14 days daily. Only slight increases in the radioactivity concentrations were observed in both plasma and tissues. After 7th administration, the rate of disappearance of radioactivity was relatively slow in the thyroid: the amount of radioactivity (ug AG-1749.eq/g) in the thyroid on day 1, 3, 5, and 7 was 0.91, 1.29, 1.22, and 1.57 ug.eq/g, and on days 3, 5, and 7 after seven days of dosing at 2 mg/kg, were 0.72, 0.67, and 0.35 ug.eq/g, respectively.

In comparison, the plasma concentrations ranged between _____ ug.eq/ml at these times. Within _____ hr after the 7th dose, 16.8% and 82.6% of the total dose were excreted in the urine and feces, respectively. Thus it appears that no appreciable amount of AG-1749 accumulates in rats after repeated dosing.

Plasma protein binding: The in-vitro binding of ¹⁴C-AG-1749 to the rat and dog plasma proteins, is 92-93% (8/11/88 amendment) and to the human plasma proteins is about 97% at the concentration range of _____ ug/ml. After oral dosing of ¹⁴C-AG-1749, radioactivity was 92% protein-bound in rats and 80-92% in the dogs.

Excretion: In the rat, after oral dosing at 2 mg/kg (18.8 uCi/kg), it is almost completely eliminated within 72 hr, largely in feces (81%), and 17.8% in urine (total of 98.8%); and 98.9% is excreted within 96 hr of dosing. In the dog, at the corresponding times, 94.5% is excreted (83% in feces, 11.9% in urine). In the biliary cannulated rats given ¹⁴C-AG-1749 id., 59.6% of the dose is excreted in 24 hr in the bile suggesting that the fecal radioactivity in the intact rats is due partly to the biliary excretion.

Metabolism: Basically, seven metabolites as well as unaltered AG-1749 have been identified or postulated (see attached sponsor's figure on the next page). It appears that in rats and dogs, AG-1749 is metabolized by the

In addition, conjugation with glucuronic and/or sulfuric acid of metabolites II, IV, V, VI, and IX occurs in both rats and dogs.

In the rat plasma, the metabolites III, IV, and VI have been identified; while in the dog plasma, unaltered AG-1749 and IV were relatively large components. In both rats and dogs, only small amounts of unaltered AG-1749 were detectable in the urine and feces with the remainder consisting of metabolites suggesting that AG-1749 was completely metabolized in rats and dogs. Further, in rats, relatively large amounts of II, III, IV and V were excreted in the urine, II and IV in the feces, and IV and IX in bile. In the dog, appreciable amounts of IV, V, VI, and IX were excreted in the urine, and II and IV in the feces. In addition, metabolites II, IV, V, VI, and IX were excreted as glucuronides, and/or sulfate in the urine.

In summary, ¹⁴C-AG-1749 administered orally is rapidly absorbed, is widely distributed in tissues, almost completely metabolized and is eliminated in feces, via hepatobiliary route, with the remainder appearing in the urine.

Pharmacokinetics of AG-1749 in Man: The $t_{1/2}$ s in the rat, dog, and man (dose: 30 mg/person=0.6 mg/kg) were _____ hr, respectively. the C_{max} values were _____ ug/ml, _____ ug/ml, and _____ ug/ml (after a 0.6 mg/kg dose and levels determined at day 1 and 7), and the AUCs were 2.6, 9.3, and 2202/2517 ng.h/ml, respectively, suggesting that AG-1749 does not accumulate in either species. In man no parent unchanged drug was found in the urine at any dose, although the hydroxylated sulfide metabolite, AG-1907, was detected up to 1.5% of the dose. AG-1908 was identified in the urine and plasma, while the plasma also contained small amounts of dealkylated AG-1977.

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REVIEWER'S ADDENDUM TO THE ABOVE REVIEW:

In the above review, the methodology used in the study were not clear, therefore this addendum was prepared.

Methods: Male Wistar rats (Jcl:Wistar, Japan) weighing between 186 to 354 g and male dogs weighing 11.5 to 12.5 kg were administered a dose of 2 mg/kg of ¹⁴C-Lansoprazole. The suspension of the compound was prepared after diluting it with unlabelled lansoprazole and suspended in 1% gum arabic for oral and intraduodenal (in biliary cannulated rats) administration. It was dissolved in DMSO, if given intravenously. After 0.25, 1 and 4 hr of its administration, blood samples from these animals were collected for determination of erythrocytes and plasma protein bindings. For determining the excretion pattern of the compound, the urine, feces and bile samples of rats and dogs, were collected after 4, 8, 24, 48, 72 and 96 hr of the administration of the compound. Biliary cannulated rats were administered lansoprazole intraduodenally, the urine, feces and bile samples were collected after 2, 4, 8 and 24 hr after the administration of the compound. These fluids were subjected to HPLC technique to fractionate the metabolites in these fluids.

Pharmacokinetics/Drug Metabolism Study in Rats and Dogs
(Study A-29-876/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Pylorus-ligated rats (n=3) were given ¹⁴C-AG-1749 (2 mg/kg) via gavage or intraduodenally. Serial blood samples were collected at 5, 15, 30 minutes, 1, 2 and 4 hours after drug administration. Levels of radioactivity in plasma samples were monitored by LSC methods.

Results: Very minimal amount (range 2.3-11.3 ng equivalent/ml) of radioactivity was detected in plasma samples when the drug was given intragastrically. However, radioactivity levels in plasma samples were substantial when the drug was given I.D. (C_{max} = ng equivalent/ml, T_{max} = 30 min) and the $t_{1/2}$ of the radioactivity was 1.4 hr. This experiment suggests that drug is mainly absorbed from the small intestine.

Distribution (Study A-29-876/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Rats (male or female Wistar) were given ¹⁴C-AG-1749 (2 mg/kg) orally (gavage). Three rats/time point were sacrificed and various tissues were collected to monitor tissue distribution of the radioactivity. The time points were 5, 30 min., 2, 6 and 24 hr. after the drug administration.

Results: The radioactivity was distributed throughout the body, in most of the tissues the T_{max} was 30 min. The concentrations of radioactivity in stomach, intestine, kidney, liver and thyroid were higher than that found in plasma. After 24 hr., radioactivity in various tissues were very low except significant level of radioactivity was seen in thyroid (about 57 fold higher than that seen in plasma).

Table 1: Levels of ^{14}C in tissue of rats after oral administration of [^{14}C]AG-1749

	Level of ^{14}C (μg AG-1749 equivalent/ml or g)				
	5 min	30 min	2 h	6 h	24 h
Plasma					
Brain					
Spinal cord					
Pituitary					
Eye ball					
Harder's gland					
Submaxillary gland					
Thyroid					
Thymus					
Heart					
Lung					
Liver					
Spleen					
Pancreas					
Adrenal gland					
Kidney					
Testis					
Skeletal muscle					
Epididymal fat					
Stomach					
Intestine					

Dose, 2 mg/kg. The mean values $\pm$ S.D. (N=3). * Mean value for two rats.

Pharmacokinetics of AG-1749 in Animals and Man
(Report A-29-1411/IND Amendment dated 3/13/92/
Pharmacology Review dated 11/3/92)

The $t_{1/2}$ s in the rat, dog and man (dose: 30 mg/kg/person = mg/kg) were hr, respectively. The C_{max} values were $\mu\text{g/ml}$, $\mu\text{g/ml}$ and $\mu\text{g/ml}$ (after a mg/kg dose and levels determined at day 1 and 7), and the AUCs were 2.6, 9.3 and 2202/2517 ng.h/ml, respectively, suggesting that AG-1749 does not accumulate in either species. In man no parent unchanged drug was found in the urine at any dose, although the hydroxylated sulfide metabolite, AG-1907 was detected up to of the dose. AG-1908 was identified in the urine and plasma, while the plasma also contained small amounts of dealkylated AG-1977.

10-Day Toxicokinetics of AG-1749 in Mice
(Abbott Report R&D 90/380/IND Amendment dated 3/13/92/
Pharmacology Review dated 11/3/92)

Groups of male mice, 45/dose group, were treated po with AG-1749 at doses of 150, 300, 600, 1,200, and 2,400 mg/kg/d, once daily for 10 days. Five mice from each dose group were sacrificed at 0.25, 0.5, 1, 1.5, 2.5, 4.0, 6.0, 10.0 and 24 hours post-last-dose and blood was collected. Urine was also collected from the 1,200 and 2,400 dose group mice on day 7. The levels of the drug were determined by TLC/liquid scintillation. Urines of treated mice were found to be purple in color and 5 mice died during the study. The AUCs were: 1.429, 3.951, 6.951, 14.877 and 27.421 ug.eq.h/ml at doses of 150, 300, 600, 1,200 and 2,400 mg/kg, respectively. The drug levels in the plasma followed a linear relationship with correlation coefficient $r=0.9995$, ie. the AUCs were dose-dependent and there was no accumulation, suggesting that the doses recommended for the repeat carcinogenicity study by the Agency were appropriate.

RATS:

1. Single Dose-Pharmacokinetics of AG-1749 in the Female Rats
(Takeda, Report A-29-696/IND Amendment dated 7/27/90/
Pharmacology Review dated 1/2/91):

Doses tested were 5, 15, 50, and 150 mg/kg in groups of female rats, 50 each. Blood samples were collected from 5 rats each at 5, 15, 30 min, and 1, 2, 3, 4, 6, 8, and 24 hr post-dosing. AG-1749 and its metabolites were analyzed by HPLC with sensitivity of 0.005 ug/ml. In the female rats also, the plasma levels (see next page) increased with increasing dose but the rate of absorption and metabolism was slower at higher doses.

Pharmacokinetics in Rats (n=5) After Single Doses of AG-1749

Dose, mg/kg	T _{max} hr	C _{max} ug/ml	t _{1/2} hr	AUC _{0-24 hr} ug.h/ml
5				
15				
50				
150				

DISTRIBUTION

In Vivo Binding To Plasma Protein: (Study A-29-876/IND
Initial Submission/Pharmacology Review dated 9/6/93)

After oral administration of ¹⁴C-AG-1749 (2 mg/kg) to rats (n=3) and dogs (n=3), about 91-95% and 80-92% of plasma radioactivity respectively was bound to the proteins.

Metabolism

The metabolic pattern of AG-1749 after oral administration in rats and dogs were similar to that seen after I.V. dose (see above).

Distribution of Radioactivity Into Erythrocytes of Rats and Dogs
(Study A-29-876 Initial Submission/Pharmacology Review dated 9/6/93)

Methods: Rats (n=3) and dogs (n=3) were given a single oral (gavage) dose of ¹⁴C-AG-1749 (2 mg/kg). Blood samples were collected from tail vein of rats at 0.5, 2 and 6 hours after drug administration. In dogs, blood samples were collected from cephalic vein at 0.25, 1 and 4 hours after drug administration. The radioactivity in whole blood and plasma samples were measured by LSC. The percentage distribution in erythrocytes was calculated by using hematocrit value.

Results: Approximately 28-52% of the whole blood radioactivity in rats were found in erythrocytes, while only 11-18% of the whole blood radioactivity was seen in dogs erythrocytes. Thus the data indicated that penetration of drug and its metabolites in dog erythrocytes is less compared to that seen in rats.

Placental Transfer Study (Study A-29-876/IND Initial
Submission/Pharmacology Review dated 9/2/93)

Methods: Pregnant rats (20th day of gestation, n=3/group) were given a single oral (gavage) dose of ¹⁴C-AG-1749 (2 mg/kg). At 0.5, 2 and 6 hr. after drug administration maternal plasma, placenta, fetal plasma and fetal tissue were collected and radioactivity in the samples were measured by LSC.

Results: Significantly higher levels of radioactivity were seen in fetal plasma than maternal plasma. The data indicated that Lansoprazole crosses placenta.

Table 9 Fetal distribution of AG-1749 and its metabolites in pregnant rats after oral administration of [^{14}C]AG-1749

Sample	^{14}C -Labeled materials	Level of ^{14}C (μg AG-1749 equiv./ml)		
		30 min	2 h	6 h
Maternal plasma	Total ^{14}C AG-1749 Metabolites			
Placenta	Total ^{14}C			
Amniotic fluid	Total ^{14}C			
Fetal plasma	Total ^{14}C AG-1749 Metabolites			
Fetal tissue	Total ^{14}C			

Dose, 2 mg/kg. Female rats (20th day of gestation, N=3) were used. Total ^{14}C ; the mean values $\pm$ S.D., AG-1749 and metabolites; the values for the pooled samples.

Localization of Lansoprazole in the Gastric Mucosa of the Rat
(Report A-29-1200/IND Amendment dated 5/1/91/
Pharmacology Review dated 10/18/91)

The distribution of H^3 -Lansoprazole was studied autoradiographically in the male rats (JCI:SD, 7wks old) by giving orally 10 mg/kg (4.13 $\mu\text{Ci}/\text{mg}$) and sacrificing rats 1, 8, and 24h later. Most silver grains were distributed to the cytoplasm of the parietal cells (29 ± 9 , 43 ± 7 , and 27 ± 12 grains/cell at 1, 8, and 24h, respectively) suggesting that maximum concentration of Lansoprazole occurred at 8h and declined thereafter. No silver grains were seen in the chief or endothelial cells.

Distribution Into Gastric Mucosa of Rat
(Study A-29-1779/ IND Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Detailed methodology was not provided.

Results: The concentrations of radioactivity in gastric mucosa were higher than those found in plasma at various time points (the time points were 0.25, 2, 4 and 6 hours after I.V. administration of the drug). Furthermore, unchanged drug, metabolite M-I and metabolite M-VI were the major components in the gastric mucosa.

Table 4 Distribution of AG-1749 and its metabolites into gastric mucosa of rats given intravenous dose of [¹⁴C]AG-1749

Tissue	Time after dosage (h)	Level of ¹⁴ C (μg AG-1749 equivalent/ml or g)				
		Total ¹⁴ C	AG-1749	M-I	M-VI	Others
Plasma	0.25					
	2					
	4					
	6					
Gastric mucosa	0.25					
	2					
	4					
	6					
Gastric muscle layer	0.25					
	2					
	4					
	6					
			ND	ND	ND	ND

Dose, 2mg/kg. Total ¹⁴C; the mean values ± S.D. (N=3), AG-1749 and metabolites; values for the pooled samples. ND; Not determined.
* Mean value for two rats.

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Tissue Levels After Repeated Oral Dosing in Mice
 (Report A-29-936/IND Amendment dated 8/30/90/
 Pharmacology Review dated 10/18/91)

Sponsor conducted this study to demonstrate that there was no difference between 5d/wk and 7d/wk regimens of drug administration. ¹⁴C-labeled Lansoprazole (5.38 MBq/mg, 98.8% pure, MBq=megabecquerel) was administered po to groups of male mice, 3 each, at doses of 1.5 and 50 mg/kg for 26 days, 5d/wk or 7d/wk. At termination, mice were sacrificed and tissue levels of the radioactivity were determined. The differences between the two regimens were clear at 50 mg/kg, when the 24h radioactivity levels were consistently lower with the 5d/wk regimen than with the 7d/wk regimen. It is important to note that in most tissues the 72h tissue levels with 5d-treatment schedule were significantly lower than 24h levels with 7d-treatment: e.g. the drug levels in the thyroid at 24h with 7d treatment were 19.70 ug/g in comparison to 9.51 ug/g at 72h after 5d treatment schedule, suggesting 5d/schedule is inappropriate for conducting carcinogenicity studies. Overall the drug levels were lower with the 5d/wk than with the 7d/wk regimen regardless of the time of measurement. Data on the tissue levels of the metabolites were not presented. Statistical analyses were probably not performed since significant differences were not shown in the table (see attached sponsor's table below).

Table 2: Levels of ¹⁴C in tissues of mice 24 and 72 h after repeated oral administration of [¹⁴C]AG-1709 for 26 days

Tissue	Levels of ¹⁴ C (as AG-1709 metabolite or p)		
	Single dose 24 h	Repeated doses	
		(7 days/week) 24 h	(5 days/week) 72 h
Plasma			
Brain			
Serum cord			
Pituitary			
Eye ball			
Thyroid gland			
Submaxillary gland			
Thyroid			
Thymus			
Spleen			
Lung			
Liver			
Spleen			
Pancreas			
Adrenal gland			
Kidney			
Testis			
Subcutaneous fat			
Stomach			
Intestine			

Mean, 20 mg/kg/day. Data are the mean values ± S.E. of three mice given [¹⁴C]AG-1709 orally
 1 h before a meal for 26 days. * Data values for the eyes.

Distribution of AG-1749 in the Mouse Bone Marrow
(Takeda Report A-29-1411/IND Amendment dated 3/13/92/
Pharmacology Review dated 11/3/92)

The distribution of C14-AG-1749 (sp. activity 5.61 MBq/mg, 99% pure, mixed with cold AG-1749) was examined in the JcL ICR male mice. After oral dosing at dose of 50 and 5,000 mg/kg (suspended in 5% gum arabic), the radioactivity in the plasma, bone marrow and other tissues was determined at 0.25, 1, and 4 hours post-dosing by TLC/liquid scintillation. High concentration of the total radioactivity (C14) were present in the plasma by first sampling at 0.25 hr (see sponsor's tables on the next page). After a 50 mg/kg dose, the radioactivity levels in the bone marrow, plasma, and heart were: 3.96, 11.29 and 3.88 ug.eq/g, respectively. The levels in the liver, stomach, kidneys were higher than those in the plasma. At 5,000 mg/kg, maximum radioactivity at 1 hr in the bone marrow was: 233 ug/g and distribution in the tissues was similar as with the lower dose.

Plasma Drug Levels: The plasma levels of the parent AG-1749 (see next page) after a dose of 50 mg/kg were 0.81, 0.27 and 0.03 ug.eq/ml (7, 5.5, and 2.7% of the total radioactivity, respectively) at 0.25, 1, and 4 hour, respectively, while after 5,000 mg/kg, the plasma levels at 0.25, 1 and 4 hr were 43, 47, and 8 ug.eq/ml (12, 14, and 9% of the total radioactivity), respectively.

In summary, the levels of the parent drug and its metabolites increased with dose and then declined with time in both plasma and tissues. Appreciable levels of the drug were distributed to the mouse bone marrow which formed the basis for the conduct of the micronucleus test in the mouse where AG-1749 was found to be non-clastogenic.

Table 1 Concentration of ^{14}C in tissues of mice given [^{14}C]AG-1749 orally

Dose (mg/kg)	Tissue	Concentration (μg AG-1749 eq./ml or g)		
		0.25h	1h	4h
50	Plasma			
	Bone marrow			
	Heart			
	Lung			
	Liver			
	Kidney			
	Stomach			
5000	Plasma			
	Bone marrow			
	Heart			
	Lung			
	Liver			
	Kidney			
	Stomach			

Mean values $\pm$ S.D. (n=5). * Not.

Table 2 Concentration of AG-1749 and its metabolites in plasma of mice given [^{14}C]AG-1749 orally

Dose (mg/kg)	Compound	Concentration (μg AG-1749 eq./ml)			AUC ^a ($\mu\text{g}\cdot\text{h}/\text{ml}$)
		0.25h	1h	4h	
50	Total ^{14}C				
	AG-1749 Metabolites				
5000	Total ^{14}C				
	AG-1749 Metabolites				

Mean values $\pm$ S.D. (n=5). * 0-4h. Figures in parentheses denote % of the AUC for total ^{14}C .

Metabolism of ^{14}C -Lansoprazole Following a Single Oral or I.V. Dose (0.5 mg/kg) to Dogs
(Report PPRd/88/344/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Beagle dogs (2/sex) were given a single oral (gavage) dose (0.5 mg/kg) of ^{14}C -Lansoprazole. Two weeks later, these animals were given similar dose of ^{14}C -Lansoprazole via cephalic

vein. Blood samples (about 10 ml) were collected from jugular vein just prior to drug administration, 0.1 (I.V. dose only), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, 96 and 120 hours after oral or I.V. administration of the drug. Urine and feces samples were collected over 24 hr. period for up to 5 days. About 3 months later, one of the female dogs bile duct was cannulated, and was given a single I.V. dose (0.5 mg/kg) of ^{14}C -Lansoprazole. Bile samples were collected from 0-2, 2-4 and 4-6 hours after drug administration. In all samples radioactivities were measured by LSC. Drug and its metabolites were measured by HPLC methods.

Results: Sponsor has not calculated any pharmacokinetic parameters. The C_{max} after oral administration was reached at 0.5 hr. after the drug administration. Subsequently, plasma radioactivity declined very rapidly. By the end of 24 hours only about 4% of C_{max} levels were seen in the plasma. The plasma radioactivity also declined very rapidly in dogs which were given similar dose via I.V. route. Two hours after drug administration, plasma concentrations of radioactivity were comparable whether drug was given via oral or I.V. route. During the first 3 hours after oral or I.V. administration of ^{14}C -Lansoprazole, about 60-80% of radioactivity seen in whole blood was associated with the plasma portion, but by the end of 12 and 24 hours after drug administration, the radioactivity levels seen in red cells and plasma were comparable (46% hematocrit assumed).

Levels of Radioactivity in Red Cells and Plasma

<u>Hours After Dose</u>	<u>Ratio of Radioactivity in Red Cells/Plasma</u>	
	<u>Oral</u>	<u>I.V.</u>
0.5	0.38	0.21
1.0	0.36	0.22
3.0	0.48	0.43
6.0	0.59	0.24
12.0	0.80	0.67
24.0	0.78	0.70

Calculation is based on a hematocrit of 46%

Irrespective of route of administration, by the end of 5 days about 23-27% and 64-68% of the administered radioactivity were excreted in urine and feces respectively. Most of the excretion occurred during the first 24 hours after the drug administration.

Complete absorption (87%; range: 73-92% including cagewash) of radiolabeled Lansoprazole via oral route was evident in dogs since excretion pattern and amount (% of dose) of radioactivity after I.V. and oral dose were comparable. The fecal excretion in dogs were mainly biliary. About 43% of the administered I.V. radioactivity was excreted into bile within first 6 hours. No free drug was seen in bile, feces and urine samples, indicating that drug was extensively metabolized in dogs. A total of 7 metabolites and 3 unidentified peaks) were seen in bile, feces and urine. The distribution pattern of metabolites were as follows:

Table 19

Mean Summary of the Distribution of Metabolites in the Excreta
of Male and Female Dogs after Oral and/or Intravenous
Administration of a 0.5 Mg/Kg Dose of ABBOTT-65006-¹⁴C

<u>Metabolite</u>	<u>% of the ¹⁴C-Dose</u>				
	<u>Bile</u>	<u>Feces</u>		<u>Urine</u>	
	<u>(I.V.)</u>	<u>(P.O.)</u>	<u>(I.V.)</u>	<u>(P.O.)</u>	<u>(I.V.)</u>
	M.D.	1.2	0.4	M.D.	M.D.
	6.0*	33.4	36.1	1.5	1.6
	8.0*	M.D.	M.D.	M.D.	M.D.
	3.7*	3.5	6.5	0.8	0.3
	6.2*	M.D.	M.D.	M.D.	M.D.
	8.1*	1.9	1.6	M.D.	M.D.
	M.D.	9.4	3.4	M.D.	M.D.
	8.5	8.6	8.3	14.3	19.9
Others	2.1	5.2	2.9	2.1	1.6
	—	—	—	—	—
<u>Total Dose</u> <u>Excreted</u>	42.6	63.3	59.4	18.8	23.4

* Free and conjugated

Note:

Metabolic Fate of AG-1749 After I.V. Administration
in Male Rats and Male Dogs
(Report A-29-1779/IND : Initial Submission/
Pharmacology Review dated 9/6/93)

Methods: Male Wistar rats (n=58) and male beagle dogs (n=3) were given 2 mg/kg/day radio-labelled Lansoprazole (^{14}C -AG-1749) for 7 consecutive days. Blood samples were collected from the tail vein of rats and from the cephalic vein of dogs. The sample collection times were 5, 10, 15, 30 min, 1, 2, 3, 4, 6, 8, and 24 hr after the drug administration on day 1, 3, 5 and 7 of the study. Three rats per time point were used, after blood collection from rats, animals were sacrificed and various tissues were collected for monitoring tissue distribution of the radioactivity. Urine samples were collected over 0-4, 4-8, 8-24, 24-48, 48-72 and 72-96 hour periods after the drug administration in rats and dogs. Additionally, feces were collected over 24 hr. period for up to 96 hr. after the drug administration. In all samples, radioactivity was measured by liquid scintillation counter (LSC). ^{14}C -AG-1749 and its metabolites were measured by HPLC and TLC methods.

Results: After I.V. dose to rats, the plasma level of the unchanged drug decreased rapidly with $t_{1/2}$ value of 0.3 hr. In dogs the plasma level of the unchanged drug decreased bi-phasically with $t_{1/2}$ (alpha) of 0.6 hr. and $t_{1/2}$ (beta) of 4.1 hr. On the basis of AUC values, about 35.2% of administered radioactivity corresponds to unchanged drug in plasma, while in dogs about 40% of administered radioactivity corresponds to unchanged drug (in rats AUC $_{0-24}$ and in dogs AUC $_{0-24}$ were used for calculation). When rats were given Lansoprazole for 7 consecutive days, then concentration of radioactivity in plasma (C_{max}) increased after 3rd dose and there was a slight increase in $t_{1/2}$ (beta) of radioactivity, and steady state was reached after the 3rd dose.

Table 1 Concentrations of ^{14}C in plasma of rats given intravenous dose of [^{14}C]AG-1749 daily for 7 days

No. of dosing	Pharmacokinetic parameters			
	C_{min}^a ($\mu\text{g}/\text{ml}$)	$t_{1/2}$ (h)		AUC^a ($\mu\text{g}\cdot\text{h}/\text{ml}$)
		α	β	
1				
3				
5				
7				

Dose, 2mg/kg/day. The mean values $\pm$ S.D. (N=3). Concentrations are expressed as μg AG-1749 equivalent/ml. ^a 24 h after dosing.

Distribution:

Methods: Distribution study after I.V. (single or multiple) dose was conducted in rats. For methods see above.

Results:

The radioactivity was distributed throughout the body rapidly. At 5 min. after I.V. dose, concentrations of radioactivity found in liver, adrenal gland, harder's gland, thyroid, pancreas, stomach and sub-maxillary gland were higher than that found in plasma. After 72 hr., radioactivity in various tissues were very low, except significant level of radioactivity was seen in thyroid. Distribution pattern of radioactivity after 7 days of treatment were similar except the levels of radioactivity in respective tissues at 24 hr. after the drug administration were slightly higher than those found after single dose. Again findings of relatively high concentrations of radioactivity in the thyroid at 24 hr. post 7th dose was evident. Additionally, six days after the last dose, concentrations of radioactivity in various tissues were minimal except in thyroid.

Table 2 Concentrations of ^{14}C in tissue of rats given intravenous dose of [^{14}C]AG-1749

Tissue	Level of ^{14}C (μg AG-1749 equivalent/ml or g)					
	5 min	30 min	2 h	6 h	24 h	72 h
Plasma						
Brain						
Spinal cord						
Pituitary						
Eye ball						
Harder's gland						
Submaxillary gland						
Thyroid						
Thymus						
Heart						
Lung						
Liver						
Spleen						
Pancreas						
Adrenal gland						
Kidney						
Testis						
Skeletal muscle						
Epididymal fat						
Stomach						
Intestine						

Dose, 2mg/kg. The mean values $\pm$ S.D. (N=3).

Table 3 Concentrations of ^{14}C in tissue of rats 24h given intravenous dose of [^{14}C]AG-1749 daily for 7 days

Tissue	Level of ^{14}C (μg AG-1749 equivalent/ml or g)				
	Days on drug ^a				6 days after the 7th dosing
	1	3	5	7	
Plasma					
Brain					
Spinal cord					
Pituitary					
Eye ball					
Harder's gland					
Submaxillary gland					
Thyroid					
Thymus					
Heart					
Lung					
Liver					
Spleen					
Pancreas					
Adrenal gland					
Kidney					
Testis					
Skeletal muscle					
Epididymal fat					
Stomach					
Intestine					

Dose, 2mg/kg/day. The mean values $\pm$ S.D. (N=3). ^a 24 h after dosing.

then the drug (and its metabolites) crosses red cell stroma of rat more easily than in dogs and humans. It should also be noted that this finding is contradictory to previous findings (see results of plasma protein binding). Sponsor should be asked to reconcile these discrepancies, particularly in rats i.e. if 92-96% of the drug is bound to plasma then how can 78-79% of the drug is sequestered in the red cells of rats.

Metabolic Fate of AG-1749 in Mice, Rats and Dogs
(Report A-29-876/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Absorption

Methods: This study was conducted to determine absorption, distribution, metabolism and excretion of ¹⁴C-AG-1749 in male mice (Crj: CD-1), male or female rats (Jcl: Wistar) and male beagle dogs (CLEA Japan). Mice were given 1.5 mg/kg of ¹⁴C-AG-1749, while rats and dogs were given 2 mg/kg of ¹⁴C-AG-1749. The route of administration was oral (gavage) or I.V. Blood samples were collected from the heart in mice (3 mice/time point) at 5, 10, 20, 30 (I.V. only), 45 (I.V. only) minutes and 1, 1.5 (I.V.

only), 2, and 4 hr. after drug administration. In rats (n=3), blood samples were collected from the tail vein or abdominal aorta serially at 5, 10, 15 (I.V. only), 30 minutes and 1, 2, 3, 4, 6, 8 and 24 hr. after drug administration. In dogs (n=3) blood samples were collected from cephalic vein at 15 min., 1 and 4 hr. after drug administration.

Results: The radiolabeled drug (^{14}C -AG-1749, pH 7.0) was absorbed very rapidly in both rats and dogs. The $T_{\max}$ in rat was 0.5 hr. and in dog was 0.25 hr. for both radioactivity as well as for parent drug. It should be noted here that parent drug corresponded to about 2.6% of total radioactivity in rats and 9.3% in dogs (based on $\text{AUC}_{0-\infty}$ values). In rat, the $t_{1/2}$ for the drug was 1.1 hr. and in dog $t_{1/2}$ (alpha) and $t_{1/2}$ (beta) values were 0.6 and 11.0 hours respectively. The $t_{1/2}$ of the parent drug found in this study is much different than that found in report # A-29-1359 (i.e. 0.4-0.6 hours). In rats, no difference in pharmacokinetic profile was seen when administered dose pH was changed from 7 to 9. Based on AUC values, about 27%, 37% and 63% of the administered radioactivity (^{14}C -AG-1749) of oral dose were absorbed in mice, rat and dogs respectively.

Table 4 Area under the plasma level-time curves (AUC) of mice, rats, and dogs after oral and intravenous administrations of [^{14}C]AG-1749

Species	Route of administration	Dose (mg/kg)	AUC of ^{14}C ($\mu\text{g} \cdot \text{h}/\text{ml}$)	P.O./I.V. ratio*
Mice (N=3)	Intravenous	1.5		0.27
	Oral	1.5		
Rats (N=3)	Intravenous	2		0.37
	Oral	2		
Dogs (N=3)	Intravenous	2		0.63
	Oral	2		

* The mean values $\pm$ S.D.: mice; 0-4 h, rats; 0-24 h, dogs; 0-72 h. † Calculations from the mean plasma concentrations.

* The ratios of the mean values of AUC after the oral and intravenous administrations.

However, if one measures the parent drug levels in plasma as a function of time, then AUC value indicated that absolute bioavailability of the parent drug was 4%, 4% and 22% in mice, rats and dogs respectively.

Table 5 Bioavailability of oral [¹⁴C]AG-1749 in mice, rats, and dogs

Species	Route of administration	Dose (mg/kg)	AUC ^x (μg · h/ml)	Bioavailability ^z (%)
Mice (N=3)	Intravenous	1.5		4
	Oral	1.5		
Rats (N=4)	Intravenous	2		4
	Oral	2		
Dogs (N=3)	Intravenous	2		22
	Oral	2		

^x The mean values or the mean values ± S.D.: mice; 0-4 h, rats and dogs; 0-24 h. ^z Values for the pooled samples. ^z = [AUC(oral)/AUC (intravenous)]x100.

Reviewer's Addendum

In this study, sponsor has used a high quantity of microsomal (1500 mg/g) without any justification. Therefore, the activity and stability of the microsomes used may be questionable.

29. Hepatic Drug Metabolizing Activity in Mice and Rats after 14-Days of Oral Treatment with Lansoprazole: (Study # R&D/93/719)

Testing Laboratories:

Study Start and Completion Dates: September 16, 1993 and October 134, 1993.

GLP Requirements: A Statement of Compliance with GLP Regulations was included.

Animals: (i) Female Crl:CD-1 BR mice weighing 20 to 30 g and (ii) Crl:CD.BR female rats weighing about 150 g.

Drug Batch No.: 62-214-AL

Methods: Thirty female mice were divided into 3 groups (10/group) and were given an oral gavage dose of 300 mg/kg/day of lansoprazole for 14 days (in 5 % gum Arabia at pH 7.0). In other second part of the study, 30 female rats were divided into 3 groups (10/group) and were given the same oral gavage dose of 300 mg/kg/day of lansoprazole for 14 days (in 5 % gum Arabia at pH 7.00). The vehicle control, test compound and phenobarbitone were administered in a volume of 10 ml/kg/day) and volume of suspensions were administered in a volume of 10 ml/kg/day) and the selected doses of phenobarbitone and lansoprazole were equivalent to 200 umol/kg/day of these compounds. The purpose of the study was to compare the induction of hepatic mucosal drug metabolizing cytochrome P4502B (CYP2B) potential of phenobarbitone or lansoprazole in rat and mice. The dose of the study was selected on the observation of hepatomegaly at an oral dose of 15 to 150 mg/kg/day for 5 to 30 days. The animals were killed after 24 hr of the last dose of the compound, their liver removed, weighed and homogenized for the preparation of mitochondrial supernatant (S-9 fractions). The pooled liver preparations of processed for the cytochrome P-450 activity and 7-pentoxoresorufin O-dealkylase activity (7-PRODase) by fluorescence spectrophotometric methods. The liver of the individual animal was removed and weighed to assess the effect of phenobarbitone and the compound.

Results:

1. Body Weight and Relative Liver Weight: The body weight changes in the animals included in lansoprazole or phenobarbitone treatment groups were not seen. A treatment related effects were seen in liver weights of mice included in 300 mg/kg/day treatment group. In rats, a dose of 150 mg/kg/day (p.o.) of lansoprazole was reported to increase the relative weights of liver significantly ($p < 0.05$ and 0.001 in animals included in lansoprazole and phenobarbitone treated groups).

2. Total Cytochrome P-450 and 7- Pentoxeresorufin O-Dealkylase Activity: Cytochrome P-450 activity was increased by 93 and 45 % in phenobarbitone and lansoprazole treated group's mouse, respectively (On umol/kg basis, lansoprazole activity was one fourth that of phenobarbitone in mouse). The total cytochrome P-450 activity was increased by 120 and 40 % in rats included in rats included in phenobarbitone and lansoprazole treatment groups, respectively (On umol/kg basis, lansoprazole activity was one third that of phenobarbitone in rats).

In lansoprazole treated mice, an increase of 306 % in the induction of 7-PRODase activity was reported (VS 1607 % in phenobarbitone treated animals). Lansoprazole activity was 1/5 th of phenobarbitone in mice. In rats, lansoprazole activity was 1/36 of phenobarbitone activity.

In summary, the liver enzyme induction activity of lansoprazole was weaker than phenobarbitone. Total cytochrome P-450 activity and 7-PRODAase activities were about half and one fifth in the mouse. These activities were one third and thirty seventh in rats. Therefore, lansoprazole was a weak inducer of liver enzymes.

Secretion in the Milk: (Study A-29-876/IND
Submission/Pharmacology Review dated 9/6/93)

Initial

Thirty minutes, 2 and 6 hr. after oral administration of ¹⁴C-AG-1749 to female rats (14 days after parturition), significant amount of radioactivity was detected in milk and most of the radioactivity corresponded to metabolites.

Excretion

Methods: Rats (n=3) and dogs (n=3) were given a single oral dose of ¹⁴C-AG-1749 (2 mg/kg). Urine samples were collected during 0-4, 4-8, 8-24, 24-48, 48-72 and 72-96 hr. after drug administration. Feces were collected every 24 hr. up to 96 hr. after the drug administration. Radioactivity levels were measured by LSC.

Results: In rats, about 18% and 81% of the administered radioactivity were excreted in urine and feces respectively over 0-72 hr. after single dose administration. The corresponding percentages in dogs were 12% and 84% respectively. In both species most of the excretion occurred during 0-24 hr. period. The excretion pattern after 7 daily doses in rats were similar to that seen after single oral dose.

Biliary Excretion and Reabsorption (Study A-29-876/IND Initial Submission/Pharmacology Review dated 9/6/93)

Methods: Biliary-cannulated rats were given a single dose of ¹⁴C-AG-1749 (2 mg/kg) intraduodenally. Bile samples were collected at 2, 4, 8 and 24 hr. after drug administration. To determine enterohepatic cycling of the biliary radioactivity, the radioactive bile was administered intraduodenally to other three bile-cannulated rats.

Results: Within 24 hr. after drug administration about 60%, 13% and 20% of the dose were excreted in bile, urine and in feces respectively. When radioactive bile was given (I.D.) to bile-cannulated rats, then within 24 hr. about 20% and 24% of dose were excreted in bile and urine respectively. These data indicate that the drug undergo enterohepatic cycling.

The absorption, distribution, metabolism and excretion (ADME) of lansoprazole were investigated in rats, mice, dogs and in a limited amount in monkeys. The reviews of these studies in each of the animal species are summarized below.

RATS

a. Absorption and Pharmacokinetics:

An oral dose of 2 mg/kg of ¹⁴C-lansoprazole (in 5% gum Arabia at pH 7.0) was absorbed rapidly by 37% in rats within 2 hrs of its administration. It was metabolized quickly with $T_{1/2}$ of 3 hrs in the plasma. The bioavailability was only 4 % in rats. The plasma concentration (C_{max}) of the compound was proportional to dose but ratios of AUC increased from 14 to 64 at 5 to 150 mg/kg dose. The plasma half life of the compound varied from 20 min to 1.2 hr. Lansoprazole was extensively (up to 96%) bound in rat plasma protein (up to 92-96% in vitro studies). The plasma concentrations were dose dependent, i.e., from ng.hr/ml at mg/kg doses. Its half life varied from 1.2 hr. The plasma concentration of AUC_(0-24hr) after multiple doses were similar and dose proportional. The circulating metabolites in rats were,

The plasma level of these metabolites were high in rats and their half life ($T_{1/2}$) were hrs. The plasma concentration of for (+)-isomer was fold higher than (-)-isomer after 2 mg/kg dose.

b. Distribution and Accumulation:

After an oral dose of 2 mg/kg, lansoprazole was found in rat intestines, stomach, liver, kidney, and thyroid from 10 to 25 times. It was also found in rat brain. Tissues to plasma ratios varied from 10 to 25 times of the tissues.

Orally administered lansoprazole in pregnant rats (on 20th day of gestation) gave 2 to 5 fold increase of drug in fetal plasma than maternal plasma at 0.5, 2, and 6 hr after the administration of the drug. The fetal metabolites were not characterized by sponsor.

In lactating rats, lansoprazole given at 14th day of parturition attained 6 to 11% of plasma concentration.

After an I.V. dose of lansoprazole was distributed in tissues, there was no change in the drug which was detected. It was eliminated in feces via hepatobiliary route. The unchanged lansoprazole entered the gastric mucosa and maintained a high concentration. metabolites were major components in the gastric mucosa.

d. Metabolic Pathways: Orally or parenterally administered lansoprazole was metabolized by

e. Excretion: Orally administered lansoprazole was eliminated in 12-25% urine and about 68-84% was excreted in the feces via bile. Free (2-3%) and (2.2%) metabolites representing 15 to 33% were detected in feces of rats. All of the metabolites are not characterized. In rat bile metabolites were present.

MICE

An oral dose from 1.5, 5, 15, and 50 mg/kg of lansoprazole was quickly absorbed in a dose dependent manner given. The plasma concentrations of 30, 230, 450, and 1840 ng/ml were seen within 20 to 25 minutes. The plasma concentrations were elevated disproportionately with larger doses. About 27% of the oral dose of 1.5 mg/kg was absorbed which has a bioavailability of 4% suggesting rapid metabolism. Its half life ($T_{1/2}$) was variable, which showed an increase with the increasing doses. It was 0.2 hr and 0.9 to 1.1 at 1.5 and 15 to 50 mg/kg doses.

The larger doses of lansprazole used in 10-day toxicity study were absorbed disproportionately, and plasma concentrations of ng.h./ml were observed in 150 and 2400 mg/kg/day doses respectively.

b. Distribution and Accumulation:

After a single dose of 1.5 mg/kg, tagged radioactivity with lansoprazole was distributed in thyroid, stomach, pituitary, liver, adrenals and intestines. A repeated dose of 1.5 and 50 mg/kg/day for 26 days produced accumulation in plasma and all body tissues examined. Two to 6 folds greater plasma concentrations were seen in mice treated with 50 mg/kg/day dose for 26 days. ¹⁴C-levels were 5 to 35 folds higher than plasma levels in thyroid, liver, pituitary, stomach, adrenals, and kidneys.

DOGS**Absorption and Pharmacokinetics:**

After a single oral dose of 2, 5, 15 and 50 mg/kg of lansoprazole, a non-linear peak plasma concentrations (C_{max}) of 350, 375, 753 and 3470 ng/ml were reported in 2, 5, 15 and 30 mg/kg. Plasma half life was from hr. After a multiple dose of 5 mg/kg/day for 7 days C_{max} and AUC levels decreased from ng/ml and ng.h/ml on day ng/ml and ng.h/ml on day

Intravenously administered lansoprazole at a dose of 2 mg/kg, also showed a decrease of plasma concentration from (on day 1) to (day 9) ng.h/ml. The half life was also decreased from 0.039 (on day 1) to 0.013 (on day 9)hr. A repeated I.V. dosing of 3 mg/kg for 2-8 days produced a 30% decrease in AUC value (ng.h/ml on day 1 and 28 respectively).

Plasma concentration of (+) stereoisomer of lansoprazole after an I.V. dose of 2 mg/kg/day were 2 to 3 folds greater than (-)-stereoisomer. C_{max} levels were 2-3 folds greater for (+)-stereoisomer than for (-)stereoisomer. Their levels (AUC 0-8 hr) after oral and I.V. dosing were 283 and 1425 ng.h/ml for (+)isomer and 138 and 859 ng.h/ml for (-)isomer. The bioavailability of two isomers was 20 to 16% for (+) and (-)isomers respectively. Lansoprazole had equal bioavailability from 50 mg plain and enteric coated granules in capsules. Peak plasma concentration was reactive within 66 minutes. After the ingestion of enteric coated tablet, a period of lag time of 1 hr was required and T_{max} was 2.9 hr. At T_{max} , the plasma concentrations attained with these 3 formulations were similar. The unchanged compound (small amount), (24% of dose), (4.6% of the dose), (2.1% of dose), were found in urine of rat and (26.2% of the dose) in the feces.

b. Protein Binding:

In vitro studies, 92 to 96% of added lansoprazole was (10 to 1000 ng/ml) bound to dog plasma proteins. An oral dose of 2 mg/kg was bound to plasma proteins up to 80 to 92% in 0.25, 1, and 4 hr. (In vivo studies the drug with plasma proteins was the metabolites of lansoprazole).

c. Distribution:

About 23 to 30% of ¹⁴C-lansoprazole (10 to 1000 ng/ml) tagged radioactivity penetrated into red blood cells. After an oral dose of 2 mg/kg of lansoprazole 11 to 17% of the drug was identified in 0.25 to 4 hr. Therefore affinity of metabolite to plasma is different from parent drug.

d. Metabolic Pathway:

Lansoprazole was showed to be metabolized rapidly in dogs as no free drug was found in dog urine, bile and feces. Ten metabolites were isolated from these fluids or excretion.

e. Excretion:

After an oral dose of 0.5 or 2.0 mg/kg to dog, 68 to 84% of the administered radioactivity excreted in feces and 12 to 25% of the radiolable was excreted in urine. These were similar to the excretion pattern observed in I.V. dosing (2 mg/kg) study with lansoprazole. The majority of fecal radioactivity (43%) administered by I.V. route was detected in 0-6hr bile samples. Therefore most of the compound excreted in feces was resulted from biliary secretion of ¹⁴C-dose. (Bile samples obtained from anesthetized dog with biliary catheter).

Urinary metabolites consisted of Significant amounts of (about 15% of dose) were found in feces after 2 mg/kg dose. Metabolites and free drug were present in bile, which constituted about 4 to 8% of the dose. Sponsor did not account for 60% of radioactivity.

After I.V. dose of the lansoprazole, levels of the drug in gastric mucosa were higher than plasma and gastric muscle layer and persisted for 6 hr. Unchanged parent drug, were components of gastric mucosa and persisted longer than in plasma.

MONKEYS

Pharmacokinetics parameter of plasma concentration (C_{max} and AUCs) were determined in monkey after a single and multiple doses. A non-dose proportional plasma concentration of C_{max} and AUC (ng.h/ml) were reported after a single dose. The half life after multiple dosing was 3.4 hr.

Humans and Interspecies comparison:

Lansoprazole by an oral route of administration was absorbed less in mice, rats and dogs than humans, and its bioavailability was 4, 22, and 80% in rats, dogs, and humans respectively.

Orally administered lansoprazole was administered extensively in humans by a metabolic pathway common to rats and dogs. Orally administered compound was eliminated mostly by urinary excretion in man. Plasma level of (+) enantiomer was higher than (-) enantiomer in rats, dogs, and humans. The degree of stereoselectivity was different since C_{max} and AUC for (+)-isomer were 9-14 times higher than (-)-isomer in humans. These were 1 to 3 folds greater in rats and dogs.

In rats, the drug undergoes degradation in intestine before reaching liver where it is metabolized further. Thus, lansoprazole was absorbed more efficiently in dogs than rats but AUC and C_{max} decreased with multiple dosing. The decrease in plasma level was due to an increase in metabolism and clearance of the drug by the induction of drug metabolizing enzymes.

In summary, lansoprazole in 5% gum Arabia (5%) or in gelatin capsules was absorbed within 2 hrs of its administration in 27, 37, and 63 to 87% in mice, rats, and dogs respectively. The administered compound was 4, 4 and 22% in rats, mice and dogs. It attained a dose dependent plasma concentration (AUC_{0-24hr}) of ng.h/ml after an oral dose of mg/kg in mice; ng.h/ml in rats mg/kg/day doses), and ng.h/ml in dogs ng.h/day). About 92 to 96% of the compound was reported to bind with rat and dog plasma protein respectively. The half life of lansoprazole was 1.2, 1.0, 3 hr in mice, rats, dogs, and monkeys respectively. After a multiple dose of the capsule, the plasma concentrations were similar in rats and mice showing that this compound was not accumulated. Lansoprazole was biotransformed into its active forms, AG-2000 and AG-1778 in parietal cells. In formulation, It is in racemate mixture, and the (+)-isomer form of lansoprazole had been reported to be weaker than (-)-isomer. It was metabolized by hydroxylation, sulfonation, and 4-hydroxylation. The metabolites isolated and purified from rat and dog plasma are:

Pharmacokinetic data of the compound has not been studied by sponsor. Their half life were 1, 3 and 11 hr in mice, rats and dogs. An oral dose was accumulated in the rat intestine, stomach, liver, kidney, thyroid, and brain. Tissue to plasma ratios varied from 2 to 35% respectively. A dose of 150 mg/kg/day in 5 days/week treatment groups of lansoprazole in rats, induced microsomal enzymes, microsome proteins as cytochrome 450 and cytochrome b₅ levels. Orally administered compound was excreted in urine (12 to 25%) and feces (68 to 80%). Only 1% of unchanged drug had been detected in plasma of rats and dogs. Metabolites to Metabolites free and conjugated equivalent to 0.6 to 3.5% were found in urine of rats and dogs. In dog bile all but hydroxymethyl metabolites were detected. The and were major metabolites in rats and dogs respectively in 6 to 11, 2 to 8, and 4% respectively.

In humans, it was extensively absorbed in plasma, and excreted in urine and bile (fecal excretion). It was demonstrated to bind with plasma proteins and the binding was higher than animals (rats, mice, and dogs). Its bioavailability was up to 80% in humans. It was metabolized in intestines before reaching liver. The rapid metabolism (in liver and RBI) in rodent was accounted for a low bioavailability in the animals.

TOXICOLOGY

ACUTE TOXICITY:

The acute toxicity studies of lansoprazole (AG-1749) submitted earlier, were reviewed in Pharmacology review of IND (Initial submission) dated August 12, 1990 and in Pharmacology review of IND (Initial submission) dated September 2, 1993. These reviews were acceptable, combined and have been reproduced below:

Methods: The acute oral/i.v. toxicity of AG-1749 was determined in 4-wk old (Jcl:ICR) mice and 5-wk old (Jcl:Wistar) rats. Lansoprazole suspension in 1% gum arabic was used for oral study while it was administered in "PEG" vehicle (pH 10.3-10.5 - for details see vehicle formulation). The rate of injection was ml/min for mice and ml/min for rats. The volume of administration in mice and rats were ml/kg. In i.v. studies, there was no vehicle control group. All animals were observed for toxic signs and mortality daily for 14 days. All animals were sacrificed at the termination of the study. The results of the acute toxicity are tabulated on the following pages:

Table
Acute Toxicity of AG-1749

Species	Route	Doses tested (mg/kg)	Highest Non-lethal Dose (mg/kg)	LD ₅₀ mg/kg	Signs/Symptoms
Mice	po	2,500, 5,000	5,000	--	No deaths; excretion of dark brown or dark purple urine.
	sc	2,500, 5,000	5,000	--	Abdominal stretching, recovered within 30 minutes.
	ip	2,500, 5,000	5,000	--	No deaths, abdominal stretching, decreased locomotor activity, respiratory depression, hypotonia of the abdominal muscles; recovery within 7-24 hr.
	iv	44, 58, 75 & 98	58	75.7 (F) 80.1 (M)	Respiratory depression, ataxia and hyporeactivity. Most deaths were within 30 min of drug administered.
Rats	po	2,500, 5,000	5,000		No deaths.
	sc	2,500, 5,000	5,000		No deaths.
	ip	2,500, 5,000	2,500	--	50% deaths from respiratory depression 2 days after dosing; at necropsy, discoloration of the liver and moderate pulmonary hyperemia were noted. Survivors recovered within 2 days of dosing.
	iv	44, 58, 75, 98	44 (M) 58 (F)	69.3	Respiratory depression, ataxia, and hyporeactivity were observed. Most deaths were in 30 min. of its administration.

Acute Toxicity of Related Compounds and Metabolites of AG-1749 and Partially Degraded AG-1749 in Mice (Takeda Report A-29-576/

IND Amendment dated 7/27/90/
Pharmacology Review dated 1/2/91)

Conducting Lab: Takeda's Drug Safety Research Labs, Osaka, Japan.

Dates of Conduct: Initiated 9/7/89; completed 1/29/90.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical:

A number of agents were tested (see list below).

Animals:

Mice, Jcl:ICR SPF, 4 wk old; males 21-25g, females 18-25g; 4 wk old; 5/sex/dose.

Doses:

Related compounds I, II, III, and IV at 1250, 2500, and 5000 mg/kg, po or ip; metabolites at 2280, 2960, 3850, and 5000 mg/kg; and partially degraded compound at 1250, 2500, and 5000 mg/kg. The concentration of solution was 1%, and injected in a constant volume of 0.1 ml/kg.

Methods and Results: Groups of mice were given the test agents po or ip and LD₅₀s were estimated. No animals died and thus the LD₅₀ of the test agents was more than 5000 mg/kg. However, the decreased locomotor activity, respiratory depression, hypo-irritability, ataxic gait and flattened posture were seen in mice at 5000 mg/kg with the partially degraded AG-1749. Similar signs of toxicity were also seen at 2500 and 5000 mg/kg doses of a related compound II and metabolite VI at 2280 mg/kg, but not at higher doses. In most cases, the signs of toxicity appeared within 4-6 hr of dosing and disappeared within a day in some cases and within 1-5 days in others (R-III, -metabolite-I).

In summary, the LD₅₀s of the partially degraded AG-1749, compounds related to AG-1749, and its metabolites were greater than 5000 mg/kg showing hypo-irritability, decreased locomotor activity, depressed respiration as signs of toxicity, and thus these agents were not more toxic to mice than the parent compound, AG-1749. For comparison, the parent compound at 5,000 mg/kg produced 50% deaths from respiratory depression 2 days after dosing and at necropsy, discoloration of the liver and moderate pulmonary hyperemia were noted. Survivors had recovered within 2 days of dosing.

6. ACUTE/SUBCHRONIC/CHRONIC TOXICITY:

2-Week I.V. Toxicity Study in Rats
(Report A-29-504/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Evaluation Laboratories
Takeda Chemical Industries, Ltd.,
Osaka, Japan

Study Started: February 1, 1989

Study Completed: June 30, 1989

GLP Requirements: Not mentioned

Animals: Jcl: Wistar rats (6 weeks old; males: 151-167g and
females: 122-136g)

Drug Batch No.: M610-016

Placebo Batch No.: ZR655-05

Methods: In this study dose selection was based on preliminary 1-week i.v. toxicity study in rats in which doses of 0 (vehicle: mannitol, pH 12-12.1), 30, 60 and 100 mg/kg/day were used. Bradypnea, decreased activity, and necrosis at the injection sites were seen in mid and high dose treated rats. Three out of 4 males died in high dose group within 4 days. At 60 mg/kg, thymus and prostate weights were decreased by 36% and 31% respectively, when compared to their respective control values. Histopathological examination revealed atrophy of the thymus and s.c. hemorrhage at the injection site in 60 and 100 mg/kg treated rats. The no effect dose in this study was 30 mg/kg/day. Based on these findings 30 mg/kg/day was chosen as the highest dose for subsequent 4-week I.V. toxicity study.

In the main study groups of 5 male and 5 female rats were given I.V. injection of Lansoprazole at daily doses of 0 (saline), 0 (vehicle: "mannitol", pH 10.5), 1, 3 and 10 mg/kg/day for 2 weeks. The volume of administration was fixed at 5 ml/kg/day (4 ml/min). The highest tested dose level in this study is about 33 times the intended human dose (15 mg/person or about 0.3 mg/kg). All animals were observed daily for clinical signs and mortality. Body weights were recorded on days 0, 3, 7, 10 and 14 of the study and food intakes were recorded weekly. Ophthalmoscopic examinations were performed on all control and high dose treated rats once pretest and once during week 1 of the study. All animals were starved for about 20 hr. after the last dose, and blood samples were collected from abdominal aorta for hematological and serum chemistry tests. Four-hour urine samples

were also collected between 21-24 hr. after dosing for urinalysis. All surviving animals were sacrificed at the end of the study period and subject to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: None
2. Mortality: None
3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were seen.
4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.
5. Vital Signs/Physical Examination/Ophthalmic Examination: No treatment related effects were seen.
6. Organ Weights: Stomach weights were increased by 16-23% in all treated rats (reflection of pharmacologic effect).
7. Gross Pathology: No treatment related effects were seen.
8. Histopathology: No treatment related effects were seen.

The data indicated that the highest tested dose was no effect dose in this study.

REVIEWER'S ADDENDUM:

A part of materials and methods from the review of the above study was deleted because it described the 4-week subacute toxicity study as the basis of the dose selection. This was incorrect and inaccurate.

4-Week I.V. Toxicity Study in Rats
(Report A-29-805/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Evaluation Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Study Started: January 12, 1990

Study Completed: June 27, 1990

GLP Requirements: A Statement of Compliance with GLP regulation was included.

Animals: Jcl: Wistar Rats (6 weeks old, males: 132-151g and females: 106-124g)

Drug Batch No.: M610-018

Placebo Batch No.: 004

Methods: Groups of rats (10/sex/group) were given I.V. injection of Lansoprazole at daily doses of 0 (saline), 0 (vehicle: "mannitol" pH 10.6-10.8), 3, 10 and 30 mg/kg/day for 4 weeks. The dose selection was based on 2-weeks I.V. toxicity study (see above). The rate of administration was 4 ml/min. The volume of administration for saline, vehicle and high dose groups was 10 ml/kg/day. The volumes of administration in low and mid dose groups were 1 and 3.3 ml/kg/day respectively. All animals were observed for clinical signs and mortality daily. Body weights were recorded on day 1, 3, and 7 of the study and twice a week thereafter. Food intakes were recorded weekly. Ophthalmoscopic examinations were performed on 5 rats/sex/group once pretest and on day 24 (before dosing) of the study. All animals were starved for about 20 hr. after the last dose and blood samples were collected from abdominal aorta for hematological and serum chemistry tests. On day 25 the 4-hr. urine samples were also collected from 5 rats/sex/group for urinalysis. All surviving rats were sacrificed at the end of the study period and subjected to complete necropsy. Histopathological examinations were performed on all tissues from control, vehicle control and high dose group. Additionally, liver, kidney, stomach and injection site from all rats in low and mid dose groups were also examined microscopically. In this study hepatic drug metabolizing enzymes (amino-N-demethylase and aniline hydroxylase) activities were also monitored.

Results:

1. **Observed Effects:** No treatment related effects were seen.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** Body weight gains in high dose treated males were decreased by 14% and this was accompanied with a reduction of 6% in food consumptions.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.
5. **Blood Chemistry/Urinalysis:** At 30 mg/kg/day, serum cholesterol levels were increased by 13% compared to the control values. Plasma T₁ and T₂ levels were normal. No treatment related effects were seen in urinalysis.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related effects were seen.
7. **Organ Weights:** Stomach weights were increased by 10-15% in all treated rats compared to the control values. In high dose group, thymus weights were decreased by 21% and 30% in males and females respectively compared to their respective control values.
8. **Gross Pathology:** Thymus atrophy was evident in high dose treated rats (males = 5/10 and females = 7/10).
9. **Histopathology:** Eosinophilia and hypertrophy of the chief cells of the stomach were seen in treated rats (males: control = 0/10, vehicle control = 0/10, low dose = 1/10, mid dose = 2/10 and high dose = 3/10; females: control = 0/10, vehicle control = 0/10, low dose = 0/10, mid dose = 1/10 and high dose = 1/10). No other treatment related abnormality was observed (including liver).
10. **Activity of Hepatic Drug-Metabolizing Enzymes:** In high dose treated male rats, hepatic aminopyrine-N-demethylase and aniline hydroxylase activities were increased by 21% and 13% respectively when compared to their respective control values.

In this study the data indicated that 10 mg/kg/day was the no effect dose.

4-Week Oral Toxicity Study in Rats
(Report A-29-167/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan.

Dates of Conduct: Initiated 1/16/85, completed 8/9/85.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-001; suspended in 1% gum arabic solution, tested periodically for stability.

Animals: Rats, Jcl:Wistar; 10/sex/dose; 5 wks old males (103-112g) and 94-105g females.

Doses: 0 (control, 1% gum arabic solution), 15, 50, and 150 mg/kg/d given, po, by gavage in constant volumes of 5 ml/kg/d, once daily, 7 days/wk for 4 weeks. The dose groups are designated as low (L), mid (M), and high (H) groups, respectively, and the respective solution concentrations were 1%, 3%, and 5%, respectively. The doses were selected on the basis of a 2-wk oral toxicity study, the outcome of which was not provided.

Methods: The test agent or placebo was administered to the rats as indicated above and the animals were observed for morbidity and mortality. The specific observations are mentioned in each respective section below. At termination of the study, the animals were necropsied, the organs were weighed and examined macro- and microscopically. However, all the organs from all the treated animals were not examined and these are discussed in the histopathology section.

Results/Observations: General behavior observed daily was normal.

Mortality: None.

Body weights recorded on days 0, 1, 3, 7, then twice weekly, showed dose-dependent decrease in the body weight gains among the male rats from the first week of study. For example the percent body weight gain at termination in the control, low, mid and high dose males was 159, 154, 145, and 133%, respectively, significant ($p \leq 0.05$ or 0.01) at mid and high doses. Among the females, the weight gains were 70, 41, 67, and 62%, respectively.

Food consumption, recorded once weekly was reduced parallel to the reduction in body weight gains in the mid and high dose male rats; the food consumed by the male rats during week 4 was 155, 152, 147, and 139 g/animal/week in the control, low, mid, and high dose groups, respectively. There was a slight but significant reduction in food intake among the high dose females (102 g versus 108 g/animal/wk in the control) during the week 3 of the study.

Ophthalmoscopic examinations of 5 animals/sex in the control and high dose groups before dosing and after 23rd dose revealed no abnormal findings. The mid and low dose animals were not examined.

Urinalyses performed during wk 3 on 5 rats/sex/group were within normal limits.

Hematology performed at termination on all rats showed the following changes: hemoglobin content was slightly decreased in the 150 mg/kg/d males, and MCV was slightly reduced in the 150 mg/kg/d females. There were no other significant agent-related changes.

Clinical chemistries were performed at termination on all rats. Liver metabolizing enzymes such as aminopyrine-N-demethylase (APND) and anilin hydroxylase (AH) were altered as follows. At 150 mg/kg/d, among the males, the APND activity (mU/mg protein) increased by 16% of the control. In the female rats, the activities of both APND and AH increased by 30 and 48%, respectively, suggesting enzyme induction at 150 mg/kg/d.

Organ weights (see table 2 below): Most changes (increases or decreases) were dose-dependent in some cases: e.g. thymus weights decreased by 1.2, 23, and 42% as compared to controls in the low, mid and high dose group male rats, while in the females, the decreases were: 8, 21, and 44.5%. The liver weights increased by 1.3, 7, and 15.5% in the males, by 3.2, 8.7, and 21.7% in the females at the corresponding doses. The adrenal weights increased by 11% of controls only in the high dose females, and the lung weights increased by about 9% each in the high dose males and mid and high dose females. Thyroids were not weighed.

Table 2
Organ Weights as % of Body Weights of Rats (g/100 g bw) Treated with AG-1749

Treatment	Brain	Heart	Lungs	Liver	Kidneys	Spleen	Thymus (x10 ⁻³)
MALES (n=9-10/group)							
Control	0.76	0.36	0.40	3.59	0.85	0.24	169
15 mg/kg/d	0.76	0.38	0.43	3.64	0.87	0.25	167
50 mg/kg/d	0.78	0.39	0.43	3.84**	0.87	0.25	130
150 mg/kg/d	0.81	0.39*	0.44*	4.15**	0.92*	0.27*	98**
FEMALES (n=10/group)							
Control	1.13	0.38	0.46	3.10	0.84	0.25	201
15 mg/kg/d	1.12	0.39	0.48	3.20	0.86	0.24	185
50 mg/kg/d	1.13	0.40	0.50**	3.37**	0.89*	0.25	159**
150 mg/kg/d	1.15	0.42**	0.50**	3.77**	0.92**	0.24	111**

* or ** = significantly different from control at p ≤ 0.05 or 0.01.

Pathology

Gross pathology: A slight atrophy of the thymus cortex was observed in only one male rat in the 150 mg/kg/d group.

Histopathology: In the control and high group, all the major organs/tissues including the liver, kidney, stomach, thymus, and thyroid were examined histologically; in the mid and low dose groups, only the liver, kidneys, stomach, and thymus were examined histologically.

Liver: A slight hypertrophy of the centrilobular hepatocytes was seen in 8 of 10 male and 8 of 10 female rats in the high dose group. No such changes were seen in any other treated rat or the controls.

Stomach: Very slight vacuolization of the parietal cells was seen in 8 of 10 high dose females. Eosinophilia at the apical portion of the chief cells was seen all groups, but was more marked in the high dose animals.

Adrenal gland: Slight hypertrophy in the zona fasciculata was seen in the high dose females.

Thymus: No significant changes were seen except hemorrhage, hemosiderin deposition, crystal of hemoglobin, and granulum with hypertrophy of the cortex was seen in one high dose male rat.

Thyroids: Although these were stated to have been examined, no detailed histological description was provided.

Enzyme histochemistry was performed on one male rat in the control group and two male rats in the 150 mg/kg/d groups at termination and activities of acid phosphatase (ACP), alkaline phosphatase (AP), and succinate dehydrogenase (SDH) were determined. No significant changes were seen in any animal.

Electron Microscopic examination of the liver and stomach of one control male and two high dose male rats at termination showed the following:

Liver: There was a slight increase in the smooth endoplasmic reticulum of the centrilobular hepatocytes in the high dose male rats. No such determinations were made on rats in the low, mid, and high dose males and females.

Stomach: Slight dilatation of tubulovesicles was seen in the parietal cells at high dose; slight dilatation, fusion, and destruction of tubulovesicles, slight increase of lysosomes, dilatation of intracellular canaliculi, and a decrease of the microvilli were seen in the parietal cells at 150 mg/kg/d. In the chief cells that showed eosinophilia by light microscopy, there was a slight increase of small and dense secretory granules.

Myelogram was normal.

In summary, AG-1749 at oral doses of 15, 50 and 150 mg/kg/d for 4 weeks produced dose dependent decrease (9 & 16%) in the body weight gains and a parallel decrease in food consumption in the mid and high dose male rats. The females were unaffected. The liver metabolizing enzymes activities of APND increased by 16% of the control in the high dose males, while in the females, the activities of APND and AH increased by 30 and 48%, respectively. The weights of the liver, spleen, and kidneys increased, mostly in the mid and high dose animals, while that of the thymus decreased, with significant effects at 150 mg/kg/d. At 150 mg/kg/d, there was a slight increase (hypertrophy) in the smooth endoplasmic reticulum of the centrilobular hepatocytes in the liver. In addition, the parietal cells of the stomach were affected: dilatation of tubulovesicles, increase in the lysosomes, decrease in the microvilli were seen, but these were probably the exaggerated pharmacologic effects of the agent. The 50 mg/kg/d dose could be considered as the no-effect dose and 150 mg/kg/d as the maximum tolerated dose for the rat. However, still higher doses could have been tested in this 4-week toxicity study without compromising the study.

13-Week Oral Toxicity Study in the Rat
(Report A-29-168/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan.

Dates of Conduct: Initiated 3/2/85, completed 5/28/86.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-002); suspended in 1% gum arabic solution, pH 6.9-7.1; periodically tested for stability.

Animals: Rats, Jcl:Wistar; 10/sex/dose; 5 wks old males (108-119g) and 94-107g females.

Doses: 0 (control, 5% gum arabic solution), 5, 15, and 50 mg/kg/d given, po. by gavage in constant volumes of 5 ml/kg/d, once daily, 7 days/wk for 4 weeks. The concentration of solutions were 0.3, 1 and 5% (w/v) for the respective test groups. The doses were selected on the basis of the 4-wk oral toxicity study (reviewed above) that showed 7-15% decrease in the body weight gain at 50 and 150 mg/kg/d doses, slight hepatotoxicity and vacuolization of parietal cells in the stomachs of mid and high doses rats, however, without any deaths.

Methods: The test agent or placebo was administered to the rats as indicated above and the animals were observed for morbidity and mortality. The specific observations are mentioned in each respective section below. At termination of the study, the animals were necropsied, the organs were weighed and examined macro- and microscopically. However, all the organs from all the treated animals were not examined as discussed below in histopathology section.

Results/Observations: General behavior observed daily was normal.

Mortality: None.

Body weights were recorded on days 0, 1, 3, 7, then twice weekly from wk 2 to 5, and finally once weekly after 5 weeks. There were sporadic changes in body weights of treated rats, but without any significant trend in any direction.

Food consumption, recorded once weekly also did not show any specific trend even though there were occasional significant changes in the treated rats.

Ophthalmoscopic examinations of 5 animals/sex in the control and high dose groups before dosing and at wk 13 just before dosing revealed no abnormal findings. The mid and low dose animals were not examined.

Urinalyses during wk 13 on 5 rats/sex/group were within normal limits.

Hematology and blood chemistry performed at termination on all rats showed the following changes. There were no significant changes other than a slight decrease in the MCV values in the high dose (50 mg/kg/d) females. No other significant changes, were seen in any animal that could be attributed to AG-1749 treatment. Various blood chemistry parameters were altered but without any specific dose-related change in any one parameter. There was a slight but significant increase in the A/G ratio of up to 7-8% in the high dose male and female rats.

Serum gastrin: Blood was collected from 9-10 rats/sex/group after they were starved of food for 20 hrs, and serum gastrin levels determined by a radioimmunoassay. In the high dose male rats, the serum gastrin levels were 1276±1139 pg/ml (controls, 219±55 pg/ml); in the females, the levels were: 487±229 and 707±385 pg/ml after 15 and 50 mg/kg/d doses, versus 173±47 pg/ml in the controls.

Organ weights: All the major organs were weighed. Thymus weights decreased by about 21% in the high dose females; the heart, lung and liver weights increased by 10, 9, and 7%, respectively, in the high dose males; while in the female rats in the high dose group, the weight of the heart, lungs, liver, and kidneys increased by about 7-11%.

Gross pathology: None was discussed.

Histopathology: In the control and 50 mg/kg/d groups, all the major organs/tissues including the liver, kidney, stomach, thymus, and thyroid were examined histologically; in the low (5) and mid (15 mg/kg/d) groups, only the liver, kidneys, and stomach were examined histologically.

Liver: A very slight hypertrophy of the centrilobular hepatocytes was seen in 8 of 10 male and 6 of 10 female rats in the 50 mg/kg/d group. No such changes were seen in any other treated rat or the controls.

Stomach: In the 50 mg/kg/d group rats, slight to very slight degree of necrosis of the chief cells was seen in 7 of 10 male rats, and 6 of 10 females rats; slight hypertrophy of the parietal cells in the glandular stomach were seen in 2 of 10 male rats, and 5 of 10 female rats. No such lesions developed in the control, low or mid dose rats. The argyrophil (ECL cell) density increased significantly (38 and 58% of the controls in males and females, respectively) at 50 mg/kg/d. However, the control group had low ECL count, and the morphology and organization of ECL cells were not described by the sponsor.

Enzyme histochemistry were performed on one male rat in the control group and two male rats in the 50 mg/kg/d groups at termination and activities of acid phosphatase (ACP), alkaline phosphatase (AP), and succinate dehydrogenase (SDH) in the liver and stomach were determined. No significant treatment-related changes were seen in any animal.

Electron microscopical examination was performed of the liver and stomach of one control male and two high dose male rats at termination.

Liver: No significant changes were seen in any treated rat.

Stomach: A slight dilatation of tubulovesicles was seen in the parietal cells at 50 mg/kg/d dose. In addition, many small and dense secretory granules and swirl-like arrangement of the rough endoplasmic reticulum in the apical cytoplasm of the chief cells were seen in the same group in which histopathology revealed eosinophilia of the chief cells.

Mvelogram was normal.

In summary, AG-1749 given orally at 5, 15 and 50 mg/kg/d for 13 weeks produced a 482% increase in the serum gastrin levels in the male rats at 50 mg/kg/d, and 181% and 308% increase at 15 and 50 mg/kg/d doses in the female rats. At 50 mg/kg/d, the weights of the heart, lung and liver increased by about 7-11% in both male and female rats but without accompanying histological changes. The target organs affected were the same as before: the stomach and liver. At 50 mg/kg/d, a slight hypertrophy of the centrilobular hepatocytes was seen in 80% male and 60% female rats. A slight degree of necrosis of the chief cells and slight hypertrophy of the glandular parietal cells was also seen in the stomachs of both male and female rats. No such lesions developed at any other dose. The ECL density increased by 38-58% in the male and female animals at 50 mg/kg/d. However, the above gastric effects were considered as physiologic responses to the exaggerated pharmacologic effects of the drug. Thus, the no-toxic effect dose for the rat was 15 mg/kg/d while the higher dose, 50 mg/kg/d could be considered as the MTD since there were no significant effects on body weight gain and mortality.

3-Month Oral Toxicity Study in Rats
(with 1 Month Recovery)

(Report TA-90-151/IND Amendment dated 4/12/91/
 Pharmacology Review dated 6/27/91)

Conducting Lab:Dates of Conduct: Initiated 6/1/90, completed 11/1/90.GLPs Statement: In compliance with FDA's GLPs.MATERIALS AND METHODS

Chemical: Lansoprazole, lot# 42-057-AL, Takeda lot M610-022); suspended in 5% gum arabic solution, tested periodically for stability.

Animals: Rats, Crl:CD BR strain; 6 wks old at start; 15/sex/dose; plus 11/sex/group as satellite groups ([1] 6 for drug levels, [1] 5 for hormone levels). Body weights not given.

Doses: 0 (control vehicle), 50, 150, 300, and 600 mg/kg/d given, po, by gavage in constant volumes of 10 ml/kg/d, once daily, 7 days/wk for 3 months. The dose groups are designated as low (L), mid (M), mid high (MH), and high (H) groups, respectively. The solution concentrations for the respective doses were 50, 150, 300, and 600 mg/10 ml. The doses were selected on the basis of previous toxicity studies.

Methods: The experimental design was somewhat complicated and therefore sponsor's table (p24) is attached below.

Treatment Group	Dose (mg/kg/day)	No. per Group					
		3-Mo. Study		Satellite Subgroup 1		Satellite Subgroup 2	
		M	F	M	F	M	F
T ₀	0 (vehicle) ^a	15 ^b	15 ^b	6 ^c	6 ^c	5 ^d (5) ^e	5 ^d (5) ^e
T ₁	50	10	10	6	6	5 ^d	5 ^d
T ₂	150	10	10	6	6	5 ^d	5 ^d
T ₃	300	15 ^b	15 ^b	6	6	5 ^d (5) ^e	5 ^d (5) ^e
T ₄	600	15 ^b	15 ^b	6	6	5 ^d (5) ^e	5 ^d (5) ^e

- ^a Vehicle - 5% gum arabic solution in 0.9% NaCl (1000 ml)
- ^b Five males and five females in groups T₀, T₁, and T₂ were held for a 1-month recovery period.
- ^c Six males and six females in groups T₃ and T₄ were held for a 1-month recovery period.
- ^d Five males and five females in groups T₀, T₁, and T₂ were used for toxicology, clinical, and general blood determinations at the end of the 3-month treatment period.
- ^e Another five males and five females in groups T₃ and T₄ were designated for toxicology, clinical, and general blood determinations at the end of the 3-month recovery period.

Briefly, groups of rats were treated with Lansoprazole or vehicle for 3 months. Five rats of each sex from the control, 300 and 600 mg/kg groups were maintained for 1 month of recovery period; and 6 rats/sex/group were used for determining the drug levels on day 1 and 83 (termination), and 5 rats/sex/group were used for determining the hormone levels on day 90 and 123 (end of recovery period). [The plasma drug and hormone levels were determined by Hazleton Labs, Vienna, Virginia]. Rats were observed for signs of toxicity and mortality. The specific observations such as body weights, food intake, clinical and hematology are mentioned below in each respective section. At termination of treatment and also at the end of 1-mo recovery, the animals were necropsied, the organs were weighed and examined macro- and microscopically. A full histopathology of only the control and high dose animals was performed. The tissues/organs of the animals in the satellite groups 1 & 2 (drug-and hormone level part) were not examined histologically.

Results/Observations: A number of clinical symptoms such as emaciation, salivation, ptosis and squinting of the eyes were seen in the treated rats. However, none of these appeared to be dose-and/or drug-related. Since controls also developed many of these symptoms, the development of any of these cannot be attributed to the drug. For example, the incidence of emaciation (weak and thin-looking animals) in the male rats was: 0, 10, 20, 13, and 13%, and in the females: 0, 0, 30, 33, 87% in the control, 50, 150, 300, and 600 mg/kg/d groups, respectively. Although in terms of percentages these values appear impressive, in terms of number of animals that were emaciated, a very small number of these animals (1-2 rats/group) were emaciated. The frequency at which this symptom was observed was also not provided. In addition, there was no significant reduction in food consumption accompanied by growth retardation, thus attaching any significance to this symptom at this time appears to be unwarranted. The incidence of squinting of the eyes among the male rats was: 20, 40, 20, 20, and 33%, and 7, 20, 0, 7, and 7% among the female rats, both not dose-related. The incidence of ptosis in the males was: 40, 20, 0, 27, and 7%, neither dose-related nor drug-related since 40% of controls also developed this symptom; and in the females: 7, 20, 20, 33, and 7% in the control, 50, 150, 300, and 600 mg/kg/d groups, respectively.

Mortality: A total of 10 rats (1M C, 2M, 1F MH, and 4M, 2F H groups) died or were sacrificed during the study, mostly from dosing accidents.

Body weights were recorded once weekly during treatment and recovery periods (see table 1 on the next page). If analyzed on the basis of terminal weight gain, growth retardation among the male rats was 10, 17, 37, and 62% at 50, 150, 300, and 600 mg/kg/d doses [significant at $p/0.05-0.01$ at 150, 300, 600 mg/kg/d], while in the females, it was 2, 11, 25 and 58% in the low, mid, mid high and high dose groups, respectively, significant at 300 and 600 mg/kg/d. But if compared with their own initial weights, then the percent decrease in body weight gain of the male rats was 2, 7, 35, and 63% in the low, mid, mid high and high group, and 1, 10, 26, and 59% among the respective treatment group females. In the latter analysis, significant treatment effects were seen at 300 and 600 mg/kg/d doses in both male and female rats suggesting that the dose of 150 mg/kg/d was well tolerated from growth point of view. During recovery, the animals in the 300 and 600 mg/kg/d groups gained body weights more than controls (males: 192 and 289% of the controls; females: 122 and 65% of the controls).

Table 1.
Body weight changes in rats treated with Lansoprazole for 3 Months

Treatment	Body weight, g			% Gain compared to initial weight	%Change compared to controls
	Initial	Terminal	Gain		
<u>MALES</u>					
Control	195	517	322	165	-
50 mg/kg/d	173	467	288	161	-2.4%
150 mg/kg/d	173	439	266	153	-7.2%
300 mg/kg/d	190	393	204	107	-35%
600 mg/kg/d	196	317	121	61	-63%
<u>FEMALES</u>					
Control	147	273	125	85	-
50 mg/kg/d	145	258	122	84	-1.0%
150 mg/kg/d	145	257	111	76	-10.5%
300 mg/kg/d	149	244	94	63	-26%
600 mg/kg/d	147	199	52	35	-59%

Food consumption was recorded once weekly. Data (see table 2 below) show that food intake was not significantly affected if compared against their own pre-drug level of food consumption.

Table 2
Food Consumption in Rats Treated with Lansoprazole for 3 Months

Treatment	Food consumption, g		
	On day 1	On day 91	Change, g
MALES			
Control	29.3	29.8	0.5
50 mg/kg/d	26.8	28.3	+1.7
150 mg/kg/d	22.5	27.5	+5.0
300 mg/kg/d	21.8	28.3	+6.5
600 mg/kg/d	22.5	24.2	+1.7
FEMALES			
Control	23.8	19.9	-3.9
50 mg/kg/d	23.8	21.1	-2.7
150 mg/kg/d	20.5	19.8	-0.7
300 mg/kg/d	23.8	21.1	-2.7
600 mg/kg/d	16.9	17.4	+0.5

Among the treated male rats, food consumption increased while in the females it decreased slightly (about 3% at 50 and less than 1% at 150 mg/kg/d) but not at higher doses. Thus, data on food consumption were without any meaningful value although sponsor stated that it decreased among the females at 600 mg/kg/d. Food consumption during recovery among the treated animals was comparable with controls.

Ophthalmoscopic examinations of each rat just prior to sacrifice was essentially normal except 2 low and 1 high dose rats had keratitis, and 2 mid dose rats developed epiphora of the eyes with no clear drug-related effects.

Urinalyses performed at termination were within normal limits.

Hematology performed at termination on all rats showed the following changes: reduced Hct (males: 1, 10, 10, 17%; females: 3, 10, 14, 17%), reduced Hb levels (males: 2, 11, 13, 19%; females: 4, 12, 16, 19%), and reduced MCV values (males: +2%, 7, 5, 11%; females: 3, 7, 11, 14%) in 50, 150, 300, and 600 mg/kg/d groups, respectively. In addition the mean myeloid:erythroid ratios decreased (by 20 and 44% at 300 and 600 mg/kg/d, respectively) but only in the male rats. However most changes were not significant toxicologically, and the animals had recovered from these changes during recovery.

Clinical chemistries were performed at termination on all rats. Although there were decreases or increases in the levels of alkaline phosphatase (AP), GGT (gamma-glutamyl transpeptidase), or triglycerides, these were not found in both sexes and were not dose-dependent. Only glucose levels decreased by 28 and 32% in the males, and 34 and 33% in the females at 300, and 600 mg/kg/d, respectively.

Drug levels (Satellite group 1): Blood samples were collected one hour after dosing on day 1 and near-the end (day 90) from 3 rats/sex in the control and 600 mg/kg/d groups. Data on plasma levels of the drug are presented from the sponsor's table (see table 3a). Because they did not provide data from males and females separately, these are re-computed in table 3b below. Although the drug did not accumulate, as stated by sponsor, data show that the plasma levels did not increase dose-dependently.

Table 3a
Drug Levels* in Rats Treated with Lansoprazole for 3 Months

Treatment	Day 1	Day 83
Control	0	0
50 mg/kg/d	0.472±0.171	1.239±1.309
150 mg/kg/d	0.774±0.497	0.781±0.380
300 mg/kg/d	1.397±0.825	1.042±0.825
600 mg/kg/d	1.245±0.677	0.751±0.355

N=6 animals (3m,3f)/group. *µg/mL.

Table 3b
Drug Levels* in Rats Treated with Lansoprazole for 3 Months

Treatment	Day 1		Day 83	
	Males	Females	Males	Females
Control	0	0	0	0
50 mg/kg/d	0.421±0.165	0.526±0.194	0.364±0.123	2.113±1.405
150 mg/kg/d	0.730±0.521	0.818±0.582	0.496±0.154	1.065±0.307
300 mg/kg/d	1.149±1.180	1.644±0.726	0.437±0.182	1.634±0.788
600 mg/kg/d	0.918±0.830	1.572±0.370	0.480±0.229	0.689±0.332

N=3 rats/sex/group, values are mean±standard deviation re-computed from the sponsor's table on page 106 that provided raw numbers for male (odd numbered) and female rats (even numbered). *µg/mL

There was a large variability within the doses tested, sex, and animals, the latter being possibly due to small number (3/sex/group) used. The plasma levels did not increase with dose, but tended to plateau at 300 and 600 mg/kg doses. Overall there were higher levels in the female rats than in males.

Hormone levels (Satellite group 2): The levels of testosterone, corticosterone and TSH did not change with treatment, while those of estradiol could not be estimated because of insufficient volumes of the serum collected. Thyroxine (T_4) levels decreased significantly in the males by 8, 29, 46, 63%; in the females by 0, 7, 20, 49% at 150, 300 and 600 mg/kg; gastrin levels increased (males: 68, 54, 65, 52%; females: 113, 126, 117, 96%) at the corresponding doses. T_3 levels decreased in both males (36, 21, 21%) and females (0, 12, and 27%) at 150, 300, and 600 mg/kg doses, respectively.

Organ weights: Most increases or decreases were seen at 150, 300, and 600 mg/kg doses but without a clear dose-relationship. However, these changes were not seen in both sexes in all cases. For example, the relative liver weights increased significantly in the males by 21 and 56% at 300 and 600 mg/kg/d, while in the female rats, these increased by 28, 44, and 71% at 150, 300, and 600 mg/kg/d, respectively. In the case of kidneys, there was an 28% increase at 600 mg/kg in the males, while in the females, the increase was 17, 26, and 35% at 150, 300 and 600 mg/kg/d doses. On the other hand, the thymus weights decreased in both male and female rats with the ranges of 11-26%, 62-55%, 62-57%, and 55-61% at 150, 300, and 600 mg/kg/d, respectively. The relative stomach weights increased dose-dependently (as an expected pharmacologic effect) by 33, 44, 55, and 100% in the males, and 33, 48, 59, and 121% in the females at 50, 150, 300, and 600 mg/kg/d doses, respectively. At the end of recovery period, stomach weights in both male and female rats were still higher while in the case of other organs, the weights had returned to normal or were different from controls only slightly.

Pathology

Gross pathology: Most gross changes seen were in the thymus, testes, uterus and liver and these were discolorations and/or adhesions to the abdominal wall of the mid high and high dose-treated male and female rats.

Histopathology: Most common finding was the atrophy in the testes, thymus and uterus. The incidence of testicular atrophy was 1/10, 2/10, 2/10, 9/10, and 10/10 in the control, 50, 150, 300, and 600 mg/kg/d dose groups, respectively. These and others (shown in table 4) are briefly discussed below. However, data on microscopic findings presented by sponsor in Table 17, pages 403 through 437 were found to be erroneous. According to the experimental design, 5 animals/sex from the control, 300 and 600 mg/kg dose groups were treated for 3 months and then maintained for a period of one month, while the data presented show the number to be 15, and thus the sponsor's calculated incidence (percentages) values are incorrect. Therefore the individual rat pathology data in table 12, pages 302-331 were reviewed, and the incidence of histopathological findings recalculated. Data on the incidence of testicular, uterine and thymic atrophy and stomach effects are also summarized in table 4. Most animals had recovered from these effects during recovery.

Testes: Epididymal hypospermia (53% and 26%), and or aspermia (6% and 40%) were seen at 300 and 600 mg/kg doses, respectively, while testicular atrophy was seen mostly in the 300 and 600 mg/kg groups. As mentioned above, the incidence of testicular atrophy was 1, 2, 2, 9, and 10 in the control, low, mid, mid high and high groups. But the control and 2 male rats each in 50 and 150 mg/kg dose groups also showed testicular atrophy, the latter were considered, by sponsor, as incidental occurrences and not treatment-related because these were unilateral and one 150 mg/kg rat had also developed a inflammatory diffuse granuloma. On the other hand, the mid high and high dose rats had developed testicular atrophy that was characterized as follows: among the 300 mg/kg/d male rats, diffuse atrophy was seen in 2/9 and multifocal bilateral atrophy in 7/9 rats. Among the 10 high dose (600 mg/kg) male rats, 8/10 rats showed diffuse atrophy (unclear whether unilateral or bilateral) and 2/10 rats developed multifocal atrophy (unclear whether uni- or bilateral). In addition, there were 2 high dose rats that had developed hyperplasia of the interstitial cells, one with minimal and another with mild degree of hyperplasia, both of which were not discussed by the sponsor. Sponsor also did not provide the degree of atrophy seen in the male rats.

Liver: Hepatic cytomegaly (males: 20%, and 53%, females: 33%, and 53%) and subtle yellow brown pigmentation was seen at 300 and 600 mg/kg doses.

Bone marrow: The incidence of bone marrow hypocellularity with lower number of myeloid elements in males was 0, 12% and in females 20% and 40% at 300 and 600 mg/kg/d, respectively.

Stomach: The microscopic changes included chief cell eosinophilia, mucosal gland necrosis and hyperplasia (seen in all treated rats), glandular dilatation, parietal cell vacuolation, gastritis, and edema in some. The severity of one or more of these changes varied with the dose.

50 mg/kg: Minimal changes in eosinophilic chief cells, glandular necrosis, and glandular dilation;

150-300 mg/kg: Above changes were more severe in degree;

300-600 mg/kg: Higher degree of mucosal gland hyperplasia, parietal cell vacuolation, edema, gastritis; increase in enterochromaffin cell population in the antral stomach, but without any foci of EC cells.

Spleen: The following incidence of capsular inflammation (males: 0/15, 1/10, 7/10, 6/15, 10/15; females: 0/15, 0/9, 4/10, 5/15, and 9/15) and fibrosis (males: 0/15, 0/9, 0/10, 4/15, and 2/15; females: 0/15, 0/10, 0/10, 4/15, and 2/15) was seen at 150, 300 and 600 mg/kg.

Thymus: The incidence of thymic atrophy in males was: 7, 8, 7; and in females: 6, 9 and 7 at 150, 300 and 600 mg/kg/d, respectively.

Thyroids: No significant changes were noted in the treated rats.

Table 4
Histopathological Findings in Rats Treated with Lausoprazole
For 3 Months Followed by 1 month of Recovery
(Numbers shown denote the number of rats with findings)

Observation	Control		50 mg/kg		150 mg/kg		300 mg/kg		600 mg/kg	
	M	F	M	F	M	F	M	F	M	F
Number treated:	15	15	10	10	10	10	15	15	15	15
Number examined:	10	10	10	10	10	10	10	10	10	10
Number in recovery:	5	5	-	-	-	-	5	5	5	5
TESTES:										
Atrophy	1	-	2	-	2	-	9	-	10	-
Recovery	0	-	NS	-	NS	-	3	-	5	-
UTERUS:										
Atrophy	-	0	-	0	-	0	-	0	-	7
Recovery	-	0	-	NS	-	NS	-	0	-	0
THYMUS:										
Atrophy	0	0	0	0	7	6	8	9	7	7
Recovery	0	0	NS	NS	NS	NS	1	0	1	1
STOMACH:										
Dilated glands	0	1	3	5	9	9	7	8	9	7
Recovery	2	0	NS	NS	NS	NS	4	4	5	3
Edema	0	0	1	0	0	0	0	3	4	7
Recovery	0	0	NS	NS	NS	NS	0	0	0	0
Hyperplasia-glandular mucosa	0	0	1	3	5	9	7	10	10	10
Recovery	0	1	NS	NS	NS	NS	0	2	0	0
Hyperplasia, non-glandular mucosa	2	0	0	3	2	1	4	4	3	2
Recovery	0	1	NS	NS	NS	NS	0	2	1	1
Necrosis	0	0	5	5	10	9	9	6	5	4
Recovery	0	0	NS	NS	NS	NS	4	5	5	4
Gastritis	0	1	1	0	0	0	1	0	2	3
Recovery	0	0	NS	NS	NS	NS	0	0	1	0
Chf. cell, eosin	0	0	10	10	10	10	9	10	10	10
Recovery	0	0	NS	NS	NS	NS	4	5	5	4

*- For recovery, there were 5 rats/sex in the control, 300 and 600 mg/kg groups, whereas in the sponsor's table 17, pages 403-437, the denominator is 15; instead it should be 5. NS-not scheduled, as explained above.

Kidneys: The incidence of nephritis in the male rats was 0/15, 0/10, 2/10, 3/15 and 5/15 at doses of 150, 300 and 600 mg/kg/d, respectively. At 600 mg/kg, there was mild and multifocal chronic nephritis. In addition, mild and multifocal tubular epithelial basophilia was more commonly seen at 600 mg/kg as compared to controls while less common at lower doses. At 150 mg/kg, intracytoplasmic yellowish-brown pigmentation, considered to be lipofuscin, was seen in the renal tubular epithelial cells.

Most changes seen in the organs at the end of treatment were not seen at the end of recovery, suggesting the animals had recovered from the toxic effects, except in the case of gastric and testicular effects from which the animals did not recover. In addition, the renal effects were still seen at recovery, but only in the 600 mg/kg dosed rats, while the lower dosed rats recovered from the renal effects.

In summary, when Lansoprazole was given po to rats at doses of 50, 150, 300 and 600 mg/kg for 3 months followed by a one month of recovery, the animals tolerated well both 50 and 150 mg/kg/d doses without developing any significant toxicity. Although there was no increased mortality, the doses of 300 and 600 mg/kg produced significant toxicity. The animals' growth was retarded: the percent reduction in weight gain as compared to controls was 35% and 63% in the males and 26% and 59% in the females at 300 and 600 mg/kg/d, respectively. At 150 mg/kg/d, the percent reduction in weight gain was only 7% in the males and 10% in the females, and at 50 mg/kg/d, the percent reduction was 1-2% in male and females, suggesting the animals tolerated well both 50 and 150 mg/kg doses. The testosterone, corticosterone, and TSH levels remained unchanged, gastrin levels increased, and thyroxine levels decreased at 150, 300, and 600 mg/kg/d doses but without any accompanying histopathology. There was a significant increase in the incidence of testicular and thymic atrophy, nephritis (the latter only the males) was seen at 300 and 600 mg/kg/d. The testicular atrophy was mostly (7/9) bilateral and multifocal in the 300 mg/kg group, while it was diffuse (unclear whether uni- or bilateral) in 8/10 rats in the 600 mg/kg/d group. In addition, two high dose rats developed hyperplasia of the interstitial cells in the testes which were not discussed by sponsor. The gastric toxicity was evidenced by necrosis and gastritis, seen more commonly in the 150, 300, and 600 mg/kg/d dosed animals. But the rats recovered from most toxic effects except from the testicular and renal effects. Thus, the maximum tolerated dose (MTD) for the rat was 150 mg/kg/d while no-toxic dose could not be determined with certainty although 50 mg/kg was devoid of any significant toxicity.

13-Week Oral Toxicity Study in the Rat
Followed by 4, 13, and 26 Week Recovery
(Report A-29-452/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan.
Dates of Conduct: Initiated 6/9/86, completed 1/5/89.
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-004); suspended in 5% gum arabic solution, pH 6.9-7.1; periodically tested for stability.
Animals: Rats, Jcl:Wistar; 4 wk old males (93-113g). A total of 80 male rats were assigned to 2 groups of 40 controls and 40 treatment groups and then into subgroups of 10 each as follows: at the end of 13 weeks of treatment, 10 each for 4, 13, and 26 weeks of recovery periods, respectively.

Doses: 0 (control vehicle) and 50 mg/kg/d given, po, by gavage at a constant volume of 5 ml/kg/d, once daily, 7 days/wk for 13 weeks. The doses were selected on the basis of the 13-wk oral toxicity study that slight hepatotoxicity and vacuolization of parietal cells in the stomachs of mid and high doses rats.

Methods: The test agent or control was administered to the rats for 13 weeks as indicated above and the animals were observed for morbidity and mortality. At the end of 13 week dosing, 10 animals from both control and treatment groups were sacrificed, and then additional 10 animals from each recovery point of week 4, 13, and 26 were sacrificed; their organs: the liver, submaxillary glands, thymus and stomach were weighed and examined microscopically. Stomachs were also examined electron microscopically. During dosing and recovery, the animals were weighed on days 0, 1, 3, 7, then twice weekly up to week 5, once weekly up to 14 weeks of recovery and once per 4 weeks thereafter. The serum gastrin levels were also determined.

Results/Observations: General behavior observed daily was normal.

Mortality: One control rat died after 88th dose, necropsy showing swollen liver and spleen, marked atrophy of the thymus, hemorrhagic lung and foamy material in the trachea and bronchus suggesting leukemia as the cause of death.

Body weight gain by the treated rats was less (range of 14-16%) than those of the controls throughout 13 weeks of dosing. During recovery, the growth of these rats (weight gain range of 20-33%) was comparable with controls, but they did not recover fully by 26 weeks after discontinuing the drug treatment.

Serum gastrin: At the end of dosing period, the serum gastrin levels in the treated rats (404 ± 262 pg/ml) were significantly higher ($p < 0.05$) than controls (155 ± 53 pg/ml), but were normal by week 4 of the recovery period.

Organ weights: Thymus weights decreased by 17% in the treated rats; the relative liver and stomach weights increased by 11%, and 18%, respectively, as compared to controls. By the end of 13 and 26 weeks of recovery periods, the relative liver and stomach weights were comparable with controls.

Histopathology:

Liver: A slight hypertrophy of the centrilobular hepatocytes was seen in the treated rats at the end of dosing, while at the end of 4, 13, and 26 weeks of recovery, no such effects were seen.

Stomach:

At the end of dosing period, necrosis of the chief cells was sporadically seen in the treated rats. Eosinophilia, slight hypertrophy of the parietal cells and hyperplasia of the chief cells were also seen in the treated rats' stomachs. The number of argyrophil cells (mainly EC and ECL cells) in the oxyntic glandular mucosa tended to increase by 72%, while the argentaffin cells density (mainly EC cells) remained unaffected in the treated group. The G-cells in the pyloric region were also significantly increased (143%) when compared to controls. However, no details on the morphology of the ECL cells were provided.

At 4 wk recovery: Slight hypertrophy, hyperplasia, and eosinophilia of the chief cells, and hypertrophy of the parietal cells were still seen. But there was no necrosis of the chief cells. The number of argyrophil cells was still high (39%) as compared to controls. But the number of other argentaffin and G-cells were normal.

At 13 wk recovery: Still slight hypertrophy, hyperplasia, and eosinophilia of the chief cells was sporadically seen, and the parietal cells were normal. The distribution pattern and the number of argyrophil were comparable with the controls, while the argentaffin cells were still high (190% of controls).

At 26 wk recovery: There were no abnormal findings of any kind suggesting complete recovery.

Electron Microscopic examination of the stomach showed the following:

At the end of 13 wk dosing period: In the treated rats, hypertrophy of the chief cells, parietal cells, and basal-granulated cells (mainly ECL cells) was seen in the oxyntic glandular mucosa of the corpus. The number of secretory granules of the chief cells and tubulovesicles and microvilli of the parietal cells also increased. In addition, in the basal-granulated cells, secretory vesicles and small vacuoles were increased.

At 4 wk recovery: The chief cells were markedly reduced in the cell size. Although slight hypertrophy of the parietal cells was still noted, the tubulovesicles and microvilli had returned to normal in appearance and number. The other cells were normal.

At 13 and 26 wk recovery periods: No abnormal findings were noted suggesting complete recovery.

In summary, the oral dosing of rats with AG-1749 at 50 mg/kg/d for 13 weeks caused retarded growth. The target organs of toxicity were the liver and possibly the stomach evidenced by hypertrophy of the hepatocytes in the liver, and necrosis of the chief cells in the stomach. There was evidence of exaggerated pharmacologic effects such as hypertrophy and hyperplasia of the parietal cells resulting in increased stomach weights; and increased number of argyrophil and argentaffin cells in the treated rats. But the animals recovered from these effects by at least 26 weeks after discontinuing the drug treatment. The chief cell hyperplasia was reversible by the end of 13-week recovery. Therefore, even though 50 mg/kg/d dose produced retarded growth, some liver toxicity and mainly exaggerated pharmacologic effects, these effects were reversible within 26 weeks after discontinuing the drug.

3-Month Oral Gavage Dose-Range-Finding Study
with AG-1749 in Rats
(Report A-29-174/IND Amendment dated 8/24/90/
Pharmacology Review dated 9/5/90)

The study, dated 10/10/86, was conducted by Bio-Dynamics Inc. Groups of rats, 15/sex/group, were given AG-1749 at doses of 5, 15, 50, and 150 mg/kg/d, po via gavage, once daily for 3 months. No animals died during the study. At 150 mg/kg/d, body weight gain of the male rats by termination was retarded by 21% while at the lower doses growth retardation was minimal. Food consumption increased by about 7% but only at 150 mg/kg/d. No such changes occurred among the females. Some hematological changes were seen at 50 and 150 mg/kg/d, but not in both sexes of the rat and their clinical importance was insignificant. The relative liver weights increased dose-dependently in the 15, 50 and 150 mg/kg/d females and in the males (by 10%) only at 150 mg/kg/d but was not associated with any histopathologic changes. In the male rats, thymus weights increased dose-dependently (5, 14, 39, and 55% at 5, 15, and 50 mg/kg/d, respectively) and in the females, by 43% at 150 mg/kg/d. Thymic atrophy was seen in both male and female rats at all doses. Thus, 150 mg/kg/d was the MTD for the rat.

6-Month Oral (gavage) Toxicity Study in Rats with AG-1749
(Report A-29-345/IND Amendment dated 4/25/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Date of study initiation was not clearly indicated, but it appears to be 12/23/86 when the test agent was first received; Completed 2/29/88.

GLPs Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS:

Chemical: AG-1749, lot #610-005; in 5% gum arabic solution; tested periodically for homogeneity and stability.

Animals: Rats, CD (Sprague-Dawley), 42 days old; males 220 g, and females 152 g; 12/sex/treatment group.

Doses: 0 (Control), 2, 10, and 50 mg/kg/d, given po (gavage) once daily, 7 days/week for 6 months at a constant dose volume of 5 ml/kg. Doses were selected on the basis of a previous 13-wk toxicity study in rats. The groups are identified in the review text below as Groups I, II, III, and IV, respectively.

Methods: The animals were given the control vehicle or the test-drug as indicated above for 190-191 days (6 months) and were observed periodically (see below under specific parameter) for signs of toxicity. Body weights and food intake were recorded pre-test, weekly for first 13 weeks, every four weeks thereafter and then terminally. A number of clinical laboratory tests were performed on 10/animals/sex/group at initiation, and at 3, 6, and/or 12 months of the study. At termination, all the animals were necropsied and examined for gross changes; only the control and 50 mg/kg/d animals' selected organs/tissues were subjected to detailed histopathology that included organ weights, a significant deficiency in the study. The stomachs and thymus glands of all the treated animals were examined microscopically.

Results/Observations:

General appearance observed twice daily of all treated rats was unremarkable.

Mortality: One low dose male rat died during blood collection. There were no other treatment-related deaths.

Ophthalmic examinations on pretest day, and month 6 revealed no abnormal treatment-related findings.

Body weights recorded weekly during treatment showed that the body weight gain of the treated and control rats was comparable with a few sporadic increases and decreases that were not dose-related.

Similarly, the food consumption by rats recorded once weekly was not significantly reduced by the drug treatment.

The urinalyses for both treated and control rats performed on pretest, month 3, and 6 were within normal limits.

Hematology performed on pretest, month 3, and 6 showed slight but significant decreases in the mean hematocrit (Hct), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) values only in the high dose (50 mg/kg/d) male rats at 3 months: 42%, 59 ± 2 cubic u, and 18.1 ± 0.6 uug as compared to controls (44%, 61 ± 2 , and 18.8 ± 0.7 uug), respectively. These changes were not seen at 6 months. Other hematologic parameters were unaffected.

Clinical chemistries did not reveal any significant treatment-related changes in any parameter.

Organ weights: There was a significant ($p < 0.01$) and dose-related increase in the stomach weights: 21% and 30% in the males and 37% and 48% in the females at 10 and 50 mg/kg/d doses. The relative weights of the thymus in the treated rats decreased by 33%, but significantly in the high dose male rats only. In the high dose male rats, the heart weights increased by about 12%, as compared to controls, but not in any other treatment group. In the high dose females, the relative kidney and liver weights increased by about 14% and 18%, respectively, as compared to controls. No other significant treatment-related changes occurred in any other organ.

Histopathology: The most significant changes were seen in the stomach and thymus of the treated rats:

Stomach:

Males: There was hypertrophy of the chief (zymogenic) cells in 11 of 12 (91%) and 12 of 12 rats examined (100%) in the 10 and 50 mg/kg/d groups, respectively. The incidence of hyperplasia of squamous cells in the forestomach glandular mucosa was 8/12 (66%), 5/12 (41%), 11/12 (91%), and 12/12 (100%) in the Gr 1 (control), II, III, and IV, respectively.

Females: The incidence of chief cell hypertrophy was 9/12 (75%) and 12/12 (100%) in the 10 and 50 mg/kg/d groups, respectively as compared to none in the low dose and controls. The incidence of squamous cell hyperplasia was 8/12 (66%), 8/12 (66%), 12/12 (100%) and 11/12 (91%), in the control, low, mid and high dose groups, respectively. In addition, 11 of 12 (91%) high dose female rats' stomachs had dilated glands containing eosinophilic material.

No attempt was made to determine serum gastrin levels. The description of the morphological changes in the gastric endocrine cells was also not provided.

Thymus: The incidence of thymic atrophy in the male rats was 5/11 (45%), 5/12 (41%), 9/12 (75%), and 12/12 (100%); and in females, it was 5/12 (41%), 6/11 (54%), 8/10 (80%), and 9/12 (75%) in the control, low, mid and high dose groups, respectively. In addition, a few female rats also had cysts in the glands.

No other significant treatment-related histopathological changes were seen in any other organ.

In summary, AG-1749 at oral doses of 2, 10, and 50 mg/kg/d for 6 months decreased slightly the hematocrit and corpuscular hemoglobin in the rats at high dose but only at 3 months and not thereafter. The stomach weights increased at 10 and 50 mg/kg/d, and in the glandular mucosa, there was dose-related hypertrophy of the chief cells and hyperplasia of the squamous cells. The thymus weights decreased dose-dependently, and was accompanied by thymic atrophy in both male and female rats at the mid and high doses only. The sporadic increases in the kidney, liver and heart weights were neither dose-related nor were affected histologically. The gastric effects were probably exaggerated pharmacologic effects, while the significance of thymic atrophy in the rat is unclear. Therefore, the no-toxic effect dose for the rat could be 50 mg/kg/d, but higher doses could have been tested without compromising the study outcome.

12-Month Oral (gavage) Toxicity Study in Rats with AG-1749
(Report A-29-438/IND , Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Date of study initiation was not clearly indicated, but it appears to be 11/25/86 when the test agent was first received; completed 12/23/88.

GLPs Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS:

Chemical: AG-1749, lot #610-005; in 5% gum arabic solution; tested periodically for homogeneity and stability.

Animals: Rats, CD (Sprague-Dawley), 43 days old; males 210 g, and females 153 g; 30/sex/treatment group.

Doses: 0 (Control), 1.5, 5, 15, and 50 mg/kg/d, given once daily, 7 days/week for at least 1 year at a constant dose volume of 5 ml/kg. Doses were selected on the basis of a previous 13-wk toxicity study in rats (reviewed above). The treatment groups are identified in the review text below as Groups I, II, III, IV, and V, respectively.

Methods: The animals were given, via oral gavage, the control vehicle or the test drug at the above indicated doses, once daily, 7 days/week for at least 1 year and were observed periodically (see below under specific parameter) for morbidity and mortality. Body weights and food intake were recorded pre-test, weekly for first 13 weeks, every four weeks thereafter and then terminally. A number of clinical laboratory tests were performed on 10/animals/sex/group before initiating drug treatment, and at 3, 6, and/or 12 months of the study (also see under specific sections). At termination, all the animals were necropsied and examined for gross changes; only the control and 50 mg/kg/d animals' selected organs/tissues were subjected to detailed histopathology that included recording organ weight changes, a significant deficiency in the study.

Observed effects:

General appearance observed twice daily of all treated rats was unremarkable except the incidence of chromodacryorrhea and lacrimation, maloccluded incisors and scabs (from rubbing against cage) was high in the high dose rats.

Mortality: A total of 17 rats (13M, 4F) died prior to termination: there were 2, 4, 1, 4, and 2 (a total of 13) males and 1, 1, 1, 0, and 1 females in groups I, II, III, IV and V, respectively. The cause of death was not discussed by the sponsor. Further, the time of death was not indicative of any drug-related effect.

Ophthalmic examinations on pretest day, and month 6, and 12 showed increased incidence of conjunctivitis and focal retinopathy in the 50 mg/kg/d dose group rats at both 6 and 12 month intervals of the study. The lower dose (5 mg/kg/d) male and female rats also developed conjunctivitis at month 12. The incidence of acute/subacute inflammation of the retrobulbar tissue in the males was 3/28 (10%) and 7/28 (25%), and in the females, it was 1/28 (3.5%) and 0/28 (0%) in the control and high dose groups, respectively.

Body weights recorded weekly showed that the body weight gain of the high dose (50 mg/kg/d) treated male rats were 487 ± 76 , 497 ± 76 , and 513 ± 85 g (significant decrease) at week 39, 43, and 48, respectively, as compared to the controls: 537 ± 89 , 555 ± 90 , and 573 ± 92 g. The body weight gain of these rats at week 52 and those of other groups throughout the study were comparable with controls. No such effects on the body weight gain of female rats were seen.

Food consumption by the rats recorded once weekly for the first 13 weeks and once monthly thereafter was not significantly reduced by the treatment; instead there was a slight but significant increase in food intake by the group IV and V male and female rats at various times of the study.

The urinalyses for both treated and control rats performed on pretest, month 3, 6, and 12 were within normal limits.

Hematology performed on pretest, 6, and 12 months showed the following changes in the mean hemoglobin and hematocrit values that were possibly related to treatment. At 6 months, in the males, Hb and Hct values decreased (15.3 ± 0.6 g/dl, and $44 \pm 2\%$) as compared to controls, 16.2 ± 0.5 , and $46 \pm 1\%$. Other hematologic parameters were unaffected. At 12 months termination, also in the males, Hgb and Hct values decreased (15.0 ± 1.6 g/dl, and $39 \pm 5\%$) as compared to controls, 14.0 ± 3.0 , and $42 \pm 9\%$. Other hematologic parameters were unaffected. The females were unaffected.

Clinical chemistries did not reveal any significant treatment-related changes in any parameter.

Organ weights: The relative stomach weights increased in a dose-related manner: among the male rats, the increase was 8, 22, 29, and 48% of the controls, and among the females, the increase was 13, 38, 38, and 52% of the controls in groups II, III, IV and V, respectively. The increases were significant ($p < 0.01$) at 5, 15, and 50 mg/kg/d doses. The relative organ weights of other organs did not increase in a dose-related manner, but only in the high dose group. For example, the relative weights of thymus in the control group (Gr I) and Gr V (50 mg/kg/d) male rats were 4.03 ± 2.99 g and 2.15 ± 0.88 g, respectively; that of the liver increased only in the Gr V female rats (3.21 ± 0.60 g as compared to controls (2.78 ± 0.40 g); the absolute kidney weights, and not relative weights increased in the Gr V females (2.98 ± 0.45 g versus 2.69 ± 0.45 g in the control). No other significant changes occurred in any other treatment group.

Histopathology: The most significant histopathological changes were seen in the stomach and thymus of the treated rats.

Stomach: There was hypertrophy of the chief cells in all male and female rats in the 50 mg/kg/d group; and also in those rats that died during the study. There were dilated gastric glands, some containing eosinophilic material, in all treated rats, but most significantly at the high dose. Though these changes were also seen in the control rats (25 of 28, 86%), the incidence was high in the 50 mg/kg/d male rats (27 of 28, 96%). All of these male rats contained eosinophilic material. The incidence of hyperplasia of squamous cells in the forestomach glandular mucosa was 85% in the controls, and 100% in the high dose, and 100% in the rats that died during the study (4/4 in Gr III; 2/2 in Gr IV). Among the females, the incidence of dilated glands was 93% and 100% in the control and high doses, respectively; while that of dilated glands

containing eosinophilic material was 18/29 (62%) and 28/28 (100%) in the corresponding groups. The incidence of hyperplasia of the glandular squamous cells in the control and high dose female rats was 25/29 (86%) and 28/29 (96%), respectively. But the sponsor did not evaluate the effects of AG-1749 on the endocrine cells of the GI tract.

Thymus: The incidence of involution/atrophy comparable in the control and high dose female rats: 27/28 (96%) and 26/27 (96%), respectively. In the male rats also, it was 100% in both control and high dose groups.

In summary, when rats were treated with AG-1749 at oral doses of 1.5, 5, 15, and 50 mg/kg/d for 12 months, 17 rats died during the study, but the cause of death was neither determined nor discussed by the sponsor. The body weight gain of the 50 mg/kg/d male rats decreased by about 10% of controls during the latter part of the study, and the rats did not recover by week 52. The females were unaffected. Although the incidence of gastric effects (dilated glands, hyperplasia of squamous cells etc.) in the high dose rats was high (up to 100%), it was comparable with controls (26-90%). But the animals in the other treatment groups were not examined histologically. The incidence of thymic involution/atrophy (96-100%) in the high dose female and male rats was comparable with controls. Essentially the treatment with AG-1749 at doses of up to 50 mg/kg/d did not produce any specific target organ toxicity in the rat.

REVIEWER'S ADDENDUM:

In an addendum to the study dated 12/16/1988, sponsor submitted the histopathological findings of stomach. These are reviewed below: A dose related increase in the glandular mucosal chief cells hypertrophy was reported. The incidences among (i) males were 0, 3.3, 86.7, 96.7 and 100% included in 0, 1.5, 5, 15 and 50 mg/kg/day treatment groups and (ii) females were 0, 3.3, 63.3, 96.7 and 100 % included in 0, 1.5, 5, 15 and 50 mg/kg/day treatment groups, respectively. The testicular interstitial cell hyperplasia incidences were 10 and 26.7 % in animals included in 1.5 and 50 mg/kg/day treatment groups respectively, and testicular interstitial cell tumors were reported in 10 % of the animals included in 50 mg/kg/day treatment group. Focal diffuse retinopathy was seen in 2 animals included in 25 mg/kg/day treatment group and 1 animal included in 50 mg/kg/day treatment group, respectively.

These are tabulated on the following page:

ABNORMALITY		TREATMENT GROUPS (mg/kg/day)					HISTORICAL CONTROL (%)
		0	1.5	5	15	50	
Glandular Mucosal Chief Cell Hypertrophy	M	0/30	1/30	26/30	29/30	30/30	--
	F	0/30	1/30	19/30	29/30	30/30	--
Interstitial Cell Hyperplasia (Testicular)		0	3/30	0	0	8/30	0.4 (Range 0-7.8)
Interstitial Cell Tumor (Testicular)		0	0	0	0	1/30	--
Focal Retiopathy	M	0	0	0	1/30	0	--
	F	0	0	0	1/30	1/30	

Therefore, lansoprazole increased the hypertrophy of the gastric chief cells in animals of both sexes included in 15 and 50 mg/kg/day treatment groups. Lansoprazole at 50 mg/kg/day for 12 months produced testicular interstitial cell hypertrophy and interstitial cell tumor in rats included in this study.

In another addendum dated 1/31/90, sponsor re-evaluated the severity of chief cells necrosis in the animals included in lansoprazole treatment groups and concluded that the severity and intensity in group 1 and 2 were minimal and the incidences of eosinophilic chief cells in glandular mucosa were similar to those of chief cells in hypertrophy. The intensity and severity of the chief cell necrosis in 15 and 50 mg/kg/day treatment groups was moderate to severe and did not change.

13-Week Oral Toxicity Study in Mice
(Report # -126-049/IND Amendment dated 4/18/91/
Pharmacology Review dated 6/27/91)

Conducting Lab:

Dates of Conduct: Initiated 4/6/90, completed 3/27/91.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: Lansoprazole AG-1749), lot# 38-006-AL, suspended in 5% gum arabic solution, tested periodically for stability.
Animals: Mice, CD-1, 8 wks old at start; 10/sex/dose; males 26-30g, females 20-23g.
Doses: 0 (control vehicle), 150, 300, 600, 1,200, and 2,400 mg/kg/d given, po, by gavage in constant volumes of 10 ml/kg/d, once daily, 7 days/wk for 13 weeks. The respective solution concentrations were 150, 300, 600, 1200 and 2400 mg/10. The basis of dose selection was not given.

Methods: Groups of mice were treated with control vehicle or Lansoprazole, po via gavage at the indicated doses, once daily, 7d/week, for 13 weeks. Mice were observed for signs of toxicity and mortality. The specific observations such as body weights, food intake were recorded once weekly and are further mentioned below in each respective section. At the end of treatment, prior to sacrifice, hematology and clinical chemistries were performed, and then the animals were necropsied, the organs (adrenals, brain, heart, kidneys, liver, gallbladder, ovaries, pituitary, testes, stomach) were weighed. Adrenals, bone marrow, ovaries, testes, heart, kidneys, stomach, liver, lung, prostate, pituitary; spleen, thymus and uterus from all control and high dose mice and all gross lesions from all mice were examined microscopically. Sponsor also re-stated (probably inadvertently?) that the bone marrow, liver, pituitary, gastric fundic mucosa, testes with epididymis from all mice were examined microscopically although they stated earlier that these organs/tissues were examined from only the control and high dose mice.

Results/Observations:

General appearance was unremarkable except 10-50% animals in the 150 mg/kg group and 40-100% animals in other treatment groups had dark blue urine, --- probably due to the excreted drug in the urine.

Mortality: A total of 9 mice died or were sacrificed: 1M, 1F in the 600 mg/kg group, 1F in the 1200 mg/kg and 3M, 3F in the 2400 mg/kg group. The exact cause of death could not be determined except that most animals died from dosing accidents.

Body weights were recorded once weekly. When compared to their initial body weights (day 0), the weight gains by day 90 (wk 13) were: 13, 21, 17, 17, 17, and 21% for the males, and 27, 27, 32, 26, 22, and 30% for the females in the control, 150, 300, 600, 1200 and 2400 mg/kg/d groups, respectively. There was no dose-relationship. And when compared to controls, the body weight gains increased (and not reduced) except in the case of female mice in the 600 and 1200 mg/kg dose groups where growth was retarded by 4 and 18%, respectively. See table 1 below.

Table 5
Body weight changes in rats treated with Lansoprazole for 3 Months

Treatment mg/kg/d	Body weight, g			% Gain compared to initial body wts.	%Change compared to controls
	Initial	Terminal	Gain		
		<u>MALES</u>			
Control	30.0	34.0	4.0	13	
150	29.0	35.0	6.0	21	-
300	30.0	35.0	5.0	17	+61
600	29.0	34.0	5.0	17	+31
1200	30.0	35.0	5.0	17	+31
2400	28.0	34.0	6.0	21	+31
					+61

Body weight changes in rats treated with Lansoprazole for 3 Months

Treatment mg/kg/d	Body weight, g			% Gain compared to initial body wts.	%Change compared to controls
	Initial	Terminal	Gain		
<u>FEMALES</u>					
Control	23.0	28.0	5.0	27	-
150	22.0	28.0	6.0	27	0
300	22.0	29.0	7.0	32	+18
600	23.0	29.0	6.0	26	-4
1200	23.0	28.0	5.0	22	-18
2400	23.0	30.0	7.0	30	+11

Food consumption increased in all treatment groups, but by a maximum of about 8-10% in the females and by about 0-6% among the male mice without any dose-relationship.

Hematology determined at termination showed reduced erythrocytes (8-17%), hematocrit (Hct, 11-16%) and hemoglobin (Hb, 11-18%) in all treatment groups male mice and female mice (erythrocytes, 3-10%; Hct, 5-12%; and Hb, 5-12%). However, these changes were without any clear dose-relationship and were of toxicologic insignificance.

Clinical chemistry: There were occasional increases or decreases in some parameters but were of toxicologic insignificance and were not dose-related in both sexes of mice. The total proteins, however, were reduced in all treatment groups, the reduction in the male mice being by 3, 13, 16, 15, and 16% of the controls, and in females: 5, 12, 17, 13.5, and 15% in the 150, 300, 600, 1200, and 2400 mg/kg/d groups, respectively.

Pathology:

Gross pathology: Most gross changes such as thickened glandular wall, gastritis, and erosions of the glandular mucosa were seen in the stomach, and smaller testes in the treated mice.

Organ weights are summarized in Table 6 on the next page. The liver/gall bladder weights increased dose-dependently in both sexes: (males: by 4, 16, 21, 36, and 45%; females: by 11, 13, 22, 35, and 49% of the controls in the 150, 300, 600, 1200, and 2400 mg/kg dose groups, respectively), and toxicologically significant at only 2400 mg/kg and possibly at 1200 mg/kg (see under histopathology).

Similarly, the relative stomach weights increased significantly in both males and females by about 62-76%, 74-92%, 76-91% and 76-81% of the controls at 300, 600, 1200 and 2400 mg/kg/d, respectively. The testicular weights decreased significantly (by 46 and 52% of the controls) at 1200 and 2400 mg/kg. In addition, there were sporadic increases or decreases in the relative organ weights of adrenals, pituitary, ovaries, and kidneys, but were neither dose-related nor seen in both sexes and thus were not considered drug-related.

Table 6
Relative Organ Weights of Mice (g/100 g bw) Treated with LANSOPRAZOLE

Treatment	Liver/gallbladder		Stomach		Testes	Ovaries
	Males	Females	Males	Females	Males	Females
Control	4.16	4.39	1.13	1.41	8.37	1.3
150 mg/kg	4.34	4.89	1.64**	1.92**	7.49	1.7*
300 mg/kg	4.83**	4.95	1.83**	2.48**	8.15	1.5
600 mg/kg	4.66	5.38**	1.97**	2.71**	7.11	1.5*
1200 mg/kg	5.66**	5.91**	1.99**	2.69**	4.55**	1.6
2400 mg/kg	6.04**	6.55**	1.96**	2.56**	4.05**	1.5

**=significantly different from control at $p \leq 0.01$.

Histopathology: The organs affected most were: the stomach, testes, and liver. The incidence of specific effects is summarized in table 7 (see next page).

Stomach: There was vacuolation of epithelial cells and cystic mucosal crypts in the fundic gland region of the glandular mucosa in all the treated male and female mice, but without dose-dependency. The pyloric region was not affected. Some mice in 300, 600, 1200 and 2400 mg/kg groups developed gastric erosions (1-3 mice) and gastritis (2-5 mice), but the incidence was not dose-dependent.

Liver: The incidence of mild degree of hepatocellular hypertrophy was seen in most treated mice (0, 9, 5, 8, 4, 5 in the control, 150, 300, 600, 1200, and 2400 mg/kg/d groups), while the moderate degree was seen in only the high dose (2400 mg/kg) group. The trace amounts of brown pigment in the hepatocytes were seen in 7 of 10 males in the 2400 mg/kg group, 1 of 10 females in the 1200 mg/kg group, and 4 of 10 females in the 2400 mg/kg group. In addition, 10-30% of mice in each group also had trace or mild generalized hepatocellular vacuolation. No such effects were seen in the control mice.

Bone marrow: In the male mice from 600, 1200 and 2400 mg/kg groups and female mice from 1200 and 2400 mg/kg, the marrow was characterized by the absence of trace iron staining of the reticuloendothelial cells explaining the reduction in the number of erythrocytes, Hb and Hct values noted above.

Testes: A moderate degree of tubular atrophy (whether uni- or bilateral was not provided) was seen in 3 each of 10 mice in the 1200 and 2400 mg/kg groups; a mild degree of tubular atrophy was seen in 2, 0, 1, 1, 2 and 5 male mice of 10 each in the control, 150, 300, 600, 1200 and 2400 mg/kg groups, respectively. A moderate degree of aspermia was also seen in 2/10 and 4/10 mice in the 1200 and 2400 mg/kg groups, respectively. The severity of the testicular lesion was not dose-related.

Table 7
Microscopic Findings in Mice Treated with Lansoprazole For 13 Weeks

Observation	Control	150	300	600	1200	2400
MALES						
Number of animals	10	10	10	10	10	10
Bone marrow:						
Iron stain -ve	6	4	7	10	10	10
Liver:						
hepatocyte						
hypertrophy, mild	0	9	5	8	4	5
moderate	0	0	0	0	0	3
Stomach/fundus:						
erosions, mild	0	0	0	0	1	1
moderate	0	0	2	0	0	0
hyperplasia, mild	0	7	7	6	8	7
moderate	0	3	3	4	2	5
vacuolar change	0	10	10	9	10	10
cystic mu.crypts	0	5	8	7	8	5
gastritis chronic						
mild	0	0	0	0	0	3
Epididymides:						
rel.aspermia						
moderate	0	0	0	0	2	4
Testes:						
tubular atrophy	2	4	5	7	8	10
trace	0	4	4	6	3	2
mild	2	0	1	1	2	5
moderate	0	0	0	0	3	3
FEMALES						
Bone marrow:						
Iron stain -ve	6	8	8	9	9	10
Liver:						
hepatocyte						
hypertrophy, mild	0	5	3	1	4	5
Stomach/fundus:						
erosions	0	1	4	0	0	0
hyperplasia	0	10	10	10	10	8
vacuolar change	0	10	10	9	10	10
cystic mu.crypts	0	4	10	7	10	9
gastritis chronic	0	0	3	3	2	2

In summary, when Lansoprazole was administered orally by gavage to mice at doses of 150, 300, 600, 1200 and 2400 mg/kg/d for 13 weeks, it produced no significant effects on their survival. The effect on growth was inconsistent: for example, the weight gain of the 150 mg/kg and 2400 mg/kg male mice was 61% each of the controls, while those of 300, 600, and 1200 mg/kg male mice was 31% each of the controls. Among the females, only the 1200 mg/kg/d mice had retarded growth by 18% while others (300 and 2400 mg/kg/d) gained by 18 and 11%, respectively. The gastric toxicity evidenced by gastritis and erosions in the glandular mucosa was seen in some mice at 300 mg/kg/d and higher doses but was not dose-dependent. The smaller testes, moderate degree of tubular atrophy, and moderate aspermia were seen at 1200 and 2400 mg/kg and not at lower doses. A mild to moderate degree of hepatocellular hypertrophy was seen in most treated mice. No such effects were seen in the control mice suggesting that these were drug-related effects. Thus, the no-toxic dose was 600 mg/kg/d, while 300 mg/kg/d was the MTD at which there were no growth retardation, no increased mortality and no significant target organ toxicity.

Reviewer's Addendum:

The following inaccuracies were noted in the review and the corrections have been provided below: (1) Table 5 (Pg. 95) was inaccurately titled as body weight changes in rats. It pertains to mice. (2) There are errors in calculations under column "% body weight gain compared to initial body weight" and % change compared to control for female mice. The correct calculation showed that lansoprazole did not retard body weight of treated female mice. This effect is similar to male mice. (3) A moderate aspermia in a 1200 and 2400 mg/kg/day treatment groups mice relate to epididymides and not testes. (4) 600 mg/kg/day was inaccurately described as a "no toxic dose" and 300 mg/kg/day as 'MTD' in mice. A dose of 600 mg/kg/day is MTD in this study.

3-Month Oral Gavage Toxicity Range Finding Study in Mice (Study # A-29-221/ -86-3048)

Conducting I:

Dates of Start and Completion: January 14, 1986 and January 16, 1987

GLPs Compliance: A statement of compliance with FDA's GLPs was enclosed.

Drug: Lansoprazole (M610-003) suspended in 5% gum arabic (the final pH adjustment of the suspension was not given) and tested periodically for stability was used during the study.

Animals: Eighty CD-1 strain 6 weeks old mice (40/sex) with mean weight of 29 G (males) and 22 g (female) were used in the study.

Doses Used: Lansoprazole was administered at doses of 0 (control, 5% gum arabic solution), 5, 50 and 150 mg/kg/day orally by gavage in a volume of 15 ml/kg/day for 3 months.

Methods: The animals were divided into 4 groups (10/sex/group) and were administered a dose of lansoprazole or placebo as indicated above. This purpose of the study was to find the dose range for the carcinogenicity study in mice. In the present study, animals were observed twice daily for morbidity and mortality throughout the study. The body weights, food intake were recorded twice pretest and once a week during study. At the end of treatment and prior to sacrifice, blood samples for hematology and clinical chemistries were collected. Animals were necropsied and postmortem examination was performed on the animals died spontaneously, accidentally or killed at the termination of the study. Adrenals, brain, heart, kidneys, liver, ovaries, pituitary, testes and stomach were separated and weighed. These organs, submaxillary glands thymic region and thyroid, gross lesions, masses were examined microscopically. Adrenals, bone marrow, ovaries, testes, heart, kidneys, stomach, liver, lung, prostate, pituitary, spleen, thymus and uterus from all control and high dose mice and all gross lesions from all mice were preserved.

Results:

1. **General Observations:** The general appearance of animals was unremarkable.

2. **Mortality:** Two mice (1 each in mid and high dose treatment groups) died during the study.

3. **Body Weight Changes:** Lansoprazole treatment up to 150 mg/kg/day did not produce a dose dependent or treatment related retardation in percent body weight gain of the animals. The initial body weight of animals were: 28.4, 29.3, 29.9 and 28.3 g (males) and, 21.2, 22.2, 21.7 and 22.2 for female included in the study. The terminal body weight (at terminal were: 33.0, 34.0, 35.6 and 34.3 g for males and 28.8, 27.5, 27.3 and 28.3 g for females of the study. was observed in either male or female mice during the study. Food consumption was also not affected during the study and was 6.9, 7.8, 8.0 and 7.3 g for females and 4.9, 4.6, 4.3 and 4.8 g for males belonging to 0, 5, 50 and 150 mg/kg/day treatment groups respectively.

4. **Hematology and Urinalysis Changes:** No change of statistically significance were seen among the hematology parameters.

5. **Clinical chemistry:** There were no dose or treatment related changes in blood chemistry parameters of mice.

6. Histopathological Changes: The congestion of the stomach of the animals included in 50 and 150 mg/kg/day treatment groups was noted. The incidences of hyperplasia and hypertrophied glandular mucosa were 0, 1, 1 and 6 among females and 1, 0, 3 and 6 among males animals included in 0, 15, 50 and 150 mg/kg/day treatment groups. The incidences of congestion of glandular mucosa were not different from the control animals. Fatty degeneration of B/X zone were 10 vs 6 among 150 mg/kg/day treatment group female animals. There was no difference in males.

In summary an oral dose of 50 and 150 mg/kg/day of lansoprazole for 3 months, produced treatment related gastric hyperplasia and hypertrophy of the stomach. No other adverse effects were seen, therefore, the highest dose of the study did not produce toxicity, the target organs of toxicity were not identified. An 'MTD' for the mouse carcinogenicity study has not been identified from this study.

2-Week I.V. Toxicity Study in Dogs
(Report A-29-505/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Evaluation Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Study Started: February 23, 1989

Study Completed: June 30, 1989

GLP Requirements: Not mentioned

Animals: Beagle Dogs (6 months old; males: 8.6-11.8 kg and females: 8.4-10.1 kg)

Drug Batch No.: M 610-016

Placebo Batch No.: ZR 655-05

Methods: Groups of 3 male and 3 female dogs were given I.V. injection of Lansoprazole at daily doses of 0 (saline), 0 (vehicle): "mannitol" pH 11.0), 1, 3 and 10 mg/kg/day for 14 days. The volume of administration was 2 ml/kg/day for control, vehicle control and high dose group, and 0.2 ml/kg and 0.6 ml/kg

were the volume of administration in low and mid dose respectively. The injection rate was fixed at 3 ml/min. The highest tested dose level in this study is about 33 times the intended human dose (15 mg/person or about 0.3 mg/kg). All animals were observed daily for clinical signs and mortality. Body weights were recorded weekly and food intakes were recorded daily. ECG were monitored during pretest and 1 hr. after the 9th dose. Blood samples were collected from cephalic vein during pretest, day 7 and day 14 of the study for hematological and serum chemistry tests. Twenty-four hr. urine samples were collected during pretest and day 3 of the study for urinalysis. At the end of the study period all dogs were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. Observed Effects: None
2. Mortality: None
3. Body Weight/Food Consumption/Water Consumption: No treatment related effects were seen.
4. Hematology/Coagulation/Bone Marrow: No treatment related effects were seen.
5. Blood Chemistry/Urinalysis: No treatment related effects were seen.
6. Vital Signs/Physical Examination/ECG: No treatment related effects were seen.
7. Organ Weights: No treatment related effects were seen.
8. Gross Pathology: No treatment related effects were seen.
9. Histopathology: Hemorrhage, connective tissue proliferation and inflammatory cell infiltration were seen at the injection site in vehicle treated and drug treated dogs.

The data indicated that the highest tested dose was no effect dose in this study.

4-Week I.V. Toxicity Study in Dogs
(Report A-29-806/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Evaluation Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Study Started: January 12, 1990

Study Completed: July 17, 1990

GLP Requirements: A Statement of Compliance with GLP Regulations was included.

Animals: Beagle Dogs (9 months old, males: 10.1-13.7 kg and females: 9.1-11.1 kg).

Drug Batch No.: M 610-018

Placebo Batch No.: 004

Methods: In this study dose selection was based on preliminary 1-week i.v. toxicity study in dogs (# A-29-1300) in which doses of 0 (saline), 0 (vehicle: mannitol, pH 12.0-12.1), 30 and 100 mg/kg/day were used. Emesis, salivation, ptosis, flaccid nictating membrane and decreased activity was seen in high dose treated dogs. Hemorrhage and edema at the injection sites were seen in vehicle and drug treated dogs and these changes were severe in high dose treated dogs. High dose treated female dogs lost 5% of its weight by the end of 7 days of treatment. Necrosis of the chief cells and parietal cells in stomach was observed in high dose treated dogs. Thrombus in the lungs were seen in males treated with 30 and 100 mg/kg/day dose levels. Sponsor concluded that 100 mg/kg/day would exceed the maximum tolerated dose. Hence, 30 mg/kg/day was chosen as the highest dose for subsequent 4-week I.V. toxicity study.

In the main study groups of 3 male and 3 female dogs were given I.V. injection of Lansoprazole at daily doses of 0 (saline), 0 (vehicle: "mannitol" pH 10.9-11.1), 3, 10 and 30 mg/kg/day for 4 weeks. The volume of administration was 6 ml/kg/day for control, vehicle control and high dose group, and 0.6 and 2 ml/kg were the volume of administration in low and mid dose respectively. The injection rate was fixed at 3 ml/min. All animals were observed daily for clinical signs and mortality. Body weights were recorded weekly and food consumption were recorded daily. ECG recording and ophthalmoscopic examinations were performed on all dogs at pretest. On week 4 ECG recordings were also obtained from all saline, control, vehicle control and high dose treated dogs before and after administration of the dose. On week 4, ophthalmoscopic examinations were also done on these dogs. Blood samples were collected from cephalic vein during pretest, day 7, 14 and 29 of the study for hematological and serum chemistry tests. Eighteen hr. urine samples were collected during pretest and on day 27 of the study for urinalysis. At the end of study

period all animals were sacrificed and subjected to complete necropsy and histopathological examinations. In this study hepatic drug-metabolizing enzymes (amino-N-demethylase and aniline hydroxylase) activities were also monitored.

Results:

1. **Observed Effects:** Swelling at the injection site and reddening of the mucous membranes were seen in 1/3 males and 1/3 females of high dose group.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** No treatment related effects were seen.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.
5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related effects were seen.
7. **Organ Weights:** In high dose treated females, liver weights were increased by 36% compared to the control value. Additionally, liver weight was also increased (39%) in 1 out of 3 high dose treated male dogs.
8. **Gross Pathology:** At the injection sites moderate to marked hemorrhage, s.c. edema were seen in high dose treated dogs.
9. **Histopathology:** At the injection sites S.C. hemorrhage, edema/thrombus were seen in all treated dogs and severity was dose related. Vacuolization in the parietal cells of stomach was seen in treated dogs (males: saline control = 0/3, vehicle control = 0/3, low dose = 3/3, mid dose = 3/3 and high dose = 3/3; females: saline control = 0/3, vehicle control = 0/3, low dose = 2/3, mid dose = 3/3 and high dose = 3/3). Single cell necrosis of the parietal cells (males: saline control = 0/3, vehicle control = 0/3, low dose = 0/3, mid dose = 2/3 and high dose = 2/3; females: saline control = 0/3, vehicle control = 0/3, low dose = 0/3, mid dose = 2/3 and high dose = 3/3) were seen in treated dogs. Additionally, 1/3 male and 1/3 females each of mid and high dose group had hypertrophy of the parietal cells. Single cell necrosis of chief cells were also seen in 1/3 mid dose treated male dogs and in 1/3 males and 1/3 females of high dose group.

In this study target organ of toxicity was stomach. The lowest dose (3 mg/kg/day) can be considered a no effect dose.

4-Week Oral Toxicity Study in Dogs
(Report A-29-169/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan.
Dates of Conduct: Initiated 3/4/85, completed 8/9/85.
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-001); periodically tested for stability.
Animals: Dogs, Beagle, 3/sex/dose; 8 mo old males (9.4-11.3 kg) and 9.2-10.5 kg females.
Doses: 0 (placebo capsule), 30, 100, and 300 mg/kg/d given, po, (1 capsule/dog/day); once daily, 7 days/wk for 4 weeks. The doses were selected on the basis of a 2-wk oral toxicity study. But the study was not provided.

Methods: The test agent or placebo was administered to the dogs as indicated above and the animals were observed for clinical signs of toxicity. The specific observations including body temperature, heart rate, and ophthalmic changes are mentioned in each respective section below. At termination, the animals were necropsied, the organs were weighed and examined macro- and microscopically. However, all the organs from all the treated animals were not examined and thus are discussed in the respective section.

Results/Observations: General behavior observed daily was normal except in one male dog in the 300 mg/kg/d group that had developed decreased locomotor activity, vomiting and diarrhea, the latter were occasionally seen in the treated dogs.

Mortality: None.

Body weights were recorded on days 0, 3, 7, 10, 14, 17, 21, 24, and 28 days of treatment. No significant drug-related changes in the body weight gain were seen in the treated dogs except in one 300 mg/kg/d group male dog that tended to lose weight during the study.

The food consumption, recorded once weekly did not decrease significantly except in one 300 mg/kg/d male dog mentioned above, and in one additional female dog in the 30 mg/kg/d group.

Body temperature was normal.

Heart rate increased in the 30 mg/kg/d animals at 1 and 2 weeks after dosing, but no such changes were seen in the high dose dogs.

Ophthalmoscopic examinations of all the control and high dose group dogs before dosing and after 28th dose revealed no abnormal findings. The mid and low dose animals were not examined.

Urinalyses performed before dosing and after the 3rd dosing wk on the 6-hr and 18-hr samples were within normal limits.

Hematology and blood chemistry performed before dosing, after the 1st, 2nd, and 4th dosing weeks on all treatment and control dogs showed the following changes: RBC counts, Hct, and Hb decreased in the 300 mg/kg/d dogs after 1 wk of dosing; WBC increased with 300 mg/kg/d at wk 4; however, these changes were toxicologically insignificant since these were within physiological limits. Blood chemistry changes were: decrease in the albumin at wk 1, and A/G ratio after wk 4; ALP increased after 2 wks of dosing at 300 mg/kg/d. Coagulation test was within normal limits.

Organ weights determined at termination showed the following changes: the absolute and relative to body weight prostate weights reduced by 67% and 41% at 30 and 300 mg/kg/d, respectively. No other changes were significant in any treatment group.

Gross pathology: None described.

Histopathology:

Liver: A slight dilatation of the centrilobular sinusoid and slight atrophy of the hepatocytes at the same site were observed in 1M and 1F dogs in the 300 mg/kg/d group.

Stomach: Occasional vacuolization and necrosis of some parietal cells was observed at 300 and 100 mg/kg/d doses.

Large intestine: Focal hemorrhage, congestion, and pigmentation of the lamina propria were seen in the same 300 mg/kg/d male dog as mentioned above.

Enzyme histochemistry was performed on one male dog in the control group and two male dogs in the 300 mg/kg/d groups at termination and activities of acid phosphatase (ACP), alkaline phosphatase (AP), and succinate dehydrogenase (SDH) were determined. There were no significant changes in activities of any enzymes except a 190% increase in the AP activity in the treated dogs (127 ± 117 U/L v/s 43 ± 7 U/L in the controls), but this was due to two outlier values of 363 and 102 U/L as compared to 69, 54, 86, and 85 U/L in the remaining dogs. In the stomach, the activities of these enzymes were normal.

Electron Microscopic examination was performed of the liver and stomach of one control male and two high dose male dogs at termination.

Liver: Slight proliferation and dilatation of the smooth endoplasmic reticulum, decrease in the glycogen in the centrilobular hepatocytes, and the formation of the blebs in the bile canaliculi were seen in the same 300 mg/kg/d male dog.

Stomach: Dilatation and fusion of the tubulovesicles and dilatation of the intracellular canaliculi, which corresponded to the vacuolization observed by light microscopy, were seen in the parietal cells at 300 mg/kg/d. There was no necrosis in the parietal cells.

Myelogram showed decreased number of erythroid series cells and an increase in the myeloid series cells in both male and female dogs in the 300 mg/kg/d group but not in any other control or treated dog.

In summary, AG-1749 at oral doses of 30, 100 and 300 mg/kg/d to dogs for 4 weeks reduced the relative prostate weights by 67% and 41% at 30 and 300 mg/kg/d, respectively but not at 100 mg/kg/d. At 300 mg/kg/d dose, there was a slight dilatation of the centrilobular sinusoid and atrophy of the hepatocytes in one male and one female dog. Occasional vacuolization and necrosis of some parietal cells was seen at 100 and 300 mg/kg/d doses. Most gastric and hepatic effects were as seen in only one high dose male dog. The number of erythroid and myeloid series cells increased in both male and female dogs at 300 mg/kg/d. The no-toxic effect and MTD of AG-1749 for the dog were 30 and 100 mg/kg/d, respectively. However, the effects of AG-1749 on the serum gastrin levels and GI endocrine cells in the dog were not determined.

13-Week Oral Toxicity in the Dog
(Report A-29-170/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himurocho, Takatsuki, Osaka, Japan.

Dates of Conduct: Initiated 9/4/85, completed 8/8/86.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-002); tested periodically for stability.

Animals: Dogs, Beagle, 3/sex/dose; 9 mo old males (10.4-12.7 kg) and 9.6-13.0 kg females.

Doses: 0 (placebo capsule), 5, 15, and 50 mg/kg/d given, po, (1 capsule/dog/day); once daily, 7 days/wk for 13 weeks. The doses were selected on the basis of the 4-wk oral toxicity study reviewed above.

Methods: The test agent or placebo was administered to the dogs as indicated above and the animals were observed 3x daily for clinical signs of toxicity. In addition, the animals were observed once weekly for their general condition. The specific observations including body temperature, heart rate, and ophthalmic changes, are mentioned in each respective section below. At termination of dosing, all the animals were necropsied, the organs were weighed and examined macro- and microscopically.

Results/Observations:

General behavior observed daily was normal except transient salivation was seen in 2 of 3 high dose male and female dogs after 4 weeks of dosing. Diarrhea was observed in some high dose animals.

Mortality: None.

Body weights recorded twice before dosing, once/week for the first 5 weeks, then once weekly thereafter showed no significant treatment-related changes in the body weight gains of the dogs.

Food consumption was recorded daily. Except in one high dose male dog where it decreased throughout the study, it remained unremarkable in the others.

Body temperature recorded at week 4, 9, and 13 was normal.

Heart rate increased in the 50 mg/kg/d animals at 4 and 9 weeks after dosing, but not thereafter suggesting that these changes were possibly coincidental.

Ophthalmoscopic examinations of all the control and high dose group dogs before dosing and after week 4, 9, and 12 of dosing revealed no abnormal findings except trauma-related white foci at week 12 in one high dose male dog, and brown foci in one high dose female dog after 4th week of dosing. Histologically, these were normal.

Urinalyses performed before dosing and after 12 wk of dosing on the 6-hr and 18-hr samples were within normal limits in all dogs except in one high dose male dog that showed casts and epithelial cells in the sediment.

Hematology and blood chemistry performed before dosing, during week 4, 9 and 13 on all the dogs showed that RBCs, Hct, and Hg values slightly decreased in one 50 mg/kg/d dog. According to sponsor (p 332, v2), there was significant increase in the SGPT levels in the 50 mg/kg/d male dogs, but review of the table VII-1,2,3,4) shows outlier values in one dog resulting in the high means. Overall, there was no significant increase in any enzyme activity associated with drug treatment. The serum gastrin levels determined after starving the dogs for about 17 hr, increased dose-dependently (by 47, 217, and 223%) in the low, mid, and high dose male dogs, but by only 13, 29, and 24% of the controls in the females dogs. The activity of aminopyrine-N-demethylase, the hepatic drug-metabolizing enzyme, increased slightly (49%) at 50 mg/kg/d. The coagulation test was normal.

Organ weights: Except slight decrease in the prostate weights at high dose, there were no other significant drug-related changes.

Gross pathology: None described.

Histopathology:

Liver: Deposits of yellow-green pigments in hepatocytes, and slight hypertrophy and vacuolization of the epithelium of the interlobular bile ducts were seen in the 50 mg/kg/d male dogs. Similar changes were also seen in the ductal epithelium of male dogs in the 15 mg/kg/d group.

Stomach: Occasional vacuolization and necrosis of some parietal cells was observed at 50 mg/kg/d dose. No ECL density was affected at 50 mg/kg/d.

Prostate: Slight atrophy of the epithelium was seen in one high dose male dog, and cystic dilatation of the acini in one 15 and one 50 mg/kg/d male dogs.

Enzyme histochemistry was performed on one male dog in the control group and two male dogs in the 300 mg/kg/d groups at termination and activities of ACP, AP, and SDH determined. There were no abnormal findings in the liver or the stomachs of any treated dogs.

Electron Microscopic examination was performed of the liver and stomach of one control male and two high dose male dogs at termination.

Liver: There was a slight proliferation of the smooth endoplasmic reticulum, a bile-like substance with moderate electron density in the bile canaliculi, and vacuolization of the epithelial cells of the interlobular bile duct seen in 2 high dose male dogs.

Stomach: Vacuolization of the parietal cells, perhaps due to dilatation of the tubulovesicles were seen at 50 mg/kg/d.

Mvelogram was normal.

In summary, when AG-1749 was given to dogs for 13 weeks at doses of 5, 15 and 50 mg/kg/d, the high dose (50 mg/kg/d) produced (a) hepatic effects such as pigment deposits in the hepatocytes, hypertrophy and vacuolization of the bile duct epithelium; (b) vacuolization and necrosis of the gastric parietal cells; (c) and significantly increased the serum gastrin levels, the latter also at the mid dose (15 mg/kg/d). The high dose also increased the activity of the liver metabolizing enzyme by about 49%. Thus, the no-toxic dose and the MTD for the dogs was 15 mg/kg/d.

13-Week I.V. Toxicity Study in Dogs
(Report A-29-1861/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories:

Study Started: June 1, 1992

Study Completed: November 6, 1992

GLP Requirements: A Statement of Compliance with GLP Regulations was included.

Animals: Beagle Dogs (8-11 months old, males: 8.6-12.8 kg and females: 7.8-10.8 kg).

Drug Batch No.: M 610-050

Placebo Batch No.: TWN 6417 and TWL 7417

Methods: Groups of 4 male and 4 female dogs were given I.V. injection of Lansoprazole at daily doses of 0 (vehicle: "mannitol" pH 10.9-11.1), 3, 10 and 30 mg/kg/day for 13 weeks. The volume of administration was 6 ml/kg/day for control, vehicle control and high dose group, and 0.6 and 2 ml/kg were the volume of administration in low and mid dose respectively. The injection rate was fixed at 2 ml/min. All animals were observed 3 times daily for clinical signs and mortality. Body weights, food and water consumptions were recorded weekly. Ophthalmoscopic examinations were performed on all dogs at pretest and twice during treatment period (week 4 and 13). Blood samples were collected from cephalic vein during pretest and twice during treatment period (week 6 and 13) for hematological and serum chemistry tests. Eighteen hr. urine samples were also collected during above mentioned period for urinalysis. Additionally, blood samples were also collected from all dogs before drug administration and 5, 10, 15 and 30 min and 1, 2 and 4 hr after drug administration on days 1, 7, 14, 21, 49 and 90 of the study for measuring plasma drug levels. Plasma gastrin levels were also determined in all animals at 4 hr after dosing on day 1, 7, 14, 21, 49 and 90 of the study. On day 90 of the study plasma gastrin levels were also measured at 2 and 6 hr after drug administration. At the end of study period all

animals were sacrificed and subjected to complete necropsy and histopathological examinations. In this study hepatic drug-metabolizing enzymes activities were also monitored and livers (2/sex/group) were examined under electron microscope.

Results:

1. **Observed Effects:** Two out of 4 males and 2 out of 4 females in high dose group were diagnosed to have Idiopathic Necrotizing Arteritis Syndrome (beagle pain syndrome) which according to sponsor was not related to the treatment. These dogs consumed less food, showed decreased spontaneous activity and one female and one male become emaciated from week 5/7 of the study. Results from these 4 affected dogs were excluded from statistical analysis. Swelling at the injection sites were seen in 1/4 males and 1/4 females of mid dose group and in 1/4 female of high dose group.
2. **Mortality:** None
3. **Body Weight/Food Consumption/Water Consumption:** No treatment related effects were seen except significant loss in body weight, decreased food and water intakes were evident in dogs (males: # 25 and # 27 and females: # 30 and # 32) of high dose group which were diagnosed to have Idiopathic Necrotizing Arteritis Syndrome.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen. Significant decreases in number of red cells (37-40%), hematocrit (38-46%) and hemoglobin (42-49%) and increases in WBC (174-200%) were seen in dogs (males: # 25 and # 27 and females: # 30 and # 32) of high dose group which were diagnosed to have Idiopathic Necrotizing Arteritis Syndrome, when compared to the pretest values.
5. **Blood Chemistry/Urinalysis:** No treatment related effects were seen. Significant increases in serum alkaline phosphatase activities (males 540% and females 95% over baseline values) and serum total bilirubin (males 14% and females 16%; compared to pretest values), and decreases in serum albumin/globulin ratios (males 57% and females 49%; compared to pretest values) and serum creatinine levels (males 41% and females 51%; compared to pretest values) were seen in dogs (males: # 25 and # 27 and females: # 30 and # 32) of high dose group which were diagnosed to have Idiopathic Necrotizing Arteritis Syndrome.
6. **Vital Signs/Physical Examination/Ophthalmic Examination:** No treatment related effects were seen.

7. Organ Weights: In high dose treated females, relative weights of liver, kidneys and adrenals were increased by 20%, 14% and 14% respectively when compared to their respective control values. Additionally, relative weights of various organs (lungs, liver, kidneys and adrenals etc) were increased in dogs having Idiopathic Necrotizing Arteritis Syndrome.

8. Gross Pathology: At the injection sites slight s.c. edema were seen in 1/4 males (# 27) and 1/4 females (#29) of high dose group. One male dog of high dose group (#25) had enlarged liver, bilateral scarring along with hemorrhage in cortex of kidney and another female dog of high dose group (#30) had discoloration of cardiac muscle and spleen, thinning of left ventricle wall, yellowish aorta wall, yellowish white nodule on the surface of spleen, scarring in kidney cortex. Both dogs (# 25 & # 30) were diagnosed to have Idiopathic Necrotizing Arteritis Syndrome.

9. Histopathology: Inflammation and fibrosis of the vein wall and perivenous tissues and intimal thickening were seen in all treated dogs and severity was dose related. Venous thrombosis (occlusive recanalized) were seen in mid and high dose treated dogs. Histopathological changes in various organs (aorta, lung, thyroid, thymus, esophagus, duodenum, jejunum, stomach, ileum, liver, spleen, heart, kidneys, mediastinal adipose tissue and bone marrow) consistent with Idiopathic Necrotizing Arteritis Syndrome were seen in 4 affected dogs of high dose group (males: # 25 and # 27 and females: # 30 and # 32). No histological changes ("hypertrophic or hyperplastic") were seen in gastric fundus of any dog.

10. Electron Microscopic Examination: Electron microscopic examination of liver revealed dilation of Disse's space, deposition of microfibrils in Disse's space, atrophy of hepatocytes and vesicularization of smooth endoplasmic reticulum in hepatocytes in dog # 25 (high dosed male with Idiopathic Necrotizing Arteritis Syndrome) and # 30 (high dosed female with Idiopathic Necrotizing Arteritis Syndrome). Additionally, increased number of lipid droplets in hepatocytes were also seen in dog # 30.

11. Plasma Drug Levels: Plasma drug levels increased with increasing dosages.

Plasma Levels of AG-1749

Day	Sex	AUC (mcg.h/ml)		
		Low Dose (n=4)	Mid Dose (n=4)	High Dose* (n=2)
1	M F			
7	M F			
14	M F			
21	M F			
49	M F			
90	M F			

* = mean of two dogs

12. Plasma Gastrin Levels: Plasma gastrin levels were highly erratic and no conclusion can be made.

Table 11 Plasma gastrin level (pg/ml)
Male

Group	Animal No.	Day	0	7	14	21	49	90		
		Time (hour)	4	4	4	4	4	2	4	6
Control	1									
	2									
	3									
	4									
		Mean	126.5	119.6	138.5	140.0	132.3	108.0	177.7	176.5
		±S.D.	36.9	24.2	9.5	17.7	30.3	14.9	73.6	42.2
AG-1749 3 (mg/kg)	9									
	10									
	11									
	12									
		Mean	128.8	228.7	127.6	246.7	147.7	246.4	390.9	223.5
		±S.D.	28.7	249.0	24.5	188.4	86.3	265.0	204.8	98.3
AG-1749 10 (mg/kg)	17									
	18									
	19									
	20									
		Mean	207.7	296.1	381.0	292.0	513.8	375.0	422.9	283.4
		±S.D.	131.4	271.6	425.0	317.2	385.3	290.2	111.2	116.0
AG-1749 30 (mg/kg)	26									14
	28									63
		Mean	133.7	129.6	643.5	504.9	769.4	550.1	426.6	407.4

Not significantly different from the control.

Table 11 (Continued)
Female

Group	Animal No.	Day	0	7	14	21	28	35	42	49	56	63	70
		Time (hours)	4	4	4	4	4	4	4	4	4	4	4
Control	3												
	4												
	7												
	8												
		Mean	112.9	123.8	130.9	134.7	141.4	148.6	154.6	164.6	158.2		
		± S.D.	16.1	48.1	29.6	24.0	32.7	11.8	37.4				
AG-1749	13												
3 (mg/kg)	14												
	15												
	16												
		Mean	121.2	269.4	258.4	386.7	400.1	177.7	333.8	228.8			
		± S.D.	13.6	123.4	196.0	119.6	424.7	86.3	95.1	55.6			
AG-1749	21												
10 (mg/kg)	22												
	23												
	24												
		Mean	228.1	314.1	793.2	112.4	1216.3	147.0	137.9	233.8			
		± S.D.	168.8	179.0	1010.4	11.7	2003.6	33.9	65.8	145.6			
AG-1749	29												
30 (mg/kg)	31												
		Mean	95.2	704.9	728.0	217.5	185.1	142.4	255.2	168.1			

Not significantly different from the control.

13. Hepatic Drug-metabolizing Enzyme Induction: Drug had no statistically significant effects on hepatic drug-metabolizing enzyme activities.

Table 12 Hepatic drug-metabolizing enzyme activity
Male

Parameter	control (0mg/kg)	AG-1749 (10mg/kg)	AG-1749 (30mg/kg)
Body weight (kg)			
Liver weight (g)			
Liver weight/body weight (g/kg)			
Microsomal protein content (mg/g liver)			
Cytochrome P-450 content (nmol/mg protein)			
Cytochrome b5 content (nmol/mg protein)			
Aminopyrine N-demethylase activity (nmol/min/mg protein)			
Aniline hydroxylase activity (nmol/min/mg protein)			
p-Nitrobenzoate O-demethylase activity (nmol/min/mg protein)			
p-Nitrophenyl glucuronyltransferase activity (nmol/min/mg protein)			

Data are expressed as the mean values ± S.D. of four animals in the 3 and 10 mg/kg groups and as the mean values of two animals in the 30 mg/kg group. Figures in parentheses are expressed as the ratio of drug treatment relative to control. Not significantly different from the control.

6-Month Oral Toxicity Study in Beagle Dogs with AG-1749
(Report A-29-348/IND Amendment dated 4/25/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Date of study initiation not given, but it appears to be 12/24/86 based on the date when test substance was first received; Completed 3/10/88.

GLPs Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS:

Chemical: AG-1749, lot #610-005; in gelatin capsules; tested periodically for stability.
Animals: Beagle dogs, 5 months old; males 7.8 kg, and females 6.0 kg; 4/sex/treatment group.
Doses: 0 (Control), 2, 10, and 50 mg/kg/d, given once daily, 7 days/week for 6 months. The basis of dose selection was the 13-wk toxicity study. The treatment groups are identified in the review text below as Groups I, II, III, and IV, respectively.

Methods: The animals were given the control (empty capsule) or the test drug at the above indicated dose levels, once daily, 7 days/week for 6 months and were observed periodically (see below under specific parameter) for signs of toxicity. Body weights and food consumption were recorded pre-test, weekly and also terminally. A number of clinical laboratory studies including EKGs and hematology were conducted on all animals before initiating drug treatment, and at month 3 and at termination (also see under specific sections). At termination, all the animals were necropsied, the organs were weighed, and examined macro- and microscopically.

Results/Observations:

General appearance of all treated dogs was essentially unremarkable.

Mortality: None.

Ophthalmic examinations and EKGs recorded on pretest day, month 3 and at termination did not reveal any abnormal findings except for a few AV nodal escape beats in one of 4 male and 1/4 high dose female dogs at 6 months.

Body weights recorded weekly and at termination of the treated dogs were comparable with those of controls with a few minor deviations.

Similarly, food consumption by the treated dogs recorded once weekly during the study weeks was also comparable with controls.

Hematology performed on pretest, month 3 and at termination showed the following changes that were not clearly related to dose or length of treatment:

at 3 months: in the males, the mean hemoglobin (Hb) values decreased by about 16%, 19%, and 17%, and the Hct values decreased by about 14%, 20, and 20% in the low, mid and high dose groups, respectively. No such changes occurred in the females. WBCs increased by about 57% in the 50 mg/kg/d females but not in the males. There were no effects on the differential count of white blood cells.

at 6 months: in the males, the Hb, MCV and MCH were lower only at high dose as compared to controls.

Clinical chemistries did not reveal any significant treatment-related changes in any parameter.

Serum gastrin levels and endocrine cell morphology were not monitored.

Urinalyses performed on pretest, month 3 and at termination showed increased levels of urobilinogen values in both male and female dogs treated with 10 and 50 mg/kg/d doses only at termination but not at 3 months. No such effects were seen at 2 mg/kg/d.

Organ weights: There were no significant decreases or increases in any one organ related to dose or length of treatment.

Histopathology:

Stomach: Among the male dogs, the vacuoles in the stomach parietal cells were seen in 4/4 and 4/4 dogs in the 10 and 50 mg/kg/d groups, while in the females, the incidence was 3/4, 4/4 in the corresponding groups. The severity (not further described) was the greatest in the high dose group. In addition, there was biliary hyperplasia/proliferation in the livers of 2 and 10 but not in the 50 mg/kg/d male dogs. Females did not develop such effects.

In summary, the treatment of beagle dogs with AG-1749 at doses of 2, 10, and 50 mg/kg/d for 6 months produced essentially no significant toxicity other than increased stomach weights from hyperplasia and hypertrophy of the glandular cells. Therefore the no-toxic dose and the MTD was 50 mg/kg/d. However, the serum gastrin levels and the GI tract endocrine cell morphology were not monitored.

12-Month Oral Toxicity Study in Beagle Dogs with AG-1749
(Report A-29-439/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Date of study initiation not given; Completed 12/29/88.

GLPs Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS:

Chemical: AG-1749, lot #610-005; in gelatin capsules; tested periodically for stability.

Animals: Beagle dogs, 6 months old; males 7.3 kg, and females 6.4 kg; 4/sex/treatment group.

Doses: 0 (Control), 1.5, 5, 15, and 50 mg/kg/d, given once daily, 7 days/week for 1 year. The basis of dose selection was not provided. The treatment groups are identified in the review text below as Groups I, II, III, IV, and V, respectively.

Methods: The animals were given the control (empty capsule) or the test drug at the above indicated dose levels, once daily, 7 days/week for 1 year and were observed periodically (see below under specific parameter) for signs of toxicity. Body weights and food consumption were recorded pre-test, weekly for first 13 weeks, every four weeks thereafter and before termination. A number of clinical laboratory studies including EKGs and hematology were conducted on all animals before drug treatment initiation, and at 3, 6, and 12 months of the study (also see under specific sections). At termination, the animals were necropsied, the organs were weighed, and examined macro- and microscopically.

Results/Observations:

General appearance of all treated dogs was unremarkable.

Mortality: Two male dogs (groups IV and V) died during the study: one group IV male dog died on day 119, but was ataxic, lethargic, did not eat well, and lost body weight before death; and one group V male did not eat well, lost body weight, developed watery stools, vomiting, and lethargy, and died on day 223 of the study. The cause of death in both cases could not be ascertained.

Ophthalmic examinations on pretest day, and month 3, 6, and 12 did not reveal any abnormal findings.

EKGs recorded on pretest day, and month 3, 6, and 12 did not reveal any abnormal findings.

Body weights recorded weekly of treated dogs were comparable with those of controls.

Food consumption recorded once weekly for the first 13 weeks and once monthly thereafter was also within normal limits.

Similarly urinalyses for both treated and control dogs performed on pretest, month 3, 6, and 12 were within normal limits.

Hematology performed on pretest, month 3, 6, and 12 showed the following changes that were not clearly related to dose or length of treatment:

at 3 months: in the males, WBCs increased significantly ($p \leq 0.05-0.01$) in groups IV and V (18.3 and 15.9×10^3 cumm) versus 10.0×10^3 cumm in the control; in the females, WBCs increased in group V (14.7 versus 7.8×10^3 cumm);

at 6 months: WBCs increased only in the V males dogs: 16.9 versus 10.9×10^3 cumm in the control;

at 12 months: WBCs increased only in the V females dogs: 12.3 versus 7.3×10^3 cumm in the control;

The neutrophil counts increased in the group V male dog that died on day 223, and monocytes were increased in the IV group dog that died on day 119 of the study. Otherwise, there were no remarkable significant treatment-related hematologic changes.

Clinical chemistries did not reveal any significant treatment-related increases in any parameter.

Organ weights: There were no significant decreases or increases in any one organ related to dose or length of treatment except a few sporadic changes such as: prostate weights decreased (10.1 , 10.2 , 7.0 , 7.4 , and 6.9 g in groups I, II, III, IV, and V, respectively) but not significantly; the thymus weights decreased in the male dogs (8.5 , 7.3 , 5.8 , 5.2 , and 5.4 g in the respective groups). There were no such changes in the female dogs.

Histopathology: The most significant finding was the presence of vacuoles in the stomach parietal cells. Sponsor stated (p 31, v 12.6) that the incidence of vacuoles in the stomach parietal cells in the male dogs was: 0, 2, 3, 4, and 4; in the female dogs, the incidence was 1, 2, 2, 4, and 4 in the groups I, II, III, IV and V, respectively. But detailed review of the sponsor's tabulated data (table 11) showed the following suggesting inconsistencies in reporting.

Sex	Treatment group				
	Gr I	Gr II	Gr III	Gr IV	Gr V
<u>Males dogs</u>					
with vacuoles	0	0	0	1	1
Dogs examined	0	0	0	1	1
	0%	0%	0%	100%	100%
<u>Females dogs</u>					
with vacuoles	1	2	2	4	4
Dogs examined	4	4	4	4	4
	25%	50%	50%	100%	100%

These data suggested that the incidence of vacuoles in the stomach parietal cells was somewhat dose-related, particularly in the females, while in the males, it was 100% (in 4 of 4 dogs as per sponsor or as per the re-evaluated table) at both 15 and 50 mg/kg/d doses. It was not clear why all the male dogs in the lower groups and those that died during the study were not examined for similar effect.

In addition, the incidence of lung alveolar wall fibrosis in the female dogs was 0/4, 1/4, 0/4, 2/4, and 1/4 in the respective treatment groups. No such fibrosis was seen in the male dogs.

In summary, when beagle dogs were given oral doses of AG-1749 at 1.5, 5, 15, and 50 mg/kg/d for 12 months, AG-1749 increased the WBC counts, mainly at 15 and 50 mg/kg/d doses during the first 3 months of the study, but later this was seen only in one sex. The prostate and thymus weights (latter in males only) decreased but non-significantly and without any histological changes. The incidence of vacuoles in the stomach parietal cells was seen at 15, and 50 mg/kg/d doses, even though all the male dogs were not examined. The no-toxic effect dose for the beagle dog was 5 mg/kg/d while the MTD could not be determined because of two deaths (one each in the 15 and 50 mg/kg/d groups), even though the cause of death could not be determined. Still higher doses should have been tested to identify specific target organ toxicity.

4-Week Oral Toxicity in Cynomolgus Monkeys
(Report A-29-1911/IND Amendment dated 9/30/93/
Pharmacology Review dated 12/16/93)

Testing Laboratories:

Study Started: June 11, 1992

Study Completed: August 17, 1993

GLP Requirements: Study conducted according to GLP regulations of Japan.

Animals: Cynomolgus Monkeys (2.5-5 years old; males: 2.33-4.51 kg and females: 2.11-3.86 kg).

Drug Batch No.: Z 528S24 (enteric coated).

Methods: Groups of C. monkeys (3/sex) were given 5, 15, 50 and 150 mg/kg/day of enteric coated formulation of AG-1749 in gelatin capsules orally for 4 weeks. The control animals received the empty gelatin capsules in similar fashion. The dose selection was based on one-week oral preliminary toxicity study. Report of the preliminary toxicity study was not submitted to us. All animals were observed for clinical signs 3 times a day. Body weights, food intakes and water consumptions were recorded weekly. Ophthalmoscopic examinations and ECG recording was done on all animals at pretest and once during week 4 of the study. Blood samples were collected from external jugular vein during pretest, and before dosing on day 6 and 25 of the study for hematological and serum chemistry test. Eighteen hour urine samples were collected during pretest and twice during dosing period (week 1 & 4) of the study for urinalysis. Additionally, blood samples were also collected from all monkeys before drug administration and 0.5, 1, 2, 4, 6, 8 and 24 hours after drug administration on day 1, 7 and 27 of the study for measuring plasma drug levels. Plasma gastrin levels were also determined in all animals at 2 hours after dosing on day 1, 7 and 27 of the study. Serum testosterone and LH levels were also determined in all males once before drug administration and on day 26 of the study. In plasma samples drug concentrations were measured by UV/HPLC methods. Plasma gastrin, testosterone and LH levels were determined by radioimmunoassay. At the end of study period all animals were sacrificed and subjected to complete necropsy and histopathological examinations.

Results:

1. **Observed Effects:** Diarrhea/soft stools were seen in high dose treated monkeys. Compound like substance in the feces was seen in high dose treated monkeys of both sexes and in mid dose treated females.
2. **Mortality:** None.
3. **Body Weight/Food Consumption/Water Consumption:** Body weight gains data were not consistent. However there was an indication of body weight loss (11-16%) in animals treated with 150 mg/kg/day (control group females lost 4% of their weights). No significant affect on water consumptions were evident and food consumption data were not quantifiable.
4. **Hematology/Coagulation/Bone Marrow:** No treatment related effects were seen.
5. **Blood Chemistry/Urinalysis:** Total protein, albumin and A/G ratio were decreased by 12% (only in males), 21-26%, 30-34% in high dose treated monkeys (both sexes) respectively, when compared to their respective control values. Urinalysis were normal.

6. Vital Signs/Physical Examination/Ophthalmic Examination/ECG: No treatment related effects were seen. A T wave of -0.04 mv was seen in one high dose treated male (#27) and of -0.01 mv and -0.03 mv were seen in high dose treated females #29 and #30 respectively. One male (#9) of low dose group also had negative T wave (-0.02 mv). Sponsor considered this negative T waves of spontaneous origin and not related to the drug administration. Negative T wave is associated with hypokalemia in the absence of any change in QRS complex. Hypokalemia was not seen in blood chemistry test, therefore this finding can be ignored.

7. Plasma Drug Levels: Plasma drug levels increased with increasing dosages.

PLASMA LEVELS OF AG-1749 (n=3)					
Days	Sex (M/F)	AUC (mcg.h/ml)			
		5 mg/kg	15 mg/kg	50 mg/kg	150 mg/kg
1	M				
	F				
27	M				
	F				

8. Plasma Gastrin Levels: Plasma gastrin levels were significantly increased in 50 and 150 mg/kg treated females.

TWO-HOUR GASTRIN RESPONSE TO AG-1749 (pg/ml)				
Groups	Sex (M/F)	Day 1	Day 7	Day 27
Control	M			
	F			
AG-1749 (5 mg/kg)	M			
	F			
AG-1749 (15 mg/kg)	M			
	F			
AG-1749 (50 mg/kg)	M			
	F			
AG-1749 (150 mg/kg)	M			
	F			

9. Serum Testosterone and LH Levels in Males: Serum testosterone levels were highly erratic (range: Pretreatment at 8:00 am: ng/ml, at 13:00 pm: ng/ml and at 15:00 pm: ng/ml). Similarly serum testosterone levels were highly erratic on day 26 of the study. Therefore, no conclusion can be made.

Mean +/- SD (Range) of Testosterone Levels (ng/ml) on Day 26 in Males

	<u>1 hr Before</u> <u>Dosing</u>	<u>4 hr After</u> <u>Dosing</u>	<u>6 hr After</u> <u>Dosing</u>
Control			
AG-1749 (5 mg/kg)			
AG-1749 (15 mg/kg)			
AG-1749 (50 mg/kg)			
AG-1749 (150 mg/kg)			

Serum LH levels were not affected by the treatment.

10. Organ Weights: The relative weights of the liver were increased by 35% compared to control values in high dose treated females.

11. Gross Pathology: Reddish mucosa in the stomach was seen in one high dose treated male (#31), and white yellowish substance was seen in large intestine of 2 high dose treated male monkeys (# 26 and # 31). Thickening of stomach mucosa was seen in 2 high dose treated females.

12. Histopathology: Vacuolization of parietal cells in the stomach were evident in treated monkeys (males: control = 0/3, 5 mg/kg = 1/3, 15 mg/kg = 3/3, 50 mg/kg = 3/3 and 150 mg/kg = 2/3; females: control = 0/3, 5 mg/kg = 3/3, 15 mg/kg = 3/3, 50 mg/kg = 3/3 and 150 mg/kg = 2/3). Additionally, hypertrophy of parietal cells in the stomach were seen in 2/3 high dose treated males. Very slight hyperplasia of argyrophil cells in the fundus was observed in 1/3 males and 1/3 females of 50 mg/kg dose group and 2/3 females of 150 mg/kg dose group.

13. Hepatic Drug-Metabolizing Enzyme Induction: Aminopyrine-N-demethylase activities were increased by 41-47% in 15, 50 and 150 mg/kg treated females and by 27% in 50 and 150 mg/kg treated males. However, these increases are not statistically significant when compared to control values.

The data indicated that stomach is the target organ of toxicity. The low dose (15 mg/kg/day) can be considered as the no effect dose since it only produced vacuolization of parietal cells in the stomach.

CARCINOGENICITY STUDIES:

18-Month Oral Carcinogenicity Study in Mice (First Study)
(Study # 87-3158; A-29-680)

This study was done in 5 groups of CD-1 mice (70/sex/group) at oral doses of 0, 1.5, 5.0, 15.0 and 50 mg/kg/day of lansoprazole (Batch Nos: 42-057-AL; M610- 038, 049 and 50) administered via gastric intubation in a volume of 15 ml/kg on 5 days/week schedule for 18 months. The study was conducted by on November 25, 1986 and was completed on March 21, 1990.

During this study, lansoprazole was administered on a 5 days/week schedule, therefore animals were not treated continuously for the entire duration of the study. It was given intermittently. The half life ($t_{1/2}$) of the drug has been reported to be 0.9 to 1.1 hr in mice, therefore blood levels of the compound could significantly be decreased during the non-treatment period of 2 days/week of the study. The survival rate of mice was also not optimal as 45.7, 58.6, 74.3 and 71.4 % males; and 55.7, 67.1, 54.3, 55.7 and 45.3 % female animals died during the study. The basis of dose selection for the study was not provided and the required 13-week toxicity study in mice was not conducted/submitted before the initiation of the present carcinogenicity study. Therefore the study was invalid and unacceptable. Sponsor was asked by the Division to first conduct 90-day dose ranging subchronic toxicity study in mice and identify the maximum tolerated dose.

Sponsor subsequently submitted the results of 3-month dose ranging study in CD-1 mice by administering gavage doses of 0, 150, 300, 600, 1200 and 2400 mg/kg/day of lansoprazole. A dose of 600 mg/kg/day was identified as MTD. The Division in its letter dated July 26, 1991, recommended that a dose of 600 mg/kg/day (an MTD) should be employed as the highest dose in the proposed study. However, sponsor had initiated the following carcinogenicity study without waiting the Division's response. Sponsor conducted an additional carcinogenicity study by using only 0 and 600 mg/kg/day dose of lansoprazole separately and submitted latter with the present NDA.

COVERSHEET FOR THE ORAL CARCINOGENICITY STUDY IN MICE

1. Study #: TD91-025 (R & D/93/547)
2. Name of Laboratory:
3. Strain: Crl:CD-1 BR
4. No./sex/group: (i) 70 mice/sex/group in main carcinogenicity study, and (ii) 30 mice/sex/group in satellite groups
5. Doses (0, L, M, H): 0, 15, 75, 150 and 300 mg/kg/day (0, 45, 225, 450 or 900 mg/mm²)
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 106
9. Week/site for first tumor:

	<u>Male</u>	<u>Female</u>
0	59/Alveolar Bronchiolar Adenoma	57/Alveolar Bronchiolar Carcinoma
15	52/Alveolar Bronchiolar Adenoma	64/Alveolar Bronchiolar Carcinoma
75	14/Multiple Malignant Adenoma (Lymphoblastic)	59/Ovarian Hemangioma
150	64/Alveolar Bronchiolar Adenoma	41/Osteosarcoma
300	15/Multiple Histocytic (Lymphoblastic)	42/Multiple Malignant Lymphoma Sarcoma
10. No. alive at termination:		

<u>Group</u>	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
0	38	54.3	32	45.7
15	41	58.6	30	42.9
75	30	42.9	25	35.7
150	22	31.4	34	48.6
300	29	41.4	24	34.3

Statistical Methods Used: The significant results were analyzed by Bartlett's test and non-significant results were tested further by using Dunnett's test for pairwise comparison of each dosage group with control. If Bartlett's test was significant, the Welch t-test with Bonferroni correction was used for pairwise comparison. A one-tail trend analysis is applied to the mortality data. Peto analysis of tumor incidences and method of Westfall and Young adjusted for the multiplicity of tests were used to compute adjusted p-values for tumors. Exact permutation test of trend was used in analysis of tumor of fewer than 10 incidences.

12. Tumor and Non-tumor Data For each Tissue: See Appendix I.

2-Year Oral Carcinogenicity Study in Mice
(Study # R & D/93/547; TD91-025,

Testing Laboratory:

Dates of Start and Completion: February 20, 1991 and October 28, 1993.

GLP & OAU Requirements: Statements of compliance were included.

Batch Nos: 42-057-AL (week 1-21), M610-038 (week 22-30), -049 (week 31-89) and -050 (week 90-106)

Animals: Albino Swiss CD-1 mice (570/sex) about 5 weeks old and weighing between 18 to 26 g (females) and 22 to 30 g (males) were used. A group of 5 mice/sex were selected for viral screening and were subsequently killed and necropsied.

Methods: The animals were divided into 5 main study groups (70/sex/group) and 5 satellite groups (30/sex/group). These groups were treated with lansoprazole suspension in gum arabic (pH 7.0) according to the following schedule (Table taken from the Sponsor submission (Volume 2.31, pp 025). Another group of 70 mice/sex were used as vehicle control group B.

Table 4.2.2. Group Assignment						
Group	Dosage (mg/kg/day)	Main Groups		Group	Satellite Animals ^a	
		Males	Females		Males	Females
1	Vehicle Control A	70	70	7	30	30
2	15	70	70	8	30	30
3	75	70	70	9	30	30
4	150	70	70	10	30	30
5	300	70	70	11	30	30
6	Vehicle Control B	70	70			

^aUp to 10/sex utilized at 12 months and 18 months for blood drug level assays.

The dose selection of the study was based on the results of 18-month oral gavage carcinogenicity study (Takeda Study # A-29-680 in which the compound was administered 5 days/week) and a 13-week toxicity study in mice. In the former study, a dose related hyperplasia of gastric glandular cells was seen in animals of 15 and 50 mg/kg/day treatment groups. In 13-week study, a dose of 150 mg/kg/day produced drug related chronic gastritis, hepatocellular hypertrophy and vacuolation. A retardation of body weight gain, erosion of glandular mucosa, mild to moderate testicular tubular atrophy were seen in animals belonging to 1200 mg/kg/day or more treatment groups. No such effects were seen in animals included

up to a 600 mg/kg/day treatment groups, thus a dose of 600 mg/kg/day was a MTD. The Division in letter dated July 26, 1991, recommended to sponsor to employ 600 mg/kg/day as the highest dose in 2-year carcinogenicity study in CD-1 mice. Sponsor did not include 600 mg/kg/day as the highest dose in the present study but conducted a 2-year carcinogenicity study in mice by using a dose of 600 mg/kg/day of lansoprazole. This study has been reviewed as a separate study (Study # R&D/93/731;

. In the present study, the hematological parameters were determined on the blood samples of 10 mice/sex/group from main study group at month 12, 18 and 24 of the study. The drug plasma levels were determined on the blood samples of the animals included in the satellite groups at month 12 and 18 of the study collected 15 min post dosing. During week 87 to 91, additional blood samples of 10 mice/sex/group of satellite group were collected at 30 and 60 min and 2.5 and 4.5 hr post dosing for the determination of drug plasma concentrations. At termination, the blood samples of 2/3rd of the surviving animals were collected for serum gastrin, LH and FSH estimation. Blood samples of the remaining 1/3rd were used for plasma somatostatin estimation (Study # TD 91-025). Prior to the start of the study, 5 animals/sex had undergone a viral screening for pneumonia virus of mouse, retrovirus type 3, encephalomyelitis virus, Sendi, mouse hepatitis virus, lymphocytic choriomeningitis virus, ectromelia virus and polyoma virus. In addition, 30 male animals were maintained untreated with study animals for subsequent viral screening test at month 6, 12, 18 and 24 of the study. Due to positive results for mouse hepatitis at month 12 of the study, an additional test for viral screening was done on March 31, 1992 on 5 male animals. Other unassigned animals of the study were maintained for 4 weeks and then sacrificed. According to sponsor (Appendix R, vol 2.41 pp 338), no separate body weight record and no balance accuracy was maintained for the animals replaced during 4 week of the study. But, in all 39 animals (distributed in all the groups) were reported to have died and were replaced during 4 weeks. The body weights of animals were recorded weekly for 14 weeks and once 2- weeks thereafter during the study. All animals survived, died or killed due to moribund condition, were sacrificed at the termination of the study. These were examined for external and internal abnormalities, palpable masses. The abdominal, thoracic and cranial cavities were examined, organs were removed, cleaned and fixed in formalin. Eyes were fixed in glutaraldehyde and testes were fixed in Bouin's fixative for their examination. Gastrointestinal tract was examined using trans-illumination. Stomach, esophagus and duodenum were removed as one unit and a cut was made along the greater curvature of the stomach and duodenum. It was pinned, serosal side down and then fixed in 10 % neutral buffered formalin. Beginning March 1993, stomach were fixed in Bouin's fluid and washed with water. One to 2 sections from an area adjacent to

pylorus were taken, and fixed in 50 to 70 % alcohol. The remaining portion of the stomach were fixed in 10 % neutral formalin. The photographs of stomach lesions were also taken at the termination of the study. The microscopic examination of the glandular and non-glandular fundic stomach, and pyloric segment was conducted. In all 23 sections were reported to cut and stained in either hematoxylin-eosin, Grimelius, Fontana-Masson or Sevier-Munger stain. These were sent to EPL (Dr. Banas), Vienna for microscopic evaluation. All other tissues were examined at IRDC (Drs. J.J. Eighmy and R.G. Geil). A peer review of glandular and non-glandular stomachs from all animals was conducted at Dr. J.F.

Hardisty of EPL conducted a peer review of livers and gallbladders from all animals in all groups and, 10 % of all other tissues and organs in the vehicle control and 300 mg/kg/day treatment groups. These were evaluated on 1 to 4 grade scale (trace, mild, moderate and severe condition). The body weights, food consumption and hematological values were analyzed by one-way analysis of variance. The significant results were tested for homogeneity of variance by Bartlett's test. The non-significant results were analyzed by using Dunnett's test and for the significant results, the Welch t-test with a Bonferroni correction was used for pair wise comparison.

Results:

1. Stability and Homogeneity of Suspension: The stability test on the suspension were done for the duration of day 8 (7 days under refrigeration and 1 day of room temperature) and 13 (12 days under refrigeration and 1 day of room temperature) during the study. The concentration of lansoprazole in this test varied from % on day 8 of the test, and % on day 13 which indicated that lansoprazole suspension was stable up to 13 days of the study. The suspensions were also homogeneous as no less than % of the calculated amount of lansoprazole was present in these suspensions. This was also within % of the desired concentration. The coefficient of variation of 6 assays results/dose were less than % and the calculated amount of lansoprazole was also within % of the desired concentration.

2. Microbial Screening of Animals: Mice used in the study were positive for the presence of mouse hepatitis virus at different interval during the study. This viral infection has been shown to increase death of animals by producing hepatic degenerative cytoplasmic organelles and necrotic vesicles in hepatocytes (Sabolovic-N, Gerard, A et al: Biomed. Pharmacother. 38: 159-67, 1984), therefore a trend of an increase number of deaths in 300 mg/kg/day treatment group was attributed chiefly to the infection and lansoprazole may play little effect on the increase in the number of mortalities during the study.

3. Observed Effects: A treatment related pale skin areas were seen in 0, 1.4, 23.9, 28.8 and 22.1 % males (from week 13-14) and 2.9, 0, 20.3, 31.7 and 27.4 % females (from week 23 to 31) belonging to 0, 15, 75, 150 and 300 mg/kg/day treatment groups respectively. Purple colored urine was excreted in all mice included in 300 mg/kg/day treatment group from day 3 of the study. Labored breathing, body cold to touch and slight decrease in activity was seen in both sexes, from week 53 to the termination of the study.

4. Mortality: The number of animals died during the study were 45.7, 44.3, 41.4, 57.1, 68.6 and 58.6 % males and 54.3, 45.7, 57.1, 62.9, 51.4 and 65.7 % females included in control 1, control 2, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively. The incidences of mortality among control and treated females were high during the study which indicated that animals died of other than drug treatment related reasons. Since mice were positive of mouse hepatitis virus from month 6 of the study, therefore the deaths might be due to the infection. Statistical reviewer, Daphne Lin, Ph.D., in her review dated June 17, 1994 also indicated a linear trend in the intercurrent mortality rate among females ($p=0.016$) and males ($p=0.0011$) mice during the study. This has been tabulated below:

Weeks	Table Intercurrent Mortality Rate											
	Control 1		Control 2		15 mg/kg		75 mg/kg		150 mg/kg		300 mg/kg	
	M	F	M	F	M	F	M	F	M	F	M	F
0-50	1/70	1/70	-	-	0/70	10/70	7/70	1/70	6/70	1/70	6/70	7/70
51-80	6/69	11/69	-	-	10/70	19/70	20/63	22/69	23/64	19/69	17/64	25/63
81-104	25/63	26/58	-	-	19/60	21/51	13/43	22/47	19/41	16/50	18/47	14/38
Term.	38	32	-	-	41	30	30	25	22	34	29	24

Therefore, increased mortality in all the groups (controls and treated) groups was due to virus infection. The increased number of deaths in 75, 150 and 300 mg/kg/day treatment groups animals may be due to the virus infection. Lansoprazole treatment did not produce death of mice during the study.

5. Body Weight and Food Intake Changes: No treatment related change in the body weight gain was observed in male or female mice included in low, mid and high dose treatment groups. Food intake of the animals was not altered by lansoprazole treatment during the study. At the time of termination of the study (week 104), the body weights were 37, 36, 37, 38, 38 and 37 g among males, and 34, 32, 34, 33, 33 and 33 g among female animals included in control 1, control 2, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively. The daily food consumption at termination was 4.6, 4.7, 4.5, 4.7, 4.6 and 4.9 g in males and 4.7, 4.6, 4.8, 4.5, 4.9 and 5.0 g in female animals included in control 1, control 2, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively.

6. Hematological Changes: Erythrocytic parameters (red blood cell counts, hemoglobin and hematocrit) were decreased in animals of 75 to 300 mg/kg/day treatment groups. These changes were not clinically important. Red blood cell morphology, i.e., incidence of polychromasia and anisocytosis were increased in a dose dependent manner in the rats included in 15 to 300 mg/kg/day treatment groups in blood sample drawn at month 12 of the study. But these blood changes were not seen at month 18 and 24 of the study, therefore were not important clinically.

7. Drug Plasma Concentration: The pharmacokinetics of the compound was determined from the plasma samples drawn at _____ min after the administration of the compound. Plasma peak concentrations of lansoprazole were achieved within _____ min in females and males, respectively. The peak concentrations were treatment related and slightly higher in females than males and $t_{1/2}$ of the drug was higher in males than females.

The plasma concentrations (AUC_{0-8}) of a dose of 300 mg/kg/day of the compound was _____ ug.hr/ml in male and female mice respectively. These concentrations were achieved in _____ hr in male and female animals. A clinical dose of 30 mg/day (_____ mg/kg/day) was reported to achieve a peak plasma concentration of _____ ug.h/ml in _____ hr with T_{max} of _____ hr. This is equivalent to CL/F value of 14 L/hr which is low compared to hepatic plasma flow (about 50 l/hr). This indicate that the maximum dose employed in the carcinogenicity study achieved 1.5 and 4.7 times higher plasma concentration than the clinical dose.

Serum gastrin levels of animals survived the study, were increased in a dose proportional manner in both males and females. These were: 54, 77, 147, 157 and 330 pg/ml in males and 31, 77, 158, 235 and 493 pg/ml in 0, 15, 75, 150 and 300 mg/kg/day treatment groups respectively. Serum somatostatin levels were not increased during the study and these were: 40, 44, 37, 51 and 57 pg/ml in males and 38, 47, 32, 52 and 46 pg/ml in females belonging to 0, 15, 75, 150 and 300 mg/kg/day treatment groups respectively. Serum LH concentrations were:

_____ ng/ml in males and _____ ng/ml in females. FSH concentrations were: _____ in males and _____ in females included in 0, 15, 75, 150 and 300 mg/kg/day treatment groups respectively. Therefore, serum luteinizing (LH) and FSH levels in males or female animals treated with the compound were not significantly affected during the study.

8. Gross Pathology: A dose dependent thickening of glandular gastric mucosa in 3, 35, 56, 59 and 62 males, and 5, 25, 56, 64 and 65 females was seen in animals belonging to 0, 15, 75, 150 and 300 mg/kg/day treatment groups respectively. This observation of thickening of the gastric mucosa was also seen in animals died during the study. Nodular thickening of glandular stomach (from trace to severe) was seen in 3, 2, 35, 56, 49 and 62 males and, 6, 5, 25, 47, 66 and 66 female animals included in control 1, control 2, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively. Corneal opacities or cloudiness was seen in 13, 14, 25, 12, 22 and 18 animals included in control 1, control 2, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively.

9. Histopathological Changes:

Non-Neoplastic Changes: In EPL report of Dr. Banas, lesions of glandular stomach consisting of ECL cell hyperplasia, hypertrophy and/or hyperplasia of the mucosa, diverticula of the hyperplastic mucosa into the stomach were seen in a dose dependent manner in animals included in the treatment groups. ECL cell hyperplasia, (diffuse and nodular) were increased in intensity and severity in a dose dependent manner in both sexes. The incidences of ECL diffuse hyperplasia were 4, 12, 44, 51 and 60 in males and 6, 13, 44, 53 and 54 in females included in 0, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively. The incidences of micronodular ECL cell hyperplasia were 1, 0, 1, 6 and 16 in males and 0, 1, 4, 6 and 7 in females respectively in 0, 15, 75, 150 and 300 mg/kg/day treatment groups, respectively.

In the same report, squamous cell papilloma in non-glandular stomach of 1 and 2 females included in 75 and 150 mg/kg/day treatment groups were reported. Squamous cell carcinoma was seen in 1 female included in 300 mg/kg/day treatment group. The carcinoids in 150 and 300 mg/kg/day treatment group were invasive. The carcinoids in other groups were not invasive.

The lesions in the glandular gastric mucosa, submucosal edema, squamous metaplasia at an area near the ridge, fibrosis of the mucosa, hyperesinophilic chief cells and mineralization of the mucosa were seen in animals included in 75 to 300 mg/kg/day treatment groups. The affected portion was characterized by proliferation of epithelial cells (i.e., foveolar cells) into the fundus and the downgrowth. If this growth extended to subserosa, it was termed diverticulum and was present in animals from 75 to 300 mg/kg/day treatment groups. Intestinal metaplasia was seen in pyloric portion of the stomach of 2

males, i.e., 1 in each of 75 and 300 mg/kg/day treatment groups and, 1 female belonging to 15 mg/kg/day treatment group. Since the incidences were not dose related, these were considered as not treatment related effect.

Neoplastic Changes:

Stomach: Squamous cell papillomas were seen in non-glandular stomach of 1 and 2 females included in 75 and 150 mg/kg/day treatment groups and in 1 male animal belonging to 300 mg/kg/day.

Liver: Hepatocellular adenoma was seen in 6 out of 70 mice belonging to 300 mg/kg/day treatment group. The incidence of necrosis of centrilobular liver and hepatocellular neoplasms were in a dose related manner as seen in the following table:

The incidences were within the historical control data of the mice provided by sponsor.

TABLE						
Incidences of Tumors in Mice*						
TUMOR	SEX	Treatment Groups (Dose mg/kg/day)				
		0	15	75	150	300
a. STOMACH						
Non-glandular						
Squamous cell Papilloma	M	0	0	0	0	1
	F	0	0	1	2	0
Squamous cell Carcinoma						
	M	0	0	0	1	1
a. LIVER						
1. Hepatocellular Adenoma	M	7/70	6/70	4/70	3/70	8/70
	F	1/70	0/70	1/70	0/70	1/70
2. Hepatocellular Carcinoma	M	3/70	4/70	4/70	3/70	5/70
	F	0/70	2/70	0/70	0/70	0/70
Combined Tumor (1+2)	M	10/70	10/70	8/70	6/70	13/70
	F	0/70	2/70	0/70	0/70	1/70
b. Testis						
Retes Adenoma						
	M	0/70	0/70	1/70	1/70	1/70

* No of animals/group = 70/sex

In summary, lansoprazole treatment up to 300 mg/kg/day dose for 12 months produced an increase in the combined incidence of hepatocellular adenoma and carcinoma. An increase in the incidences of retes testis adenoma (a rare tumor) was also seen. Animals used in the study were infected with mouse hepatitis

virus thus the observed increased number of deaths were due to infection and not treatment related. The microscopic changes observed during the study could be the result of viral infection and/or due to lansoprazole. The study data will be considered in the context of 600 mg/kg/day treatment group in the following study (study # R&D 93/731).

COVERSHEET FOR THE ORAL CARCINOGENICITY STUDY IN MICE

1. Study #: TD91-276/ (R & D/93/731)
2. Name of Laboratory:
3. Strain: Charles River CD-1 mice
4. No./sex/group: 70 mice/sex/group in main carcinogenicity study and 30 mice/sex/group in satellite groups of the study.
5. Doses: 0 and 600 mg/kg/day (0 and 1800 mg/mm²)
(The study was conducted by using only 2 groups, i.e., control and 600 mg/kg/day treatment groups. The low and mid doses were not used in the study and the results of study # R & D 93/547 were considered in this study.)
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 101 and 104 weeks for female and male animals.
9. Week/site for first tumor:

	<u>Male</u>	<u>Female</u>
0	57/Multiple Malignant Lymphoma (Lymphoblastic)	27/Multiple Malignant Lymphoma (Lymphoblastic)
600	57/Multiple Histocytic Sarcoma	71/Multiple Histocytic Sarcoma

10. No. alive at termination:

<u>Group</u>	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
0	39	55.7	30	42.9
600	16	22.9	12	17.1

11. Statistical Methods Used: 1. Two sample t-tests were used to determine the significant change in body weights, food consumption and food efficacy of 600 mg/kg/day dose group with the mean of vehicle control group. The F-test for homogeneity of variance was performed and if F-test was significant, the t-test with unequal variances was used, otherwise t-test with pooled variance was used. Data for male and female animals were analysed separately and level of significance was $p < 0.05$

12. Tumor and Non-tumor Data For each Tissue: See Appendix I.

2-Year Oral Carcinogenicity Study in Mice
R & D/93/731;

Test: Oral:

Date: Start and Completion: September 12, 1991 and December 15, .

GLP & OAU Requirements: Statements of compliance were included.

Batch Nos:

Animals: Four hundred (200/sex) Charles River CD-1 mice about 6 weeks old and weighing between 19 to 24 g males and 18 to 23 g females were used.

Methods: This was a complimentary study to the above mouse carcinogenicity study (R & D/93/547) because the dose selection in that study was not appropriate. Sponsor did not conduct the previous mouse carcinogenicity study by using the identified MTD in 3-month toxicity study. The study was conducted by employing the arbitrary doses of 0, 15, 75, 150 and 300 mg/kg/day of lansoprazole. The highest recommended dose of 600 mg/kg/day (Division's letter dated July 26, 1991) was not used. Therefore, sponsor was asked to conduct the present study with an identified MTD of 600 mg/kg/day (1800 mg/kg/mm²) of lansoprazole. The animals were divided into 2 main study groups (70/sex/group) and 2 satellite groups (30/sex/group). These groups were treated with lansoprazole suspension in gum arabic (pH 7.0) according to the following schedule (Table taken from the Sponsor submission (Volume 4.2, pp 026). Only one group of animals was used as a vehicle control.

Table 4.2.2.
Group Assignment

Group	Dosage (mg/kg/day)	Males	Females
1	Vehicle Control A	70	70
2	600	70	70
3	Vehicle Control B (Satellite Group ^a)	30	30
4	600 (Satellite Group ^a)	30	30 ^b

^aUp to 10/sex utilized at 12 months and 18 months for blood drug level assays.

^b10 mice reassigned to Group 2 during study week 21

In the previous 2-year oral gavage carcinogenicity study in mice, lansoprazole was administered at a dose of either 0, 15, 75, 150 or 300 mg/kg/day (i.e., 15, 60 or 240 mg/m²) for 2 years. An increase in the incidences of ECL hyperplasia in the fundic and pyloric portion of the stomach was reported in

animals included in with 75 mg/kg/day or higher treatment groups. The incidences of necrosis of centrilobular liver and hepatocellular neoplasms of liver were seen in animals included in 300 mg/kg/day treatment group. In the present study, the hematological parameters were determined in 10 randomly selected animals/sex of main study groups (i.e., from 600 mg/kg/day treatment and vehicle control groups) at 12, 18, 20 (females only) and 24 months of study and at the time of killing the animals. The animals were not fasting at the time of drawing the blood samples. The plasma drug levels were determined in satellite groups of animals 60 min postdosing at 12 and 18 months of the study. In week 82 to 86, samples were collected at 15 and 30 min and 2.5 and 3.5 hr postdosing. The animals were not fasting at the time of drawing the blood samples. Serum somatostatin, FSH, LH and gastrin levels were determined in all the animals of the study (main and satellite study groups). The data of the satellite group was combined and discussed with the study group (Study # TD91-276). All surviving animals killed at the time of termination of the study, those died or were killed in extremis during study were examined thoroughly for palpable masses and other abnormalities. The organs were removed, cleaned and examined as in the previous 2-year carcinogenicity study in mice (reviewed above). The remaining untreated animals were maintained during the study and were used. Beginning week 21, 10 females from satellite group were transferred to 600 mg/kg/day treatment group of main study. These groups were examined histopathologically along with other animals. Microbiological screening was conducted on 5 animals/sex selected at randomly before the start of the treatment. Viral screening was also done at month 6, 12, 18 and 24 of the study on 5 males of the sentinel group. According to sponsor, a few sections of gallbladder were either missed or lost during the histopathological evaluation. Inflammatory, degenerative and hyperplastic changes were graded according to severity.

Results:

1. Stability and Homogeneity of Suspension: The stability of the suspension tested on day 8 (7 days under refrigeration and 1 day of room temperature) and 13 days (12 days under refrigeration and 1 day of room temperature), showed that the lansoprazole suspensions were stable as their concentration varied from % on day 8 of the test, and % on day 13. This indicated that lansoprazole was stable up to 13 days of the study. The suspensions were also homogeneous as not less than % of the calculated amount of lansoprazole was present at the time of observation on day 13.

2. Microbial Screening of Animals: The animals included in the study showed a positive test for the presence of mouse hepatitis virus at various intervals during the study. MHV infection has been shown to increase death of animals by producing hepatic

degenerative cytoplasmic organelles and necrotic vesicles in hepatocytes (Sabolovic-N, Gerard, A et al: Biomed. Pharmacother. 38: 159-67, 1984). One of the male (# 43727) had a positive test for minute virus of mice at month 12 of the study. The importance of this infection was not provided by sponsor.

3. Observed effects: Animals included in treatment groups were reported to have labored breathing, cold to touch and decreased activity. Corneal opacity was seen in males (56.3 % vs 34.6 % control), and females (66.7 % vs 23.2 % in control) mice included in 600 mg/kg/day treatment group. Excretion of purple colored urine was seen in 75 % males and 55.6 % females included in 600 mg/kg/day treatment group. None of the control animals excreted purple colored urine.

4. Mortality: The number of animals died during the study were high in both control and treatment groups. But the number of dead animals in 600 mg/kg/day treatment group were higher than the control group, i.e., 54 males included in the treatment group while 31 animals in control group died in the study. Among females, 59 in 600 mg/kg/day treatment group died vs 40 in the control group. A significantly high number ($P < 0.05$) of male and female animals died in the treatment group. According to FDA statistician reviewer, D.Lin, Ph.D., the mortality among males and females were significantly ($p < 0.0001$) higher than the control.

5. Body Weight and Food Intake Changes: No treatment related changes in the body weight gain was observed in male or female mice during the study. The body weights were 35 and 37 g (males) and 33 and 33 g (females) in animals belonging to control and 600 mg/kg/day treatment groups, respectively at week 104 and 100 of the study. Food intake of the animals was also not altered by lansoprazole treatment during the study. These were 5.4 and 5.3 g/animal/day in control and lansoprazole treated females, and 5.4 and 5.5 g in males belonging to control and lansoprazole treatment groups.

6. Hematological Changes: At month 12, 18 and 24 of the study, the red blood cell counts, hemoglobin and hematocrit were decreased significantly in animals included in 600 mg/kg/day treatment groups. But, these changes were of only statistical importance.

7. Pharmacokinetic and Hormone Data: A dose of 600 mg/kg/day for 2 years maintained a plasma concentration of _____ ug.hr/ml in male and female mice with a half life of _____ min in male and female animals. The peak plasma concentration (C_{max}) was seen in _____ hr in male and female animals. (Table taken from vol 4.5, pp 269):

Table 3. Pharmacokinetic Parameters From 18-Month Mean Data for Study TD91-276

Dose (mg/kg/day)	C _{max} (ng/mL)	T _{max} (hr)	AUC ₍₀₋₄₎ (ng·hr/mL)	AUC _(0-∞) (ng·hr/mL)	% AUC _(0-∞) of AUC ₍₀₋₄₎	t _{1/2} (min)
Male						
600	2622	2.5	9077	13284	31.7	127.9
Female						
600	3808	1.0	11289	16074	29.8	127.1

A linear peak plasma concentration (AUC_{0-∞}) of ng·hr/ml was observed in males and ug·hr/ml in females belonging to 15, 75, 150, 300 and 600 mg/kg/day treatment groups, respectively. The peak reached within 1 hr of its administration after 18 months of its administration. Therefore lansoprazole maintained a linear plasma concentration of the compound during the study.

The plasma somatostatin was not affected during the study and the concentrations were: pg/ml in animals included in control and 600 mg/kg/day treatment groups respectively. The serum FSH concentration was ng/ml in control and 600 mg/kg/day treatment groups respectively. Gastrin levels in the treated animals was about 14 times higher than the control group animals, i.e., these were pg/ml in control and 600 mg/kg/day treatment groups respectively. LH concentration were decreased in treated animals and these were ng/ml in control and 600 mg/kg/day treatment groups respectively.

8. Gross Pathology: At autopsy, a treatment related change in the liver color characterized by pale brown (degenerative changes), tan or dark brown discoloration due to bleeding in 7 out of 54 males and 13 out of 59 females was reported in animals died during the study. In 21 out of 70 males belonging to 600 mg/kg/day treatment group, a reduction in the testes size was reported. This was confirmed with a degeneration of seminiferous tubules. In 600 mg/kg/day treatment group, no palpable tumors were reported during the study.

6. Histopathological Changes: The microscopic evaluation of the stomach, liver and gall bladder was performed by Dr. Banas of EPL. A treatment related incidence of diffuse ECL cell hyperplasia was present 18 and 34 out of 70 male and female mice respectively, in 600 mg/kg/day treatment group. In the treated group of mice, incidences of micronodular ECL cell hyperplasia were, 24 males and 23 females out of 70 animals/sex respectively. Micronodular cell hyperplasia was not seen in animals belonging to control group. Lesions in the glandular stomach consisting of gastric mucosa, diverticulum of the hyperplastic mucosa and glandular dilatation and vacuolation of gastric mucosa was seen. In EPL report of Dr. Banas, an increase in incidence of hepatocellular adenoma was seen in males (5 in control group vs 15 in treated group out of 70 animals) and female (1 in control group vs 4 in 600 mg/kg/day treatment group animals) mice. The incidence of hepatocellular carcinoma (9 vs 3 in control group) in male mice were more than females (4 vs 1 in control group). The non-neoplastic lesions were necrosis of centrilobular liver and hepatocellular neoplasms were seen in the lesions of the glandular gastric mucosa, submucosal edema, squamous metaplasia at an area near the ridge, fibrosis of the mucosa, hyperesinophilic chief cells and mineralization of the mucosa were seen. No gall bladder lesions were reported. The incidences of gastric lesions and testicular adenoma are shown in the following table (The incidences have been shown in combination with the preevious study # R & D 93/547:

TABLE
Incidences of Liver Tumors in Mice

TUMOR	SEX	# of Groups (Dose mg/kg/day)						
		Study # R&D 93/547				Study # R&D 93/731		
		0	15	75	150	300	0	600
LIVER								
1. Hepatocellular Adenoma	M	7/70	6/70	4/70	3/70	8/70	5/70	15/70
	F	0/70	0/70	0/70	0/70	1/70	1/70	4/70
2. Hepatocellular Carcinoma	M	3/70	4/70	4/70	3/70	5/70	3/70	9/70
	F	0/70	2/70	0/70	0/70	0/70	1/70	4/70
Combined Tumor(1+2)	M	10/70	10/70	8/70	6/70	13/70	8/70	24/70
	F	0/70	2/70	0/70	0/70	1/70	2/70	8/70
TESTES								
Retes Testies Adenoma	M	0/70	0/70	1/70	1/70	1/70	0/70	3/70
Stomach								
Invasive Gastric carcinoid	M	0/70	0	0	1/70	1/70		

In summary, lansoprazole attained a dose related plasma peak concentrations, incidences of micronodular ECL cell hyperplasia in mice included in 300 and 600 mg/kg/day treatment groups. It produced a dose related testicular degeneration and intestinal metaplasia in mice included up to a treatment of 75 to 600 mg/kg/day treatment groups. Gastric diverticula, intestinal metaplasia in the pyloric area, hepatic adenoma and carcinoma were seen among animals included in treated animals. The gastric carcinoids in the animals were not observed in the study, which could be due to the high mortalities of animals in this study. The treatment produced treatment related testes adenoma, hepatocellular adenoma and carcinoma in animals included in lansoprazole treatment groups.

COVERSHEET FOR THE FIRST ORAL CARCINOGENICITY STUDY IN RATS

1. Study #: 86-3028/A-29-681
2. Name of Laboratory: for Takeda
Chemical Industries Ltd., Osaka, Japan
3. Strain: Sprague Dawley CD Rats (Charles River, New York)
4. No./sex/group: 60/sex/group approximately 7 week old rats with mean weight of 258 g for males and 177 g for females were divided in 5 groups
5. Doses (O, L, M₁, M₂, H): 0 (vehicle), 1.5, 5, 15 and 50 mg/kg/day (0, 8.85, 29.5, 88.5, or 295 mg/mm²) 5 days a week for 24 months
6. Basis for dose selection stated: No
7. Interim sacrifice: No
8. Total duration (weeks): 2 years
9. Week/site for first tumor:

	<u>Male</u>	<u>Female</u>
0	57/Pituitary pars distalis adenoma	56/Pituitary pars distalis adenoma
1.5	61/Lymphoret. granulocytic leukemia	52/Leg fibrosarcoma
5	58/Pituitary pars distalis adenoma	52/Pituitary pars distalis adenoma
15	61/Pituitary pars distalis adenoma	37/Pituitary pars distalis adenoma
50	44/ Hepat. Reticuloendothel.	46/Malignant Mammary tissue carcinoma

10. No. alive at termination:

<u>Group</u>	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
0	20	33.3	19	32.0
1.5	19	31.7	26	43.0
5.0	17	28.3	28	47.0
15.0	17	28.3	28	47.0
50.0	16	26.7	24	40.0

Statistical Methods Used: One-way analysis of variance and multiple comparison procedure, if required was used for significant change in body weights and food consumption. Bartlett's test was used for finding if variances were equal. Parametric or non-parametric analyses were used for finding the significant changes. The significance of difference from the mean of the control group was determined by using Dunnett's test. Trend test for the results in a dose level was used. The test of equal variance (Bartlett's test) was used, and 1 and 5 % significance was determined.

12. Tumor and Non-tumor Data For each Tissue: See Appendix I.

2-Year Oral Carcinogenicity Study in Rats (First Study)
(Study # 86-3028; A-29-681)

Testing Laboratory:

Dates of Start and Completion: September 13, 1986 and January 25, 1990.

GLP & OAU Requirements: Statements of compliance were included.

Batch Nos: M610-004, 5 and 7.

Animals: Six hundred (300/sex) Sprague Dawley CD Rats approximately 7 weeks old and weighing between 215-297 g (males) and 149 to 204 g (females) were used.

Methods: These animals were divided into 5 groups (60/sex/group) and were administered an oral gavage dose of either 0, 1.5, 5.0, 15.0 or 50 mg/kg/day (5 days a week) of lansoprazole (pH 7.0) in 5 % gum acacia in a volume of 5 ml/kg for 2 years. The basis for the dose selection of the study was not provided. Each of the animals was observed twice daily for mortality and overt signs of toxicity but the signs were recorded/week. Detailed physical examination for signs of local or systemic toxicity and palpation of masses was made pretest and weekly during the study. The body weights and food consumption of animals were recorded weekly for 13 weeks and once/4 weeks thereafter during the study. Hematology parameters were determined on the fasting blood samples obtained from orbital sinus from all the animals at termination of the study or just before the sacrifice of the animal. The blood samples were preserved in methanol. At the termination of the study, serum gastrin concentrations were determined on the blood samples of all of the surviving animals. All animals which died during the study or killed due to moribund condition and surviving animals of groups I and V sacrificed at the termination of the study, were examined for external gross and internal abnormalities and masses. The organs, tissues, mucosal surfaces of these animals excepting those which died accidentally during the study, were separated, cleaned, examined macroscopically and microscopically. The stomach of all of the animals opened, stapled to cardboard and immersed in 10 % formalin afterwards. Testes of males included in 1.5 to 50 mg/kg/day treatment groups were also examined. Gross lesions Tissues/organs of animals of other treatment groups i.e., 1.5, 5 and 15 mg/kg/day treatment groups were examined

histopathologically. All other organs separated, preserved and examined histopathologically were brain, spinal cord, lung, trachea, diaphragm, psoas muscle, tongue, heart, liver, pancreas, esophagus, stomach, intestines, salivary glands (submandibular, sublingual and parotid), kidney, spleen, testis/ovary, prostate, epididymis, vagina, mammary glands, adrenal, thyroid and parathyroids, thymus, pituitary gland, eyes, skin, bone with marrow and gross lesions and tissue masses. The special stains of Geimelius or Fontatna-Masson for stomach and Wright (Camco) stain for blood, were used in this study. The results were analyzed and the statistical significance of the changes of body weight, survival rate and tumor incidences were compared with control as described above. The significance of the change in the treated animals from the control animals was determined by statistical test of one way analysis of variance by using Bartlett's test for equal variances by a parametric or non-parametric procedure. Cox test, tarone test and Kaplan Meir method were used for survival data analysis. The Chi square test, Armitage test for monotonic trend tests were used to determine the significance of tumors incidences in the animals.

Results:

1. Stability and Homogeneity of Suspension: The stability test of the suspensions done on day 10 (from 4 to 10°C - under refrigeration condition) showed that the suspensions were stable up a period of 10 days and the concentration of lansoprazole in these suspensions were % in group II, group IV and group V respectively. The value for group III suspension was not provided in the text of the sponsor and the Appendix 15 for stability was not provided by sponsor. The stability of suspension of group IV treatment group could not be assessed. The homogeneity test on group I, II, III, IV and V suspensions showed that 83.8, 105, 103 and 101 % mean nominal values of the suspension, respectively were present. Therefore lansoprazole suspensions were stable and homogeneous up to 10 days after their preparations.

2. Microbial Screening of Animals: The animals included in the study were not tested for the detection of viral infection during the study.

3. Observed effects: Sponsor did not submit the table for physical changes of the animals during the study but stated that the animals included in the treatment groups were comparable with those of control group animals. The changes were claimed to be due to the age of rats.

4. Mortality: The percent mortality among control (0), 0.5, 5, 15 and 50 mg/kg/day treatment groups were: 66.7, 68.3, 71.7, 71.7 and 73.3 in males and 70, 60, 55, 55 and 60 in females, respectively. The number of deaths among treated animals during the study were not statistically different from those in the control group animals. The surviving/mortality data of the study was re-evaluated by C.Thomas, Ph.D.. He used Kaplan-Meier survival curve and concluded that the previously observed mortalities of animals were not different as reported earlier. These were: 67, 68, 70, 72 and 73 % among males and 68, 57, 53, 53 and 60 % in females.

5. Body Weight and Food Intake Changes: In male rats; body weight retardation of 19.3 % was seen in animals included in 50 mg/kg/day treatment group, no changes in body weight were seen in animals of the other groups. A retardation of 14.9, 22.3, 17.4 and 19.8 % in the body weight gain among females included in 1.5, 5, 15 and 50 mg/kg/day treatment groups respectively was also seen. The food consumption of animals of the study was not affected. Initially, these were, 22.2, 21.9, 22.3, 21.8 and 22.4 g/day in males, and 22.3, 22.5, 22.5, 22.7 and 22.2 g/day in females included in control, 0.5, 5, 15 and 50 mg/kg/day treatment groups respectively. But it gradually decreased after week 10 of the study and was stabilized at 27.7, 29.3, 28.3, 28.9 and 26.4 g for males, and 20.8, 20.7, 20.0, 20.5 and 20.6 g for females after week 29 of the study.

6. Hematological Changes: No dose or treatment related changes in the hematological parameters of both sexes were observed during the study.

7. Serum Gastrin Changes: Serum gastrin levels were increased in treated animals (both male and female mice). These were 123.6, 147.2, 153.7, 174.9 and 374.6 pg/ml respectively in 0, 1.5, 5, 15 and 50 mg/kg/day treatment groups males respectively. In females, gastrin levels were 109.2, 142.4, 111.1, 199.1 and 415.1 pg/ml respectively in 0, 1.5, 5, 15 and 50 mg/kg/day treatment groups. The serum gastrin were increased slightly up to 15 mg/kg/day treatment groups but a significant increase in this hormone level was seen in animals 50 mg/kg/day treatment group. Hypergastrinemia was attributed to the persistent inhibition of acid secretion.

8. Organ Weight Changes: The organ weights were not recorded during the study.

9. Gross Pathological Changes: The thickness of the stomach wall was increased in 1, 1, 4, 6 and 6 males out of 60 animals in each group of control, 1.5, 5, 15 and 50 mg/kg/day treatment groups, and in 1, 1, 1, 1 and 5 out of 60 animals in each of the 0, 1.5, 5, 15 and 50 mg/kg/day treatment group females.

10. Histopathological Changes: The non-neoplastic changes like dose related hypertrophy and eosinophilia of the chief cells of the stomach from minimum to severe intensity was seen in 0, 3, 36, 47 and 49 males, and 0, 3, 23, 34 and 44 females belonging to 1.5, 5, 15 and 50 mg/kg/day treatment groups. The severity of these lesions was dose related and their grading were 0.00, 1.33, 1.47, 2.34, 2.59 in males, and 0, 1.00, 1.57, 1.91 and 2.41 in females included in control, 1.5, 5, 15 and 50 mg/kg/day treatment groups respectively. A dose related incidences of intestinal metaplasia of the glandular mucosa of stomach were in 0, 0, 3.4, 5.0 and 10.3 % males, and 1.7, 0, 0, 0 and 10.2 % females of the respective groups. The metaplasia was present in pyloric region as detected with HE and Alcian blue PAS stain (report # A-29-840). The incidence of single cell necrosis and eosinophilic content of gastric mucosa was also dose related.

One female animal (# 5543) of 50 mg/kg/day treatment group was originally stated to have gastric adenocarcinoma. Its morphology was re-examined at the Drug Safety Research Laboratories, Takeda Chemical Industries Ltd., Osaka (Japan-Study # A-29-968). Based on a positive reaction with argyrophilic staining (with Grimelius/Sevier-Munger stain) of the tumor and a negative reaction with Fontana-Masson staining, the tumor was re-evaluated as of ECL cell origin. Electron microscopic examination did not show glandular or mucin secreting cells, therefore the diagnosis of gastric adenocarcinoma was changed to gastric carcinoid tumor (Amendment dated February 18, 1994 - reports # A-29-935 and A-29-968).

The incidences of hepatocellular carcinoma were 1.7, 1.7 and 3.3 % in female animals belonging to 5, 15 and 50 mg/kg/day treatment groups respectively. These were not seen in male animals included in the study.

A dose related testicular interstitial cell hyperplasia were 3.3, 5.0, 1.7, 13.3 and 33 % respectively in animals included in 0 (control), 1.5, 5, 15 and 50 mg/kg/day treatment groups respectively. These incidences were significantly high in animals belonging to 15 and 50 mg/kg/day treatment groups. A significant ($p < 0.001$) increase from a slight to minimum intensity, of testicular interstitial cell tumors was seen in rats included in 15 and 50 mg/kg/day treatment. Their incidences were 1.7, 3.3, 3.4, 10 and 20 % respectively in animals included in 0 (control), 1.5, 5, 15 and 50 mg/kg/day treatment groups, respectively. These tumors were reported to displace, atrophied and replace seminiferous tubules. The reviewing pathologist, C.Lin, Ph.D., concluded that lansoprazole treatment produced interstitial tumors.

In summary, lansoprazole (at a dose of 50 mg/kg/day (for 5 days/week) for 2 years produced a dose related gastric hyperplasia and intestinal metaplasia, testicular interstitial cell hyperplasia and tumors. since the animals were treated for 5 days per week during the study, therefore it was not a valid study for predicting the carcinogenic potential of the compound.

COVERSHEET FOR THE CARCINOGENICITY STUDY IN RATS

1. Study #: A29-1863; 1148/CA
2. Name of Laboratory: Takeda Chemical Industries, LTD, Japan
3. Strain: Sprague Dawley (CrI:CD^R SD BR) rats
4. No./sex/group: 60 rats in each of the Control 1 (O₁), Control 2 (O₂), 5, 15 and 50 mg/kg/day treatment groups). Four satellite groups consisting of 25 (15 males and 10 males) animals in Control 1 (O₁), Control 2 (O₂), 5, 15 and 50 mg/kg/day treatment groups).
5. Doses (O, L, M, H): 0, 5, 15 and 50 mg/kg/day or (0, 29.5, 88.5 and 295 mg/mm²) plus a 50/0 mg/kg/day (295/0 mg/mm²/day) treatment group.
6. Basis for dose selection stated: Yes
7. Interim sacrifice: No
8. Total duration (weeks): 97 weeks for males and 104 weeks for females
9. Week/site for first tumor:

<u>Male</u>	<u>Female</u>
O 14/Spleen lymph Malignancy	44/Brain Glioma
L 19/Bone Marrow Leukemia	25/Bone Marrow Leukemia
M 25/Bone Marrow leukemia	58/Pituitary Gland Adenoma/ Brain Granu. cell Tumor
H 45/Pancreatic Adenocarcinoma	28/Brain Glioma
H 32/Bone Marrow Leukemia (Recovery Gr)	

10. No. alive at termination:

	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
O ₁	19	31.6	14	23.3
O ₂	20	33	21	35.0
L	23	38.3	18	30.0
M	19	31.7	22	36.7
H	27	45.0	28	46.7

11. Statistical Analysis: Treatment related changes in body weight, food consumption, hematological parameters, LH and testosterone were determined by Bartlett's chi Square test and then by one-way analysis of variance and significance was determined by Bartlett's and Dunnett's test. Survival was estimated by Kaplan-Meier technique, the heterogeneity and dose related trend was determined using Tarone's test. Tumor analysis was done by Peto's method. Treatment groups animals were compared with animals of control group by sex.

12. Tumor and Non-tumor Data For each Tissue: See Appendix V.

2-Year Carcinogenicity Study in Rats
(Study #: A29-1863/1148/CA)

Testing Laboratory: Takeda Chemical Industries, LTD, Yamaguchi, Japan

Dates of Start and Completion: December 20, 1990 and October 13, 1993.

GLP & OAP Requirements: Statements of compliance to Japanese GLP was included.

Batch No: M610-038

Animals: Cr1:CD^R(SD)BR (SPF) 6 weeks old rats weighing between 162-236 g (males) and 134 to 203 g (females) were used.

Methods: Seven hundred and sixty (420 males and 340 females) were divided into 5 main and 4 satellite groups (60/sex/group). The animals in main groups were administered a dose of either 0 (2 groups), 5, 15 and 50 mg/kg/day of lansoprazole (in gum arabic suspension at pH of 6.9 to 7.1 and in a volume of 5 ml/kg) for 2 years. The compound was administered in stomach via catheter daily for 97 weeks in females and 104 weeks in males. One group (recovery group) of 60 male rats was treated for 78 weeks during the period of 2 years. Animals of satellite groups were used for gastrin, testosterone, LH hormone assays and drug levels, and 5 animals in each of these groups were used as replacement animals. The dose selection of this study was based on 3-month toxicity study in which a dose of 150 mg/kg/day produced hyperplasia and a retardation of more than 20 %. It was identified as the MTD and the Division recommended to sponsor this as a high dose in the study. Necrosis of chief cells, squamous cell hyperplasia and hyperkeratosis in the stomach was observed in animals included in 50 and 150 mg/kg/day treatment groups. The dose selection of the present study was also based on the experience of an invalid carcinogenicity study

in rats in which the rats were treated for 5 day/week (Study # A-29-681). In the present study, the animals were observed twice daily for abnormal signs, general behavior and toxic effects. The palpable masses in each animal were recorded weekly. The animals were weighed weekly for the first 14 weeks and then every 4 weeks thereafter during the study. The food consumption was recorded weekly during the first 14 weeks, 4 weeks thereafter till the termination of the study and during week 52 and 104. Hematological parameters in non-fasted 5 animals/sex/group of the satellite groups were determined on a blood sample drawn 2 hr post dosing and plasma concentrations of the compound were determined at 0.25, 0.5, 1, 2, 4, 8 and 24 hr post dosing at month 6, 9, 12, 18, 22 (for males) hr and month 24 (females groups and recovery group) months of the study. Serum gastrin was estimated by radioimmunoassay technique (Gastrin RIA kit II Dianabot) in the blood samples drawn at 2 and 4 hr after the drug administration. Serum testosterone and LH were estimated in rat blood samples drawn at 2 and 4 hr after the drug administration by radioimmunoassay technique using testosterone-¹²⁵I kit, (Soren Biomedical). At the time of necropsy of the animals/sacrifice, if the animal killed in extremis, the blood samples for hematological examination were collected. Plasma concentration of lansoprazole was determined by a high performance liquid chromatographic (HPLC) technique on the blood samples collected from 10 animals/dose/satellite group at 0.5 hr after 3 months, and at 0.5, 1, 2, 3 and 5 hr post dosing in month 6, 12, 18 and 24 months of the study. 24-Hr urine samples from 15 animals/group were collected for determining urinary LH levels during 12 months of dosing. 24-Hr urinary creatinine, urinary LH to creatinine ratio were also determined. Gross pathological examination on all animals that died spontaneously, killed in a moribund or the surviving animals sacrificed at end of the study. The animals which died during the study or found in a moribund condition were examined for palpable masses, necropsied and thoracic and abdominal cavities were examined fully. Organs were removed, examined and placed in fixative. The stomach was inflated and fixed in 10 % neutral formalin. Stomachs opened for its examination, and sections containing lesser curvature of fundus and forestomach were also examined. All tissues and organs of animals in control and high dose groups and from rats those died spontaneously were embedded in paraffin and examined microscopically. The stomach sections were examined after H and E staining, Grimelius staining, Fontana-Masson staining or Savier-Munger stainings. The ophthalmoscopic examination was done weekly during this study. The stomachs, testes, liver and eyes and all gross neoplastic lesions from mid and low dose groups and recovery group were examined histopathologically. The proliferative lesions were classified according to Standardized System of Nomenclature and Diagnostic Criteria,

1991 of NTP. All tissues excepting eyes were fixed in formalin. Eyes were fixed in 3 % glutaraldehyde and testes with epididymides in Bouin's fixative. The following organs were placed in 10 % formalin: bone, rib, lungs, liver, pancreas, kidneys, spleen, prostate, testes, ovaries, adrenal, pituitary glands, brain, mammary glands, pituitary glands, lymph nodes (mesenteric) salivary glands, seminal vesicles, thyroids with parathyroids uterus, vagina and trachea, brain, caecum, colon, duodenum, esophagus, exorbital lacrimal gland, ear, heart, ileum, jejunum, nasal turbinate, rectum skeletal muscle, spinal cord, trachea and urinary bladder. The body weight, food consumption, hematological parameters changes in various groups, during the study were analyzed by using Bartlett's test for homogeneity. If the variances were homogenous a one-way analysis of variance was applied. If the tests were significantly different, the Dunn-Bonferroni's method was performed to compare each dosed group with the control. If the variances were heterogenous, the Kruskal-Wallis test was used to test overall group differences. The tumor analysis and test for hyperplasia of testicular interstitial cells, gastric argyrophil cells and hepatocytes was done by employing Peto test and fisher's exact test. Mortality data and tumor data were analyzed using computer program 'MULTTEST'.

Results:

1. Observed effects: Staining on nasal and eye furs was observed in a dose related manner. Reddish tears and corneal opacity were observed in 9, 3, 5, 9, 8 and 18 males of the control 1, control 2, low, mid, high and high dose recovery treatment groups respectively. These observations were not seen in female animals of the study. The palpable masses were not different in animals belonging to various treatment groups and control group.

2. Mortality: Forty one, 40, 37, 41 and 33 (and 42 in 50 mg/kg/day treatment recovery group) males, and 46, 39, 42, 38 and 32 females out of 60 animals/sex/group of 0, 0, 5, 15 and 50 mg/kg/day treatment group died during the study. Sponsor terminated the treatment in males at the time when their survival rate was around 33 % which was at week 98 of the study. No significant increase in mortality was seen among animals of high dose treatment group and control group. The cause of death of animals in treated or control group was pituitary adenoma and/or nephropathy. The mortality rate is shown in the following table (taken from sponsor's submission vol 2.54, pp 043):

Sex Dose (mg/kg/day)	Male						Female				
	0	0	5	15	50	50 *	0	0	5	15	50
Animals examined	60	60	60	60	60	60	60	60	60	60	60
Termination (week)			98			105			105		
Decedent											
Found dead	37	35	34	35	30	37	33	25	23	28	24
Sacrificed in moribund condition	4	5	3	6	3	5	13	14	19	10	8
Total	41	40	37	41	33	42	46	39	42	38	32
Terminal kill	19	20	23	19	27	18	14	21	18	22	28
Survival rate (%)	32	33	38	32	45	30	23	35	30	37	47

*: Recovery group

3. Body Weight and Food Intake Changes: The final body weights were 874, and 553 g for male and female rats at the termination of the study. The final weights were taken after week 98 in males and week 104 for females in the study. A retardation of body weight gain of 0.2, 0 and 16.4 % in males, and 12.4, 6.5 and 21.0 % in female rats included in 5, 15 and 50 mg/kg/day treatment groups, respectively, was observed. The daily food consumption of animals in 50 mg/kg/day treatment group was reduced by 13.1 % during the study. The absolute food consumption were 28.9, 29, 27.8 and 25.1 g in males at the termination of the study. The daily food consumption of the animals included in recovery group was decreased and this was 23.3 g/animal at the termination of the study. In females the daily food consumption was 24.3, 20.4, 24.3 and 23.9 g/animal in the control, 5, 15 and 50 mg/kg/day treatment groups respectively. Therefore the body weight reatardation in the females was not related to the food intake.

4. Hematology/Blood Coagulation/Bone marrow Changes: No treatment related hematological changes were seen in the treated groups of animals. Bone marrow cytological alterations were not determined during this study.

5. Plasma Drug Levels: A treatment related peak plasma concentration of 384, 770 and 2571 ng.hr/ml in males, and 239, 839 and 2154 ng.hr/ml in females belonging to 5, 15 and 50 mg/kg/day treatment groups, respectively. Plasma peaks were attained in 30, 30 and 120 min in males and in 15, 30 and 120 min in females after its administration. There was a lack of dose proportionality at the highest dose as Cmax and AUC values in male animals included in 50 mg/kg/day treatment group were only 3.53 and 6 times at a dose which was 10 times greater. In females the plasma concentrations were proportional as AUC value

of 50 mg/kg/day dose was 9.2 times than the 5 mg/kg/day dose. Toxicokinetic data of the study are shown in the following table (taken from sponsor's submission vol. 2.53, pp 464):

Table 9 Plasma levels of AG-1749 - Group mean values
- Pharmacokinetic parameters of AG-1749 administered daily by gavage -

Compound : AG-1749 AG-1749 AG-1749
Dose (mg/kg/day): 5 15 50

1) Male

Time	Dose (mg/kg)	month 6	month 9	month 12	month 18	month 22
T_{max} (hr)	5 15 50					
C_{max} (ng/ml)	5 15 50					
AUC_{0-24} (ng · hr/ml)	5 15 50					
AUC_{0-24} (ng · hr/ml)	5 15 50					

2) Female

Time	Dose (mg/kg)	month 6	month 9	month 12	month 18	month 24
T_{max} (hr)	5 15 50					
C_{max} (ng/ml)	5 15 50					
AUC_{0-24} (ng · hr/ml)	5 15 50					
AUC_{0-24} (ng · hr/ml)	5 15 50					

Values of C_{max} and AUC are indicated as mean ± S.D..

Number of animals are indicated in parenthesis.

1): C_{max} values on month 9 of female groups are indicated the plasma levels after dosing for 1 hour.

- : Not determined

A clinical dose of 30 mg/day (0.6 mg/kg/day) was reported to achieve a peak plasma concentration of ug.h/ml in hr with T_{max} of hr . This is equivalent to CL/F value of 14 L/hr which is low compared to hepatic plasma flow (about 50 l/hr). These indicate that the maximum dose employed in the carcinogenicity study achieved only times higher plasma concentration than the clinical dose.

Serum gastrin levels were significantly increased in animals included in treatment groups than in the control group. The serum gastrin level data at month 24 (2 hr response) of the study is given in the following table (taken from vol 2.54, pp 474):

Table 10. Serum gastrin levels - Group mean values (mean $\pm$ S.D.)
Two-hour gastrin response to AG-1749 (at termination)

Group	:	1	3	4	5
Compound	:	Control	AG-1749	AG-1749	AG-1749
Dose (mg/kg/day)	:	-	5	15	50
Group	Number of Animals	Gastrin (pg/ml)	Group	Number of Animals	Gastrin (pg/ml)
1 M	10	115.2 $\pm$ 28.5	1 F	10	142.6 $\pm$ 74.1
3 M	10	376.5 $\pm$ 280.4**	3 F	10	364.4 $\pm$ 85.9**
4 M	7	843.4 $\pm$ 421.9**	4 F	10	812.5 $\pm$ 334.0**
5 M	10	1341.5 $\pm$ 568.2**	5 F	10	1147.2 $\pm$ 601.1**

** : Significantly different from control, $p < 0.01$

These were highest after 6 months of treatment. These were 202, 1201.0, 648.4 and 2619.7 pg/ml in males, and 836, 1586.6, 2183.3 and 2223.8 pg/ml in females included in 5, 15 or 50 mg/kg/day treatment groups respectively. The concentration of gastrin decreased slightly at 12 and 18 month observation periods. These were pg/ml in males and pg/ml in females at 18-month

observation time point. The gastrin levels were not significantly changed at the termination of the study. 24-Hr serum gastrin levels of males and females included in 50 mg/kg/day treatment group were higher than the animals in control group pg/ml in males and females vs pg/ml in control group males and females). In recovery males, gastrin levels were comparable with those of control group animals pg/ml in control males). Females were not included in the recovery group.

Serum testosterone levels were not altered after 2, 4 and 6 hr after a dose of 5, 15 or 50 mg/kg/day of lansoprazole for 6 months. The serum testosterone level (ng/ml) at month 22 of the study is given in the following table (taken from vol 2.54, pp 480:

Table 11		Serum testosterone levels (ng/ml) month 22 - Group mean values (mean $\pm$ S.D.)				
Group	Compound	Dose (mg/kg/day)	1(2) Control	3 AG-1749 5	4 AG-1749 15	5 AG-1749 50
Group	Number of animals	Hours after administration				
		2	4	6		
1H(2H)	10	0.43 $\pm$ 0.53	0.35 $\pm$ 0.43	0.36 $\pm$ 0.50		
3H	10	0.62 $\pm$ 0.47	0.72 $\pm$ 0.85	0.66 $\pm$ 0.86		
4H	9	0.32 $\pm$ 0.30	0.23 $\pm$ 0.17	0.34 $\pm$ 0.32		
5H	10	0.38 $\pm$ 0.33	0.33 $\pm$ 0.36	0.22 $\pm$ 0.23		
No significant difference.						

Its concentrations were not statistically different in the animals included in 5, 15 and 50 mg/kg/day treatment groups respectively

Serum LH concentrations in males were not altered during 24-month treatment with lansoprazole. These concentrations were also not changed after an acute treatment of a dose of lansoprazole at month 6, 12 and 18 of the study. The serum LH level (ng/ml) at month 22 of the study is given in the following table (taken from vol 2.54, pp 485):

Table 12-1 Serum LH ^a levels (ng/ml) month 22 - Group mean values (mean ± S.D.)						
Group	Compound	Dose (mg/kg/day)	1(2) Control	3 AG-1749 5	4 AG-1749 15	5 AG-1749 50
Group	Number of animals	Hours after administration				
		2	4	6		
1H(2H)	10	1.10 ± 0.39	1.15 ± 0.13	1.13 ± 0.21		
3H	10	1.17 ± 0.28	1.16 ± 0.16	1.09 ± 0.23		
4H	9	1.23 ± 0.22	1.30 ± 0.34	1.19 ± 0.14		
5H	10	1.20 ± 0.19	1.13 ± 0.27	1.14 ± 0.25		

No significant difference.
^a : Calibrated against a reference standard preparation (rat LH-PK-2, NIH).

Lansoprazole treatment did not produce an effect on the excretion of urine and its creatinine, LH and LH/Creatinine ratios. These are shown in the following table of sponsor's submission (vol 2.54 pp 486).

Table 12-2 Urine LH levels - Group mean values (mean±S.D.) month 12 (Day 1)

		1	3	4	5		
Group		Control	AG-1749	AG-1749	AG-1749		
Compound		-	5	15	50		
Dose (mg/kg/day)		-	5	15	50		
Group	Number of animals	Urine volume (g)	Creatinine concentration (mgX)	LH ^a concentration (ng/ml)	Total LH ^a (ng)	LH ^a /Creatinine ratio (× 10 ³)	
1M	14 §	31±10	91±25	0.20±0.05	6.4±3.0	2.6±1.7	
3M	13 §	31±12	95±26	0.22±0.04	7.2±4.1	2.6±1.3	
4M	12 §	39±10	69±10	0.24±0.05	9.2±2.0	3.7±1.6	
5M	15	36±8	77±21	0.24±0.04	8.6±2.4	3.4±1.1	

No statistical significance

1 : Calibrated against a reference standard preparation (ratLH-RP-2, NIH)

§ : See text for the reason of decrease in the number of animals.

Therefore, lansoprazole did not affect the synthesis, metabolism or excretion of testosterone and LH during the study.

5. Physical Examination, Viral Screen: Ophthalmoscopic examination showed that 1/16, 2/16, 1/19, 2/16, 2/23 and 2/29 males had cataract while there were no incidences of cataract in females included in this study. The viral screening of the rats were not conducted during the study.

6. Gross Pathology: During autopsy, the incidence of thickening of the glandular mucosa of the stomach were seen in a dose dependent manner as 5, 18, 25 and 29 males included in 0, 5, 15 and 50 mg/kg/day treatment groups were seen. The incidences in female rats were 5, 16, 26 and 38 in 0, 5, 15 and 50 mg/kg/day treatment groups. These are shown in the following table. These animals were also seen to bear yellowish white nodules in glandular stomach. The enlargement of testes with white to yellow foci was observed in 1 and 2 males included in 15 and 50 mg/kg/day treatment groups respectively.

The incidences of yellow/red nodules in liver of 2 cm or less were observed in 1/120, 1/60, 1/60 and 4/60 in males and, 1/120, 4/60, 2/60 and 9/60 females included in control, 5, 15 and 50 mg/kg/day treatment groups. In the recovery group, 4/60 males were seen to have nodule.

7. Histopathological Changes:

Non-Neoplastic Changes: Foci of altered hepatocytes were seen in male and female animals of control and treatment groups of the study. The distribution of alteration of the hepatocytes did not show any definite pattern of effect, excepting a trend of an increase in eosinophilic foci of altered hepatocytes were seen in a dose dependent manner. Lansoprazole treatment produced diffused retinal atrophy. A dose dependent increase in the incidences of 0, 2.0, 2.0, 10.0 and 21 % were seen in female rats included in 0, 5, 15 and 50 mg/kg/day treatment groups. A trend of an increase of incidences in retinal atrophy was seen in male rats, i.e., 9.0 % in treated vs 0 in control animals (see following Table).

A treatment related increase in retinal atrophy in 2 (females) and 3 (1 male and 2 female) rats included in 15 and 50 mg/kg/day treatment groups was reported. The outer nuclear layers were reduced to a smaller number and outer plexiform layer was reduced. A dose related incidence of testicular interstitial cell tumors (unilateral plus bilateral) were reported in 0, 1, 2, 7 and 12 animals in control, 5, 15 and 50 mg/kg/day treatment groups respectively.

TWO YEAR RAT (SD) CARCINOGENICITY STUDY OF LANSOPRAZOLE
(Study # 1148/CA)
Takeda Study

ABNORMALITY		DOSE (mg/kg/day)				
		0	5	15	50	50/0
No. of Incidences*						
Retinal Atrophy	M	1	0	0	6	1
	F	0	1	7	15	--
Micronodular Liver	M	4	3	6	8	6
	F	1	5	3	9	--
Foci of Altered Hepatocytes	M					
	Clear	0	3	0	0	0
	Baso.	21	27	26	19	12
	Eosin	43	47	45	57	52
	F					
	Clear	13	4	5	0	--
Bilateral Diff Cataract	Baso.	36	42	27	26	--
	Eosin	22	30	41	49	--
Bilateral Atrophy	M	0	0	0	6	1
	F	0	1	1	7	15

* Each group consisted of 60 animals/sex

Neoplastic Changes:

Stomach:

The incidences of intestinal metaplasia in male animals included in treatment groups were higher than the control animals (33.3 % vs 18 % in the control). This trend was not seen in female animals included in the study. The lesions in the intestinal metaplasia composed of a single or a few glands which are morphologically similar to that of the small intestines. These cells contain goblet cells, epithelial cells with brush border and occasional Paneth cells. No cellular atypia or epithelial tumors (adenoma/adenocarcinoma) could be found in these lesions. Gastric E-cell carcinoid were increased ($p < 0.001$ to 0.002) in a dose dependent manner. These were 1.7, 1.7, 6.67 and 15 % among males and 1.7, 10, 25.0 and 40 % among females included in 5, 15 and 50 mg/kg/day treatment groups respectively. The morphology of the carcinoid cells was similar to the hyperplastic eosinophilic cells.

Liver:

Hepatocellular adenoma incidences in male rat were 8.0, 0, 3.0, 7.0 and 12.0 % in animals included in control, 5, 15 and 50 mg/kg/day treatment groups respectively. These incidences were greater than the mean of historical and strain control values but were within the strain control values.

Testis:

Testicular interstitial cell adenoma was seen in a dose dependent manner and the incidences were 1.7, 3.4, 11.7, 20 and 23.3 % in rats included in Control, 1.5, 5.0, 15, 50 and 50/0 (Recovery group) mg/kg/day treatment groups. The incidences in 15 and 50 mg/kg/day treatment groups were more than the strain and sponsor historical control data. These are shown in the following table.

TWO YEAR RAT (SD) CARCINOGENICITY STUDY OF LANSOPRAZOLE
(Study # 1148/CA)
Takeda Study

TUMOR		DOSE (mg/kg/day)*					Incidences (%)	
		0	5	15	50	50/0		
Pancreatic Acinar cell Adenocarcinoma	M	0	0	0	1	--		
	F	0	0	0	1	--		
Intestinal Metaplasia	M	13	12	26	23	15		
	F	10	10	3	11	--		
Hepatocellular Adenoma	M	5	0	2	4	7	3.0	4.2
	F	1	0	1	4	--		
		p=0.035**						
Gastric ECL-Cell Carcinoid	M	0	1	4	9	3	0	
	F	0	6	15	24	--	0	
		p<0.0002						
		p<0.0001						
Testicular Interstitial Cell Tumor		3	5	4	11	14	2.60	4.68
		p<0.0001						
Uterine Squamous Cell carcinoma		0	0	0	0	2		
		p< 0.0001						
Uterine Endometrial Stromal polyps		2	4	5	6	--		

* No. of animals/group = 60/sex

Uterus:

Two out of 60 animals included in 50 mg/kg/day treatment group had carcinoma of uterine squamous cell carcinoma. Uterine endometrial polyps were increased in a dose related manner. But these incidences were within the historical control incidences.

In summary, lansoprazole at a dose of 50 mg/kg/day produced a dose related gastric cell E-cell carcinoid and intestinal metaplasia in both male and female rats, and testicular interstitial cell tumors in males. It did not affect the hemostasis of testosterone and LH hormone as testosterone, LH and FSH levels were not affected. Lansoprazole treatment increased gastrin levels in a dose dependent manner. The highest dose of lansoprazole was found reported to achieve a peak plasma concentration equivalent to recommended clinical dose of 30 mg/day (mg/kg/day), therefore the desired plasma concentration of the compound (times the clinical dose) were not reached in this study, therefore the study is not acceptable and is not valid.

COVERSHEET FOR THE CARCINOGENICITY STUDY IN RATS

1. Study #: TA91-024/126-051, R&D/93/546
2. Name of Laboratory:
3. Strain: Charles River CD¹ Sprague Dawley
4. No./sex/group: 70
5. Doses (O, L, M₁, M₂, H): 0 (2 groups), 5, 25, 75 and 150 mg/kg/day *or (0, 29.5, 147.5, 442.5 and 885 mg/mm²) plus a 150/0 mg/kg/day (885/0 mg/mm²/day) treatment 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 ₁	30/Skin Fibrosarcoma	27/Transitional Cell Carcinoma
O ₂	58/Skin Neurolomoma	51/Skin Sebaceous Carcinoma
L	27/Mononuclear cell Leukemia	69/Pituitary Adenoma
M ₁	46/Pituitary Benign Meningioma	41/Neurofibrosarcoma of Skin
M ₂	20/Skin Fibrosarcoma	9/Malignant Meningioma
H	38/Mononuclear cell Leukemia	63/Mammary Gland Fibroadenoma
RH	47/Testicular Interstitial cell Tumor	

10. No. alive at termination:

	<u>Male</u>	<u>% Survival</u>	<u>Female</u>	<u>% Survival</u>
O ₁	30	43.8	24	34.3
O ₂	28	40.0	28	40.0
L	25	35.7	43	61.4
M ₁	27	38.6	28	40
M ₂	31	44.3	39	55.7
H	27	38.6	42	60

11. Statistical Analysis: Treatment related changes in body weight, food consumption, hematological parameters were analyzed using a one-way analysis of variance and significance was determined by Bartlett's test and/or Dunnett's test. Test for pairwise comparison was done by Welsh t-test with Bonferroni correction. Treatment groups animals were compared with animals of control group by sex.

12. Tumor and Non-tumor Data For Each Tissue: See Appendix 4.

2-Year Carcinogenicity Study in Rats
(Study #: TA91-024/126-051, R&D/93/546)

Testing Laboratory:

Dates of Start and Completion: February 20, 1991 and November 2, 1993.

GLP & OAP Requirements: Statements of compliance were included.

Batch No: M610-049, M610-050 (after week 88 of the study)

Animals: One thousand six hundred and ten (840 males and 770 females) Charles River CD^R Sprague Dawley rats about 4 weeks old and with mean body weights of 168 g (males) and 136 g (females) were used.

Methods: These animals were divided into 6 main study and 5 satellite groups. Each main group consisted of 70/sex/group and satellite groups consisted of 30/sex/group. An additional group (group 12) of 70 male rats was also included in the study. These animals were administered lansoprazole by the following schedule as shown in the following table (taken from sponsor's submission vol 2.59, pp 036):

Table 4.2.2.-1 Group Assignment								
Group	Dosage (mg/kg/day)	Main Groups		Group	Satellite Animals ^a		18-Month	
		Males	Females		Males	Females	Group	Males
1	Vehicle Control A	70	70	7	70	70		
2	5	70	70	8	70	70		
3	25	70	70	9	70	70		
4	75	70	70	10	70	70		
5	150	70	70	11	70	70		
6	Vehicle Control B	70	70				12	70 ^b

^aUp to 10-30/sex utilized at 3, 6, 12, 18 and 24 months for blood drug level and hormone assays.

^bThe 70 male animals in Group 12 (150/0 mg/kg/day) were treated with lansoprazole for 18 months, then were administered vehicle alone for the remaining 6 months. Group 12 rats were used for testicular, ocular and gastric histopathologic examinations and hormone assays. Group 12 animals were subject to a complete necropsy and all scheduled tissues were saved.

Lansoprazole suspension was administered in a concentration of mg/ml in a volume of 10 ml/kg. The animals in group 12 were treated with a dose of 150 mg/kg/day of lansoprazole for 18 months and vehicle alone for period of 6 months (recovery period). The dose selection of this study was

based on the results of 3-month toxicity study (Study No. TA90-152, R&D/90/339) in which a dose of 150 mg/kg/day produced a retardation of 21 % body weight gain and decreased thymus size without any histopathological changes in rats of both sexes. The doses of 300 and 600 mg/kg/day produced reduced testes and testicular atrophy. Based on these observations, sponsor was suggested to use a dose of 150 mg/kg/day as an MTD for 2-year rat carcinogenicity study (Division letter dated July 26, 1991). Animals of satellite group were used for hormone assays (Report # R&D/93/515) and necropsied. The ophthalmic examination of the animals in vehicle control and 150 mg/kg/day treatment groups was conducted at week 95 of the study. Testicular, ocular and gastric histopathologic examinations were done in animals of group 7. Animals of group 12 will be used testicular and gastric histopathological and hormonal assays. In the present study, the animals were observed twice daily for abnormal signs, general behavior and toxic effects, and palpable masses in each animal were recorded weekly. The animals were weighed weekly for the first 14 weeks and then every 2 weeks thereafter during the study. The food consumption was recorded weekly during the first 14 weeks, and 4 weeks thereafter till the termination of the study. Hematology parameters were determined on non-fasted 10 animals/sex/group of the main groups at 3, 6, 12, 18 and 24 months (if possible) of the study at the time of autopsy of the animals and at the time of sacrifice, if the animal killed in extremis. Plasma concentration of lansoprazole was determined by a high performance liquid chromatographic (HPLC) technique on the blood samples collected from 10 animals/dose/satellite group 30 min 4 hr after treatment at month 3, 6, 12, 18 and 24 of the study. The blood samples from month 3 and 6 were collected at 4 hr post dosing. At month 3, 6, 12, 18 and 24 of the study, 30 animals/group were used for determined Plasma/serum hormone assays. A 4 hr post dosing blood samples of blood were collected at 12, 18 and 24 months from 10 males/group. A 24-hr urine samples were collected from satellite groups 7 to 11 and the hormone assays were conducted. Plasma somatostatin; serum estradiol, FSH, gastrin, LH, LH/testosterone ratio, prolactin and testosterone and; urinary 24 hr total FSH and LH were estimated in frozen samples by a radioimmunoassay technique. Serum testosterone was determined by the double antibody radioimmunoassay kit (testosterone-¹²⁵I kit) for rat testosterone estimation. The animals which died during the study or found in a moribund condition were examined for palpable masses, necropsied and thoracic and abdominal cavities were examined fully. At the termination of the study (week 104-106), organs from all the animals were removed, examined and placed in fixative. The gastrointestinal tract was examined by using transillumination. All tissues excepting eyes, testes and stomach were fixed in formalin. Eyes were fixed in 3 % glutaraldehyde and testes with epididymides in Bouin's fixative.

All organs and tissues from all animals included in control, 5, 25, 75, 150 and 150/0 mg/kg/day treatment groups were separated. In addition, stomachs, testes, eyes, liver from female animals and all gross sections of the lesions sections from 150/0 mg/kg/day treatment group were prepared for examination. Four sections of stomachs (2 sections/fundic and pyloric part) were embedded, cut into sections and stained either with H and E, Grimelius, Fontana-Masson or Sevier-Munger stain. The following organs were examined histopathologically: bone, rib, lungs, liver, pancreas, kidneys, spleen, prostate, testes, ovaries, adrenal, pituitary glands, brain, mammary glands, pituitary glands, lymph nodes (mesenteric) salivary glands, seminal vesicles, thyroids with parathyroids uterus, vagina and trachea, brain, caecum, colon, duodenum, esophagus, exorbital lacrimal gland, ear, heart, ileum, jejunum, nasal turbinate, rectum skeletal muscle, spinal cord, trachea and urinary bladder. The microscopic examination on the sections of stomach was performed by an Experimental Pathology Laboratory (EPL) scientist, Deborah Banas. In one of Protocol Revision Sheet # 15 dated January 28, 1993, sponsor stated that the preliminary microscopic evaluation data of the stomach by IRDC pathologist, were deleted from the computer files. The hard copies were retained. The eyes from the terminally sacrificed rats were examined during the study. The eyes of the animals killed or died during the study, were not examined. Treatment related changes in body weight, food consumption, hematological parameters were analyzed using a one-way analysis of variance and significance was determined. If the results were significant, Bartlett's test for homogeneity of variance was performed. If it was non-significant, the Dunnett's test for pairwise comparison for each of the treatment groups with control group was used. Mortality data were analyzed by Cox test and Tarone test was used for trend test. Peto test was used for analyzing the tumor data. Test for pairwise comparison was made and the significance (Welsh t-test) was determined by using Bonferroni adjustment. The control and treatment groups animals were compared with each other at 0.05 level of significance. The Kruskal-Wallis test was used to test overall group differences.

Results:

1. Homogeneity and Stability of Suspension: The homogeneity test for uniform dispersion of the substance in 5, 25, 75 and 150 mg/kg/day treatment groups suspensions showed a mean nominal values of 98, 99, 102 and 94 % lansoprazole. Therefore, lansoprazole suspensions were homogeneous up to 10 days of their preparations. The stability test of the suspension done on day 8 and 13 (from 4 to 10°C - under refrigeration condition) showed that the suspensions contained 86 to 101% and 95 to 101%

of the initial test article. The initial stability test conducted on March 27, 1991 did not show the presence of lansoprazole in 0.5, 2.5 and 75 mg/kg/day treatment groups. But the suspensions of 150 mg/kg/day treatment group was positive for the lansoprazole. But the subsequent analysis of the suspensions on day 8 and 13 revealed that the suspensions were within the acceptable limits of 86 to 101 % for the compound. Therefore compound was stable during the study.

2. Microbial Screening of Animals: The animals included in the study did not show positive test for viral infection during the study.

3. Observed effects: Hypersalivation was reported in 32 to 57 % and 53 to 82 % animals belonging to 75 and 150 mg/kg/day treatment groups respectively. This was observed within 5 to 20 min of the administration of the compound on study day 16 and 11. These incidences were decreased as the study progressed. Enlargement of testes was seen from week 78 to week 104 as the incidences were 10.7, 30.8 and 54.7 % in week 78, 91 and 104 of the study.

4. Mortality: Forty, 45, 43, 39 and 43 out of 70 males, and 46, 27, 42, 31, 28 and 42 females out of 70 females in each of the 0, 5, 25, 75 and 150 mg/kg/day treatment group died during the study. These are shown in the following Table (taken from vol 2.59, pp 44). Since the number of mortalities in the treatment groups were not different from the control groups, deaths were not related to the drug.

Table 5.2.1.1.		
Dosage Level (mg/kg/day)	Mortality ^a	
	Male %	Female %
Vehicle Control A	40/70 57	46/70 66
5	45/70 64	27/70 39
25	43/70 61	42/70 60
75	39/70 56	31/70 44
150	43/70 61	28/70 40
Vehicle Control B	42/70 60	42/70 60
150/0	47/70 67	

^a Number dead/number initiated
^b Treated for 18 months with lansoprazole and for 6 months with vehicle alone

5. Body Weight and Food Intake Changes: A dose related retardation of body weight gain of 0.2, 1.1, 14.3 and 20.4 % in females, and 0, 0, 10.3 and 19.0 % in males included in 5, 25, 75 and 150 mg/kg/day treatment groups, respectively was seen. The food consumption of males was reduced by 1.2, 0.4, 4.1 and

13.5 % during the study. The mean food consumption was 19.8, 18.2, 19.9, 19.2, 19.3 and 20.3 g/male/day, and 24.4, 24.1, 24.3, 23.4, 21.1 and 24.1 g/female/day in 5, 25, 75 and 150 mg/kg/day treatment groups, respectively. Food efficacy of rats during the study was affected in rats included in the study.

6. Hematology/Blood Coagulation/Bone Marrow Changes: No treatment related or statistically important hematological changes were seen in the treated groups of animals. Bone marrow cytological alterations were not determined during this study.

7. Blood Drug Concentrations: Lansoprazole blood concentrations were done in Drug Metabolism Department,

Lansoprazole was reported to attain a treatment related peak plasma concentration within 30 to 60 min of its administration in both sexes of rats. $T_{1/2}$ of a dose up to 50 mg/kg/day was about 20 to 40 min in both sexes but it was about 60 to 75 min at a dose of 75 to 150 mg/kg/day. Female rats attained a higher plasma concentration than male rats. Toxicokinetic data of the study are shown in the following table (Extracted from sponsor's submission vol. 2.72, pp 10 to 23):

Observ. Time (Months)	Dose (mg/kg/day)	Sex (ng/ml)	C _{max} (hr)	T _{max} (hr)	T _{1/2} AUC _{0-∞} (ug.h/ml)
6	5	M			
		F			
	25	M			
		F			
	75	M			
		F			
	150	M			
		F			
12	5	M			
		F			
	25	M			
		F			
	75	M			
		F			
	150	M			
		F			
18	5	M			
		F			
	25	M			
		F			
	75	M			
		F			
	150	M			
		F			

An oral dose of dose of 75 mg/kg/day achieved a plasma peak concentration ($AUC_{0-\infty}$) of ug.hr/ml in male and female mice in min, respectively. A higher dose of 150 mg/kg/day, achieved a plasma concentration of ug.hr/ml with in the similar Tmax of in about min. A clinical dose of 30 mg/day (0.6 mg/kg/day) was reported to achieve a peak plasma concentration of ug.h/ml in hr with Tmax of hr. This is equivalent to CL/F value of 14 L/hr which is low compared to hepatic plasma flow (about 50 L/hr). This indicated that the maximum dose employed in the carcinogenicity study achieved only times higher plasma concentration than the clinical dose.

8. Serum/Plasma Hormones Changes:

Serum gastrin Concentrations: Gastrin levels in males and females were significantly higher in all treatment groups than in control group throughout the study. The highest increase of times was seen in animals included in 150 mg/kg/day treatment group (8.157 vs 2072 pg/ml in treated animals). In females included in 75 and 150 mg/kg/day treatment groups, prolactin levels were low at 12, 18 and 24 months of treatment and in at 24 month than in the control group.

Males:

a. Somatostatin: Mean plasma concentrations of somatostatin were 9.6, 11.9, 12.0 and 11.9 pg/ml in in rats included in 25, 75 and 150 mg/kg/day treatment groups respectively. At 24 month after 4 hr treatment, only a trend of an increase in the hormone was seen in animals of treatment groups. In recovery males, somatostatin levels were decreased during recovery period and were not significantly different from the animals in control group animals.

b. LH: This hormone levels in recovery males animals included in 150 mg/kg/day treatment group at 4 hr were higher (0.508 vs 0.224 ng/ml in 150 mg/kg/day treatment and control groups respectively). This was not statistically significant. The recovery males had significantly higher LH than animals included in 24 months treatment group.

c. Testosterone: At 4 hr post dosing period on 12 and 24 months of the study, testosterone levels were significantly ($p < 0.05$) lower in males of 75 and 150 mg/kg/day treatment groups than the control males. These were 0.505 and 0.468 ng/ml at 12 months and 4 hr. At 24 months and 4 hr, the testosterone levels were 0.587 and 0.356 ng/ml in 75 and 150 mg/kg/day treatment groups vs 0.926 ng/ml in control. The testosterone levels in males of

the recovery subgroup of 150 mg/kg/day treatment group at 4 hr were also found significantly lower (0.491 ng/ml vs 0.926 ng/ml in the control group). At 24 hr post dosing in month 24, the testosterone levels of animals in 150 mg/kg/day treatment group, were lower than the control group indicating that the effect was a transient one. Testosterone/LH ratio in 75 and 150 mg/kg/day treatment groups animals at 18 months and 24 hr were 1.8 and 2.3 vs 0.28 in control group. In male rats included in 75 and 150 mg/kg/day treatment groups, FSH concentration in serum samples collected at 4 and 24 post dosing were not affected. A similar trend of an increase in estradiol was also seen in these groups of male animals.

Females: The estrogen cycle in the animals was not evaluated in this study by sponsor. The results obtained are reviewed below:

a. Estradiol Levels: In females, the estradiol levels were not affected during the study excepting at 6 month and 4 hr and 18 month and 4 hr observations when these were claimed to be decreased. Estradiol levels at 6 months and 4 hr, and at 18 months and 4 hr were: 137.3, 140.4, 147, 103.6 and 101.2 pg/ml, and 96.2, 90.8, 75.6, 87.9, 92.7 pg/ml in control, 25, 75 and 150 mg/kg/day treatment groups respectively.

b. FSH: FSH levels were not affected during the study. Serum prolactin levels in 150 mg/kg/day treatment female rats were decreased from 12 to 24 months and 4 hr serum samples. These were: (i) 39.3, 40.8, 37.8, 32.7 and 24.6 ng/ml at 12 months and 4 hr, (ii) 72.2, 77.8, 72.1, 56.4 and 41.4 ng/ml at 18 months and 4 hrs and (iii) 640.2, 373.9, 394.9, 204.6 and 308.5 ng/ml at 24 months and 4 hr.

c. Somatostatin: Its levels were not different from the animals in control group animals.

d. LH and Testosterone: LH hormones levels were not affected by lansoprazole treatment. Testosterone levels in females included in the study were not affected and these were comparable with the control group of animals. Testosterone/LH ratio was also not affected during the study.

9. Gross Pathology: During autopsy, macroscopic nodules and masses in liver were observed in 4, 1, 4, 3 and 2 out of 70 female animals in each of the control, 5, 25, 75 and 150 mg/kg/day treatment groups. The number of liver cysts were: 1, 2, 2, 4, 4 and 7 in males and 1, 1, 2, 3 and 13 females belonging to control, 5, 25, 75, 150 and 150/0 (for males only) mg/kg/day treatment groups. No increase in the incidence of liver nodules/masses were seen in male rats.

Thickening of glandular stomach were seen in 32.8, 51.4, 67.1, 65.7 and 57.1% animals included in 0 (control), 5, 25, 75 and 150 mg/kg/day treatment groups, respectively. The increased thickness and nodules in the stomach wall were seen in 2.9, 22.8, 37., 31.7 and 22.8%, in 0 (control), 5, 25, 75 and 150 mg/kg/day treatment groups. No relevant pathological changes could be seen in non-glandular portion of the stomach.

The macroscopic testicular enlargement was seen in a dose related manner as 0, 1, 2, 12 and 12 out of 70 animals were in 0, 5, 25, 75 and 150 mg/kg/day treatment group. A tan to white or discolored testicular foci were reported in 3, 7, 8, 20 and 30 animals out of 70 animals in each of the control, 5, 25, 75 and 150 mg/kg/day treatment groups. Incidences of testicular interstitial cell benign tumors were significantly high ($p < 0.0001$) in 150 mg/kg/day treatment group. An increase in the number of incidences of splenic cysts were 1, 1, 4, 8 and 7 males and 1, 2, 2, 6 and 18 females included in 0, 5, 25, 75 and 150 mg/kg/day treatment groups respectively. The number of pituitary cysts were not different in treatment or control groups. Incidences of diffuse retinal atrophy were increased in a dose dependent manner and these were 5, 2, 2, 16 and 30 males, and 1, 3, 10, 23 and 47 females in 0, 5, 15, 75 and 150 mg/kg/day treatment groups, respectively.

10. Histopathological Changes:

a. Non-Neoplastic Changes: Lansoprazole caused treatment related incidences of carcinoid in the fundic portion of stomach in 3, 3, 2 and 4 males and, 5, 12, 25 and 21 female rats included in 5, 25, 75 and 150 mg/kg/day treatment groups respectively. The incidence of carcinoid are shown in the following table. The number of animals in the table do not reflect the total number of animals affected as a few of the animals had more than 1 type of carcinoid. A few of these were found to have 6 to 7 carcinoid.

TWO YEAR RAT (SD) CARCINOGENICITY STUDY OF LANZOPRAZOLE
(Study # TA 91-024)
Study

ABNORMALITY		CONTROL	DOSE (mg/kg/day)			150	150/0
			5	25	75		
No. of Incidences							
Hyperesino- philic Chief Cells	M	0	31	43	42	42	15
	F	0	26	42	48	42	-
Hypertrophy/Hyper- plasia Total	M	20	55	63	65	57	70
	F	30	62	44	15	6	--
ECL Cell Hyper- plasia (Adenomat.)	M	0	0	5	2	6	-
	F	0	16	17	24	27	-
Micronodular/ Edenomatous ECL cell Hyperplasia	M	3	31	34	47	36	29
	F	14	57	50	57	52	-
Intestinal Metaplasia	M	5	11	24	24	31	17
	F	2	8	10	18	25	-
Peripher. Retinal Atrophy (Diffuse)	M	5 p=0.0000	2	2	16	31	14
	F	1 p=0.0000	3	10	23	46	---
Cataract (Total)	M	6	5	2	9	15	14
	F	1	1	2	4	15	---
Testicular Hyperplasia	M	16	16	29	49	57	--

Lansoprazole treatment in rats increased the incidence and severity of hyperplasia/hypertrophy of gastric mucosa in the fundus portion. Glandular dilatation with increased eosinophilic material in the lumen was reported in gastric mucosa. The dilatation is minimal to multifocal in control group animals and was more severe and diffuse in the treated groups. Hyperesinophilic chief cells were noted in 31, 43, 42, 42 and 15 animals belonging to 0, 5, 25, 75, 150 and 150/0 mg/kg/day treatment groups respectively.

Treatment related diffuse fibrosis and/or atrophy of the fundic hypertrophy and glandular mucosa, fatty infiltration of mucosa and mononuclear cell infiltration were also reported in the rats included in lansoprazole treatment groups. In pyloric stomach, hypertrophy and hyperplasia of less intensity were seen in 7, 5, 10, 14, 18 and 7 animals included in Control 1, Control 2, 5, 25, 75 and 150 mg/kg/day treatment groups respectively. Intestinal metaplasia was seen in 5, 10, 19, 19, 18, and 12 males included in Control 1, Control 2, 5, 25, 75 and 150 mg/kg/day treatment groups respectively. The incidence were mostly minimal, therefore action of lansoprazole on pylorus is less intense than fundus.

In the non-glandular stomach, hyperplasia and hyperkeratosis of the limiting ridge were increased in a dose related manner. The incidence of hyperkeratosis were 8, 34, 47, 52 and 51 out of 70 animals in each of the groups of the study. Gastric hyperplasia was seen in 11, 35, 40, 52 and 58 out of 70 animals in each of the groups included in the study. The severity of the hyperplasia and hyperkeratosis were also dose dependent in the animals. A mild to moderate hyperplasia and hyperkeratosis of non-glandular stomach were seen in rats of both sexes included in 150 mg/kg/day treatment group.

The incidence of tumor were significantly lower ($p < 0.04$ - biostatistical review of Dr. D. Lin, HFD-715) among the animals included in recovery group.

Peripheral diffuse retinal atrophy incidences were increased in a treatment related manner and these were 0/25, 2/27, 12/31 and 11/21 males, and 0/18, 0/43, 5/28, 20/39 and 32/36 females belonging to 0, 15, 75 and 150 mg/kg/day treatment groups, respectively.

b. Neoplastic Change:

The incidence of hepatocellular carcinoma were: 1, 1, 0, 2, 1 and 0 out of 70 males and 1, 0, 0, 0, 5 and 0 in each of 0, 5, 15, 75 and 150 mg/kg/day treatment groups. Thymic lymphoid hyperplasia was seen in 0, 3, 9, 27 and 35 male rats included in 0, 5, 25, 75 and 150 mg/kg/day treatment groups respectively. Incidences of testicular hyperplasia were 16, 16, 29, 49 and 57 out of 70 animals examined in each of the 0, 5, 15, 75 and 150 mg/kg/day treatment groups. Degeneration of seminiferous tubules, hypospermia in the epididymis and atrophy of seminal vesicle were observed in only 75 and 150 mg/kg/day treatment groups (table 13 taken from sponsor's submission vol 2.59 pp 174). The incidences of interstitial cell tumors, hyperplasia and tubular degeneration reduced during the recovery period. The retinal atrophy was seen in the later part of the study (from week 86) and it was in a dose related manner. The increase was seen in both unilateral or bilateral incidences, therefore sponsor's contention that bilateral retinal atrophy was age related was not correct. In the present study the frequency of retinal atrophy was more in females than males.

TWO YEAR RAT (SD) CARCINOGENICITY STUDY OF LANSOPRAZOLE
(Study # TA 91-024)
Study

TUMOR		DOSE (mg/kg/day)					Historical Control (Sponsor)	Strain Control
		0	5	25	75	150		
NON-NEOPLASTIC		No. of Incidences*						
Gastric Fundus Carcinoid (Intramuc).	F	0	5	12	25	21		
	M	0	3	3	2	4	0	0
		p value= 0.001 p=0.001						
Gastric ECL Carcinoids Intramucosa	M	3	3	2	4	2		
	F	0	5	12	25	21		
NEOPLASTIC								
Hepatocellular Carcinoma	M	1	1	0	2	1		
	F	1	0	0	0	5		
Hepatocellular Adenoma	F	1	0	0	0	5	1.3 (0.7-2.9)	2.2 (1-5.5%)
	M	0	2	5	8	4	3 (1.33-10.0)	4.2 (1.3-18.2)
Interstitial Cell Testicular Cell) Tumor		4	9	39	47	36		

* Each group consisted of 70 animals/sex

In summary lansoprazole up to 150 mg/kg/day dose, produced a dose dependent retardation in the body weight gain, retinal atrophy and carcinoid tumors in the stomach fundus in both males and females rats. It also produced testicular interstitial cell (Leydig's cell) benign tumors of transient nature as were reduced during the 6 months recovery period.

REPRODUCTIVE TOXICITY STUDIES:

Segment I. Study with AG-1749 in Rats (Study of Fertility and General Reproductive Performance in the Rat
(Report A-29-292/IND Amendment dated 4/25/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Initiated 6/20/86, completed 8/21/87

GLPs Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-004, in 5% gum arabic solution; tested periodically for homogeneity and stability.

Animals: Rats, CD (Sprague-Dawley), at the study initiation, 131 males rats were 45d old, weighing 185-210 g, and 131 females, 83d old weighing 223-259 g. 26 rats/sex/group.

Doses: 0 (control vehicle), 5, 15, and 50 mg/kg/day, po via oral gavage at a constant volume of 5 ml/kg. Doses were selected on the basis of 13-wk toxicity study in rats.

Methods: Groups of 26 males and 26 females were given AG-1749 at the dose indicated on days 63 and 14, respectively, prior to breeding and until sacrifice on day 13 of gestation or lactation day 22. The estrus cycle of the females were monitored 10 days prior to breeding and until evidence of copulation. Males continued receiving treatment until all females were bred. The dams were observed twice daily for morbidity and mortality. Body weights of the males were recorded weekly until sacrifice. In the case of dams to be sacrificed on day 13 of gestation, these were weighed on days 0, 6, and 13 of gestation; the remaining were weighed on gestation days 0, 6, 13, 18, 20 and on lactation days 0, 4, 7, 14, and 22. Food intake was measured on days 0, 6, 13, and 18 of gestation.

About 50% (12/26) of the dams in each group were sacrificed on day 13 of gestation and the number of corpora lutea, implantation sites, and live and dead embryos were recorded. The dams were also examined for gross morphological changes; their organs: the heart, lungs, liver, kidneys, spleen, adrenal glands, thymus, and stomach were weighed and preserved for future examination. At this time, the F₀ males were sacrificed and examined for any signs of toxicity affecting any of the major organs mentioned above. The remaining pregnant dams, 14/group were allowed to deliver with treatment continuing throughout gestation until weaning (postpartum day 22). The offspring (F₁) pups were examined, weighed, sexed on day 0, and 4 of birth.

On lactation day 4, litter size was reduced to 8 pups/sex/dam and were allowed to remain with the dam for 3 weeks. The remaining pups were sacrificed after external examination. The F₁ pups were weighed on lactation days 0, 4, 7, 14, and 22. The dead pups were examined for external, skeletal and visceral malformations by appropriate techniques.

Developmental Indices: The pups that were retained on lactation day 4 were examined for the following developmental indices: pinna detachment (lactation day 4-5), incisor eruption (day 12), eye opening (day 14-16), and negative geotaxis on days 5, 10, and 15. On lactation day 22, all dams were sacrificed and examined as above.

Observations/Results: There were no treatment-related deaths. The appearance was unremarkable throughout the study with a few exceptions, such as hair loss and missing or malaligned incisors.

The body weight gain by the treated female rats during gestation and lactation were comparable with controls, while those of the high dose males during the latter part of the study (weeks 10, 11, 12, and 14) were significantly reduced as compared with controls.

Food consumption was unaffected with treatment.

Gestation day 13 Uterine Examination Observations: Essentially, there were no significant effects on the number of corpora lutea, group mean pre- and post-implantation loss that could be attributed to the treatment.

The mean copulatory interval was slightly increased at 50 mg/kg/d (4.7 ± 3.64 versus 2.9 ± 1.41 in the control), but was within the normal variability. The other indices such as gestation length, fertility index for the males and females, the number of dead/live pups on lactation day 0, 4, 7, and 22 were all within the normal limits.

A total of 20 F₁ pups (9C, 3L, 2M, and 6H dose) were born dead or died during lactation. In addition, 2C, 3M, and 1H dose pups were missing during lactation and presumed cannibalized. According to the sponsor, these were not related to treatment.

The developmental indices such as pinna detachment, eye opening, incisor eruption, and negative geotaxis were all within the normal limits.

No treatment-related malformations were detected.

Lactation day 22 Observations: There were no abnormal observations other than increased absolute and or relative stomach weights at all doses in the males, and at the high dose in the females on lactation day 22. The thymus weights were also decreased in the male rats at mid and high doses, and also in the dams on lactation day 22, but only at the high dose. These observations are not different from those observed in the subchronic and chronic toxicity studies with rats and dogs.

In summary, AG-1749 was administered to male and female rats at dose levels of 5, 15, and 50 mg/kg/d prior to mating and throughout gestation. AG-1749 did not reduce fertility, general reproductive performance, the length of gestation, the number of live newborn pups, and the rate of conception. Thus, for the rat, 50 mg/kg/d was the no-toxic dose, and that was the MTD for the rat.

Segment I: Fertility and General Reproductive Performance
in Rats Following Oral Administration
(Report #RD90/249/Study TA-90-146/IND
Amendment dated 3/13/92/Pharmacology Review dated 11/3/92)

Conducting Lab:

Dates of Conduct: Initiated 10/19/90, completed 1/21/91

GLP Statement: In compliance with the FDA's GLPs.

MATERIALS AND METHODS

- Chemical:** Lansoprazole (AG-1749, Abbott-65006), lot #42-057-AL, suspended in 5% gum arabic solution and tested periodically for stability.
- Animals:** Charles River CrL:CD BR Rats; males 6 wk old at start; females sexually mature (200g). Males 17/group; Females 34/group.
- Doses:** 0 (control vehicle), 15, 50, and 150 mg/kg/day, po in constant volume of 10 ml/kg, once daily for 7 days a week. The corresponding concentrations were: 1.5, 5 and 15 mg/ml. The doses selected were in response to the Division's recommendations.

Methods: The design of the study was as follows: the treated female rats were mated with the treated males and the untreated females were mated with the treated males. However, the treated females should have been mated with the untreated males to determine the effects of the drug on female fertility, a significant deficiency in the design. On the other hand, according to sponsor, due to the decreased number of implantation sites found in the high-dose females during the C-section on gestational day 13, the untreated females were mated with the treated males to determine if these effects could be duplicated by treating only males. Groups of male rats were treated for 108 days prior to mating, during and until mating was completed. The female rats were treated for 14 days prior to mating, during mating, gestation and until C-section on day 13; and 50% were also treated throughout gestation and lactation until they were sacrificed. The estrous cycle of the female rats was monitored prior to mating.

The dams to be C-sectioned on day 13 (17/group) were weighed on gestational days 0, 6, 9, 12, while the littering-dams were weighed on these days in addition to days 0, 4, 12, and 21 post-partum. Food consumption was also monitored on various days during treatment and lactation. Maternal care was observed for all the dams. Males and dams were observed for signs of morbidity and for mortality. Dams were examined for symptoms of labor or delivery, the duration of gestation and for any other clinical signs. Following the C-section on day 13, ovaries were examined for the

number of corpora lutea, and each uterus was examined for the number and distribution of viable and non-viable embryos, early resorptions, the total number of implantations, and growth of the embryos.

Of the littering dams, the day of delivery was noted, and the following were recorded: the litter size, offspring sex, weight, the number of live- and stillbirths, and gross malformations. The offspring was weighed on days 4, 12, and 21 postpartum. Further, on day 4 postpartum, each litter was culled to obtain 4 males and 4 females (total of 8 pups/litter). These were examined on various postpartum days for a number of developmental parameters on the indicated lactational days of age: pinna detachment (d4), surface righting (d5), cliff avoidance (d7), auditory startle (d12), eyelid opening (d12), airdrop righting reflex (d16), passive avoidance (d22), and activity measurement (d35).

Data on fertility index, mating index, time to mating, fetal sex, gestation length, pup birth day findings, survival indices, litter size, and pup sex were all analyzed by appropriate statistical tests.

Results:

The drug concentrations in the formulations corresponded closely to the intended levels. For example, the drug concentrations for the _____ mg/ml samples (15, 50 and 150 mg/kg/d doses) were in the ranges of _____ and _____ mg/ml, determined at week 0 and week 11, respectively.

Observed Effects:

Mortality: Four male rats died or had to be sacrificed from moribundity. The cause of death was either accidental or dosing-related accidents.

Males: At 150 mg/kg/d, the growth of male rats was retarded by about 10% throughout the study but not at the lower doses as compared to controls.

Females: Among the dams, there were no treatment-related clinical signs. The body weight gain in the two lower dose groups (15 and 50 mg/kg/d) was comparable with controls. At 150 mg/kg/d, the growth was retarded by about 5g during treatment and also tended to be lower at the end as compared to controls. Essentially, there was no effect on growth during treatment.

Food intake by the treated dams in the 150 mg/kg group was lower by about 12% compared to controls.

The regularity of the estrous cycles, gestation length, mating performance, and time to mating were unaffected by drug treatment. There were no treatment effects on the mating/copulatory rates, or fertility rates.

Treated dams selected for C-section:

The number of corpora lutea in the treated groups were comparable with controls. However, the number of absolute implantations or implantations/dam were significantly ($p < 0.05$) reduced (38.5%) at 150 mg/kg/d. In fact, there was a significant dose-related decrease in the incidence of implantation sites relative to corpora lutea: 86%, 85%, 80%, and 55% at 5, 50, and 150 mg/kg/d, respectively, (reduced trend at $p < 0.05$). The number of viable fetuses was reduced significantly ($p < 0.05$) from 13.57/dam in the controls to 8.33/dam (38.6%) at 150 mg/kg/d.

**Segment I Fertility & General Reproductive Performance
in Rats Treated with Lansoprazole (AG-1749)
(DAMS SCHEDULED FOR C-SECTION)**

PARAMETER	DOSE, mg/kg/d			
	Control	5	50	150
# Females mated	33	34	34	33
# Pregnant dams	30	32	33	26
Pregnancy rate, (%)	91	94.1	97.1	78.8
# Examined at C-section	14	15	16	15
# Implants	205	219	205	135*
Implants/litter	14.65	14.60	12.81	9.0*
# Viable fetuses	190	199	189	125*
Viable fetuses/dam	13.57	13.27	11.81	8.33*
Post-implant loss/dam	0	0	0	0
Pre-implant loss/dam	1.07	1.33	1.00	0.67
Corpora lutea/dam	17.0	17.13	15.88	16.27
Sex ratio, m/f	99/117	120/122	115/119	32/62

* = significantly different at $p < 0.05$

Treated dams selected to litter:

The length of gestation among the treated and control dams was comparable. See table below. While the number of corpora lutea in the high dose group were

comparable with controls, the litter size and the number of livebirths/dam were both reduced (34.5%) at 150 mg/kg/d. These data are further summarized below.

Segment I study (contd.): DAMS SCHEDULED FOR LITTERING

PARAMETER	DOSE, mg/kg/d			
	Control	5	50	150
# Dams littered	16	17	17	11
Length of gestation, d	21.79	22.00	22.12	23.16
Litter size	13.38	14.24	13.94	8.91*
Livebirths/dam	13.19	13.94	13.65	7.45*
Stillbirths/dam	0.19	0.29	0.29	1.45
Total pups born	214	242	234	94
total live newborns	214	237	232	82
mean live newborns/dam	13.19	13.94	13.65	7.45*
mean dead newborns/dam	16	17	17	11
Pup survival*	99	99	99	96

* = significantly different at $p < 0.05$; Pup survival index = no. live pups at day Y / total no. newborn pups on day 0 x 100. Pup survival on days 1, 4, 12, and 21 was in the range of 93.3 to 100% for all doses.

E, Offspring: Fetal weight, sex distribution, gross examinations were all within the normal limits. However, the female pup weight on days 12 and 21 of lactation was significantly reduced (17 and 20.5%, respectively). The male offspring growth remained normal.

Postnatal growth, behavioral development, survival, sex distribution and general appearance of the offspring were essentially normal.

Untreated females mated with treated males:

Among these females, there were no significant effects on food intake and gestational body weight gain. The pregnancy rates among the control, low, middle and high dosed dams were 88.2, 93.8, 100.0 and 76.5%, respectively, reduced at high dose. There was a decreasing trend in the number of early resorptions with increasing dose ($p < 0.05$), significance of which is unknown.

In summary, the female rats were treated with Lansoprazole (AG-1749) at doses of 5, 50, and 150 mg/kg/d for 14 days prior to mating, during gestation, lactation, and were mated with male rats (also treated at these doses for 108 days prior to mating and during mating). Among the treated females, the 150 mg/kg/d dose reduced the litter size (33%), decreased the number of livebirths (43%), decreased the number of implantations (34%), and decreased the number of viable fetuses/dam (38.6%). The two lower doses of 5 or 50 mg/kg/d did not produce similar effects. Among the untreated females mated with treated males, although the pregnancy rate was slightly reduced at 150 mg/kg/d, there was no other significant reproductive toxicity. On the other hand, Lansoprazole produced reproductive toxicity in the rat at 150 mg/kg/d.

I.V. Segment I. Fertility and General Reproductive
Performance Study in Male Rats
(Report A-29-1773/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Research Laboratories
Takeda Chemical Industries, Ltd.
Yamaguchi, Japan

Dates Study Started and Completed: May 16, 1991 and December
1992

GLP Requirements: Not mentioned

Animals: Jcl: Wistar rats (males: 7 week old, 190-234g; females
11 week old, 183-222g)

Drug Batch No.: Z 338201 & Z 338202 (each vial contained 30 mg
of AG-1749, 3.7 mg of NaOH, 10 mg of malumine and 60 mg of
mannitol)

Vehicle: PEG formulation (adjusted to pH 10-11 with 0.1 NaOH)

Methods: In this study male rats (10/group) were given I.V.
daily doses of 0 (saline), 0 (vehicle: "PEG formulation"), 3, 10
and 30 mg/kg/day of Lansoprazole. Male rats were treated for 9
weeks prior to mating and throughout the mating phase and until
they were sacrificed (total 13 weeks of treatment). The volume
of administration was 10 ml/kg and rate of administration was
2 ml/min. The dose selection was based on 4-week I.V. toxicity
study (see above). Treated rats were mated with untreated
females (9-10/group). All animals were observed at least once a
day. Body weight of male rats were recorded weekly and pregnant
females were weighed on days 0, 6, 13, 18 and 20 of gestation.
Food consumption in males were recorded weekly. The mating
performance and fertility of both sexes were evaluated. Males
were necropsied after 13 weeks of treatment. Testis,
epididymides, seminal vesicles and ventral prostate were weighed.
On day 20 of gestation all dams were sacrificed and examined for
number of corpora lutea, the number of implants, the number of
dead or resorbed fetuses and number of live fetuses. The live
fetuses were weighed and sexed. Live fetuses were examined for
external abnormalities.

Results: Two high dose treated males died on day 38 and 71 of
the study just after drug administration. The cause of death was
considered not to be treatment related. Decreased locomotor
activity was seen in 1/10 high dose treated rats. Body weight
gains were reduced by 14% in high dose treated male rats compared
to vehicle control values. Lansoprazole up to 30 mg/kg/day had
no abnormal effects on fertility and mating performance of
treated males. The number of corpora lutea, pre- and post-
implantation losses, number of live fetuses, weights of fetuses
and sex ratios were comparable in all groups. In this study dose
selection was adequate and 30 mg/kg/day is the maximum tolerated
dose in this species.

Segment II. Teratology Study of AG-1749 in the Rat
(Report A-29-144/IND Amendment Dated 8/12/90/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka, Japan.
Dates of Conduct: Initiated 2/7/85, completed 7/25/85.
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-001); suspended in 5% w/v gum arabic solution; periodically tested for homogeneity of the suspension and stability of the test agent.
Animals: Rats, Wistar (Jcl:Wistar); estrus virgin female rats (12-14 wk old) were mated with 14-16 wk-old males; 22-23 pregnant rats/treatment group.
Doses: 0 (control vehicle), 15, 50, and 150 mg/kg/d given, po, gavage, at concentrations of 0.3, 1 and 3% w/v, in constant dose volume of 5 ml/kg, once daily, on days 6 through 17 of gestation. The doses were selected on the basis of a 2-wk oral toxicity study in the rat that was not provided.

Methods: The pregnant rats were administered the test agent or the control vehicle po via gavage as mentioned above and were observed for signs of toxicity twice, pre-and post-dosing throughout treatment. Body weight and food consumption were recorded at intervals of 3-4 days. The dams were sacrificed on day 20 of gestation, and their ovaries, uteri, and main organs were examined. The number of corpora lutea, implants, dead embryo/fetuses (resorption, placental remnants, and macerated fetus), and live fetuses and their sex ratio were determined. The live fetuses were examined for fetal malformations and fetal weights were recorded. Approximately 2/3 of fetuses were eviscerated and fixed in 95% alcohol, cleared and stained with Alizarin red S and examined for skeletal abnormalities (major abnormality) and variations (minor abnormality); the number of sacro-lumbar vertebrae ossified was counted as a degree of ossification. The remaining 1/3 fetuses were fixed in Bouin's fluid to detect visceral abnormalities and variations according to Wilson's and Barrows' techniques. Data on the conceptuses were analyzed on the basis of litter, and not on the individuals.

Results: There were no deaths, no abnormal clinical signs, and no drug-related effects on body weight gains and food consumption by the dams during the study.

The number of corpora lutea and implants in the treatment groups were comparable with controls. The fetal or embryonic survival, the number of live fetuses, and sex ratio were also comparable with the controls. For example the total number of live fetuses (shown in Appendix 3-2, page 411) were 303, 310, 292, and 288 with the corresponding mean litter size of 13.2, 13.5, 12.7, and 13.1 in the control, low, mid, and high dose groups, respectively. However, the number of fetuses examined for skeletal and visceral abnormalities, shown in tables 5 and 6, page 400 and 401, v2, are shown as: 303, 310, 302, and 298 with the corresponding mean litter size of 13.1, 13.4, 13.1, and 13.5, respectively, with an apparent discrepancy.

The fetal weights of the male and females in the 150 mg/kg/d group were significantly decreased by 8 and 10%, respectively, as compared to the controls.

There were no major skeletal or visceral abnormalities in any treatment group. However, the mean frequency (%) of minor skeletal variations was 1.2, 2.1, 4.2, and 6.7 in the control, low, mid and high dose groups, respectively. The type and frequencies (%) of dumb-bell-shaped thoracic vertebral bodies, dumb-bell-shaped lumbar vertebral bodies in the high dose group were 3.4 and 2.3, as compared to the 0 and 0.5 in the control group, respectively. The number of sacro-caudal vertebrae ossified, as a degree of ossification, decreased from 6.2 ± 0.6 in the control to 5.0 ± 0.7 in the 150 mg/kg/d group.

In summary, when pregnant rats were treated with AG-1749 at 15, 50, and 150 mg/kg/d given on days 6 through 17 of gestation, there was a slight decrease in food consumption by the dams, and a slight decrease in the fetal body weights, but there were no significant embryo-lethal or teratogenic effects at doses as high as 150 mg/kg/d.

Segment II. Teratology Study in Rats with AG-1749
(Report A-29-366/IND Amendment dated 8/12/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Initiated 4/28/86, completed 5/18/88.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-004, suspended in 5% arabic gum solution.

Animals: Pregnant rats, Sprague-Dawley, 40/treatment group.

Doses: 0 (control vehicle), 30, 100, 300 mg/kg/day, given po (gavage) once daily on days 6 through 17 of gestation. The doses were selected from a dose-range finding study with pregnant rats where the highest dose of 300 mg/kg/d produced fetotoxicity (increased post-implantation loss, and significantly reduced fetal body weights). In addition, maternal toxicity (reduced maternal body weight gain, and food intake, and significantly increased liver, kidney and thymus weights) were observed at the high dose.

Methods: The test agent or control vehicle was administered once daily on days 6 through 17 of gestation, and the rats were observed daily for signs of toxicity. Body weights were recorded on gestation days 0, 6, 8, 10, 13, 15, 18 and 20; and of those females allowed to deliver were also recorded on lactation days 0, 4, 7, 14, and 22. Food consumption was recorded on similar days. Twenty of the 40 rats/group were subjected to the C-section on day 20, and the remaining 20 dams were allowed to deliver normally.

Of the 20 dams (F_0) that were subjected to C-section, the F_1 fetuses were removed for recording viable and non-viable fetuses, early and late resorptions, the number of total implantations, and corpora lutea, and placental weights, and teratological effects. Maternal liver, kidneys, spleen, adrenal gland, thymus and stomach from each dam thus sacrificed were weighed and examined. Fetuses were weighed, sexed, and examined for external malformations. Further, 1/3 of fetuses were examined for visceral malformations/variations (Bouin's technique) and 2/3 for skeletal malformations/variations by Dowson's technique.

The remaining 20 F_0 dams were allowed to deliver, and F_1 pups were selected from each litter and evaluated for behavioral and developmental indices. The gestation length, the number of live and dead births were recorded for each dam; and each pup (F_1) were examined for any malformations. Eight (8) pups from each F_1 litter were examined for developmental and behavioral functions. The pups were observed for 3 weeks post-delivery. On lactation day 22, 2 pups/sex were selected from each litter and retained. One male and one female pup were considered as 'behavioral pups', and the others ('reproductive pups') were mated (when 84 days old) to assess their reproductive capabilities. All F_1 pregnant females were allowed to deliver normally. On lactation day 7, the F_1 females and their F_2 offspring were sacrificed and necropsied. One-third of the F_2 pups from each litter were preserved for skeletal examinations. The fate of the remaining 2/3 pups is unclear.

Of the F_1 'behavioral pups', the following were recorded: pinna detachment (lactation day 4); negative geotaxis (day 5, 10, 15); incisor eruption (day 11-13); eye opening (day 14); testicular descent (day 22-25); vaginal opening (day 34); activity and emotionality (day 35), and various other neurobehavioral developmental parameters.

Of the 'reproductive pups', the litter size, stillborns, liver births, etc. were recorded, and so were the implantation sites in utero, and gross, skeletal, and visceral malformations/variations.

Results:

F_0 Maternal observations: One dam died during the study from dosing error. Otherwise, there were no abnormal behavioral findings.

F_0 maternal body weight gains (calculated) were reduced significantly ($p < 0.05-0.01$) on gestation days 8, 10, and 18: e.g., on gestation day 10, there was a 11 and 20% retardation in body weight gain in the mid and high dose dams, respectively. The retarded body weight gain paralleled the reduction in food intake by these dams. During lactation, the maternal body weight gains by the treated dams were comparable with controls.

Further, the dosing of F_0 dams with AG-1749 reduced maternal weight gain during gestation days 6-17. The maternal weight gain on day 10 of gestation was 44, 41, 39, and 35 g among the control, 30, 100, and 300 mg/kg/d dams, respectively. The relative liver weights increased (4.84) in the high dose dams as compared to controls (4.47); and thymus weights decreased (0.15) in the high dose dams as compared to controls (0.25).

The food intake of the F₀ dams also decreased in the treated dams: e.g. on day 8 of gestation, the control, low, mid, and high dose dams consumed 76.8, 72.8, 68.8, and 61.8 g/kg/d food, respectively.

Cesarean Section Observations: At 300 mg/kg/d dose and not at lower doses, fetal weights were reduced by about 11% suggesting mild fetotoxic effect of the drug. The remaining parameters: viable fetuses, post-implantation loss, total implantations, corpora lutea, fetal sex ratios were all within normal limits.

Organ weights: The relative thymus weights of the F₀ dams decreased by about 40%, while the liver weights increased by 8% at 300 mg/kg/d. No such changes occurred at the mid and low doses.

F₁ Fetal observations: There were no malformations seen that could be attributed to the drug.

With regard to developmental variations, a number of such variations were seen at high dose (300 mg/kg/d) and these were: unossification of the skull, hyoid, vertebrae and sternebrae, and high incidence of litters with 7th cervical rib(s) as compared to the controls (see Table 3). The incidence of 7th cervical rib was also increased in fetuses and weanlings at 300 mg/kg/d. There were no other significant teratological effects seen at any dose in the F₁ generation pups.

F₀ Parturition and F₁ Litter Observations did not reveal any significant drug effects on the pup viability at birth or during lactation, the number of live and dead pups, pup survival and pup weights. A total of 18 pups (5 of 164, 2 of 241, 7 of 196, and 4 of 241 in the control, low, mid and high dose groups, respectively) were found dead during lactation, from an undetermined cause except one high dose pup had septal defect, and another complete sinus inversion; and one high dose pup had a 7th cervical rib. In addition, 6 pups (2 in the middle and 4 in the high dose group) were missing or presumed completely cannibalized. AG-1749 did not effect the reproductive performance of F₁ offspring to yield F₂ pups.

Of the two high dose F₁ pups with visceral variations, one had absence of innominate vessel and the other hydronephrosis. Among the F₁ pups, the incidence of 7th cervical rib(s) was: 3 of 193 controls, 1 of 184 low dose, and 15 of 194 (7.7%) high dose pups. A few malformations but scattered across all treatment groups were seen. However, there was no increase in any one specific type at any one dose. For example, one of pups had skeletal malformations: one had a vertebral anomaly and the other sternoschisis. These effects were not seen at the lower 30 and 100 mg/kg/d doses.

In summary, AG-1749 administration to pregnant dams on days 6 through 17 of gestation produced developmental toxicity (decreased fetal weights and increased ossification alterations) at 300 mg/kg/d, and retarded maternal gestational weight gain by 11 and 20% at 100 and 300 mg/kg/d doses, respectively. There were no effects on parturition, lactation of the pregnant rats, and neither was there any effect on the F₁ and F₂ neonatal behavior, viability, and growth or reproductive capabilities of the F₁ generation at any dose. Further, there were no teratogenic

effects in the rat treated with AG-1749. The lowest dose 30 mg/kg/d was without any adverse effects on the development of the rat, while the higher dose of 300 mg/kg/d was maternotoxic.

Table 3
Segment II Teratology Study in Rats with AG-1749

Parameter Measured	Control	30	AG-1749, mg/kg/d 100	300
<u>F₀-Pregnancy Performance</u>				
# Females mated	40	40	40	40
Pregnancy rate, %	100	100	100	100
# Dams selected for C-section	20	20	20	20
# Viable fetuses/dam	14.6	14.2	14.2	14.6
Post-implantation loss/dam	0.8	1.5	1.0	0.9
Total implantations/dam	15.4	15.7	15.2	15.5
Corpora lutea/dam	16.5	17.7	16.8	18.0
Mean preimplantation loss	7%	11.3%	19.6%	14.2%
Mean post-implantation loss	4.9%	9.6%	6.6%	5.8%
Mean fetal body weight (g)	3.5	3.5	3.4	3.1**
Fetal sex distribution	145M, 147F	134M, 149F	140M, 143F	137M, 155F
<u>Observations (fetal malformations/variations) in F₁ Fetuses</u>				
No. litters examined	20	20	20	20
# Fetuses examined externally	292	283	282	291
# Fetuses examined visceraally	99	99	98	98
# Fetuses examined skeletally	193	184	185	194
<u>Malformations</u>				
Total # fetuses (litters) with malformations	1(1)	3(1)	4(4)	3(3)
<u>Developmental Variations</u>				
Total # fetuses (litters) with variations	70(17)	86(20)	59(18)	87(20)
7th cervical rib(s)	3(3)	1(1)	-	15(8)
<u>Observations (Skeletal) in F₁ Pups (Lactation day 22)</u>				
# Litters examined	12	18	14	18
# Fetuses examined skeletally	46	71	56	68
<u>Malformations:</u>				
Vertebral anomaly	0	0	0	1(1)
Sternoschisis	0	0	0	1(1)
Total fetuses with malformations	0	0	0	2(2)
<u>Developmental variations:</u>				
7th cervical rib(s)	4(3)	5(5)	6(5)	39(16)
...				
...				
Total fetuses(litters) with variations	11(5)	15(12)	15(12)	45(16)

Segment II. I.V. Teratology Study in Rats
(Report A-29-1783/IND Initial Submission/
Pharmacology Review dated 9/6/93)

Testing Laboratories: Drug Safety Research Laboratories
Takeda Chemical Industries, Ltd.
Osaka, Japan

Dates Study Started and Completed: Not given

Report Date: December 25, 1992

GLP Requirements: Not mentioned

Test Species: Pregnant Jcl: Wistar rats

No. of Animals: 13-14 pregnant rats/group

Drug Batch No.: 2 338201 (each vial contained 30 mg of AG-1749,
3.7 mg of NaOH, 10 mg of malumine and 60 mg of mannitol)

Vehicle: PEG formulation (adjusted to pH 10-11 with 0.1 NaOH)

Methods: Pregnant rats were given I.V. doses of 0 (saline), 0 (vehicle: "PEG Formulation"), 10 and 30 mg/kg of Lansoprazole from day 6 to 17 of gestation. The volume of administration was 10 ml/kg and rate of administration was 1.5-2 ml/min. All dams were observed daily. Body weight and food consumptions were recorded on days 0, 6, 8, 10, 13, 15, 18 and 20 of gestation. On day 20 of gestation all dams were sacrificed and examined for number of corpora lutea, the number of implants, the number of dead or resorbed fetuses and number of live fetuses. Live fetuses were weighed, sexed and examined for external abnormalities. Approximately one-third of the fetuses were examined for visceral abnormalities and the remaining two-third fetuses were examined for skeletal malformations and variations.

Results: A total of 3 dams (1 from vehicle control group and 2 from high dose group) died during the study period due to respiratory failure. These deaths were not considered to be treatment related. No treatment related findings were evident in dams except thymus weights were decreased by 40% in high dose treated dams compared to vehicle control values. There were no significant changes in pregnancy parameters (mean number of corpora lutea, number of implants, pre- and post-implantation loss, number of live/dead fetuses and weight of the fetuses). No treatment related abnormalities were observed on external, skeletal and visceral examinations in any group. Thus the highest test dose (30 mg/kg/day) was maternal toxic. However, no embryotoxic and teratogenic effects at dosage up to 30 mg/kg/day was observed.

Segment II. Teratology Study in the Rabbit
(Report A-29-354/IND Amendment dated 4/25/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Initiated 6/6/86; completed 3/30/87

GLPs Statement: In compliance with the Japanese and FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749; lot #MC10-004, suspended in 5% gum arabic solution; periodically tested for homogeneity and stability.

Animals: Pregnant New Zealand rabbits, 2.8-3.9 kg, 16/dose group.

Doses: 0 (control vehicle), 3, 10, 30 mg/kg/day, given po on days 6 through 18 of gestation in a constant dose volume of 5 ml/kg. The basis of dose selection was a dose-range finding study in rabbits that showed body weight loss and decreased food consumption at 30 mg/kg/d.

Methods: The does were given the test agent on days 6 through 18 of gestation and were observed for signs of toxicity once to twice daily throughout pregnancy. The does were weighed on days 0, 6, 9, 13, 16, 19, 23, and 29 of gestation; food intake was recorded on days 1, 6 through 18, 23, and 28 of gestation. The does were sacrificed on day 29 of gestation, and uteri of each dam were examined for implantation sites, excised and weighed. All viscera of the does were also examined. The following parameters were recorded: the number of corpora lutea, implants, viable and non-viable fetuses, early and late resorptions, and macerated fetuses. Fetuses were individually weighed, sexed, and examined externally; placentas were weighed and examined; the sex ratios were calculated. It is not clear as to how many fetuses were examined for visceral and how many for skeletal anomalies (by Bouin and Alizarin's techniques). Fetal examinations consisted of fetal/placental weights and sex ratio, abnormal litter ratio, malformation rate, and ossification variance. Whether the data on placental weight, post-implantation loss, and sex ratios were analyzed on the fetus or litter is not clear.

Results: The number of pregnant rabbits in each group were: 16, 15, 14, and 15 in the control, low, mid and high (C, L, M, H) groups, respectively. One doe died on or before day 13 of gestation from an unknown cause. Diarrhea and soft feces were noted in many mid and high dose does. Generally, the body weight gains and food intake of high dose pregnant rabbits decreased during gestation, but later recovered.

Findings at autopsy were not significant that could be related to drug treatment.

Fetal observations: The number of corpora lutea, implants, pre- and post-implantation loss, mean fetal body weights, and placental weights in the treated rabbits were comparable with those of those of the controls. The number of live fetuses were 132, 102, 102, and 92 in the C, L, M, and H groups. The other parameters are summarized in Table below.

The incidence of malformations and skeletal variations was also not significantly higher in the treated groups when compared to controls. The type and number of malformations were also not significant in the treated groups than controls.

In summary, the treatment of rabbits with AG-1749 at doses of 3, 10, and 30 mg/kg/d produced no significant maternal toxicity. There were no effects on the number of corpora lutea, implants, pre- and post-implantation loss, fetal and placental weights, and various other fetal parameters. However, the fetal mortality was high at 30 mg/kg/d as compared to controls. Therefore, the lower dose of 10 mg/kg/d was the no-toxic effect dose. AG-1749 did not produce any teratogenic effects in the rabbit at any dose, up to 30 mg/kg/d.

Table 2
Segment II Teratology of AG-1749 in the Rabbits

Parameter Measured	Control	3	Treatment, mg/kg/d	
			10	30
# pregnant does	16	15	14	14
# corpora lutea	151	146	134	127
mean # corpora lutea/doe	9.4	9.7	9.6	9.1
mean preimplantation loss	4.7	13.1	17.5	5.0
mean implantations	8.9	8.5	7.9	8.6
mean resorptions/doe	0.6	0.0	0.0	2.5
dead fetuses	0	0	1	2
# live fetuses	132	102	102	92
mean live fetuses/doe	8.3	6.8	7.3	6.6
mean live fetal weight, g				
Male	36.4	39.4	38.8	37.4
Female	36.1	37.6	36.9	36.1
sex ratio, F/M	62/70	34/68	57/45	46/46
<u>Malformations/Variations</u>				
# Fetuses Examined	132	102	102	92
# of External Abnormalities	0/132	0/102	1/102	1/92
exencephaly	0/132	0/102	1/102	0/92
exophthalmia	0/132	0/102	1/102	0/92
flexion contracture of wrist joint	0/132	0/102	0/102	1/92
club foot	0/132	0/102	0/102	1/92
brachyury	0/132	0/132	0/102	1/92
# of Visceral Abnormalities	76/132	65/102	65/101	67/91
# Skeletal Abnormalities	5/132	5/102	1/101	6/91

(Study 295-099/IND Amendment dated 11/7/88/
Pharmacology Review dated 8/12/90)

Conducting Lab:

Dates of Conduct: Initiated 5/5/86, completed 5/9/88.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-004, suspended in 5% arabic gum solution and tested periodically for homogeneity and stability.

Animals: Pregnant rats, Sprague-Dawley, 214-307 g on day 0 of gestation, 23-30/treatment group.

Doses: 0 (control vehicle), 15, 50, 150 mg//kg/day, given po (gavage) once daily on gestation days 15 through lactation day 21 in a constant volume of 5 ml/kg. The doses were selected from a 5-week toxicity study. No other details were provided for the basis of dose selection.

Methods: The F₀ dams received the control vehicle or the test agent once daily on days 15 of gestation through weaning (day 21 post-partum), instead of day 17 of gestation through day 21 post-partum. The dams were observed daily for mortality and morbidity. The maternal body weights and their food consumption were recorded on gestation days 0, 6, 13, 15, 17, 18, and 20, and lactation days 0, 4, 7, 14, and 21. The gestation length was recorded. After delivery, all pups were examined for external malformations, and the number of live-and stillbirths were recorded for each dam. The pups were weighed, sized (length), and sex-ratio determined. From each litter, 8M/8F pups were evaluated for the following developmental indices: pinna unfolding on day 4 of birth; tooth eruption, day 11; eye opening, day 14; testes descent, day 22; and vaginal opening, day 34; visual (day 22), auditory (day 33) functions; activity, emotionality and other behavioral functions on appropriate days. The remaining pups were necropsied and examined for skeletal and visceral malformations. At weaning (lactation day 21), the dams (F₀) were killed and necropsied; viscera and traces of implantation sites were examined, and their thymus, liver, stomach and adrenals were weighed.

At weaning, 2M, and 2F F₁ pups were selected from each litter/group for evaluating the following: 1M, and 1F pups were used for evaluating developmental and behavioral effects, and the remaining 1M and 1F pups for evaluating the reproductive performance when they were a minimum of 84 days old. However, instead of sacrificing F₁ pregnant dams on day 13 of gestation, they were allowed to deliver normally, gestation length was recorded, and F₂ pups were examined. Following birth of F₂ pups, the gestation length was recorded, the litters were examined for size, still and livebirths, and any gross anomalies. The F₁ dams and F₂ pups were observed for up to 7 days post-delivery, were necropsied and examined for any skeletal anomalies as explained above. The number of corpora lutea, implantation sites, and the number of live/dead fetuses were recorded. In addition, copulation, fertility, gestation, birth, and vitality indices were recorded. The thymus, liver, stomach, and adrenals of the F₁ dams were weighed.

Results:

F₀ Maternal Observations: Only one of 27 dams in the high dose group died on day 19 of lactation. There were no other deaths among the F₀ dams. There were no other abnormal clinical signs in any treated dam during the study.

Throughout gestation and lactation, the body weight gains of the dams remained within normal limits with a few sporadic changes, while the food consumption remained within normal limits during gestation, but during lactation, it decreased among the high and mid dose dams. The lowest dose had no effects on food consumption.

The length of gestation, the number of corpora lutea and the number of live/dead fetuses etc remained comparable across all dose groups. However, the pup survival on lactation days 4, 7 and 22 was lower in the high dose group (90, 92, and 95%, respectively) than in the control (100%). Further, the offspring (pups) of the treated dams, in general, were smaller by about 8-16% than those of the control dams.

The number of total newborn F₁ pups were 303, 411, 391, and 350 in the control (C), low dose (LD), mid dose (MD) and high dose (HD) groups, respectively. Among these, there were a total of 130 deaths: 27 (12C, 2LD, 1MD and 12HD) were born dead; 28 (3C, 2LD, 8MD and 15HD) were missing or were completely cannibalized; and 75 (14LD, 28MD and 33HD) died during lactation. No exact cause of death or any abnormal finding was apparent in these dead or missing pups, except one high dose pup had patent ductus arteriosus.

Lactation day 22 Uterine Observations: The mean number of total implantations per dam were comparable across treatments.

Organ weights: The thymus weights decreased significantly at 50 and 150 mg/kg/d doses, and not at 15 mg/kg/d.

F₁ Pup Morphological Observations: There were no malformations, and variations seen in any treated pup that could be considered treatment-related.

F₁ Developmental and Behavioral Observations: The parameters such as pinna detachment, incisor eruption, eye opening, negative geotaxis, hind-limb support, testicular descent, vaginal opening, pupil and corneal reflexes, pain response, auditory function, rotarod performance and shuttle avoidance were all within the normal limits.

F₁ General Observations:

The general appearance and behavior of F₁ rats was normal. Body weights of male and female rats, during the week 7-9 of the study, were slightly but significantly ($p < 0.05-0.01$) reduced in the 50 and 150 mg/kg/d groups by 8% and 9.5%, respectively. In addition, the body weights of the mid and high dose F₁ behavioral male and female rat offspring (those that were used for behavioral observations) were significantly reduced during the 17 weeks of post-delivery period. No such changes occurred at 15 mg/kg/d. The F₀ maternal body weights remained unchanged.

F₁ delivery data showed that male and female fertility were reduced at all doses, but not in a dose-related manner: the fertility index (gravid rats [females or males]/total rats [females or males]) for control, low, mid and high dose females was 90.5, 78.6, 85.2, and 71.4; and 90.5, 70.4, 81.5, and 81.8 for males, respectively.

F₂ Litter Observations did not show any significant treatment- or dose-related effects on the number of implantation sites, pups delivered or post-implantation losses. However, a total of 47 pups (5C, 18LD, 12MD, and 12HD) were either born dead or found dead on lactation day 0, died during lactation, or were missing due to possible cannibalization. There were 2 cases of hydronephrosis (1C, 1MD), distended ureters (1MD, 1HD) and one case (1LD) of hemorrhagic testes.

In summary, the treatment of F₀ pregnant dams with AG-1749 reduced the survival of F₁ offspring at 150 mg/kg/d, specially during the first 1-7 days of lactation. No such effects were seen at 50 or 15 mg/kg/d doses. The growth of the offspring was retarded dose-dependently during 14 and 22 days of lactation. No other effects on the physical or behavioral development of the F₁ pups was seen. The male and female pup (F₁ offspring) body weights were reduced at both 50 and 150 mg/kg/d doses. Further, the fertility of all the treated F₂ male and female offspring was reduced as compared to controls but not in a dose-related manner.

MUTAGENICITY:

Bacterial Mutagenicity Study on AG-1749 (Ames Test).
(Report A-29-181/IND Initial Submission/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical-
Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku,
Osaka, Japan.
Dates of Conduct: Initiated 2/18/85, completed 3/27/85.
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-001); periodically tested for stability.
Positive Controls: Acrylamide, Na-p-dimethylaminobenzenediazosulfonate,
2-aminoanthracene, 9-aminoacridine-HCl, diethylnitrosamine,
3,4-benzpyrene, N-ethyl-N'-nitro-N-nitrosoguanidine,
2-Nitrofluorene, Na-azide.
Negative Controls: Solvents such as DMSO
Concentrations: 0.03, 0.1, 0.3, 1, 3, 10, and 30 mg/ml in DMSO.
Tester Strains: Escherichia coli WP2hcr and Ames' Salmonella typhimurium
TA98, TA100, TA1535, and TA1538. For metabolic activation
in vitro, the S9 fraction was prepared from the
Sprague-Dawley rat livers.

Methods: The test compound was tested for mutagenic potential according to the method of Ames. The maximum concentration of AG-1749 tested over a range of 3-3,000 ug/plate since the highest concentration of 5,000 ug/plate induced growth inhibition while 500 ug/plate did not. The methodology was adequately described and the criteria for assessing the positive effect were that the positive control increased the number of revertant colonies at least more than 2x the negative control.

Results: AG-1749 was not mutagenic with or without metabolic activation. In comparison the positive controls were mutagenic since these increased the number of revertant colonies more than twice the controls, the criterion for assessing the mutagenic potential of an agent.

Micronucleus Test on AG-1749 in Mice
(Report A-29-251/IND Amendment dated 3/27/89/
Pharmacology Review dated 8/12/90)

Conducting Lab: Drug Safety Evaluation Laboratories, Takeda Chemical Industries, Ltd., 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka, Japan.
Dates of Conduct: Initiated 3/2/87; Completed 5/12/87
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-004); in 5% arabic gum solution; tested periodically for stability.
Positive Control: Mitomycin (lot # 294AFC) at 2 mg/5 ml/kg, ip.
Negative Control: 5% arabic gum solution.
Animals: Specific pathogenic free male mice (C3HxSWV)F₁, 7 wk old, 26-36 g; 5/dose group.
Doses: 0 (control vehicle), 1,250, 2,500, and 5,000 mg/20 ml/kg given po by gavage, or 0 (control) and 1,250 mg/20 ml/kg daily doses for 4 consecutive days.

Methods: The animals that received a single dose were sacrificed 30 hr after treatment and those that received successive dosages were sacrificed 24 hr after the last dose. The femur was removed from each animal and bone marrow cells obtained and appropriate slides were prepared for counting polychromatic erythrocytes/mouse for the presence of micronuclei. The frequency of reticulocytes, and frequency of micronucleated polychromatic erythrocytes (MNPCE) were determined in the negative control (gum arabic), positive control (mitomycin) and AG-1749 treated mice. A significant increase in the number of MNPCE is considered a positive response.

Results: The MNPCE were 0.18, 0.23 and 6.10 in the highest dose group (5,000 mg/kg AG-1749), the negative and positive control groups, respectively. Similarly, the administration of 4 successive doses of AG-1749 also did not increase the MNPCE in the mice. The % reticulocytes were also not affected by the drug treatment. These data suggest that AG-1749 is not a mutagen in this assay system, i.e. did not cause micronucleus induction and thus is not a clastogen or a spindle poison.

In summary, AG-1749 was not a clastogen in the mouse micronucleus test. However, test was deficient in that the highest dose tested (5,000 mg/kg) was not high enough to produce any one of the following (i) marked and significant increase in the micronucleus frequency in the target cell population, (ii) significant suppression of the frequency of RNA-positive erythrocytes, or (iii) compound-related signs of toxicity or significantly reduced survival. Further, the test agent was tested only in the males and not both sexes. In addition, for the multiple dosing phase, the lowest dose, 1,250 mg/kg was tested and not the highest tolerable dose, 5,000 mg/kg.

Mouse Lymphoma Assay
(Report A-29-710/IND Amendment dated 7/13/90/
Pharmacology Review dated 1/2/91)

The assay was conducted to determine the ability of AG-1749 to induce mutations to 6-thioguanine resistance in the mouse using specific mouse lymphoma cell line L5178Y.

Conducting Lab:

Dates of Conduct: Initiated 3/4/89, completed 6/20/89.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-016); 99.6% pure.

Positive Controls: 4-Nitroquinoline-1-oxide (NQO) at 0.1 and 0.15 ug/ml;
benzo(a)pyrene (BP) at 2 and 3.0 ug/ml-concentration.

Negative Controls: Solvent- DMSO

Concentrations: Experiment 1: 0, 0.5534, 1.75, 5.534, 17.5, 55.34, 175, 553.4, and 1,750 ug/ml;

Experiment 2: 0, 12.5, 25, 50, 100, 200, and 400 ug/ml;
both experiments were run in the presence and absence of
S-9 microsomal fraction prepared from the Wistar rat livers.

Tester Cells:

Mouse lymphoma cell line of L5178Y.

Methods: AG-1749 was tested for mutagenic potential according to the method of Cole and Arlett (Mutation Res. 34: 507-526, 1976). The assay acceptance criteria were: (i) the mutation frequencies in the negative control (DMSO) cultures fell within the normal range of 0.2-20 mutants/10⁶ viable cells, and (ii) at least one concentration of each of the positive control (NQO and BP) induced a clear increase in mutation frequency. The agent was considered as mutagenic if statistically significant (or at least two-fold increase) induced mutation occurred at consecutive doses in at least one experiment and dose-related increase in mutation could be confirmed by regression analysis in both experiments. It was stated in Methods that the cultures were maintained in flasks for a total of 7 days at 37°C for the expression of HGPRT mutations. But according to a more recent method (Guideline for Performing the Mouse Lymphoma Assay for Mammalian Cell Mutagenicity, Mut Res. 189:143-156, 1987), cultures are to be maintained for not more than 2 days because longer periods may result in a negative response even with positive controls. However, the positive controls did show positive mutagenic response. Other assay conditions were described in sufficient detail.

Results: AG-1749 was not mutagenic with or without metabolic activation. In comparison the positive controls (NQO and BP) were mutagenic since the mutation frequency with NQO in the absence of S-9 was 3-6x the negative controls. For BP, in the presence of S-9 fraction, the frequency rate was about 10x the negative controls. In experiment 2, where a narrow range of AG-1749 was tested, the frequency of mutations was 2-3x the negative controls in the absence of S-9 activation, and 5-7x the negative controls.

In summary, AG-1749 was non-mutagenic in the mouse lymphoma assay.

In-Vivo/In-Vitro Rat Primary Hepatocyte Unscheduled
DNA Synthesis Assay
(Report A-29-718/IND Amendment dated 7/13/90/
Pharmacology Review dated 1/2/91)

The assay was conducted to determine the DNA damage caused by AG-1749 or its metabolite by measuring unscheduled DNA synthesis induced in the rat hepatocytes in-vivo and in-vitro.

Conducting Lab:

Dates of Conduct: Initiated 9/20/89, completed 2/26/90.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-018; suspended in 5% gum arabic, pH 7.
Positive Controls: 4-Nitroquinoline-1-oxide (NQO) at 0.1 and 0.15 ug/ml;
benzo(a)pyrene (BP) at 2 and 3.0 ug/ml concentration.
Animals: Fisher 344 rats, 3/group, and their isolated hepatocytes.
Doses: AG-1749: 625, 1250, 2500, and 5000 mg/kg, po via gavage in
volumes not exceeding 15.2 ml/kg.
Positive Control: Dimethylnitrosamine (DMN): 10 mg/kg, ip.

Methods: Groups of rats, 3 each for two different time points (4 and 15 hr), were given single doses of AG-1749 as indicated above. Hepatocytes were collected via perfusion at 4 and 15 hr after dosing. The assay was performed according to the methods described by Williams (In Chemical Mutagens, v6, pp 61-79, 1980), Mirsalis et al. (Environ. Mutagenesis, 4:553-562, 1982) and Butterworth et al. Mut Res., 189:123-133, 1987).

The assay criteria were: at least 70% viability of hepatocytes; 85% viability of the attached cells; the positive control (DMN) showing a net nuclear grain (NG) value of 26+, and 80-100% of nuclei containing more than 5 NG/nucleus; grain counts obtainable from 2 of 3 rats; and where at least 3 dose levels have been tested of which the highest dose should not be more than LD₅₀. The evaluation criteria were: if the agent yielded greater than or =5 NG/nucleus, and more than or =10% of cells responding (opposed to 20% as suggested by the Guidelines [Mut Res. 189 (2):123-133, 1987]), the agent was considered positive. The guidelines also suggest that conditions such as: a dose-related increase in both NG and the % of cells in repair, are required to confirm a positive response for counts less than 5 NG.

Results: Although four doses: 625, 1250, 2500, and 5000 mg/kg were tested, the results were provided only for the three highest doses. The hepatocyte viability at 4-4.3 hr post-dosing was 87-96.6% of the total cells collected in the perfusate; the attachment efficiency was in the range of 48.5-85.7%, and the viability of the attached cells ranged between 89.2-98.2%. At 15.3 to 16.0 hr, the viability was 81.2-96.8%; attachment efficiency was 61.3-89.2%, while the viability of the attached cells was 88.1-97.7%. About 10% of the cells responded with 5 or more NG/nucleus. However, this number is much lower than recommended by the guidelines suggesting that evaluation criteria set by the testing laboratory were less stringent. On the other hand, 2 of 3 animals in each group showed positive response (5 or more NG/nucleus) to the positive control, all of which confirmed the validity of the assay.

Thus, under the conditions of the assay, AG-1749 did not induce any unscheduled DNA synthesis in the rat liver hepatocytes, i.e. did not have any mutagenic potential at a dose range of 1250-5000 mg/kg.

In-Vitro Human Lymphocyte Assay To Determine
the Chromosome Damaging Potential of AG-1749
(Report A-29-650/IND Amendment 7/31/90/
Pharmacology Review dated 1/2/91)

The assay was conducted to evaluate the chromosome damaging potential of AG-1749 using the cultured human lymphocytes in an in-vitro cytogenetics assay of Evans and O'Riordan (Mutation Res 31: 135-148, 1975).

Conducting Lab:

Dates of Conduct: Initiated 3/3/89, completed 7/10/89.

GLPs Statement: In compliance with UK GLPs, OECD's guidelines, but not of FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-016; 99.6% pure, dissolved in DMSO at a final concentration of 1500 ug/ml.
Negative Control: Dimethylsulphoxide (DMSO)
Positive Controls: Methylsulphonate (MMS), 75 and 100 ug/ml;
Cyclophosphamide (CPA), 12.5 and 25 ug/ml.
Test System: Human lymphocytes from two healthy male human volunteers.
Dose Range: Trial 1: 3.17 to 150.0 mg/ml (or 31.07 ug to 1500 ug/ml as the final concentration);
Trial 2: 10-50 mg/ml (or 100-500 ug/ml as the final concentration); both in the presence or absence of rat liver S-9 homogenate, obtained from the Aroclor-induced Histar rat liver.

Methods: The assay method used was described in sufficient details and thus is not repeated here. The highest dose selected was such that it gave 50-80% reduction in the mitotic index, based on 1,000 cells counted; 100 metaphases were counted from each culture for chromosome aberrations. Cells with 44 (ie 2n-2) or more chromosomes were considered acceptable for scoring and aberrations were classified. The total number of numerical and structural aberrations, and total structural aberrations, were counted.

The assay acceptance criteria were: (a) if the incidence of structural aberrations in the negative control cultures fell within the normal range, (b) at least 80% (160/200) of the intended cells were analyzable at each test level; and (c) the positive control induced statistically significant increase in the incidence of structural aberrations.

The evaluation criteria were if (a) statistically significant increase in the incidence of structural aberrations occurred at one or more test levels, and (b) the incidence of aberrations at such data points exceeded the normal range of 0-4 for structural and numerical aberrations, and 0-9 for total aberrations per 100 cells.

Results: Based on the degree of mitotic inhibition (not less than 50%), the test levels were selected for the final data analysis. In trial 1, in the absence of metabolic activation (- S-9), the test level of 1500 ug/ml was toxic to the cells. At 31.07, 47.80, 73.53 ug/ml, AG-1749 did not cause any mitotic inhibition, while at higher test levels (411.9, 633.8, and 975.0 ug/ml), mitotic inhibition was complete. In the presence of S-9 (metabolic activation), about 50% mitotic inhibition was seen at 267.8 ug/ml.

In trial 2, 350 ug/ml caused 52% mitotic inhibition and was thus considered as the top test level for data analysis. Thus, for trial 1, 113.1, 174, and 267.8 ug/ml, and for trial 2, 250, 300 and 350 ug/ml test levels were selected for data analysis. The assay met the criteria for validity.

In terms of chromosome aberrations, in the presence of S-9, the incidence of structural aberrations was comparable with the negative control. In the absence of S-9, there were chromosome or chromatid deletions. The number of total aberrations excluding gaps were 3, 6, 16, 31, and 31 in the negative control, 250, 300, 350 ug/ml AG-1749, and MMS, test levels in the absence of S-9. In the presence of S-9 and at lower concentrations of 113.1, 174.0, 267.8 ug/ml of AG-1749, the number of total structural and numerical aberrations were: 2, 5, and 8, respectively, compared to 1 with the solvent. The % cells with structural and numerical aberrations were 1, 2.5, and 4 compared to 0.5 with the solvent. The other types are summarized below.

Chromosomal Aberrations* with AG-1749

Type	Solvent - S-9	250	AG-1749, ug/ml; (-S-9)		MMS, 100 ug/ml
			300	350	
Cells scored	200	200	200	200	50
Chromatid deletions	1	2	11	18	9
Struct. & Num.	3	6	16	31	31
Struct. only	3	4	13	23	31
% cells w/Struct. & Num.	1.5	2.5	5	12	46
% cells w/Struct. only	1.5	1.5	3.5	8.5	46

* Struct=structural, Num=numerical aberrations.

There was statistical significance between the negative controls and 300 and 350 ug/ml test levels. No exchange-type of aberrations were seen, but deletion-type aberrations were seen suggesting some potential to cause chromosome aberrations in the human lymphocytes. Thus, the assay was repeated by Takeda Labs (see below).

In-Vitro Cytogenetic Test of AG-1749 Using Human Lymphocytes
(Report A-299-699/IND Amendment dated 7/31/90/
Pharmacology Review dated 1/2/91)

Sponsor provided data from a second in-vitro cytogenetic study, conducted by Takeda Labs, Japan, to evaluate the chromosome damaging potential of AG-1749 using the cultured human lymphocytes according to the revised method of Evans (Handbook of Mutagenicity Test Procedures, eds Kilbey et al., pp405-470, 1984).

Conducting Lab: Takeda's Drug Safety Research Labs, Osaka, Japan.
Dates of Conduct: Initiated 11/28/89; completed 3/6/90.
GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: AG-1749, lot # M610-018; 99.6% pure, dissolved in DMSO at a final concentration of 1500 ug/ml.
Negative Control: Dimethylsulphoxide (DMSO), 1% v/v.
Positive Controls: Mitomycin (MMC), 0.1 and 0.05 ug/ml in the 24- and 48-hr. treatment series, respectively.
Cyclophosphamide (CPA), 7.5 ug/ml in the 2-hr treatment series with or without S-9.
Test System: Human lymphocytes from two healthy male human volunteers.
Test Level: Experiment I & II: 15, 30, 50, 55, 60, 65, and 70 ug/ml, at 24 or 48 hr incubation times in the absence of S-9;
Experiment where cultures were incubated for 2 hr: 62.5, 125, 200, 225, 250, 275, and 300 ug/ml levels were tested in the presence or absence of S-9, obtained from phenobarbital-induced liver homogenate S-9.

Methods: The assay method used was described in sufficient detail and thus is not repeated here. Briefly, the cells were exposed to the test solutions for either 24 or 48 hr in the absence of S-9, or for 2 hr in the presence or absence of S-9. The doses were selected when the mitotic index of the treated cells relative to the control cells decreased drastically from 60-100% at 50 ug/ml to 10-20% at 75 ug/ml regardless of the treatment time or the donor; and when treated for 2 hr, the relative index was 50% around 250 ug/ml in the presence or absence of S-9. Based on the mitotic index, the high dose of AG-1749 for chromosome analysis was selected. The metaphases were inspected for polyploidy and chromosomal aberrations: breaks and exchanges (chromatid or chromosomal). Further, at each test level including the controls, 1000 metaphases were scored for polyploidy and 200 for chromosome aberrations.

Results: The MI_{50} (dose producing a 50% or more reduction in the mitotic index) of 60 ug/ml at 24 hr and 48 hr was adopted as the highest dose for chromosomal analysis. AG-1749 increased the incidence of chromosomal aberration-bearing cells significantly at the highest doses in the 24-hr series in the case of donor 1 and in the 24-hr and 48-hr series in donor 2 suggesting some donor-variability. The incidence of chromosomal breaks was high, also suggesting chromosomal damaging potential. Although sponsor suggested that this could be due to cytotoxicity, the latter was not confirmed by calculating the proliferation index (PI, the proportion of mitotic cells in their 1st, 2nd, and 3rd metaphase) for each treatment group. An increase in such index could suggest induction of cell-cycle delay or cytotoxicity of the agent. However, such an index was not calculated.

In experiments I and II (donor 1 & 2), % cells with aberrations excluding gaps are shown in table below that suggest dose-related effects since the effects were significantly different ($p \leq 0.05-0.01$) at a dose-range of 50, 60 and 65 ug/ml test levels. In the 2-hr series, with or without S-9, the numbers were comparable with negative controls.

In summary, in the Takeda study also, AG-1749 showed evidence of chromosomal damaging potential at 50-65 ug/ml test levels in the human lymphocytes.

Human Lymphocytes^a Treated with AG-1749
For 24-or 48 hr Without Metabolic Activation

Treatment	Experiment I 24-hr	Experiment II 24-hr	Experiment I 48-hr	Experiment II 48-hr
DMSO	0 (0%)	1 (0.5%)	2 (1%)	2 (1%)
15 ug/ml	1 (0.5%)	0 (0%)	2 (1%)	1 (0.5%)
30 ug/ml	3 (1.5%)	2 (1%)	1 (0.5%)	1 (0.5%)
50 ug/ml	6 (3%)	ND	ND	ND
60 ug/ml	ND	13 (6.5%)**	6 (3%)*	ND
65 ug/ml	ND	ND	ND	12 (6%)*

^a=Number of cells (%) with aberrations excluding gaps. ND=not determined;
* or **= significant at $p \leq 0.05$ or 0.01. Number of cells analyzed were 200 in each case.

In-Vivo Bone Marrow Cytogenetics Assay in Rats
(Report TA-90-267/IND Amendment dated 5/1/91/.
Pharmacology Review dated 10/18/91)

The assay was conducted to determine the clastogenic (chromosomal aberration induction) activity of Lansoprazole in the rat bone marrow cytogenetic assay.

Conducting Lab:

Dates of Conduct: Initiated 8/17/90, completed report: 12/3/90.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical:

Lansoprazole, lot# 42-057-AL; 99.6% pure.

Animals:

Rats, Sprague-Dawley, 8 wks old at start; 246-295g males, 187-246g females; 5/sex/group.

Doses:

Males: control, 200, 667, 2000 mg/kg, ip;

Females: control, 90, 300, and 900 mg/kg, ip, given in a constant volume of 10ml/kg.

Positive Controls: Cyclophosphamide, po, 60 mg/kg, dissolved in water.

Negative Controls: Control vehicle, 5% gum arabic in distilled water.

Methods: Groups of rats, 5/sex/group, were dosed with control vehicle or single doses of Lansoprazole at the indicated doses. Six, 18, and 30h post-dosing, the rats were sacrificed for bone marrow extraction. Note: approximately 2h prior to sacrifice, 2 mg/kg, ip, colchicine (to arrest mitosis) was injected. After the rats were sacrificed, tibia with epiphyses were removed, and bone marrow was extracted by using Hank's solution, extracts were centrifuged, and slides were prepared for counting the number of cells containing 2n+2 chromosomes. The location of cells bearing aberrations was identified and mitotic index (MI) based on at least 500 cells was calculated. Further, polyploidy (cells containing more than one set of homologous chromosomes) was analyzed while scoring the MI.

Evaluating criteria were based on the general published guidelines (Preston et al. 1981, Mutation Research, 87: 143-188, 1981). Briefly, gaps were not counted as significant aberrations while breaks, deletion, translocations, multiradials, rings, multicentrics etc. were counted as significant aberrations. Data were analyzed on a per animal basis, and the number of cells containing at least one structural aberration and the number of cells containing 2 or more such aberrations were counted, statistically analyzed by Kriskal-Wallis test at 95% confidence level.

Criteria for positive response were statistical significance in dose-related increase in the number of structural aberrations at at least one dose level.

Results: The positive control, cyclophosphamide produced the anticipated aberrations (males: 63.4±4.7%; females: 60.8±3.2%), while Lansoprazole did not produce any significant increase in the number of aberrations of any type at any dose.

In summary, Lansoprazole at dose levels of up to 2000 mg/kg in the males and 900 mg/kg in the female rats was not clastogenic.

Micronucleus Test in ICR Mice

(Report A-29-698/IND Amendment dated 8/30/90/
Pharmacology Review dated 10/18/91)

Conducting Lab: Takeda Labs, Japan
Dates of Conduct: Initiated 1/10/90, completed report: 2/15/90.
GLPs Statement: In compliance with OECD guidelines, but unclear whether in compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: Lansoprazole, lot# M610-018; 99.6% pure, dissolved in 5% gum arabic solution, pH 7 to give 25%w/v concentration.
Animals: Mice, ICR, 8 wks old at start; 32-42g males, 28-38g females; 5/sex/group.
Doses: 0 (control vehicle), 5,000 mg/kg Lansoprazole, po.
Positive Controls: Mitomycin, 2 mg/kg, ip.
Negative Controls: Control vehicle, 5% gum arabic in distilled water.

Methods: Mice were given single oral doses of the control vehicle, positive control or Lansoprazole at the maximum attainable dose of 5,000 mg/kg and the mice were sacrificed at 24, 48, and 72h later, their femur removed and bone marrow aspirated. Slides were prepared and the frequency of micronucleated polychromatic erythrocytes (%MNPCE) were calculated. At least 2000 polychromatic erythrocytes per mouse were examined for the presence of micronuclei, and at least 1000 erythrocytes/mouse for calculating the frequency of reticulocytes (%reticulocytes), thereby satisfying the assay criteria that was conducted according to guidelines of MacGregor et al. (Mut Res. 189: 103-112, 1987).

Results: The frequency of MNPCE in the test group male mice was 0.12, 0.14, and 0.13, at 24, 48 and 72h post-dosing, respectively. The %MNPCE in the female mice were 0.09, 0.08, and 0.11, respectively. In comparison, mitomycin produced the frequency of 7.82 and 6.24 in the male and female mice, respectively. The frequency of reticulocytes in erythrocytes tended to be 14-17% higher in the females at 24, 48, and 72h post-dosing as compared to controls, and 43% higher in the male at 48h, but 20% lower at 72h. The %reticulocytes with mitomycin was 57.1 and 52.7 in the male and female mice, respectively, the frequency lower than that found in the treated mice. The %MNPCE with the negative control (gum arabic) ranged between 0.11 and 0.22 at 24 through 72h.

In summary, Lansoprazole at 5,000 mg/kg did not induce micronuclei in the mouse erythrocytes, and thus it was not clastogenic. Although the study was repeated in both sexes of mice, and the mice were sacrificed at three time points, 24, 48 and 72h post-dosing, only one dose of 5,000 mg/kg Lansoprazole was tested.

SPECIAL STUDIES:

1. Hemolytic Potential of Lansoprazole in Rats: (Report A-29-902 & A-29-1782/IND Initial Submission/Pharmacology Review dated 9/6/93)

Methods: In study A-29-902 five male Wistar rats were given saline or 0.5 mg/kg/ of Lansoprazole (dissolved in polyethylene glycol, final concentration 3 mg/ml, pH 10-11) intravenously via tail vein as a 30 second bolus. In study A-29-1782, the above study was repeated and rats were injected with 1 mg/kg of Lansoprazole (6 mg/ml, pH 10-11). Thirty minutes after the injection, blood samples were collected from abdominal aorta. Percent neurolysis was determined spectrometrically.

Results: No hemolysis was evident.

2. Rabbits: In Vitro Hemolysis Study of Lansoprazole: (Report A-29-901 & A-29-1781/IND Initial Submission/Pharmacology Review dated 9/6/93)

Methods: Rabbit blood (0.1 ml) was added to 1 ml incubation mixture containing 0, 1.4, 3 or 6 mg of Lansoprazole (solvent for Lansoprazole was PEG) and incubated at 37°C for 5 or 30 minutes. At the end of incubation period percent hemolysis was monitored spectrometrically.

Results: No hemolysis was evident, except at 30 minutes time point 5% and 20% hemolysis were seen in systems containing 3 and 6 mg/ml of Lansoprazole respectively.

3. (a) I.V. Irritation Study in Rabbits: (Report A-29-900 & A-29-1780/IND 41,938 Initial Submission/Pharmacology Review dated 9/6/93)

Methods: Male Japanese White rabbits (n=6/group) was given a single injection of saline, Lansoprazole (9 mg or 18 mg dissolved in 1 ml PEG) or positive control (sulfobromophthalein sodium [BSP] into ear vein. The volume of administration was 3 ml and given at the rate of 1 ml/min. One, 2, 3, 5, 7, 10 and 14 days after treatment, injection sites were examined for local irritation, thrombus formation, erythema and swelling of the auricle. Two and 14 days after the injection 3 rabbits/group were sacrificed and injection sites were examined microscopically.

Results: Slight edema and inflammatory cell infiltration but no thrombus were seen in Lansoprazole treated rabbits up to 2 days after injection. These changes disappeared within 14 days after drug administration. No abnormalities were seen in saline

treated rabbits and the positive control treated rabbits showed expected results. Similar results were seen when 3 or 6 mg/ml of AG-1749 were used (report # A-29-1228) in the above experiment. Thus slight i.v. irritation was seen by AG-1749 i.v. formulation.

(b) Skin Irritation Study of AG-1749 I.V. Formulation in Rabbits

(Report A-29-2016/IND
dated 7/19/94)

Amendment dated 5/13/94/Pharmacology Review

Methods: I.V. formulation of AG-1749 contained AG-1749, sodium hydroxide, D-mannitol, methylglucamine, polyethylene glycol 400, Hcl and distilled water. In this study skin irritation potential of AG-1749 I.V. formulation was assessed. There were six rabbits in each group. Each rabbit from treatment group received occluded application of AG-1749 (3 mg/0.5 ml/site) at 4 sites

(2 intact and 2 abraded) on their back. The control group rabbits received saline and/or AG-1749 solvent in similar fashion. The patches were removed at 24 hours after the exposure. Application sites were examined at 24, 48 and 72 hours after treatment for erythema and edema.

Results: No erythema and/or edema was seen at any skin sites in the rabbits. Hence, Lansoprazole is non-irritant to the skin of rabbits.

4. Paravenous Irritation Study of AG-1749 I.V.
Formulation in Rabbits

(Report A-29-2015/IND Initial Submission/Pharmacology Review dated 8/6/93)

Methods: Male Japanese White rabbits (n=6/group) were given a single injection 0.5 ml of AG-1749 (I.V. formulation 6 mg/ml) into the hypoderm near the vena auricularis posterior. The control group rabbits were given saline in similar fashion. One, 2, 3, 5, 7, 10 and 14 days after treatment, injection sites were examined for local irritation, thrombus formation, erythema, thickness and dilatation of veins and venules. Two and 14 days after injection, 3 rabbits/group were sacrificed and injection sites were examined microscopically.

Results: Rabbits treated with AG-1749 I.V. formulation had moderate erythema, edema, slight dilatation of small vessels at the injection sites, and this effect disappeared within 7 days after injection. Histopathological examination of the injection sites revealed edema, slight deposition of fibrin and inflammatory cell infiltration and some of these findings were still present at 14 days after the treatment. No abnormalities were seen in saline treated rabbits. The data indicated that AG-1749 I.V. formulation produced paravenous irritation in rabbits.

5. Antigenicity of Lansoprazole in the Guinea Pig and Mouse
(Report A-29-1182/IND Amendment dated 6/27/91/
Pharmacology Review dated 10/18/91)

This is the same study that was submitted in the amendment #100 as the published study: Antigenicity of Lansoprazole in the Guinea pig and Mouse (Doi et al. Yakuri to Chiro 18 [suppl]: 131-139, 1990).

Conducting Lab: Drug Safety Research Laboratories, Takeda Chemical Industries, Ltd., 6-10-1, Himuro-cho, Takatsuki, Osaka Japan.

Dates of Conduct: Initiated 7/4/89, completed 11/6/89.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: Lansoprazole, lot # M610-016; suspended in 5% gum arabic.
Animals: Guinea pigs, female, Std:Hartley, 9-10 wks old when used; 10/group except controls that consisted of 5 guinea pigs. Mice, male, Slc:A/J, 15 wks old when used.

Doses: Since the dosage regimen is somewhat complicated to briefly describe in the text, the design is shown in the sponsor's table attached below.

Table 1. Antigenicity study of AS-1743 in guinea pigs

Group	Test	Adjuvant	Dose	Route	Duration of course	Interval	No. of doses	Volume	Vehicle	No. of animals
No.	examined									
1	AS-1743	—	15mg/kg	p.o.	4 weeks	1 day**	25	2 ml/kg	5% gum arabic	10
2	AS-1743	PCA	15mg/kg	i.d.	4 weeks	2 weeks	3	2 ml/kg	5% gum arabic	10
3	AS-1743-HSA	PCA	0.1 mg/animal	i.d.	4 weeks	2 weeks	3	1 ml/animal	Saline	10
4	AS-1743-APF	PCA	0.1 mg/animal	i.d.	4 weeks	2 weeks	3	1 ml/animal	Saline	10
5	Vehicle	PCA	—	i.d.	4 weeks	2 weeks	3	1 ml/animal	5% gum arabic	10
6	OVA	PCA	0.1 mg/animal	i.d.	4 weeks	2 weeks	3	1 ml/animal	Saline	5

** Administered 24h before a test

Table 2. Antigenicity study of AS-1743 in mice

Group	Test	Adjuvant	Dose	Route	Duration of course	Interval	No. of doses	Volume	Vehicle	No. of animals
No.	examined									
1	AS-1743	—	15 mg/kg	p.o.	4 weeks	1 day**	25	10 ml/kg	5% gum arabic	10
2	AS-1743	PCA	15 mg/kg	p.o.	4 weeks	2 weeks	3	10 ml/kg	5% gum arabic	10
3	AS-1743-HSA	PCA	0.1 mg/animal	p.o.	4 weeks	2 weeks	3	0.2 ml/animal	Saline	10
4	AS-1743-APF	PCA	0.1 mg/animal	p.o.	4 weeks	2 weeks	3	0.2 ml/animal	Saline	10
5	Vehicle	PCA	—	p.o.	4 weeks	2 weeks	3	0.2 ml/animal	5% gum arabic	10
6	OVA	—	0.1 mg/animal	p.o.	4 weeks	2 weeks	3	0.2 ml/animal	Saline	10

** Administered 24h before a test

Methods: The antigenicity of Lansoprazole was studied in guinea pigs and mice by using Lansoprazole-protein conjugate prepared by incubating Lansoprazole and proteins at pH 3 for 1h. Groups of mice and guinea pigs, 10/group, were first immunized by oral administration of Lansoprazole at 15 mg/kg, 25 times the intended human dose (30 mg/man) for 4 weeks (a total of 25 doses) during which period the animals were observed once daily for signs of toxicity and body weight changes. Another group of animals was used to promote antibody formation by injecting the following elicitors (5 mg/animal Lansoprazole, iv: 1 mg/animal Lansoprazole-HSA or Lansoprazole-APF; 5 mg/animal OVA) and the animals were observed for clinical signs. A separate group of animals was

used for the positive controls (FCA emulsion of Lansoprazole-HSA [human serum albumin], Lansoprazole-APF [apoferritin] and OVA [ovalbumin]). The animals were observed once daily during dosing and once/wk during the study (4 weeks) for signs of toxicity, and body weight gain. For producing systemic anaphylaxis, 2 weeks after the final dose, each animal was challenged with the elicitor mentioned above and anaphylaxis graded (negative, mild, moderate, severe, fatal etc.). The guinea-pig 24h- passive cutaneous anaphylaxis (PCA) reaction was studied by measuring specific IgG, antibodies in the guinea pig serum and IgG, antibodies in the mouse serum. In the 24h-PCA reaction, - specific IgE antibodies in the mouse serum were determined by the rat 24-h PCA reaction after challenging with the appropriate elicitor. In the passive hemagglutination PHA reaction, the agglutinating antibodies in the mouse serum were measured. And finally, in the agar gel precipitation test, the precipitating antibodies in the mouse serum were determined.

Results:

Observed effects:

General appearance and body weight gains of both species were normal.

No anaphylaxis developed in any guinea pig. There was no homologous 24-h PCA (passive cutaneous anaphylaxis) in response to either elicitor (Lansoprazole, Lansoprazole-HSA or Lansoprazole-APF). In mice, similarly no such reactions occurred. In addition, there was no PHA reaction in the mice. In both cases, the positive controls produced anticipated positive reactions.

In summary, Lansoprazole was non-antigenic in the mouse and guinea pig since neither oral (15 mg/kg) nor intradermal (15 mg/kg) administration of Lansoprazole produced any homologous passive cutaneous anaphylaxis reactions in either animal species. No hapten specific antibodies were produced in the mouse serum when mice were treated orally with Lansoprazole or treated sc with Freund's complete adjuvant (FCA) emulsion of Lansoprazole.

6. Immunotoxicology of Lansoprazole in the Rat
(Abbott Report R&D/90/278/IND Amendment dated 8/30/90/
Pharmacology Review dated 10/18/91)

Conducting Lab:

Sponsor:

Dates of Conduct: Initiated 5/14/90, completed 10/29/90.

GLPs Statement: In compliance with FDA's GLPs.

MATERIALS AND METHODS

Chemical: Lansoprazole, lot # 06-677-AL, suspended in 5% gum arabic.
Animals: Rats, Sprague-Dawley, females, 8/group.
Doses: 0 (control vehicle), 50, 150, and 300 mg/kg/d, po, for 14 days in a constant volume of 5 ml/kg.
Positive Control: Cyclophosphamide, 25 mg/kg, ip.
Negative Control: Saline, 5 ml/kg.

Methods and Results: Groups of female rats were dosed with the test agent or the positive and negative controls, once daily for 10 days. On day 11, these were challenged with sRBC, a T-dependent antigen, and treatment continued from day 11 through 14 and were observed daily for body weight changes and any

signs of toxicity. At termination, the thymus and spleen of each animal were removed and used for the following assay: spleen IgM antibody response to the T-dependent antigen, sRBC, day4 response.

Results:

Observed effects: At doses of 50, 150, or 300 mg/kg, Lansoprazole produced the anticipated dose-dependent reduction in the relative thymic weight (e.g. 24.9% and 52.5% at 150 and 300 mg/kg, respectively), but did not reduce the number of IgM spleen anti-body-forming cells in response to the T-dependent antigen, sheep erythrocytes while the positive control, cyclophosphamide (25 mg/kg) produced the anticipated response (reduction in the thymic [63%] and spleen weight [50%]) and in the number of AFC spleen cells (by 81%)) suggesting that Lansoprazole does not have any immunotoxic potential in the Sprague-Dawley rat at doses of up to 300 mg/kg.

In summary, Lansoprazole at oral doses of up to 300 mg/kg/d for 14 days did not show any immunotoxic potential in the Sprague-Dawley rat.

7. In-Vitro Effect of AG-1749 on the Leydig Cell Testosterone Synthesis: (PPRd/90/111/IND Amendment dated 8/12/90/
Pharmacology Review dated 1/2/91)

Methods: This study was done to determine if lansoprazole inhibited the synthesis of testosterone. The isolated Leydig cells from the Sprague-Dawley rats were incubated with lansoprazole for 3 hr at concentrations of 0.01, 0.0316, 0.1, 0.316, 10.0, 31.6, 100, or 316 ug/ml. Ketoconazole was used as a positive control, and testosterone levels were determined by radioimmunoassay.

Results: No data on the amounts of testosterone synthesized were determined, instead, the viability of the Leydig cells was calculated from which the inhibition concentration was calculated. The EC_{50} for the compound was 9.92 ± 1.03 ug/ml, while that for ketoconazole was 0.12 ± 0.01 ug/ml indicating that lansoprazole exerted about 100x less inhibitory effect on testosterone synthesis than ketoconazole. However, no data on the negative control were provided. In addition, the comparison with ketoconazole appeared to be unjustified since the mechanism of testosterone synthesis inhibition by ketoconazole was quite different. While ketoconazole blocks the microsomal cytochrome P-450-dependent C,7 20 lyase enzyme complex that converted

17-alpha-hydroxyprogesterone to androstenedione (see De Coster et al. Eur J. Pharmacol 29:489-493, 1985), AG-1749 appeared to act on androstenedione to testosterone step, the last step in the testosterone biosynthetic pathway (cholesterol-- pregnenolone-- progesterone-17-alpha-OH-progesterone-- androstenedione-- testosterone).

REVIEWER'S ADDENDUM: In the above study, the Leydig cells were stimulated by a maximum dose of 100 ng/ml of LH in each incubation.

8. Effect of Lansoprazole on Testosterone Production in Rat Leydig Cells In Vitro: (A-29-807/IND Amendment dated 8/12/90/Pharmacology Review dated 1/2/91)

Methods: Lansoprazole was tested from 1 to 100 ug/ml concentration for its inhibitory effects on testosterone production, and also at ug/ml concentration in the presence of testosterone precursors.

Results: Lansoprazole produced dose-dependent suppression of testosterone synthesis induced by human chorionic growth hormone (hCG), dibutyryl cAMP, or pregnenolone with IC_{50} of 10, 9, and 42 ug/ml, respectively, while the IC_{50} for the basal suppression was 38 ug/ml. It suppressed the production of testosterone by 34% when androsterone was used as a precursor, suggesting the role of 17-beta-hydroxysteroid dehydrogenase (the enzyme that converts androsterone into testosterone, the last step in the biosynthetic pathway of testosterone synthesis) in the mechanism of action of lansoprazole. But because (a) testosterone levels were also suppressed (17-51%) by lansoprazole when other precursors, pregnenolone, progesterone, and 17-alpha-OH-progesterone were used, and (b) no data on enzyme inhibition (enzyme kinetics) were provided, the suggestion of this specific enzyme's role in the mechanism of action, is not supported by the present data. On the other hand, lansoprazole suppressed the production of testosterone at a very high concentrations (IC_{50} being 38 ug/ml) which could be achieved only at a high dose of 1,900 mg/kg in the rat, if plasma concentrations increase linearly. Furthermore, the comparison with ketoconazole is inappropriate as discussed above.

9. Hormone Levels in Rats Treated with Single Dose of AG-1749 (PPRD/90/045/IND Amendment dated 8/12/90/Pharmacology Review dated 1/2/91)

Groups of male rats, 10 each, were given a single oral dose of vehicle, ketoconazole (25 mg/kg) or AG-1749 -50, 500 or 5,000 mg/kg), and blood samples were collected from the jugular vein as follows: in subgroup A, 5 rats each were used at 1.5, 2.5, and 3.5 hr; in subgroup B, 5 rats each were used at 1.0, 2.0, 3.0 and 4.0 hr post-dosing. Serum levels of LH, follicular stimulating hormone (FSH), prolactin, testosterone were determined. According to sponsor, AG-1749 decreased slightly the plasma testosterone levels at 500 and 5,000 mg/kg doses, but the data showed a large variability in the testosterone levels

within animals; and the decreases, though significant at $p < 0.05$, were not related to dose or time of measurement. For example, the decreases were 80 and 77% at 180 and 240 min with 500 mg/kg; 55% and 57% at 90 and 150 min post-dosing with 5,000 mg/kg dose. There was large variability among the controls also. Further, there was no compensatory increase in the LH levels as seen above. In summary, single oral doses of 50, 500 or 5,000 mg/kg AG-1749 did not show consistent effects on the serum testosterone or LH levels.

The following studies were submitted for the first time with the present Application and are reviewed below:

10. Effect of Metabolites of Lansoprazole on hCG-stimulated Testosterone Production in Rat Leydig Cells In vitro: (Study # A-29-1548)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

Dates of Start and Completion of Study: These were not provided with the study.

OAU and GLP Standard: Statement of compliance was not included.

Methods: Lansoprazole and its 6 metabolites, (sulfide), (benzimidazole), (5-OH sulfide), (5-OH-Lansoprazole), (sulfone) and (3'-CH₂OH sulfide) were tested for their effect on testosterone synthesis in hcg-stimulated Leydig cells. Each of these compounds were tested up to a final concentration of $\mu\text{g/ml}$ in a culture medium containing Leydig's cells (i.e., final concentration of each compound was 1 %).

Results: Lansoprazole and its metabolites inhibited testosterone production and their IC₅₀ were 11, 0.96, 6.8, 22.5 or 28.0 $\mu\text{g/ml}$ for lansoprazole, respectively. were about 11.5 and 1.6 times as active as lansoprazole while other two metabolites were about half and 2.6 times respectively less active than lansoprazole. From the graph given on page 261 of the above referred submission, were estimated to have no inhibitory activity from $\mu\text{g/ml}$ concentration respectively. However, was claimed to be an active metabolite by sponsor as it suppressed testosterone production by 70 % at a concentration of $\mu\text{g/ml}$ concentration. To support this claim, no data was provided by sponsor.

In summary, lansoprazole and its metabolites inhibited testosterone production and their IC_{50} were 11, 0.96, 6.8, 22.5 or 28.0 ug/ml respectively.

11. Plasma Clearance and Metabolism of ^3H -Testosterone in Rat After Acute and Chronic Treatment with Lansoprazole: (Study # R&D92/493; V92-015)

Testing Laboratory:

Dates of Start and Completion of Study: July 6, 1992 and September 11, 1992.

OAU and GLP Standard: Statement of compliance was not included.

Methods: Twenty four male Sprague Dawley rats weighing between 201-25 g were divided in 6 groups (4/group). In 1 group, lansoprazole was administered a single gavage oral dose of 150 mg/kg of Lansoprazole (Lot No 53-143-AL) and in other group only vehicle was given. These animals were given a dose of 0.24 mg/kg, i.v. of ^3H -testosterone in a volume of 1 ml/kg (20 ul/animal) through tail vein. The remaining 4 groups (4/group) were administered an oral dose of 0, 0, 150 or 150 mg/kg/day of lansoprazole for 21 days. A tracer ^3H -testosterone in 0.24 mg/kg dose was given to 2 groups (1 control and the other treatment group) either at 1 or 48 hr after the last dose of lansoprazole. Blood samples were collected by orbital bleeding at different intervals and radioactivity was determined by thin layered chromatography.

Results: Plasma concentrations of ^3H -testosterone in the lansoprazole treated and control groups were similar. AUC_{0-24hr} s (ng eq. hr/ml) of animals included in a single dose of lansoprazole and control group were 0.041 and 0.033 respectively. AUC_{0-24hr} s (ng eq. hr/ml) of animals treated for 21 days were: 0.021 and 0.034 in 1.5 hr blood samples on day 21; and 0.032 and 0.033 in 24 hr blood samples. The half life of ^3H -testosterone was 0.3 and 0.28 hr in animals included in treated and control groups respectively. Mean renal clearance time (MRT) were 0.55 and 0.51 hr. Total plasma clearance in the treated and control animals were 8.2 ± 3.7 and 4.608 ± 0.977 ml/hr. These were not different in the control and treated animals. Therefore, magnitude of decrease in testosterone was not significant.

12. Effect of Single Dose of Lansoprazole Treatment on Rat Serum Testosterone After hCG-Loading Doses: (Report # A-29-1352)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries, LTD., Japan

Methods: Forty male (10/group) Sprague-Dawley 8 weeks old rats were administered a single daily dose of either 0 (5 % arabic gum), 15, 50 or 150 mg/kg of AG-1749 for 1 or 2 weeks. After 1.5 hr of the last dose at week 1 or 2, hCG

(100 i.u./kg) was injected subcutaneously. Testosterone levels were determined in blood samples collected after 3 to 4 hr of hCG injection from 10 rats/time interval/group. Serum concentrations were determined using radioimmunoassay kit. The significance of the results were assessed by using Williams Multiple comparison test.

Results: After 1 and 2 weeks of continuous administration of lansoprazole, a slight but significant ($p < 0.01 - 0.05$) decrease of serum testosterone was reported in serum samples of the animals belonging to 50 and 150 mg/kg/day treatment groups. In serum samples collected during week 1, after 3 and 4 hr of the administration of hCG injection, a decrease of 20.9 to 22.9 % and 39.7 to 40.4 % of the serum testosterone was seen respectively in animals belonging to 50 and 150 mg/kg/day treatment groups. Lansoprazole at an oral dose of 15 mg/kg/day for 2 weeks did not further affect testosterone concentrations as the testosterone concentration in hCG treated animals and lansoprazole treated animals were comparable. A decrease of 36.4 to 39.7% and 40.4 to 49.5% in samples collected at 1 and 4 hr after hCG was observed in animals included in 50 and 150 mg/kg/day treatment groups. These changes in the hormone levels were within the control range seen in only hCG treated animals. Therefore, lansoprazole affected only slightly at the high dose which was not of clinical importance.

In summary, lansoprazole up to a dose of 150 mg/kg/day for 1 to 2 weeks, did not affect hCG induced testosterone synthesis.

13. Lansoprazole and Metabolites on hCG-Stimulated testosterone in Rats: (Study # 29-1733)

Testing Laboratory: Research and Development Division, Takeda Chemical Industries, LTD., Japan

Methods: Male Sprague-Dawley 10 weeks old rats were used in this study. The rats were sacrificed, testes separated, removed to isolate Leydig cells by density gradient centrifugation. Leydig cells (1×10^5) in 0.2 ml of Medium 199 were incubated with AG-1749 for 2 hr at 37°C in CO₂ and air mixture (19:1), prior to the addition of 10 ng/ml of hCG and the mixture was reincubated for 1 hr. Incubation was terminated by centrifuging and the supernatant collected, stored at -40°C till

used for assay. Test compound; AG-1749, metabolites were dissolved in DMSO while were dissolved in 1N HCl). The final concentration of DMSO in cultured medium was 1 % and final concentration of HCl was N. Testosterone levels were determined using radioimmunoassay.

Results: AG-1749 and its 4 metabolites were dissolved in DMSO and up to a concentration of ug/ml. were dissolved in strong acid (1 N HCl) medium and sponsor did not obtain the effect of the control solvents. Therefore the design of the experiment was not valid and the results of the study are not dependable and valid.

Therefore, the results of the study can not be considered for their effect on testosterone synthesis.

14. Effect on Serum Hormones in Rats included in 1-Month Oral Toxicity Study Rats: (R&D/92/024 - TA91-310)

Testing Laboratory:

Dates of Start and Completion: October 24, 1991 and April 15, 1992

QAU and GLP Standard: A statement of inspection was included.

Animals: One hundred and sixty male Crl:CD[®]BR strain rats 9 to 10 weeks old and weighing between 295 to 374 g were divided in to 4 groups of the study (40/group).

Methods: The study was conducted to examine the serum hormone levels of LH, testosterone, prolactin and FSH in male rats. In addition testicular levels of LH receptors, testosterone and urinary levels of FSH and LH were also done during the study. The dose selection for the study was based on the results of a previous carcinogenicity study in which the animals treated with a dose of 15 or 50 mg/kg/day orally for 5 day/week schedule were found to hyperplasia and ledig's cell hyperplasia seen in animals of 150 mg/kg/day treatment group. The animals in the present study were treated orally with either a dose of 0, 15, 50 or 150 mg of lansoprazole/kg/day in 5 % solution (in gum arabic, pH 7.0) and in a volume of 10 ml/kg. All animals were observed daily, their behavioural differences observed weekly and their body weights recorded twice weekly during treatment period and once during last week of the study. Blood samples (1.5 ml) from all animals at 5 (day 0 and 23) and 24 (day 2 and 27) hr post dosing, were collected for determining serum LH and testosterone concentrations by a radioimmunoassay technique.

The 24-hr urine samples on day 13, 15, 19 and 21 (immediately after the dosing of the animals) were collected for the estimation of LH and testosterone. At the termination of the study, blood samples (1.5 ml/rat) were collected for serum hormone estimation. Testes were removed and processed for isolation of LH receptors and testosterone estimation.

Results:

i. Observed Effects: AG-1749 at 150 mg/kg/day, produced lacrimation, salivation and matted hair on muzzle hind in 3, 6 and 6 rats for 3, 8 and 9 days of the study respectively. The animals included in 15 or 50 mg/kg/day treatment groups did not show any treatment related effects.

ii. Mortality: None.

iii. Body Weight/Food & Water Consumption: A retardation of 16.1 % in body weight gain in animals included in 150 mg/kg/day treatment group was reported. The body weights of animals included in other treatment groups of the study were not affected. The food and water consumption were not measured during the study.

iv. Serum/Urine Hormone Levels: Serum leutinising hormone (LH) was not affected of any biological relevance during the study of animals included in lansoprazole treatment groups were not affected. Serum testosterone concentrations in samples collected 5 or 24 hr post dosing in control animals were very different, with a great variation, therefore the difference in the mean values were not different. Sponsor conclusion that a significant decrease of testosterone concentrations were seen in 24 hr samples collected on day 2 and 27 post dosing were not correct and acceptable. Serum FSH concentrations were also not affected significantly on day 0, 2 or 23 of the study in treated animals. These were 10.75 ± 2.44 and 11.89 ± 2.2 ng/ml vs 7.63 ± 2.1 ng/ml in control group in rats included in 50 or 150 mg/kg/day treatment groups was observed on day 27 of the study.

Serum prolactin levels of animals belonging to 15, 50 or 150 mg/kg/day treatment groups, were not different from the animals included in control group affected on day 0 and 23 after 5 hr of treatment (22.10 ± 10.71 , 22.73 ± 14.53 , 19.5 ± 13.7 and 18.9 ± 11.37 ng/ml in control, 15, 50 or 150 mg/kg/day treatment groups) and; 24 hr after treatment on day 2 and 23 of the study.

This experiment therefore, showed that testosterone synthesis was not affected in animals treated up to 150 mg/kg/day for 28 days. Testicular testosterone levels were not significantly different from the animals in the control group. Thus,

sponsor's claim that lansoprazole treatment reduced testosterone concentrations was not correct because variation of the animals was very high and no conclusion could be drawn.

Testicular LH Receptors Changes: Lansoprazole treatment produced a significant down-regulation of testicular LH receptors by 18 to 22.7%. But, testicular testosterone and progesterone levels and; pituitary GnRH receptors contents were not affected by lansoprazole treatment. Therefore, sponsor's contention that lansoprazole affected hypothalamic pituitary gonad (HPG) axis was not confirmed in this study. The initial effect of lansoprazole on testosterone and LH might be a direct effect on testis.

Urinary Hormones Levels: Urinary LH concentration of animals was not affected by a treatment of up to 150 mg/kg/day of lansoprazole for 28 days. Urinary volumes in the treated animals was not affected. FSH concentration of animals included in 15, 50 or 150 mg/kg/day treatment groups was also not affected. The pattern of urinary gonadotropins excretion was similar to those of hormone content in treated animals. Lansoprazole did not alter serum LH and prolactin levels.

In summary, lansoprazole treatment produced no effects on serum and urinary hormones which were of biological relevance. The LH and receptors and testosterone were not affected during the study.

15. One Month Study of Orally Administered Lansoprazole on Serum Hormones in Male Rats: (R&D/92/204 - TA92-146)

Testing Laboratory:

Dates of Start and Completion: March 23, 1992 and August 31,, 1992

OAU and GLP Standard: No statement was included

Animals: Male Crl:CD^RBR strain rats 9 to 10 weeks old and weighing between 200 to 250 g were used.

Methods: The study was conducted to investigate the role of hormones in the 2-year rat carcinogenicity study and to determine the hormonal alteration at additional time points of 2, 8, 12, 16 and 24 hr post dosing. A dose of 150 mg/kg/day was selected as this dose was claimed to alter serum and urinary LH and testosterone levels. The doses of 1.5 and 5 mg/kg/day for 2 years (on 5 days/week schedule) did not produce testicular hyperplasia and tumor in rats but higher doses of 15 and 150 mg/kg/day produced both testicular hyperplasia and tumors. One

hundred and twenty rats were divided in 2 groups and were administered a dose of either 0 or 150 mg/kg/day for 1 month in a volume of 10 ml/kg (suspension in 5 % gum arabic pH=7.0). The animals. All animals were observed daily, their behavioural differences observed weekly and additionally 1-3 hr after the dosing 1/week during the study. Blood samples (2.0 ml) from all animals at day 0 and 23 at 2, 5, 8, 12, 16 and 24 hr post dosing, were collected for determining serum LH, FSH, testosterone and estradiol concentrations by a radioimmunoassay technique. No more than 3 blood samples/rat/day were collected during the study. Concentration of LH, estradiol, progesterone and FSH were determined by radioimmunoassay. The 24-hr urine samples on day 14, 16 and 20 were collected for the estimation of LH, FSH, testosterone and progesterone by radioimmunoassays. At the termination of the study, pituitary and both testes were removed and cleaned for the analysis of LH receptors and testosterone, estradiol and progesterone. Testicular LH receptors and pituitary GnRH receptors were determined by saturation analysis.

Results:

- i. Stability and Homogeneity Test: Analysis at 4 weeks of study indicated that the suspension was stable and 97.7 and 99.8 % of the substance was present.
- ii. Observed Effects: AG-1749 at 150 mg/kg/day, produced lacrimation, nasal discharge and salivation. An increased number of lens opacities (7 out of 24 vs 0 in control group), dark color (blood !) and discharge were seen in treated animals.
- iii. Mortality: None.
- iv. Body Weight/Food & Water Consumption: A retardation of 34.9 % in body weight gain in animals included in 150 mg/kg/day treatment group was seen.
- v. Serum Hormones Levels: Serum leutinising hormone (LH) levels in the treated animals were not affected during the study. Testosterone hormone level of animals were not affected in any serum collected at any time intervals significantly. Testosterone levels were higher at 12 hr of the study and were lower at 2 hr post dosing period of the study. No relevant and steady alteration in the hormone levels could be observed. Therefore, lansoprazole did not affect testosterone levels on day 0 or on day 23 of the study. A tendency of an increase of urinary free testosterone excretion was seen in animals included in 150 mg/kg/day treatment group. This was not statistically significant, therefore not of relevance.

There was no difference between free testicular level of LH receptors. Testicular testosterone and progesterone receptors were also not affected in the animals included in the treatment group. Relative testicular and pituitary weights were not affected during the study. Therefore, lansoprazole may not have an effect on pituitary GnRH axis of the rats.

In summary, lansoprazole treatment produced no effect on hormones like testosterone, estradiol, FSH and LH at 150 mg/kg/day for 1 month. No effect was seen on the testicular LH receptors.

16. In Vitro Inhibition of Enzymes of Leydig's Cells Steriodogenic Pathway: (Report # R&D/92/507)

Conducting Labortary:

Date of Start and Completion: November 13, 1991 and October 29, 1992

Methods: This study was conducted to determine the inhibition of testosterone synthesis by lansoprazole in rats. During this study, the effect of lansoprazole was determined on:
(i) testosterone production in the presence and absence of rat liver homogenate, S-9; (ii) in the presence of stimulators of steroidal synthesis (LH-, cAMP-) or precursors of testosterone biosynthesis (hydroxycholesterol, pregnenolone, progesterone, hydroxyprogesterone and androstendione). From the dose response curves for each of the stimulator/precursor in the absence and presence of lansoprazole at varying concentrations of hormones and lansoprazole were constructed.

In part (i) of the study, leydig cell preparations were exposed to (i) various concentrations of hCG, lansoprazole or control agent ketoconazole (0-200 ug/ml) in triplicate. A dose response curve was prepared by using maximally stimulating dose of hCG from 0 to 200 ng/ml for 2 hr.

In part (ii) of the experiment; the effect of lansoprazole in the LH-, cAMP- or steroids- stimulated Leydig cells was studied and its effect on testosterone production was determined. During this study, cells were exposed Lansoprazole (ug/ml concentration) or ketoconazole (ug/ml) for hr. Varying amount of rat LH (ng/ml) were added and suspension reincubated for an additional hr.

In cAMP or steroid precursors stimulated part of the study (part 1), alteration of leydig cell testosterone synthesis was determined upon their exposure to lansoprazole (10 ug/ml) for 2 hrs. Varying concentrations of either lansoperazle or

cAMP/steroids in the presence of a fixed amount of stimulators were used. The steroid precursors used were: 17-hydroxycholesterol, 20hydroxycholesterol, pregnenolone, progesterone, hydroxy-progesterone and androstenedione. These were added in Leydig cells suspension and reincubated for an additional 1 hr.

In Part (i) of the experiment; the effect of Lansoprazole (10 ug/ml) on P450 side chain cleavage (P450scc) enzyme in rat Leydig cell mitochondrial preparations was studied. A known amount of mitochondrial protein was incubated for min with a constant amount of lansoprazole, ketoconazole (ug/ml), with varying concentrations of unlabelled 25 hydroxycholesterol and a constant amount of triated hydroxycholesterol (3 uCi). The labelled hormones were extracted, dried, dissolved in tetrahydrofuran in hexane and separated by HPLC. Testosterone concentration were determined by a RIA. Ketoconazole (10 ug/ml) was used as a control group.

Results:

i. From a dose response curve for hCG-stimulated testosterone production, a maximally stimulated dose was approximated to be 2 ng/ml which was selected for the assay. The testosterone synthesis inhibitory studies indicated that EC₅₀ for ketoconazole and lansoprazole were 0.04 and 12 ug/ml respectively. The inhibitory effect of these agents was not altered in the presence of S-9 mix.

ii. Lansoprazole at 1 ug/ml concentration, did not inhibit rat LH-stimulated testosterone synthesis. But a higher concentration of 10 ug/ml, inhibited about 50 % of testosterone production over the entire range of rat LH concentrations. Lansoprazole at either ug/ml did not affect Km but produced a significant ($p < 0.01$) decrease of Vmax at ug/ml. During this period, Leydig cell concentration in the cell suspension, estimated by β -hydroxysteroid dehydrogenase staining, was 80 % and its viability 84 % (81 % with ketoconazole), therefore inhibitory effect of lansoprazole on testosterone production was not due to cell death.

Lansoprazole at ug/ml concentration, decreased cAMP induced testosterone synthesis at a concentration of ug/ml. Vmax in cAMP stimulated cells was significantly decreased ($P < 0.01$). Km was slightly increased with lansoprazole and was 0.045 uM vs 0.013 uM in control vehicle control.

The testosterone synthesis inhibition was not seen at 1 ug/ml concentration, equal to the plasma concentration achieved with a dose of 50 mg/kg in rats. The inhibition was seen only at a concentration of ug/ml which is about times greater

concentration of no effect dose of 50 mg/kg/day in rats. No significant difference in Vmax for the P450scc enzyme in the presence of Lansoprazole (10 ug/ml) was noticed (1.19 vs 1.17 picomol/mg protein/0.3 hr). Km value was increased insignificantly, from uM, in the treated solution. This indicated that the agent may act as a non-competitive inhibitor in this steroidal pathway.

(iii) Lansoprazole (10 ug/ml) did not produce any difference in steroid synthesis in a similar concentration resulted in the inhibition of hydroxycholesterol stimulated steroidal synthesis.

In summary, lansoprazole produced no inhibition of testosterone production in hCG-stimulated Leydig's cells at ug/ml concentration achieved by a dose of 50 mg/kg dose in rats. At 10 ug/ml concentration, lansoprazole inhibited about 50 % of testosterone production. A very high concentration of lansoprazole was required for in vitro inhibition of testosterone. Therefore no meaningful conclusion could be drawn by the experiment.

17. Effect of Lansoprazole on testosterone production in the mouse Leydig cells: (Study # A-29-975)

Lansoprazole (0.32-200 ug/ml) suppressed the basal, hCG-, dibutyryl cAMP- and pregnenolone-stimulated testosterone levels in the isolated mouse Leydig cells with IC₅₀s of 25, 8, 13, and 19 ug/ml, respectively. In comparison, the IC₅₀ of ketoconazole and omeprazole against hCG in the mouse were 0.35, and 17 ug/ml, respectively. The comparison with ketoconazole was not valid since this compound blocks the microsomal cytochrome P-450-dependent C, 7-20 lyase enzyme complex that converts 17-alpha-hydroxyprogesterone to androstenedione (see De Coster et al. Eur J. Pharmacol 29:489-493, 1985), while the mechanism of action of lansoprazole on testosterone synthesis has not been elucidated as yet.

A very high concentration of lansoprazole was required to for in vitro inhibition of testosterone, therefore this was not a valid experiment and no meaningful conclusion could be drawn.

18. Effects of Lansoprazole on Serum LH and Testosterone Levels in the Mouse: (A-29-974)

In a 4-week toxicity study, lansoprazole was given in single dose of 150 mg/kg/day and this dose did not alter significantly the serum testosterone or LH levels at 3, 4 and 5 h after the administration of the last dose (Report # A-29-974).

Table For Mean Serum Hormone Concentrations (ng/ml) in Mouse

Treatment Group	Mean Serum Concentrations (ng/ml) After Last Dosing (hr)		
	3	4	5
<u>Testosterone:</u>			
Control	2.9±2.78	0.6±0.34	0.61±0.46
150	2.5±4.82	0.66±0.29	4.69±8.30
<u>LH:</u>			
Control	26.8±16.1	32.9±38.7	75.3±117.0
150	23.4±7.1	29.3±13.8	31.2±24.9

19. On the Possible Mechanism of the Increased Incidence of Cell Tumors in the 2-Year Carcinogenicity Study of AG-1749 in SD Rats: (A-29-764/IND Amendment dated 7/27/90/Pharmacology Review dated 1/2/91)

Sponsor conducted this study to understand the possible mechanism of an increased incidence of Leydig cell tumors found in the 2-year carcinogenicity study with AG-1749 in the Sprague-Dawley rats.

AG-1749 at a single oral dose of 150 mg/kg did not alter the serum or the organ levels of testosterone or luteinizing hormone (LH) in the male rats (5/group) at 1, 4 or 24 hr post-dosing.

Lansoprazole at an oral dose of 150 mg/kg/day for 4 weeks showed a trend for a transient decrease in serum testosterone levels by 39.5% (1.04±0.52 ng/ml v/s 1.72±0.53 control) at 3.5 hr and by 44.4% (1.45±0.74 v/s 2.61±1.12 control) at 4 hr. There was no increase in testosterone levels at 4.5 and 24 hrs time interval of the study. The serum LH levels increased by 68% at 4 hr, by 52% at 5 hr. But, at 3.5 and 4.5 hr post-dosing, these were not changed. The pituitary LH levels were increased by 60% at 4 hr but not at 3.5, 4.5, and 5 hr post-dosing. Due to large variability of the change in serum testosterone levels, the changes appeared to be inconsistent, not too highly significant and sporadically seen at various times post-dosing. The relative testes and pituitary weights decreased in the male rats by 20 and 9%, respectively. The weight change of pituitary gland in female rat was not seen. Further, the prostate, uterus, and seminal vesicles weights were not changed.

In another experiment, the synthesis serum testosterone stimulated by human chorionic gonadotropin (hCG, 100 iu/kg, sc, 1.5 hr after the last dose of AG-1749), was suppressed by lansoprazole in a dose-dependent manner. After a 1-week treatment with an oral dose of 150 mg/kg, the suppression at 2-4 hr post-hCG injection was 20-28% and suppression was 45-48% with 500 mg/kg. After a 2-week treatment, the suppression was 35-46% with 150 mg/kg, and 53-61% with 500 mg/kg.

Lansoprazole was not antiandrogenic in the 3-wk old castrated rats treated with an oral doses of 50, 150, and 300 mg/kg/d for 2 weeks. The animals of this study were treated with testosterone propionate (0.15 mg/rat) 30 min after the last dose of AG-1749. TSDA-291, a known antiandrogen, was used a positive control at an oral dose of 400 mg/kg/d for 2 weeks, and 5 % gum arabic solution as the negative control. The ventral prostate and seminal vesicles weights did not change.

On the basis of an in-vitro study with Leydig cells, sponsor tried to link the observed decrease in testicular testosterone levels and increased serum LH to an inhibition of biosynthesis of testosterone. They further stated that the Leydig cell tumorigenesis seen in the invalid 78-week rat carcinogenicity study may be related to the increased serum LH levels and a decrease in the testicular testosterone levels. However, this theory could not explain the observed Leydig cell tumorigenesis in the Fisher 344 rat (Turek and Desjardins, JNCI, 63(4): 969-975, 1979, sponsor's submission, v 26 page 169) where the serum LH levels were either normal or too low to measure. Though estrogens or some antiandrogens may produce Leydig cell tumors, lansoprazole was found not to show any such activity in the rat.

REVIEWER'S ADDENDUM:

It is a non-GLP study and no data were provided, therefore this study is not a valid study.

20. Study of Lansoprazole Associated Preimplantation Loss in Female Rats: (Study # R & D 93/539, A-93-021)

Testing Laboratory:

Dates of Start and Completion of the Study: February 5, 1993 and November 3, 1993.

Animals: Two hundred and twenty (110/sex) Cr1:CDRBR mature rats weighing about 200 g were used.

Batch #: 62-214-AL.

Methods: In the previous Segment I. Fertility and reproductive toxicity study in rats (Study # TA 90-146/r&d91/299) significant reduction in number of implantations without a decrease in the number of corpora lutea were seen in rats included in 150 mg/kg/day treatment group. Therefore the present study was designed to confirm the preimplantation losses and to define the

timing and nature of the losses. One hundred and five female rats were divided into 5 groups of 15 rats and were administered lansoprazole as per the following schedule (shown in the table below):

Treatment Group	Dosage (mg/kg/day)	Treatment Period ^a	No. of Female Rats ^b
T ₀	0 (vehicle) ^c	P, M, GD 0-8	15 (15)
T ₁	150	P, M, GD 0-8	15 (15)
T ₂	150	P, M, GD 1	15
T ₃	150	GD 1-3	15
T ₄	150	GD 1-8	15

- ^a P = 14 days pre-mating. M = Mating. GD = Gestational Day
^b Fifteen female rats in groups T₀ and T₁ (within parentheses) constituted a satellite subgroup for hormonal determination.
^c Vehicle = 5% gum arabic adjusted to pH 7 with KOH

Lansoprazole was administered in 5 % gum arabic (pH 7.0) suspension orally by gavage in a volume of 10 ml/kg. All animals were observed twice daily, their weight recorded every week, and physical examination of the animals was observed 1/2 hr after the administration of the compound. Blood samples (1 ml/rat) was collected from the animals assigned for for plasma hormone estimation groups (control group and animals in a group treated 14 days prior to mating, during mating and up to gestation day 8). Estradiol, progesterone and prolactin levels were determined by RIA methods. Corpora lutea and number of implantations were counted in these animals. All surviving females were sacrificed on day 9 of gestation. The distribution of live and dead fetuses/uterine horn determined and presence of empty implantation and resorbed sites noted.

Results:

1. General Effects on Dams: No treatment related effects were seen in female rats treated with 150 mg/kg of lansoprazole during the study. No significant change in retardation of body weight gain was observed in animals. The number of corpora lutea in the animals belonging to different interval treatment groups were 17.2, 16.59, 16.85, 17.23 and 16.5 in female rats belonging to 5 different treatment groups. Preimplantation losses were also not affected and were comparable with control group of animals.

2. Effect of lansoprazole on Female Hormone Levels: Estradiol, progesterone and prolactin levels of animals belonging to the control and treatment groups were not significantly different from one another during the study. The levels of these hormones on day 14 and 21 of the gestation were as shown in the following table.

Table For Mean Effect on Corpora Lutea and Implantation Losses

	Control	Treatment Group			
		T ₁	T ₂	T ₃	T ₄
1. Incidences of Preimplantation Losses (%)	8.5	7.7	8.8	9.0	2.0
2. Implantation Sites (Mean)	15.75	15.3	15.4	15.7	16.1
3. # Corpora Lutea	17.2	16.6	16.8	17.2	16.6

In summary, administration of lansoprazole during premating, mating and gestation day (0-8, 1, 1-3 or 1-8) did not affect incidences of preimplantation losses and corpora lutea. Therefore, the adverse effects on fertility parameters, noted in the previous study (#TA 90-146) could not be confirmed in the study.

21. Estimation of Serum Testosterone and LH Levels in 19-Week Subchronic Toxicity Study in Aged Rats: (Study # A-29-1532)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

Dates of Start and Completion of Study: March 24, 1992 and July 8, 1992

OAU and GLP Standard: Statement of compliance was included.

Animals: Forty male SD rats about 65 weeks old and weighing between 630 to 793 g were used in the study.

Methods: The study was conducted to examine the influence of serum LH and testosterone concentrations on the morphology of Leydig's cells. These animals were divided in 4 groups (10/group) and administered a dose of either vehicle (0), 15, 50 or 150 mg/kg/day of lansoprazole suspension in 5 % gum arabic, orally for 19 weeks in a volume of 5 ml/kg by gavage. The doses of this study were selected on the basis of a 5-week preliminary study in which a dose of 150 mg/kg/day increased incidence of hyperplasia of Leydig cells in aged (37 weeks old) rats. In the present study, animals were observed twice daily and their physical signs recorded twice per week through out the treatment period and; weighed once per week. Blood samples from all the animals/group were collected from orbital sinus at week 0, 2, 4 and 6 after 15, 29th and 44th dosing. During this study, a

modification in the schedule of collecting blood samples was made by sponsor as lansoprazole treatment produced no effect on serum LH concentrations. The blood samples after 57th, 59th, 61st, 92nd, 94th, 98th and 100th day of dosing were collected at 4, 8, 23, 6, 2, 4 and 8 hr respectively. The basis of change of schedule of blood samples collection was not sound as lansoprazole have a $t_{1/2}$ of less than 2 hr in rats, therefore the collection of blood samples during initial 2 hr period should have been more frequent. Serum LH and testosterone were estimated by radioimmunoassays. All of the surviving animals were fasted for 20 hr, weighed, exanguinated and dissected. The testes, prostate, seminal vesicles and pituitary were examined visually and these organs were separated and weighed. The testes were fixed in 10 % formalin and embedded in paraffin, sectioned and stained with hematoxylin and eosin and examined microscopically to determine proliferative lesions of Leydig cells which were classified as either hyperplastic nodules with reduced diameter of seminiferous tubule or lesions without nodules.

Results:

i. Observed Effects: Lansoprazole treatment produced no effect in rats. But, the table of observations were not provided with the submission, therefore this claim could not be confirmed.

ii. Mortality: Seven animals (1, 3, 2 and 1 rats belonging to control, 15, 50 and 150 mg/kg/day treatment group respectively) died during the study. The death of 1 of the animals of 15 mg/kg/day treatment group was due to intubation error and the death of other 2 animals was due treatment related causes of slight to moderate degree of atrophy of seminiferous tubules epithelium.

iii. Body Weight Changes: A significant body weight loss of 13.8 % ($p < 0.01$) was seen in animals belonging 150 mg/kg/day treatment group. No effect on the body weights of the animals included in other treatment groups were seen.

iv. Serum Hormones Levels: During pretreatment period, there were much fluctuations in serum testosterone concentrations of animals of various treatment groups. The mean serum testosterone levels in control animals varied from 0.33 to 0.48, 0.33 to 0.66 and 0.64 to 0.74 ng/ml at 4, 5 and 23 hr respectively. A reduction ($p < 0.05$ to 0.01) of testosterone concentrations of the animals included in 150 mg/kg/day treatment group were seen. But these changes were only of the statistical importance and not of biological relevance. The serum concentration data of testosterone and LH hormones are shown in the following tables taken from sponsor's submission (vol 2.73, pp 262 and 263):

Table 2 Serum testosterone concentrations (ng/ml)
- Group mean values (mean $\pm$ S.E.) -

Dosing period	Hrs after dosing	Treatment groups (n=10)			
		1M Control	2M AG-1749 15 mg/kg	3M AG-1749 50 mg/kg	4M AG-1749 150mg/kg
Serum testosterone concentration (ng/ml)					
Pre	14:00	0.48±0.35	0.33±0.28	0.48±0.33	0.40±0.24
	15:00	0.66±0.38	0.50±0.25	0.69±0.33	0.52±0.30
	9:00	0.64±0.46	0.62±0.22	0.74±0.35	0.78±0.47
1st	4 ⁺	0.81±0.55	0.68±0.45	0.51±0.35	0.43±0.33 ⁺
	5 ⁺⁺	0.83±0.40	0.66±0.34	0.50±0.30 ⁺	0.40±0.23 ⁺⁺
	23 ⁺	0.47±0.31	0.28±0.19 ⁺	0.24±0.21 ⁺	0.17±0.16 ⁺⁺
15th	4 ⁺⁺	0.58±0.26	0.47±0.31	0.29±0.33 ⁺⁺	0.20±0.15 ⁺⁺
	5 ⁺	0.64±0.22	0.59±0.43	0.38±0.27 ⁺	0.37±0.28 ⁺
	23	0.28±0.18	0.37±0.26	0.35±0.36	0.17±0.11
29th	4	0.40±0.21	0.43±0.15	0.24±0.17	0.30±0.23
	5 ⁺⁺	0.54±0.27(1)	0.50±0.14	0.45±0.17	0.27±0.17 ⁺⁺
	23	0.25±0.18(1)	0.33±0.16	0.25±0.19	0.17±0.16
44th	4 ⁺⁺	0.43±0.17(1)	0.29±0.15 ⁺	0.24±0.24 ⁺	0.11±0.12 ⁺⁺
	5 ⁺⁺	0.49±0.17(1)	0.42±0.13	0.38±0.22	0.17±0.15 ⁺⁺
	23	0.31±0.10(1)	0.50±0.32	0.27±0.24	0.25±0.26
57th	4 ⁺	0.46±0.28(1)	0.46±0.32	0.33±0.26	0.22±0.21 ⁺
59th	8	0.56±0.36(1)	0.68±0.29	0.40±0.27	0.39±0.28
61st	23	0.55±0.29(1)	0.45±0.32	0.31±0.21	0.42±0.44
94th	2	0.36±0.27(1)	0.57±0.30(2)	0.41±0.28	0.37±0.34
98th	4	0.39±0.23(1)	0.48±0.42(2)	0.38±0.30(1)	0.22±0.22
92nd	6	0.35±0.15(1)	0.37±0.19(2)	0.27±0.17	0.21±0.17
100th	8	0.43±0.34(1)	0.33±0.33(3)	0.58±0.96(1)	0.35±0.51

+ : p<0.05 vs control

++ : p<0.01 vs control

+ : Significantly negative trends as for the AG-1749 treated groups. p<0.05

++ : Significantly negative trends as for the AG-1749 treated groups. p<0.01

Number in parentheses indicates the number of animals that could not be determined because of death or shortage of serum.

Table 3 Serum LH concentrations (ng/ml) - Group mean values (mean $\pm$ S.D.) -

Dosing period	Hrs after dosing	Treatment groups (n=10)			
		1M Control	2M AG-1749 15 mg/kg	3M AG-1749 50 mg/kg	4M AG-1749 150 mg/kg
Serum LH concentration (ng/ml)					
Pre	14:00	14.4 ± 7.1	15.0 ± 7.3	16.2 ± 7.2	13.8 ± 10.4
	15:00	15.5 ± 2.5	14.7 ± 5.4	15.3 ± 6.1	12.5 ± 9.2
	0:00	14.8 ± 8.8	15.4 ± 6.6	13.8 ± 3.7	10.6 ± 5.5
1st	4	12.2 ± 8.4(3)	11.6 ± 6.0	13.6 ± 5.4	10.0 ± 7.0(1)
	5	11.4 ± 8.5(1)	10.5 ± 5.1(1)	8.8 ± 3.8(1)	9.9 ± 7.4
	23	8.2 ± 2.9(1)	15.3 ± 8.2	13.0 ± 5.5	8.8 ± 3.9(1)
15th	4	11.8 ± 6.6	13.7 ± 6.7(1)	12.8 ± 7.3	10.2 ± 5.0
	5	10.8 ± 5.3(1)	13.4 ± 8.8	11.9 ± 4.0(1)	11.0 ± 4.5
	23	11.0 ± 7.2	11.7 ± 8.3	11.5 ± 5.2	8.7 ± 3.4
29th	4	15.1 ± 11.2	13.8 ± 7.0	19.5 ± 12.8	14.5 ± 8.0
	5	12.8 ± 5.9(2)	12.5 ± 9.5	14.9 ± 6.1	11.9 ± 5.0
	23	13.5 ± 10.1(1)	16.0 ± 8.8	14.8 ± 8.9	11.2 ± 5.3
44th	4	7.7 ± 4.1(2)	11.8 ± 6.2	16.3 ± 7.1*	10.7 ± 5.8* (1)
	5	12.2 ± 7.8(2)	18.0 ± 10.3(1)	15.1 ± 6.1	10.9 ± 4.4
	23	12.6 ± 8.0(2)	15.7 ± 11.3	10.2 ± 4.5	13.4 ± 6.9(1)
57th	4	15.3 ± 9.2(1)	15.2 ± 8.2	13.3 ± 5.4	11.6 ± 5.8
59th	8	14.3 ± 8.9(1)	13.4 ± 5.5	10.2 ± 2.7	11.4 ± 4.1
61st	23	12.6 ± 8.2(1)	11.4 ± 5.8	14.1 ± 7.5	11.5 ± 3.6
84th	2	17.7 ± 16.3(1)	18.8 ± 5.7(2)	14.3 ± 4.8	16.2 ± 5.9
98th	4	17.7 ± 11.1(1)	15.0 ± 7.3(2)	12.8 ± 6.4(1)	13.0 ± 8.2
92nd	6	20.6 ± 9.3(1)	20.3 ± 10.1(2)	25.8 ± 16.3	20.6 ± 9.9
100th	8	16.2 ± 10.9(1)	15.0 ± 8.3(3)	14.8 ± 6.9(1)	13.9 ± 11.9

* : p<0.05 vs control

Number in parentheses indicates the number of animals that could not be determined because of death or shortage of serum.

vii. Organ Weights: The mean absolute or relative (body weight ratio) weight of testes, ventral prostate, seminal vesicle and pituitary gland were affected during this treatment.

viii. Gross Pathology: No gross pathological changes were reported, but the table for pathological changes was not included in the submission.

ix. Histopathological Changes: Hyperplasia of Leydig cell was treatment related as 1, 4 and 5 animals included in control, 50 and 150 mg/kg/day treatment groups, respectively were found to have this hyperplasia. The number of hyperplastic lesions were also treatment related as the mean number of 1, 8 and 8.4 lesion/animal were seen in animals included in control, 50 and 150 mg/kg/day treatment groups, respectively.

In summary, lansoprazole treatment up to 150 mg/kg/day dose increased the incidences of ledig's cell hyperplasia and number of hyperplastic nodules. The testosterone and LH levels were not altered during the treatment in these animals. Therefore hyperplasia seen in these animals was not related to change in serum testosterone levels and they affect by other mechanism.

22. Effect of Testosterone Supplement on Lansoprazole Induced Hyperplasia and Leydig Cells Tumors in Aged F344 Rats: (Study # A-29-1533)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

Dates of Start and Completion of Study: March 2, 1992 and July 8, 1992

QAU and GLP Standard: Statement of compliance was included.

Animals: Sixty male F344 rats about 37 weeks old and weighing between 314 to 356 g were used in the study.

Methods: The study was conducted to examine the influence of serum LH and testosterone concentrations on the morphology of hyperplastic Leydig's cells and Leydig cell timor. Sixty animals were divided into 4 groups (15/group) and 2 of these groups were given an oral dose of 0 (vehicle) and 150 mg/kg/day of lansoprazole suspension in 5 % gum arabic. To the remaining 2 groups of animals, 2 tubes containing 4.1 mg/tube (i.e., 27.3 mg/kg) were implanted subcutaneously and then were administered a dose of either 0 or 150 mg/kg/day of lansoprazole in a volume of 5 ml/kg/day. Five animals in these groups were given lansoprazole for 23 days and the other 10 received the compound for 26 days. The dose selection of lansoprazole was made on the basis of previous studies with young SD rats while the dose of testosterone was selected on the basis of a previous study by F. Chatani (Stimulatory effect of luteinizing hormone on the development and maintenance of 5 α -reduced steroid-producing testicular interstitial cell tumors in F 344 rats. Anticancer Research 10: 337, 1990) in which they showed that a subcutaneous dose of testosterone could antagonized the Leydig;'s cells hyperplasia. This study was undertaken to determine the effect of testosterone supplement on lansoprazole induced serum

testosterone and luteinizing hormones (LH) and morphology of Leydig cells. Animals were observed and weighed daily and detailed physical examination was performed twice per week during study. Blood samples from 10 animals/group were collected at 4 and 6 hr after the administration of the compound from tail vein at week 1, 4, 13 and 26 of the dosing period. Serum samples were used to estimate LH and testosterone by radioimmunoassays. Five animals per group were sacrificed 23 weeks after dosing and the remaining after 26 weeks of treatment (26 weeks treatment). Animals were fasted for 20 hr and weighed prior to sacrifice and dissected. The testes, prostate, seminal vesicles and pituitary were examined visually and these organs were separated and weighed. The testes were fixed in 10 % formalin and embedded in paraffin, sectioned and stained with hematoxylin and eosin and examined microscopically. Proliferative lesions of Leydig cells were classified into hyperplastic nodules and proliferative lesions without nodules. The number of hyperplastic nodules of tumors of leydigs cell/cut surface of the testes were counted.

Results:

i. Observed Effects: Testes of animals included in testosterone alone or testosterone plus lansoprazole treatment groups were atrophied (on physical examination) after week 15 of treatment as 0, 1, 14 and 15 out of 15 animals in control, lansoprazole alone, testosterone alone and testosterone plus lansoprazole treated groups respectively had small testes. The number of animals affected by treatment increased with the duration and at week 19 of the study, 1, 7, 15 and 15 out 15 animals in each of the control, lansoprazole alone, testosterone alone and testosterone plus lansoprazole treated groups respectively had small sized testes. The testicular atrophy was seen up to 26 weeks of the study.

ii. Mortality: None.

iii. Body Weight Changes: A significant ($p < 0.01$) body weight loss of 5.6 and 1.8 % was seen at week 26 week of the study in animals belonging to lansoprazole and lansoprazole plus testosterone treatment groups respectively during the study. The maximum body weight loss of 6.3 and 5.4 % occurred during week 6 of the study.

iv. Serum Hormones Levels: Lansoprazole treatment did not affect mean serum testosterone concentration in rats. Animals included in testosterone plus lansoprazole treatment group were reported to have significant ($p < 0.01$ to 0.05) reduction in serum testosterone after 4 hr of lansoprazole treatment during week 1. But these were no no biological relevance as the levels were still within the range. Serum testosterone concentration

in lansoprazole plus testosterone treated animals was higher (ng/ml) than animals included in control (ng/ml) or lansoprazole alone (ng/ml) treatment group. But, serum testosterone concentration of these animals was within the $\pm$ SD, therefore not different. Lansoprazole treatment did not produce any change in testosterone concentration.

Lansoprazole treatment up to 26 week, did not affect serum LH concentration either in control or testosterone retreated rats.

v. Organ Weights: The absolute and relative weight of testes were significantly ($p < 0.05$) decreased in rats included in testosterone or testosterone plus lansoprazole treatment groups respectively. But lansoprazole treatment did not produce any effect on testes weight or pituitary weight.

viii. Gross Pathology: Lansoprazole treatment produced a decrease in testicular size, intratesticular white or red foci in rats treated with the compound up to 23 or 26 weeks. Testosterone pretreatment increased lansoprazole induced effect on testes size only as the intratesticular white or red foci were decreased in these animals.

ix. Histopathological Changes: Lansoprazole treatment increased the intensity (grade) of nodular hyperplasia of Leydig cells, i.e., slight hyperplasia was seen in control group animals and moderate intensity hyperplasia was seen in animals included in 150 mg/kg/day treatment groups of F344 rats, respectively. The number of nodular hyperplasia of Leydig cell in lansoprazole treated animals were decreased by testosterone as 2.6 and 4.5 neoplastic lesions were seen in animals treated with testosterone. These were 36 and 38.6 in control and 150 mg/kg/day of lansoprazole plus testosterone respectively. Therefore, testosterone supplement throughout the study, reduced the nodular hyperplasia.

The incidence of leydig cell tumors in F344 rats are very high and this tumor develops earlier in this strain than SD strain of rats. Therefore, the results of the study may not be applied to SD rats.

In summary, lansoprazole treatment increased both the size and number of hyperplastic nodules and tumors of Leydig cells. Testosterone implantation reversed these abnormalities, therefore testosterone may be directly implicated with hyperplastic nodules of Leydig cells.

23. Effect of Lansoprazole on Adrenal Steroidogenesis by a Loading Dose of ACTH In Rats: (Study # A-29-1547)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

Dates of Start and Completion of Study: Initiation date was not given but study report date was September 30, 1992.

OAU and GLP Standard: Statement of compliance was not included.

Methods: Seventy male 10 weeks old SD rats were divided in 7 groups (10/group). Out of these, 3 groups received a single dose of either 0, 500 or 5000 mg/kg/day of lansoprazole. Animals in group IV received a single dose of 100 mg/kg/day of ketoconazole. To the remaining 3 groups, lansoprazole was administered at a dose of 0, 150 or 500 mg/kg/day for 4 weeks. A loading dose of ACTH (10 IU/kg) was administered s.c. either 3 hr after a single dose or 3 hr after the last dose of the 4-week dosing period. Blood samples (10 rats/time period/group) for estimating the total serum corticosteroid, were collected at 15, 30 and 60 minutes after ACTH injection from rat tail artery.

Results:

A single dose of lansoprazole (up to 5000 mg/kg/day) did not affect ACTH stimulated serum corticosterone whereas Ketoconazole at 100 mg/kg/day time dependently inhibited stimulated serum corticosteroid synthesis. A prolonged treatment with a dose of 500 mg/kg/day of lansoprazole for 4 weeks, also did not affect corticosteroid levels. But a lower dose (150 mg/kg/day) of lansoprazole reduced ($p < 0.01$ to 0.05) serum corticosteroid levels by 21.9 %, 12.2 % and 14.1 % after 15, 30 and 45 min of corticosterone administration. Since it was not a dose related effect, therefore this was considered not dose related.

In summary, a single dose of lansoprazole (up to 5000 mg/kg) or a prolonged treatment of the compound for 4 days (up to 500 mg/kg/day) did not affect steroidogenesis.

24. In Vitro Interaction of Lansoprazole and its Metabolites with Taurine: (Study # A-29-2036)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

OAU and GLP Standard: Statement of compliance was not included.

Methods: This study was conducted to determine the possible interaction of lansoprazole or its metabolites with ^{14}C -taurine (1mM, 1kBq/10nmol) in presence and absence of rat liver and retina homogenates. The radioactivity was located and quantified by radioluminography on a photographic plate.

Results:

In the presence of liver S-9 and retinal homogenates, no radioactive spots except of taurine were reported. Taurine was not metabolized/degraded in the presence of hepatic or retinal homogenates. Therefore, lansoprazole and its metabolites did not interact with taurine in vitro.

25. Electroretinographic Study in Rats Treated Orally with Lansoprazole for 4 Weeks: (Study # A-29-1990/1647-vt)

Testing Laboratory: Pharmaceutical Development Division, Takeda Chemical Industries Ltd., Japan

OAU and GLP Standard: Statement of compliance was not included.

Methods: This study was conducted on September 10, 1993 and reported on February 9, 1994. The aim of the study was to determine lansoprazole effect on rat retina after 4 weeks of treatment. Twenty four rats (12/sex) were divided in to 2 groups and treated with a dose of either 0 or 300 mg/kg/day of lansoprazole for 4 weeks in a volume of 5 ml/kg/day. The dose selection of the study was not provided. The necropsy of the animals was done at 2, 4 and 24 hr after the final dosing. The animals were observed daily and weighed weekly during the study. Electroretinogram recording was made in anesthetized animal at pretreatment and 2 times during the study. Animals were killed after 4 weeks of treatment, eye ball removed and examined externally and fixed with glutaraldehyde in buffer, stained with hematoxylin and eosin and examined microscopically.

Results:

i. **Observed Effects:** According to sponsor, no effects were seen.

ii. **Mortality:** None.

iii. **Body Weight Changes:** A significant retardation in the body weight gain ($p < 0.05$) in male animals was observed during the study. But no appreciable effect on the body weight gain was seen in females during this period.

iv. Electroretinographic Observations: Amplitudes of the alpha and beta waves in female animals were less in the animals of the treatment group from the control group animals. The wavelet indices and a- and b- waves latencies were not affected by lansoprazole treatment. At week 4, the mean latencies of alpha waves were 17.91 and 16.62 msec in males; and 16.98 and 17.29 msec in females in control and 300 mg/kg/day treatment groups, respectively. The beta wave latency at week 4 was: 56.67 and 53.82 msec among males; and 54.67 and 54.98 msec in females in control and 300 mg/kg/day treatment groups, respectively. These changes were not statistically different in the control and treated groups animals on week 4 of the study. Therefore, no change was noted in the retina morphologically and lansoprazole was not toxic rat retina.

v. Histopathological Changes in Retina: In one male (# 11) animal, a mild decrease in alpha wave which was not statistically significant and different from the control animals, therefore not important therefore not was not treatment related.

In summary, lansoprazole at a dose of 300 mg/kg/day for 4 weeks did not produce any morphologically changes on the retina of the rats.

26. Electroretinographic Study in Rats treated with Oral Dose of Lansoprazole for 13 Weeks: (Study # A-29-1991/1648-vt)

Testing Laboratory: Pharmaceutical Development Division, Takeda Chemical Industries Ltd., Japan

OAU and GLP Standard: Statement of compliance was not included.

Methods: This study was conducted to study the effects of lansoprazole on the morphology and retinal functions of retina. The study was conducted [on September 10, 1993 and reported on March 1, 1994] to determine the effect of orally administered lansoprazole (Batch # M610-050 suspension in 5 % gum arabic (pH 7.0), on retina of rats treated for 13 weeks. The dose selection of the study was based on 3-month toxicity study (Study # TA90-152) in which a dose of 150 mg/kg/day was an MTD. During this study, 24 rats (12/sex) of 17 weeks of age, were divided in to 2 groups and treated with an oral dose of either 0 or 150 mg/kg/day of lansoprazole for 13 weeks in a volume of 5 ml/kg/day. The animals were observed daily and weighed weekly during the study. Electroretinographic readings were recorded in anesthetized animal (Ketamine 50 mg/kg, i.m.) at pretreatment and 24 hr after dosing in week 6 and 12 during the study. The animals were given a photo stimulation with SLS-3100 and a xenon lamp for light stimulation. The

amplitude of ERG was measured to a lowest point of a-wave and from b point (b-waves). Animals were killed after 13 weeks of treatment, eye ball removed and examined externally and fixed with glutaraldehyde in buffer, stained with hematoxylin and eosin and examined microscopically.

Results:

i. Observed Effects: According to sponsor, no effects were seen.

ii. Mortality: None.

iii. Body Weight Changes: A significant retardation ($p < 0.05$) in the body weight gain of 47.4 and 33.7 g in male and female animals respectively was observed during the study.

iv. Electro-retinographic Observations: Amplitudes of the alpha, beta waves and wavelength indices in female animals were not significantly different in the animals included in the treatment group from those of control group animals. The alpha and beta waves in rats included in treatment group were not affected during the study. These were: 329, 310.8, 321.7 and 327 uV in control group males and, 321.8, 318.5, 318.2 and 322.7 uV in treated group males at week 0, 6 and 12. In females, the amplitudes were 347.7, 376.7, 361.3 and 371.1 in control and, 354.9, 379.9, 361.3 and 353.9 uV at week 0, 6 and 12 of the study.

v. Histopathological Changes in Retina: No histopathological changes of eyes were mentioned.

In summary, lansoprazole up to a dose of 150 mg/kg/day for 13 weeks did not produce an adverse effect on the retina of the rats.

27. Electroretinogram (ERG) in Rats Treated Intravenously with Lansoprazole for 13 Weeks: (Report A-29-1992/1657-vt/IND 41,938 Amendment dated 6/13/94/Pharmacology Review dated 7/19/94)

Testing Laboratory: Drug Safety Research Laboratories
Takeda Chemical Industries Ltd.
Osaka, Japan

Study Started: September 13, 1993

Report Dated: February 22, 1994

GLP Requirements: Not mentioned.

Animals: Cr1:CD(SD) rats (14-15 weeks old, males: 412-461 g and females: 251-289 g).

Drug Batch No.: 2338711

Methods: In mouse carcinogenicity study, Lansoprazole produced retinal atrophy at 15 mg/kg/day and higher dose levels. In the present study sponsor assessed the effects of Lansoprazole (30 mg/kg/day, I.V. for 13 weeks) on retinal function and morphology in rats. Eight male and 8 female rats were given daily I.V. injection of Lansoprazole (30 mg/kg/day, 5 ml/kg/day, pH 10-11) for 13 weeks. The control group rats (6/sex) were given vehicle (not identified, adjusted to pH 10-11) in similar fashion. All animals were observed daily for clinical signs and mortality daily. Body weights were recorded at pretest, twice weekly during weeks 1-4 and weekly thereafter. Retinal function was determined by electroretinogram (ERG) recording. ERG was recorded from left eye of each rat at pretest and at 24 hr after drug administration during week 6 and 12 of the study. Briefly, animals were anesthetized and their pupils were dilated with mydriatic, then left eye of each animal was fitted with contact lens type electrode. Before recording rats were placed for 90-120 min in dark then ERG was evoked by 32 light flashes at 2-sec interstimulus intervals. Amplitude of a-wave, b-wave and wavelet index, and latencies of a-wave and b-wave were measured. At the end of treatment period, all animals were sacrificed and eyeball was subjected to complete gross pathological examinations. Left eye balls were fixed with a mixture of glutaraldehyde and phosphate buffered formalin and the right eyes were fixed with Davidson's solution. Both eyes of all animals were sectioned, stained with hematoxylin and eosin and examined histopathologically.

Results:

1. **Observed Effects:** No treatment related effects were seen.
2. **Mortality:** None
3. **Body Weights:** At the end of treatment period body weight gains were decreased by 25% in males and 30% in females at 30 mg/kg/day. Food intakes were not reported.
4. **Electroretinogram (ERG) Findings:** No treatment related effects on ERG parameters (amplitudes of a-wave and b-wave, wavelength index and latencies of a-wave and b-wave) were seen.
5. **Gross Pathology:** No abnormality was seen in eyeballs.
6. **Histopathology:** No abnormality was seen in eyeballs.

Thus no retinal toxicity was evident when 30 mg/kg/day of Lansoprazole was given intravenously to SD rats for 13 weeks.

28. Taurine Levels of Liver, Retina and Serum in Young Rats Treated with Lansoprazole for 4 Weeks: (Study # A-29-2040/6199-vt)

Testing Laboratory: Research & Development Division, Takeda Chemical Industries Ltd., Japan

OAU and GLP Standard: Statement of compliance was not included.

Methods: Lansoprazole (Batch # M610-050) was administered in 13 week old [Crj:CD (SD)] rats (30/sex) at an oral dose of either 0 or 300 mg/kg/day in gum arabic suspension for 4 weeks in a volume of 5 ml/kg/day. The dose selection of the study was based on a previous 3-month toxicity study in which 150 mg/kg/day was an MTD and 300 mg/kg/day dose produced retardation in body weight gain and treatment related liver toxicity in rats. The necropsy of the animals was done at 2, 4 and 24 hr after the final dosing. The animals were observed daily and weighed weekly during the study. Five animals per time group, i.e., at 2, 4 and 24 hr after the final dose were killed, blood collected, liver and eye tissues were separated for Na^+, K^+ -ATPase activity (umoles/mg protein/hr), taurine and its metabolites concentration by a HPLC technique.

Results:

i. Observed Effects: According to sponsor, no effects were seen.

ii. Mortality: None.

iii. Body Weight Changes: A significant retardation in the body weight gain of 43.6 and 41.9 % ($p < 0.01/0.05$) in body weight gain in male and female animals was observed during the period. This was associated with a reduction of 19.8 and 19.2 % in food consumption in males and females, respectively. The food consumption of males in control and treatment group males was 28.3 and 22.7 g/animal/day on week 4 of the study. In females, the daily food consumption was 20.3 and 16.4 g/animal/day, respectively.

v. Blood/Tissue Taurine and Related Compounds Estimation: Sponsor measured taurine levels in liver and retina on the assumption that decreased levels of taurine leads to retinal degeneration, (ii) lansoprazole affects hepatic cysteinesulfinic acid decarboxylase (CSAD) activity and (iii) taurine levels in rats is maintained by synthesis of taurine in rat liver which is dependent on cysteine decarboxylase activity. CSAD activity was about 1000 times high in rats than man. Sponsor did not provide tables for the levels of taurine and cysteinesulfinic acid in liver, retina and plasma but histograms were given. From these histograms, the following was approximated. Liver taurine levels were decreased ($p < 0.01$ to 0.05) in male and female animals after 4 and 24 hr of lansoprazole administration. Hypotaurine concentration in liver was not affected. Liver cysteinesulfinic acid were not affected during the study. Retinal taurine levels appeared to be not affected up to 24 in

males or females but hypotaurine concentration was increased in rats of both sexes during 2 hr after its administration and was decreased at 4 and 24 hr. Cysteinesulfinic acid concentration in the liver of male and female animals was increased at 4 and 24 hr of the administration of the compound. Serum concentrations of taurine were not affected in animals during the study. The effect on the retinal Na^+, K^+ -ATPase activity ($\mu\text{moles/mg protein/hr}$) in male (5.26 vs 5.32 in control) and female (4.6 vs 5.01 in control) rats was not of clinical significance.

In summary, lansoprazole treatment at 300 mg/kg/day produced no effect in liver, retina and serum taurine, hypotaurine or cysteinesulfinic acid concentrations in rats. Therefore, sponsor's contention that lansoprazole and its metabolites affected retinal taurine was not valid.

29. Hepatic Drug Metabolizing Activity in Mice and Rats after 14-Days of Oral Treatment with Lansoprazole: (Study # R&D/93/719)

Testing Laboratories:

Study Start and Completion Dates: September 16, 1993 and October 134, 1993.

GLP Requirements: A Statement of Compliance with GLP Regulations was included.

Animals: (i) Female Crl:CD-1 BR mice weighing 20 to 30 g and (ii) Crl:CD.BR female rats weighing about 150 g.

Drug Batch No.: 62-214-AL

Methods: Thirty female mice were divided into 3 groups (10/group) and were given an oral gavage dose of 300 mg/kg/day of lansoprazole for 14 days (in 5 % gum Arabia at pH 7.0). In second part of the study, 30 female rats were divided into 3 groups (10/group) and were given the same oral gavage dose of 300 mg/kg/day of lansoprazole for 14 days (in 5 % gum Arabia at pH 7.00). The vehicle control, test compound and phenobarbitone were administered in a volume of 10 ml/kg/day). The selected doses of phenobarbitone and lansoprazole were equivalent to 200 $\mu\text{mol/kg/day}$. The purpose of the study was to compare the induction of hepatic mucosal drug metabolizing cytochrome P4502B (CYP2B) potential of phenobarbitone or lansoprazole in rat and mice. The dose of lansoprazole in the study was selected on the observation of hepatomegaly at an oral dose of 15 to 150 mg/kg/day for 5 to 30 days. In this study the animals were killed after 24 hr of the last dose of the compound, their liver removed, weighed and homogenized for the preparation of mitochondrial supernatant (S-9 fractions). The pooled liver preparations were processed for the cytochrome P-4 activity

and 7-pentoxoresorufin O-dealkylase activity (7-PRODase) by fluorescence spectrophotometric methods. The liver of the individual animal was removed and weighed to assess the effect of phenobarbitone and the compound.

Results:

1. Body Weight and Relative Liver Weight: The body weight changes in the animals included in lansoprazole or phenobarbitone treatment groups were insignificant. A treatment related effects were seen in liver weights of mice included in 300 mg/kg/day of lansoprazole treatment group. In rats, a dose of 150 mg/kg/day (p.o.) of lansoprazole was reported to increase the relative weights of liver significantly ($p < 0.05$ and 0.001 in animals included in lansoprazole or phenobarbitone treatment groups).

2. Total Cytochrome P-450 and 7- Pentoxoresorufin O-Dealkylase Activity: Cytochrome P-450 activity was increased by 93 and 45 % in phenobarbitone and lansoprazole treated groups mouse, respectively (On $\mu\text{mol/kg}$ basis, lansoprazole activity was one fourth that of phenobarbitone in mouse). The total cytochrome P-450 activity was increased by 120 and 40 % in rats included in rats included in phenobarbitone and lansoprazole treatment groups, respectively (On $\mu\text{mol/kg}$ basis, lansoprazole activity was one third that of phenobarbitone in rats).

In lansoprazole treated mice, an increase of 306 % in the induction of 7-PRODase activity was reported (VS 1607 % in phenobarbitone treated animals). Lansoprazole activity was 1/5th of phenobarbitone in mice. In rats, lansoprazole activity was 1/36 of phenobarbitone activity.

In summary, the liver enzyme induction activity of lansoprazole was weaker than phenobarbitone. Total cytochrome P-450 activity and 7-PRODAase activities were about half and one fifth in the mouse. These activities were one third and thirty seventh in rats. Therefore, lansoprazole was an inducer of liver enzymes but weaker than phenobarbitone.

30. Distribution of ^{14}C -Lansoprazole and ^{14}C - Omeprazole in Occular tissues in Female Rats: (Study # A-29-2001)

Testing Laboratory: Pharmaceutical Development Division, Takeda Chemical Industries, Japan.

OAU and GLP Standard: Statement of compliance was not included.

Methods: Sixty female Crl:CD (SD) rats weighing 238-271 g and, 60 female Long Evans (LE) pigmented rats weighing 223-248 g were used in the study. The age of animals at the start of the study was about 10 weeks. Rats of each of the strains were divided in to 2 groups (30/group). An oral dose of 50 mg/kg of either ^{14}C -lansoprazole (37.8 Mbq/10ml/kg) or ^{14}C -omeprazole (34.9 Mbq/10 ml/kg) was given to 1 group of SD and 1 group of pigmented rats. After a single dose, blood samples were collected from

abdominal a 25, 1, 2, 4, 8 and 24 hr for the estimation of metabolites. At the same time retina, sclera (including pigment vit choroid, iris, ciliary body), lens and viterous body, were separated. The ocular tissue was weighed and homogenized for the determination of ^{14}C concentrations.

Results:

i. Concentration of Lansoprazole: The ^{14}C tagged radioactivity of lansoprazole in the lens, retina, viterous body and plasma were comparable with those of LE rats. But the concentration of radioactivity in sclera of SD rats was different from the LE rats. The maximum concentration was attained in 2 hr in SD rats and in 4 hr in LE rats. The concentrations of radioactivity were μg lansoprazole eq/g in SD and LE rats respectively indicating that it accumulated in melanin containing tissues. This has been shown in the following table taken from sponsor's submission (amendment dated June 30, 1994, vol. 12.2, pp 179 and 180).

The radioactivity declined gradually and could be detected after 24 hr. In pigmented rats, the plasma concentrations were slightly higher than SD rats (5.38 in SD and 8.79 μg eq/ml in LE rats). Sclera concentrations were higher from the initial observation period of 0.25 hr to the total period of 24 hr. This indicated that lansoprazole accumulated in melanin tissues of pigmented rats.

Lansoprazole and its metabolites were identified in the plasma, retina, and sclera of both SD and LE rats. The plasma to sclera ratio in albino and pigmented ratios were 0.36 and 10.07, respectively at 4 hr after the administration of the compound. Thus, indicating that the radioactivity tagged with drug has affinity for pigmented cells. In albino rats, the unchanged drug in plasma, retina and sclera was 11.2, 24.8 and 13.9 % respectively. Lansoprazole and its 9 metabolites were present in higher amount in pigmented rats than SD rats, therefore lansoprazole had an affinity for melanin. (See table for the concentrations):

Table 1 Concentration of radioactivity in plasma and ocular tissues of albino and pigmented rats given 50mg/kg oral dose of ^{14}C lansoprazole

Animal	Tissue	Concentration of ^{14}C (μg lansoprazole equivalent /ml or g)					
		0.75h	1h	2h	4h	8h	24h
Albino rat (SD)	Plasma						
	Retina						
	Sclera ²						
	Lens						
	Vitreous body						
Pigmented rat (Lung Evans)	Plasma						
	Retina						
	Sclera ²						
	Lens						
	Vitreous body						

Mean $\pm$ S.D. (N=5). ² Include pigment epithelium, choroid, iris, and ciliary body. Figures in parentheses denote ratio of tissue concentration to plasma. ³ N=4.

Omeprazole was also distributed in retina, sclera, lens, and vitreous body of SD and LE rats. The ratio of ^{14}C -concentrations were much higher in LE rats than SD rats suggesting lansoprazole and omeprazole have affinity for the melanin containing tissues.

This study indicated that lansoprazole and omeprazole distributed in ocular tissues and their distribution pattern was similar. The highest concentrations were seen in sclera indicating that lansoprazole, omeprazole and their metabolites have affinity for melanin containing tissues.

This study did not give any additional information on the role of taurine in retinal damage. Therefore, sponsor's contention that lansoprazole and its metabolites affected retinal taurine could not be validated by these studies.

31. Light Microscopic Autoradiographic Distribution of Intravenously Administered ^{14}C -Lansoprazole in Female Rats and Female Mice: (Study # CMR 93192)

Testing Laboratory:

OAU and GLP Standard: Statement of compliance was not included.

Methods: The study was undertaken to determine if lansoprazole was accumulated in the specific portion of rat eyes. Sixty female rats weighing 120-150g were given an intravenous dose of 58 mg/kg (supplemented with 30 or 60 uCi of ¹⁴C-lansoprazole at a rate of 1 to 1.5 ml/min. Female mice weighing 25 to 30 g were given the same dose of lansoprazole at the rate of 5 ml/min in a volume of 19.3 ml/kg. Two hr after the administration of the compound, the total radioactivity of 1 eye/rat (by combustion method) and the distribution (using cryostat sectioning) of lansoprazole tagged radioactivity in the other eye/rat were determined.

Results:

i. Concentration of Lansoprazole: The concentration of ¹⁴C tagged radioactivity of lansoprazole was dose proportional as the radioactivity was 2 times in animals treated with 60 uCi dose than 30 uCi dose of the compound. The radioactivity was about 2/3rd less in mouse eye than rats (on an equal uCi/g basis) (Graphs taken from sponsor's submission, vol 12.2 pp 190 'A'). Lansoprazole was accumulated in inner segment layer of retinal photoreceptor cells, and scattered label in the plexiform layer in the rats. No such accumulation was seen in mice. Only scattered and unremakable radioactivity was seen in mouse. In mouse eyes, the radioactivity was 2/3rd lesser than rat eye.

This study indicated that lansoprazole was distributed in rat retina more than the mouse retina. From radiophotographs, it was seen to be present in the rat sclera. In mouse sclera, it presented only a small to negligible amount. This study indicated that lansoprazole was accumulated either aa a parent drug or one of its metabolites.

LABELLING

The annotated labeling of lansoprazole generally conforms to the format specified under CFR 21, subpart B, 201.50 to 201.57 dated April 1991. However, the following changes should be made in the proposed labelling:

PROPOSED LABELING:**(1) Carcinogenicity, Mutagenicity and Fertility:****SPONSOR'S PROPOSED LABELING:****Carcinogenesis, Mutagenesis, and Fertility**

Rats and mice exposed continuously to 150 mg/kg/day or 300 mg/kg/day, respectively, for 24 months failed to provide evidence for a primary carcinogenic effect. Increased incidence of proliferative changes, leading to benign neoplasia in several tissues, were indicative of mechanisms secondary to hormonal perturbations within the animal model. They included gastric carcinoids secondary to sustained hypergastrinemia in female rats and Leydig cell tumors secondary to altered testosterone homeostasis in male rats. Gastric carcinoids have been shown to occur in female rats given proton pump inhibitors, albeit not in humans. The development of Leydig cell tumors in laboratory rats is enhanced by a wide variety of nongenotoxic pharmaceutical and dietary products and is confined to this species. A metabolite of lansoprazole (M-1) found in rats but not humans was shown to be the most potent inhibitor of testosterone synthesis in this species. Adverse hormonal effects from lansoprazole have been investigated but not shown to occur in humans.

Lansoprazole was not mutagenic in *in vitro* *Salmonella typhimurium* and *Escherichia coli* assays. A mouse micronucleus test at up to 5000 mg/kg (approximately 10,000 times the human dose) was negative for the induction of micronuclei. Results from a rat *in vivo/in vitro* unscheduled DNA synthesis assay in rat hepatocytes were negative. Also, a mammalian cell mutagenesis assay was negative.

In vitro cytogenetics studies showed increased levels of aberrations consisting mainly of chromatid breaks which occurred only at cytotoxic concentrations. These cytotoxic concentrations were at least 50 to 60 times expected clinical blood levels of parent drug. Therefore, such concentrations will not be reached in humans.

Lansoprazole had no effect on fertility in either male or female rats. In animals allowed to have a normal parturition, lansoprazole treatment produced a slight prolongation in gestation only at the highest dosage tested (150 mg/kg/day) which was also toxic to the dams.

REVIEWER'S COMMENTS:(a) Carcinogenicity:

Sponsor submitted 6 carcinogenicity studies with the submission. Two of these (i.e., 1 each in rats and mice were invalid as animals were treated with lansoprazole on 5 days/week schedule. In rat invalidated study, 1 female was diagnosed to have gastric carcinoid. This was changed to gastric adenocarcinoma. In valid 2-year rat carcinogenicity study, rats were treated with oral doses of 0, 5, 25, 75 and 150 mg/kg/day for 2 years. Dose related gastric carcinoids (in 0, 7.1, 17.1, 32.8 % animals included in these treatment groups were reported. The incidences of intestinal metaplasia and combined hepatocellular carcinoma/adenoma were increased in a dose dependent manner in animals of both sexes included in the study. In males rats, testicular interstitial cell tumors were also seen in a dose dependent manner. Uterine squamous cell carcinoma in females included in treatment groups were 1.4, 0, 1.4 and 5.6 % females included in 5, 25, 75 and 150 mg/kg/day treatment groups. These tumors, therefore were not confined to only females as contended by sponsor. The mechanism of these abnormalities was not related to hormonal perturbations as lansoprazole did not exert any effect on testosterone synthesis. Therefore contention of sponsor can not be accepted and the proposed labeling should be modified accordingly as suggested below in Reviewer's Recommendation part of labeling.

(b) Mutagenicity: Lansoprazole was clastogenic in in vitro tests with human lymphocytes repeatedly. It induced an increase in the number of chromosomal aberrations at 50 and 60 ug/ml levels. It was not clastogenic in vivo rat bone marrow chromosomal aberration test. Since it was positive in point mutation test, sponsor should have conducted studies to rule out the thymidine kinase site. Sponsor should indicate in the labeling of lansoprazole that it was clastogenic in vitro chromosomal aberration test in human lymphocytes.

(c) Fertility: In a Segment I. Reproductive Performance and Fertility study in rats, lansoprazole was administered at an oral dose of 5, 50 or 150 mg/kg/day for 14 days prior to mating throughout lactation period in females and/or for 108 days before mating in males. Lansoprazole was more toxic in female rats than males, therefore only males were treated during the study and mated with untreated females. No antifertility or fetotoxicity was seen in non-treated females mated with treated males. The labeling of lansoprazole should indicate that lansoprazole was maternal and fetotoxic in female rats. Orally and intravenously administered lansoprazole was not teratogenic in pregnant rats and rabbits.

The above stated changes should be incorporated in the proposed labeling as recommended below.

Recommendation: The proposed text of sponsor should be modified as stated below:

(a) Carcinogenicity, Mutagenicity and Fertility: "Lansoprazole was tested for its carcinogenic potential in 6 carcinogenicity studies. Two of these studies were invalidated and not accepted because the animals were treated for 5 days/week schedule. In the invalidated rat carcinogenicity study, one female rat had gastric adenocarcinoma which was re-evaluated and changed to gastric carcinoid. In 2-year carcinogenicity study in rats, lansoprazole produced cataract, retinal atrophy, intestinal metaplasia, gastric carcinoids and testicular cell adenomas in a dose related fashion. These incidences were more than the strain and historical control incidences. In 2-year carcinogenicity study in mouse, combined incidences of hepatic carcinoma and adenoma were increased in mice included in 75 to 600 mg/kg/day treatment groups. Lansoprazole (at ug/ml concentration) was reported to be clastogenic repeatedly, in vitro in human lymphocytes chromosomal aberrations test. It was not clastogenic in a in vivo rat bone marrow chromosomal test. In Segment I. Fertility and reproductive performance test, lansoprazole was maternal toxic at 150 mg/kg/day (250 times the proposed clinical) dose administered 14 days before mating and continued during lactation. No fertility or reproductive toxicity was reported in untreated female rats when mated with lansoprazole treated males."

"Pregnancy Category B: Lansoprazole up to 250 times the clinical dose, was not teratogenic in pregnant rats and rabbits. There are however, no adequate and well controlled studies in pregnant women. Lansoprazole should be given to pregnant women only if potential benefits justify the potential risk to the fetus"

SUMMARY AND EVALUATION:

Lansoprazole is a benzimidazole derivative which inhibits the gastric parietal cell membrane enzymes ($H^+ + K^+$ -ATPase) system. It is converted into its active forms in the acidic environment, binds to secretory canaliculi enzymes and inhibits acid secretion. It has only an insignificant effect on gastro-intestinal motility but its antisecretory effect was claimed to last for 8 hr which was about 2 times more than famotidine. It was shown to inhibit basal and stimulated gastric acid secretions in man. Antisecretory effect of lansoprazole on stimulated gastric acid secretion was about 4 to 8 times more than omeprazole and famotidine.

Based on these effects of lansoprazole on gastric acid secretion, sponsor in the present New Drug Application, proposed to use lansoprazole in gastroesophageal reflux disorders (GERD) and duodenal ulcers at a dose of 300 ug/kg/day (based on 50 kg body weight of an adult) orally before eating for up to 8 weeks which is followed by a maintenance dose of 300 ug/kg/day for a for a period of 8 weeks. In duodenal ulcer, it is recommended at an oral dose of 300 ug/kg/day up to 4 weeks is also recommended in the treatment of duodenal ulcers.

In support of the application, sponsor submitted studies on its preclinical pharmacology, absorption, distribution, metabolism and excretion (ADME), pharmacokinetics, placental transfer; acute, subacute, subchronic and chronic toxicity in the rats, mice and dogs by oral and i.v. routes; four 2-year carcinogenicity studies in the mouse and rat, oral and I.V. Segment I. fertility and general reproductive performance study in male and female rats, oral and intravenous Segment II. Teratology study in rats, Segment II. Teratology Study in rabbits, mutagenicity studies: Ames test (2 studies); in vitro chromosomal aberration assay in human lymphocytes; rat micronucleus test; mouse lymphoma forward test; rat hepatocyte UDS Assay and dominant lethal tests in male and female mice; and special toxicity studies to study hemolytic, i.v./paravenous irritation potentials in rats and rabbits, the effect of lansoprazole on synthesis and metabolism of testosterone in rats, effect on taurine metabolism and synthesis in rat and mice liver and eyes.

Absorption, distribution, metabolism and excretion (ADME) studies in mice, rats and dogs were submitted. It was administered in 5% gum Arabic (5%) in rats and mice, or gelatin capsules in dogs. About 27, 37, and 63 to 87% of the orally administered compound was absorbed in mice, rats, and dogs respectively within 2 hrs of its administration. It was claimed to be a prodrug as it found to be biotransformed into 2 active forms, AG-2000 and AG-1778 in parietal cells. It attained a dose dependent plasma concentration (AUC_{0-24hr}) in animals, i.e., an oral dose 1.5 to 50 mg/kg/day attained plasma concentration from ng.h/ml in mice. In rats, a plasma concentration of ng.h/ml was seen after an oral dose of 2 to 150 mg/kg/day. In dogs, an oral dose from 0.5 to 50 mg/kg/day attained a plasma peak of 450 to 8800 ng.h/ml. The bioavailability of the compound was 4, 4 and 22% in rats, mice and dogs, respectively. About 92 and 96% of the compound was reported to bind with rat and dog plasma protein respectively. The half life of lansoprazole in mice, rats and, dogs was 1.2, 1.2 and 1.0 respectively. In monkeys, the $T_{1/2}$ was 3 hr. After multiple doses of the compound, the plasma concentrations in rats and mice, were similar to that of the single dose suggesting that the compound did not accumulate in body tissues after its prolonged use. It is present as a racemate mixture in the formulation and,

the (+)-isomer form of lansoprazole was slightly weaker in its acid antisecretory properties than (-)-isomer. Lansoprazole was metabolized by hydroxylation, sulfonation, and 4-hydroxylation. The metabolites isolated and purified from rat plasma are: (benzimidazole). hydroxysulfide and 5-hydroxylansoprazole (AG-1908 or and an unidentified metabolite. In dog plasma, and free lansoprazole were isolated as the major metabolites. The other unidentified metabolites were in small amounts. Orally administered compound was accumulated in the rat intestine, stomach, liver, kidney, thyroid, and brain. Plasma ratios of these tissues varied from 2 to 35%. An oral dose of 150 mg/kg/day (in 5 day/week schedule) for 18 months, induced an increase of microsomal enzymes (cytochrome 450 and cytochrome b₅) and microsome proteins. Lansoprazole was excreted in urine (12 to 25 %) and feces (68 to 80 %). Only 1% of unchanged drug was detected in plasma of rats and dogs. Metabolites were detected in rat urine. Metabolites were major urinary metabolites in rat and, were found in dog feces. In dog bile, all but hydroxymethyl metabolites were detected. In man, lansoprazole was reported to be absorbed and bind extensively in plasma proteins. It was excreted in urine and bile (fecal excretion). Its bioavailability was very high (up to 80%) in humans. It was metabolized in intestines before reaching liver. The rapid metabolism in rodent was accounted for a low bioavailability in the animals. Lansoprazole was demonstrated to cross rat placental barrier and parent compound and its metabolites were detected in fetus. It was also secreted in the milk of lactating rats.

Lansoprazole and its metabolites were tested for their acute tolerance in rats and mice. In the rat, LD₅₀s (p.o. route) of the parent compound was greater than the highest tested dose of 5000 mg/kg/day. Although LD₅₀ by oral route could not be assessed from this study but a single i.v. dose of 44 to 58 mg/kg was LD₅₀ by intravenous route. The LD₅₀s of the partially degraded AG-1749 and its metabolites were also greater than the highest dose employed used in the study. The comparison of toxic potential of compound to its metabolites and other compound can not be made from this data. The chief signs and symptoms of lansoprazole toxicity were: decreased activity, respiratory depression, flattened posture, ataxia and hyporeactivity. Liver damage (discoloration of the liver) and moderate pulmonary hyperemia were also noted in these animals. LD₅₀ values for male and female mice were 80.1 and 75.7 mg/kg respectively and toxicity of lansoprazole in mice was similar to that in rats.

In 2-week intravenous subacute toxicity study in rats, lansoprazole was given at the doses of 1, 3 and 10 mg/kg/day. The highest dose of the study was identified as a 'no effect dose' in this study. The target organ of toxicity was not identified in this study.

In 4-week intravenous toxicity study in rats, lansoprazole was used at doses of 3, 10 and 30 mg/kg/day. The highest dose caused an increase in total cholesterol (13%), hepatic aminopyrine-N-demethylase levels (21%), and atrophy of thymus. In this study, increased hypertrophy of gastric chief cells, an exaggerated pharmacologic effect of the compound, was seen in all rats included in treatment groups. A dose of 10 mg/kg/day was a 'no effect dose' in this study and liver, stomach and thymus were target organs of toxicity.

Lansoprazole toxicity was assessed by administering gavage oral doses of 0, 15, 50 and 150 mg/kg/day for 4 weeks. These were given in a volume of 5 ml/kg. The highest dose of 150 mg/kg/day produced hypertrophy of the gastric endoplasmic reticulum, hyperplasia of parietal cells and increase in lysosomes. A dose of 50 mg/kg/day was a 'no effect dose' in this study and stomach was the target organ of toxicity.

In a 13-week oral subchronic toxicity study in rats, lansoprazole was administered at 0, 5, 15 and 50 mg/kg/day. Hypertrophy of the centrilobular hepatocytes and, gastric parietal cells and increase in ECL cells were seen in animals included in 50 mg/kg/day group. The highest dose of 50 mg/kg/day of the study was well tolerated and non-toxic and 15 mg/kg/day dose was assessed as a 'no effect dose' in this study.

A 13-week oral subchronic toxicity study in rats was conducted to examine the reversibility of lansoprazole effects at the recovery period of 4, 13 and 26 weeks. In this study, the compound was given at 0 and 50 mg/kg/day doses in 2 groups of animals. Hypertrophy of the centrilobular gastric parietal cells was observed in the treated animals which was gradually decreased and cells attained the normal configuration at recovery week 26. The reversal of hyperplasia of chief cells was seen to be initiated by week 13 of the study. Therefore, lansoprazole toxicity produced during 13-week toxicity was fully reversible after 26 weeks of recovery.

In a 3-month oral gavage dose-range-finding study in rats, lansoprazole was administered at 0, 5, 15, 50 and 150 mg/kg/day. A treatment related incidence of hypertrophy of chief cells (9 and 13 out of 15 females in 50 and 150 mg/kg/day treatment groups and; 10 and 14 out of 15 males in 50 and 150 mg/kg/day treatment groups respectively) of minimal to slight intensity were noted in animals included in 50 and 150 mg/kg/day treatment groups. The incidence of eosinophilia in the chief cells were 9 and 13 out of 15 males and; 10 and 14 out of 15 females of 50 and 150 mg/kg/day treatment groups, respectively. Single cell necrosis was seen in 4 out of 15 and 10 out of 15 males and 6 and 12 out of 15 females of these groups. This data suggested that 150 mg/kg/day was MTD for the rats.

In 3-month oral toxicity study in rats, lansoprazole was given by gavage for 3 months at doses of 0, 50, 150, 300 and 600 mg/kg/day in 5 groups of Sprague-Dawley rats. A subgroup of animals among the high dose treatment group, was used as a recovery groups of 1 month. A retardation of body weight gain of 35 and 26 % in males, and 63% and 59% in the females included in 300 and 600 mg/kg/d treatment groups respectively, was seen at the termination of the study. Hypertrophy and hyperplasia of chief cells was seen in animals belonging to 150 mg/kg/day treatment group. No treatment related toxicity was seen in animals included in 50 and 150 mg/kg/day treatment groups, therefore a dose of 150 mg/kg/day was a MTD. The animals included in 300 and 600 mg/kg/day treatment groups did show any change in testosterone, corticosterone, and TSH hormones but showed an increase in gastrin levels. Hyperplasia of testicular interstitial cells was seen in animals included in 600 mg/kg/day treatment group. The target organs of toxicity were stomach, testes and thymus.

In a 6-month chronic oral toxicity study in rats (# A-29-345), lansoprazole was administered at doses of 0, 2, 10 and 50 mg/kg/day. A dose related hypertrophy of the chief cells and hyperplasia of squamous cells, probably an exaggerated pharmacologic effects, were seen in animals included in 50 mg/kg/day treatment group. Therefore a dose of 50 mg/kg/day produced no significant toxicity in the rat and was identified as a maximum non-toxic dose.

In 1-year chronic toxicity study in rats, lansoprazole was administered at the doses of 0, 1.5, 5, 15 and 50 mg/kg/day. The chief cell hypertrophy was seen in 80, 83, and 100% male and; 75, 100 and 100% in female rats, respectively belonging to 5, 15, and 50 mg/kg/day treatment groups. A diffuse and linear hyperplasia of the argyrophil cells were seen animals included in 15 and 50 mg/kg/day treatment groups. There was minimal to slight single cell necrosis of the chief cells and the most pronounced necrosis was seen in the 50 mg/kg/day treatment group. Focal retinopathy was seen among rats included in 50 mg/kg/day treatment group. The squamous cell metaplasia of uterine endometrial glands were 43.3, 0, 13.3 6.6 and 83.3 % in 0, 1.5, 5, 15 and 50 mg/kg/day treatment groups, respectively. The target organs of toxicity were not identified in this study stomach, eyes and uterus (in females). The maximum dose of 50 mg/kg/day was a non-lethal and produced least toxicity. Therefore, sponsor could have used a dose higher than 50 mg/kg/day to elicit the target organs of toxicity for the study.

In 13-week oral toxicity study in mice, lansoprazole was used at doses of 0, 150, 300, 600, 1200 and 2400 mg/kg/day. An exaggeration of pharmacological effects of gastric hyperplasia, gastritis, erosions of the gastric glandular mucosa and hypertrophy of liver were reported in animals included in 150 to 600 mg/kg/day

treatment groups of the study. A reduction in the testicular weight ($p < 0.01$) and tubular atrophy in male animals belonging to 1200 and 2400 mg/kg/day treatment groups was observed. Aspermia was seen in epididymides of animals belonging to 1200 and 2400 mg/kg/day treatment groups. The target organs of toxicity for the study were stomach, liver and epididymides and a dose of 600 mg/kg/day was identified as the MTD in mice.

In 2-week subacute intravenous toxicity study in dogs, lansoprazole was administered from a dose of 0, 1, 3 and 10 mg/kg/day in a volume of 2 ml/kg/day. The highest dose of 10 mg/kg/day, was tolerated well by dogs. No target organ of toxicity could be identified in this study.

In 4-week intravenous toxicity study in dog, AG-1749 was given at daily doses of 0, 3, 10 and 30 mg/kg/day (volume of injection = 6 ml/kg/day). An increase in the absolute weights of liver by 36 to 39 % was seen (1/sex) in animals included in 30 mg/kg/day treatment group. Single cell necrosis of the parietal cell was seen in 10 and 30 mg/kg/day treatment groups. Liver, and stomach were the target organs of toxicity and a dose of 30 mg/kg/day was a MTD.

In 4-week oral toxicity study in dog, AG-1749 was given at a dose of 0, 30, 100 and 300 mg/kg/day. A reduction of 67% and 41% relative weights of prostate was reported in animals included in 30 and 300 mg/kg/day treatment groups, respectively. Occasional vacuolization and necrosis of parietal cells was seen in animals belonging to 100 mg/kg/day treatment group. There was a slight dilatation of the centrilobular sinusoid and atrophy of the hepatocytes in one male and one female dog included in 300 mg/kg/day treatment group. Gastric and hepatic toxicity were seen in only one male dog belonging to 300 mg/kg/day treatment group. An increase in the number of erythroid and myeloid cells was seen in both male and female dogs included in 300 mg/kg/day. Liver, bone marrow and stomach were the target organs of toxicity in this study. A dose of 30 mg/kg/day was a no effect dose and 100 mg/kg/day was maximum tolerated dose.

In 13-week intravenous subacute toxicity study in dogs, lansoprazole was given at 3, 10 and 30 mg/kg/day. Inflammation and fibrosis of the vein wall and perivenous tissues and intimal thickening, venous thrombosis (occlusive recanalized) were seen in 10 and 30 mg/kg/day treatment groups dogs. The lowest dose (3 mg/kg/day) was the no effect dose. A dose of 30 mg/kg/day was identified as a MTD since it produced an increase in the relative organ weights of liver (20%), kidneys (14%), adrenals (14%) and venous thrombosis at the injection sites.

In 13 week oral toxicity study in dogs, lansoprazole was administered at 0, 5, 15 and 50 mg/kg/day. The pigment deposits in the hepatocytes and hypertrophy of the bile duct and, necrosis of the gastric parietal cells and an increase in the serum gastrin levels was seen in animals included in 15 and 50 mg/kg/day treatment groups. Therefore a dose of 5 mg/kg/day was a 'no effect dose' and a dose of 15 mg/kg/day was a MTD.

In 6-month chronic toxicity study in dogs, lansoprazole was administered at a dose of 0, 2, 10 and 50 mg/kg/day in gelatin capsules. Animals included in 50 mg/kg/day treatment group experienced pharmacological effect of hyperplasia of the glandular gastric cells, thus lansoprazole was tolerated well up to 50 mg/kg/day by dogs. Serum hormones were not determined during the study.

Lansoprazole was tested for its chronic 12-month toxicity in dogs. It was administered at oral doses of 0, 1.5, 5, 15 and 50 mg/kg/day in gelatin capsules. It produced ocular toxicity of conjunctivitis, focal retinopathy in animals of 50 mg/kg/day treatment group. These abnormalities were not associated with histopathological changes, therefore not treatment related. The autopsies of animals belonging to high dose treatment group and other animals those died during the study (of other treatment groups) was performed were performed. Hyperplasia of the glandular gastric cells and dilated cells containing eosinophilic material were seen in the animals included in 50 mg/kg/day treatment group. These were exaggerated pharmacological effects. Therefore no treatment related toxicity of lansoprazole was elicited in this study and target organ of toxicity were not identified. A higher dose of the compound could be used in the study.

In a 4-week oral toxicity study in monkeys, lansoprazole was administered at doses of 0, 5, 15 and 150 mg/kg/day. Animals belonging to 150 mg/kg/day treatment group had diarrhea containing blood and/or white substance. Therefore, gastrointestinal tract is the target organ of toxicity. A low dose of 15 mg/kg/day was considered as the no effect dose since it only produced vacuolization of parietal cells in the stomach.

Sponsor submitted 6 carcinogenicity studies (3 each in mice and rats) with the submission. Two out of these studies, i.e., 1 each in rats and mice, were not valid as animals were treated for 5 days/week schedule in these studies. In this invalid mouse carcinogenicity study, lansoprazole was administered at the dose of 0, 1.5, 5, 15 and 50 mg/kg/day orally for 78 weeks. There were undue number of deaths among control and treatment groups, the animals showed only a dose related hyperplasia of gastric glandular cells (an exaggerated pharmacologic effect) and the basis of dose selection (13 week toxicity study) was not given in this study. Therefore this study was not on a scientific basis and not valid,

In the second carcinogenicity study in mice, lansoprazole was administered at doses of 0, 15, 75, 150 and 300 mg/kg/day for 2 years in mice. Animals used in the study were infected with mouse hepatitis virus thus the observed increased number of deaths were due to infection and not treatment related. Lansoprazole treatment from 75 to 300 mg/kg/day produced ECL cell hyperplasia in the fundic and pyloric portion of the stomach. An increase in the combined incidence of hepatocellular adenoma and carcinoma was not statistically significant. An increase in the incidences of rete testis adenoma (a rare tumor) was seen in animals included in 300 mg/kg/day treatment group. The study data are further considered in conjunction with the next mouse carcinogenicity study in which the animals were treated with 600 mg/kg/day during the study (study # R&D 93/731).

Sponsor conducted the above study without considering the Division's recommended MTD of 600 mg/kg/day for 2 year mouse carcinogenicity study. Therefore, sponsor requested to discontinue the 15 mg/kg/day treatment group from the above mouse carcinogenicity study. Sponsor was asked to complete the above study and conduct another study by selecting a dose of 600 mg/kg/day as the highest dose. Therefore, sponsor performed this study by selecting the doses of 0 and 600 mg/kg/day for 2 years in mice. The mortalities among 600 mg/kg/day treatment group were more than control and 300 mg/kg/day treatment groups. Gastric diverticula, micronodular ECL hyperplasia and increased incidences of intestinal metaplasia in the pyloric area were seen in animals included in 600 mg/kg/day treatment group. A combined incidences of hepatic adenoma and carcinoma were increased among animals of both sexes included in 600 mg/kg/day treatment group. The incidences of necrosis of centrilobular liver and hepatocellular neoplasms in animals included in 75, 150, 300 and 600 mg/kg/day treatment groups. Therefore, lansoprazole produced an increase in the combined instances of hepatocellular adenoma and carcinoma, intestinal metaplasia and rete testis in mice during this study.

In the first rat carcinogenicity study lansoprazole carcinogenic potential was tested at a dose of either 0, 1.5, 5, 15 or 50 mg/kg/day for 2 years at a schedule of 5 day/week. A dose related histopathological changes of gastric hyperplasia and intestinal metaplasia, testicular interstitial cell hyperplasia and tumors were reported in animals included in 50 mg/kg/day treatment group. A dose related incidences of intestinal metaplasia of the glandular mucosa of stomach were seen in males and females of the respective groups (reported to be present in pyloric region as detected with HE and Alcian blue PAS stain - report # A-29-840). One animal # 5543 belonging to 50 mg/kg/day treatment group was reported to have gastric adenocarcinoma initially. Based on its positive reaction with argyrophilic staining (with Grimelius/Sevier-Munger stain) and a negative reaction for the presence of glandular or mucin secreting cells

(-ve with Fontana-Masson staining), the tumor was re-evaluated as of ECL cell origin and the diagnosis of gastric adenocarcinoma was changed to gastric carcinoid (Amendment dated February 18, 1994 - reports # A-29-935 and A-29-968).

Sponsor conducted a study in rats in which 5 days/week treatment schedule was compared with 7 days/week schedule. The animals in this study were treated with a dose of 50 mg/kg/day in both schedules. A substantial higher levels of the drug in the thyroid, liver, pituitary and the GI tract of rats included in 7days week scheduled was reported. Therefore, the data clearly indicated that the results of 5 days/week treatment group can not be compared with 7 day/week schedule. Sponsor's claim that the 5 days per week dosing schedule can not be accepted and sponsor was informed to conduct carcinogenicity studies on 7 days/week schedule. The maintenance of peak non-fluctuating blood levels of the drug throughout the length of the study are essential for conducting such carcinogenicity studies.

In the second carcinogenicity study in rats, lansoprazole was given in 5 groups of rats at 0, 5, 25, 75 and 150 mg/kg/day for 2 years. Lansoprazole treatment produced a dose dependent retardation in the body weight gain, retinal atrophy, enlarged testes and carcinoid tumors in the stomach fundus and testicular interstitial cell tumors in males included in 0, 5, 15, 75 and 150 mg/kg/day treatment groups. Degeneration of seminiferous tubules and hypospermia was seen in a dose related manner. Sponsor attributed this adverse effect to a reduction on the synthesis of the testosterone. But a review of the studies submitted revealed that lansoprazole did not affect (1) ACTH serum corticosteroid, (2) enzymes of steroidogenic pathway in Leydig's cells in vitro, in the presence and absence of S-9 homogenate in the rat and mouse, (3) the synthesis of testosterone up to a dose of 150 mg/kg/day in the rat. In female animals, lansoprazole up to a dose of 150 mg/kg/day did not affect LH, FSH and prolactin levels in postnatal period. Therefore, lansoprazole did not affect testosterone synthesis as contended by sponsor.

Sponsor submitted few studies to understand the observed retinopathy in rats. Sponsor attribute the retinopathy to the taurine deficiency. But, a report of Purina Chow Inc., showed that the taurine contents of diet were 0.02 to 0.0298 % in diet tested in 1984 and 1992. Therefore the proposal of dietary deficiency is not valid and the retinal degeneration seen in the rats might be a direct effect of the compound. The other studies on interaction of lansoprazole with taurine showed that lansoprazole was distributed in retina but its interaction with taurine could not be confirmed by the submitted studies, therefore sponsor contention that the retinal degeneration (seen after 84 weeks in rats) might be related

to deficiency of taurine in food or age of animals is not valid. Retinal degeneration after a prolonged treatment with the drug may be a result of a direct toxic effect of lansoprazole with retinal cells. It should be regarded as a serious adverse effect on the prolonged use of the compound.

In another carcinogenic study in rats, the animals were divided in main groups and satellite groups and were administered a dose of either 0 (2 groups), 5, 15 and 50 mg/kg/day of lansoprazole (in a volume of 5 ml/kg as a suspension) for 98 weeks in females and 105 weeks in males. A recovery group of 60 male rats was treated for 78 weeks and these were allowed a recovery period of 26 weeks. Animals of satellite group were used for gastrin, testosterone and LH hormone assays. Lansoprazole treatment produced a dose related thickening of glandular mucosa of the stomach, gastric carcinoid tumors and bilateral enlarged testes. An increase in testicular interstitial cell tumors in males and uterine endometrial stromal polyps in females.

Sponsor conducted special studies to determine the antiandrogenic effects of lansoprazole in the young castrated rats. The synthesis of testosterone was not significantly affected in these rats. In vitro studies, lansoprazole inhibiting testosterone synthesis at a very high concentration (equivalent to plasma level of 38 ug/ml). This level could be achieved only after a very large dose, i.e., at a dose of about 1,900 mg/kg/day which is 3167 times higher than the recommended dose of 0.6 mg/kg/day. When tested for its effects on the synthesis of testosterone in the rat Leydig cells in-vitro, it was 100 % less potent (EC_{50} of 9.92 ug/ml or 618 nM) than ketoconazole (EC_{50} of 0.12 ug/ml or 228 nMol). In a study on the action lansoprazole on testosterone synthesis enzymes, it was shown not to inhibit 17-alpha-OH-steroid dehydrogenase, the enzyme that converts androstenedione into testosterone in the last step of the testosterone biosynthetic pathway. Testosterone synthesis was suppressed even when other intermediate steps precursors were used. There was no data of any of the in-vitro enzymatic assays were provided to show that AG-1749 inhibits this particular enzyme. Therefore, AG-1749 acts probably, on the last step of the pathway (androstosterone to testosterone). However, the data on testosterone and LH indicated lansoprazole did not interfered with testosterone synthesis.

In Segment I. Reproductive and Fertility toxicity study in rats, lansoprazole was administered at a dose of either 0, 5, 15 or 50 mg/g/day in oral gavage. Male rats were treated 80 days prior to mating and during mating and female rats were treated 14 days prior to mating, during mating and day 13 of gestation or lactation day 22. No maternal, feto- or embryo- toxicity was reported in the animals. The dose selection of the 2nd Segment I. study was based on the Agency's recommendations (a HFD-180 response to amendment

dated August 24, 1990). In the second Segment I study, AG-1749 was tested at oral doses of 5, 50, or 150 mg/kg/day. Male rats were treated with lansoprazole 108 days prior to and during mating, and females were treated 14 days prior to mating, during mating, and gestation. Due to the decreased number of implantation sites at 150 mg/kg/day dose, the untreated females were mated with treated males to determine if these effects could be duplicated by treating the males only. However, the treated females were not mated with untreated males to determine the drug effects on female fertility. There were no effects on the dam growth during treatment, but the number of implantations (135 versus 205 in controls) and live fetuses (125 versus 190 in controls) were significantly reduced among 150 mg/kg/day treatment group. The female pup weights during lactation (days 12 and 21) were significantly reduced by 17 and 20.5%, respectively. Among the untreated females (mated with treated males), there was no significant reproductive toxicity, suggesting that the effects seen in the treated females were not as result of treating the male rats, but were as a result of treating the female rats. Thus, a dose of 150 mg/kg/day of AG-1749 (Lansoprazole) produced reproductive toxicity in the rat.

In I.V. Segment I. fertility and general reproductive performance study in male rats, doses of 3, 10 and 30 mg/kg/day were used. There were no abnormal effects on the fertility and mating performance of treated male rats (females were not treated in this study) at doses up to and including 30 mg/kg/day of Lansoprazole.

In I.V. Segment II. teratology study in rats doses of 10 and 30 mg/kg/day were used. No teratogenic effects was observed in rat fetuses up to a dose of 30 mg/kg/day of lansoprazole.

AG-1749 was non-mutagenic in battery of in vitro and in vivo mutagenicity tests like, Ames test, in-vivo/in-vitro rat hepatocyte unscheduled DNA synthesis assay and mouse lymphoma assay. In HGPRT test, the dose-range of lansoprazole was too narrow and the test was done not in accordance with the guidelines. In this test, lansoprazole did not show positive results and the positive control showed only a weak positive results. Lansoprazole produced a dose-dependent increase in the number of chromosomal aberrations in human lymphocytes repeatedly. But it was negative in in vivo rat bone marrow test. However, a statement that "AG-1749 produced chromosomal aberrations in the human lymphocytes repeatedly should be included in the appropriate portion of the labeling.

I.V. or S.C. administration of Lansoprazole to rabbits produced slight local irritation.

The labelling of lansoprazole conforms to the format specified under CFR 21, subpart B, 201.50 to 201.57 dated April 1991. However, the suggested changes described in the text, should be incorporated.

RECOMMENDATIONS:

1. From preclinical standpoint, the application may be approved for the indications in which it will be used for a short period of 4 to 8 weeks. The prolonged use of lansoprazole may pose a safety risks of tumorigenicity in patients.
2. Lansoprazole may be approved for (i) Reflux esophagitis and ii) Zollinger Ellison's Syndrome. But the maintenance therapy should be within 6 months. A prolonged use could pose a danger of intestinal metaplasia in man. These were seen in 2 year rat and mouse carcinogenicity studies. An increase incidences of hepatocellular adenoma and carcinoma were noticed in rats and mice.
3. Sponsor should be asked to change the proposed label of Prevacid[®] as suggested in the text of the review.

Yash Chopra
1.9.1995

Yash, M. Chopra, M.D., Ph.D
Pharmacologist, HFD-180

cc:

Original NDA

HFD-180

HFD-181/CSO

HFD-180/Dr. Chopra

HFD-180/Dr. Choudary

HFD-180/Dr. Fredd

HFD-345/Dr. James

HFD-502/Dr. Taylor

HFD-502/CAC

YM/6/20/94/3258C

YMC/hw/1/9/95

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MEMORANDUM

(Review)

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

DATE: March 6, 1995

FROM: Supervisory Pharmacologist
Division of Gastrointestinal and
Coagulation Drug Products, HFD-180

SUBJECT: NDA 20,406 (Prevacid/Lansoprazole Delayed-Release
Capsules) - Amendment (# 039) Dated February 24, 1995 -
Sponsor's Revised Draft Labeling

TO: NDA 20,406

In the Agency's approvable letter, the Sponsor was asked to submit final printed labeling (FPL) of the furnished draft labeling. In the amendment dated February 24, 1995, Sponsor provided a "revised draft labeling" instead of the FPL. The preclinical pharmacology portions of the Agency's draft labeling (referred to as Agency's Initial Version) and the Sponsor's proposed revisions of these portions are reproduced below. Our evaluation of the Sponsor's versions and our recommendations for Agency's final labeling (referred to as Agency's Final Version) follow.

1. Clinical Pharmacology

Pharmacodynamics

Endocrine Effects

Agency's Initial Version:

"In 24-month carcinogenicity studies in Sprague-Dawley rats with daily dosages up to 150 mg/kg, proliferative changes in the Leydig cells of the testes, including benign neoplasm, were increased compared to control rates."

Sponsor's Version:

Evaluation: Sponsor changed the last word "rates" to "rats" under the assumption that it was a typographical error. It was not a typographical error. In the original Sponsor's labeling, it was shown as "were increased over control incidence". In the

Agency's initial version this was changed to "were increased compared to control rates". The change was handwritten by the Office Director.

Recommendation: Agency's initial version should be retained.

Agency's Final Version: "In 24-month carcinogenicity studies in Sprague-Dawley rats with daily dosages up to 150 mg/kg, proliferative changes in the Leydig cells of the testes, including benign neoplasm, were increased compared to control rates."

2. Precautions

Carcinogenesis, Mutagenesis, Impairment of Fertility

Agency's Initial Version:

"In two 24-month carcinogenicity studies, Sprague-Dawley rats were treated orally with doses of 5 to 150 mg/kg/day, about 1-40 times the exposure on a body surface (mg/m^2) basis, of a 50 kg person of average height (1.46 m^2 body surface area) given the recommended human dose of 30 mg/day ($22.2 \text{ mg}/\text{m}^2$). Lansoprazole produced dose-related gastric enterochromaffin like (ECL) cell hyperplasia and ECL cell carcinoids in both male and female rats. It also increased the incidence of intestinal metaplasia of the gastric epithelium in both sexes. In male rats, lansoprazole produced a dose-related increase of testicular interstitial cell adenomas. The incidence of these adenomas in rats receiving doses of 15 to 150 mg/kg/day (4 to 40 times the recommended human dose based on body surface area) exceeded the low background incidence (range %) for this strain of rat. Testicular interstitial cell adenoma also occurred in one of thirty rats treated with 50 mg/kg/day, (13 times the recommended human dose based on body surface area) in a 1-year toxicity study.

In a 24-month carcinogenicity study, CD-1 mice were treated orally with doses of 15 to 600 mg/kg/day, 2 to 80 times the recommended human dose based on body surface area. Lansoprazole produced a dose related increased incidence of gastric ECL cell hyperplasia. It also produced an increased incidence of liver tumors (hepatocellular adenoma plus carcinoma). The tumor incidences in male mice treated with 300 and 600 mg/kg/day, (40 and 80 times the recommended human dose based on body surface area) and female mice treated with 150 to 600 mg/kg/day (20 to 80 times the recommended human dose based on body surface area) exceeded the ranges of background incidences in historical

controls for this strain of mice. Lansoprazole treatment produced adenoma of rete testis in male mice receiving 75 to 600 mg/kg/day (10 to 80 times the recommended human dose based on body surface area).

Lansoprazole was not genotoxic in the Ames test, the ex vivo rat hepatocyte unscheduled DNA synthesis (UDS) test, the in vivo mouse micronucleus test or the rat bone marrow cell chromosomal aberration test. It was positive in in vitro human lymphocyte chromosomal aberration assays.

Lansoprazole at oral doses up to 150 mg/kg/day (40 times the recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats."

Sponsor's Version

**"Carcinogenesis, Mutagenesis, and
Fertility**

Purged page

4

Note: we request this addition to more completely represent the data.

Note: we request this addition as we believe this qualifier adds important information.

Evaluation:

In the portion dealing with mouse carcinogenicity study, Sponsor changed the dose ratios between mouse dose range and human dose from . . . In Agency's initial version, the mouse mg/kg doses were converted to mg/m² using a conversion factor of 3. This is based on a body weight of 0.02 kg and a surface area of 0.0066 m² (Freireich, E.J., et al, Cancer Chemother. Rpts. 50: 219-244, 1966). A conversion factor of 3 for the mouse has been adopted by the CDER Supervisory Pharmacologists since 1993. No error was made in Agency's calculation of dose ratios for mouse carcinogenicity study doses and the proposed clinical dose. Sponsor's assumption of an error in our calculations is unfounded and therefore Sponsor's ratios should be changed to the original ratios provided by the Agency in the Agency's initial version.

In the Sponsor's version, the increased incidences of liver tumors at 300 mg/kg/day in males and 150 mg/kg/day in females were deleted with appended notes that there were no increases.

There were increased incidences at 300 mg/kg/day in males (22.8% incidence in treated versus 15.7% incidence in combined controls) and 150 mg/kg/day in females (5.7% incidence in treated versus 1.4% incidence in controls).

In the mutagenicity portion, Sponsor added "in vitro mammalian cell mutagenicity test" to the negative genotoxicity tests. This description is nonspecific. The conducted assay was mouse lymphoma (L5178Y) cell/HGPRT forward mutation assay. The HGPRT locus in this cell system is not suitable to evaluate the clastogenic component of a genotoxic compound (Moore et al, Mutagenesis 4: 394-403, 1989). Sponsor's test involved an inappropriate locus and substrate and therefore reference to this test should be deleted. Sponsor qualified the statement of positivity in vitro human lymphocyte chromosomal aberration assays as occurring at cytotoxic concentrations. This is inappropriate since the positive results were repeatable at different concentrations and cytotoxicity is not involved in the tests. Therefore, the misleading qualifier should be deleted.

Recommendation: Sponsor's proposed changes are not acceptable. Agency's initial version should be retained.

Agency's Final Version:

"Carcinogenesis, Mutagenesis, Impairment of Fertility

In two 24-month carcinogenicity studies, Sprague-Dawley rats were treated orally with doses of 5 to 150 mg/kg/day, about 1-40 times the exposure on a body surface (mg/m²) basis of a 50 kg person of average height (1.46 m² body surface area) given the recommended human dose of 30 mg/day (22.2 mg/m²). Lansoprazole produced dose-related gastric enterochromaffin like (ECL) cell hyperplasia and ECL cell carcinoids in both male and female rats. It also increased the incidence of intestinal metaplasia of the gastric epithelium in both sexes. In male rats, lansoprazole produced a dose-related increase of testicular interstitial cell adenomas. The incidence of these adenomas in rats receiving doses of 15 to 150 mg/kg/day (4 to 40 times the recommended human dose based on body surface area) exceeded the low background incidence (range %) for this strain of rat. Testicular interstitial cell adenoma also occurred in one of thirty rats treated with 50 mg/kg/day (13 times the recommended human dose based on body surface area) in a 1-year toxicity study.

In a 24-month carcinogenicity study, CD-1 mice were treated orally with doses of 15 to 600 mg/kg/day, a 2 to 80 times the recommended human dose based on body surface area. Lansoprazole produced a dose related increased incidence of gastric ECL cell

hyperplasia. It also produced an increased incidence of liver tumors (hepatocellular adenoma plus carcinoma). The tumor incidences in male mice treated with 300 and 600 mg/kg/day, (40 and 80 times the recommended human dose based on body surface area) and female mice treated with 150 to 600 mg/kg/day (20 to 80 times the recommended human dose based on body surface area) exceeded the ranges of background incidences in historical controls for this strain of mice. Lansoprazole treatment produced adenoma of rete testis in male mice receiving 75 to 600 mg/kg/day (10 to 80 times the recommended human dose based on body surface area).

Lansoprazole was not genotoxic in the Ames test, the ex vivo rat hepatocyte unscheduled DNA synthesis (UDS) test, the in vivo mouse micronucleus test or the rat bone marrow cell chromosomal aberration test. It was positive in in vitro human lymphocyte chromosomal aberration assays.

Lansoprazole at oral doses up to 150 mg/kg/day (40 times the recommended human dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats."

3. Pregnancy

Evaluation: The subheadings under this category do not follow the correct sequence.

Recommendation: In the Sponsor's version the "pregnancy category B "should follow" teratogenic effects".

Agency's Final Version:

Pregnancy:

Teratogenic Effects. Pregnancy category B.

Teratology studies have been performed in pregnant rats at oral doses up to 150 mg/kg/day (40 times the recommended human dose based on body surface area) and pregnant rabbits at oral doses up to 30 mg/kg/day (16 times the recommended human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to lansoprazole.

There are, however, no adequate or well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

4. Nursing Mothers

Agency's Initial Version:

Nursing Mothers

"Lansoprazole or its metabolites are excreted in the milk of rats. It is not known whether lansoprazole is excreted in human milk. Because many drugs are excreted in human milk, because of the potential for serious adverse reactions in nursing infants from lansoprazole, and because of the potential for tumorigenicity shown for lansoprazole in rat carcinogenicity 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."

Sponsor's Version:

Nursing Mothers

Evaluation: The Agency's initial version complies with the specific requirements contained in 21 CFR, 201.57. It identifies that lansoprazole or its metabolites are excreted into the milk of rats, expresses lack of data in human and points out the known tumorigenicity in animal studies and provides appropriate precautionary statement. Sponsor's version does not conform to the above specifications. It avoids mentioning the tumorigenic risks.

Recommendation: Sponsor's version should be refused. Agency's initial version should include the missing words "and mouse" before carcinogenicity studies.

Agency's Final Version:

Nursing Mothers

Lansoprazole or its metabolites are excreted in the milk of rats. It is not known whether lansoprazole is excreted in human milk. Because many drugs are excreted in human milk, because of the potential for serious adverse reactions in nursing infants from lansoprazole, and because of the potential for tumorigenicity shown for lansoprazole in rat and mouse carcinogenicity 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.

5. Overdosage

Agency's Initial Version:

Oral doses up to 5000 mg/kg in rats (approximately 1300 times the recommended human dose based on body surface area) and mice (about 675 times the recommended human dose based on body surface area) did not produce deaths or any clinical signs. Lansoprazole is not removed from the circulation by hemodialysis.

Sponsor's Version:

Overdosage

Note: an apparent error was made in conversion (see Appendix F).

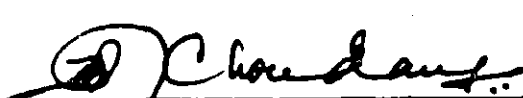
Note: we request this paragraph be added back in as we believe it contains important safety information for the physician.

Evaluation: Under the mistaken notion that the Agency's calculations were in error for mouse data, Sponsor changed the dose ratios for mouse to human and for rat to human. These should be corrected. Sponsor is adding a new sentence. "In one reported case of overdose, the patient consumed 600 mg of lansoprazole with no adverse reaction." This sentence was not included in Sponsor's original submission.

Recommendation: The dose ratios between the rat and mouse acute toxicity doses and the human dose on a surface area basis, should be restored to the numbers in Agency's initial version. The newly added sentence about the clinical overdose experience is acceptable subject to verification.

Agency's Final Version:

Oral doses up to 5000 mg/kg in rats (approximately 1300 times the recommended human dose based on body surface area) and mice (about 675 times the recommended human dose based on body surface area) did not produce deaths or any clinical signs. Lansoprazole is not removed from the circulation by hemodialysis. In one reported case of overdose, the patient consumed 600 mg of lansoprazole with no adverse reaction.

 3/8/95
Jasti B. Choudary, Ph.D., B.V.Sc.
Supervisory Pharmacologist

cc:
Orig. NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Fredd

JBC/hw/3/7/95
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Patent/Exclusivity
Inf.

13.0 PATENT INFORMATION

We, TAP Pharmaceuticals Inc. (TAP), certify that the drug lansoprazole is claimed in U.S. Patents as listed below. Takeda Chemical Industries, Ltd., of Japan has licensed lansoprazole as covered by these patents to TAP.

<u>U.S. Patent No.</u>	<u>Expiration Date</u>	<u>Coverage</u>
4,628,098	12/08/03	Compound
4,689,333	08/25/04	Pharmaceutical formulations containing lansoprazole, and a method of treating gastritis
5,013,743	05/07/08	Use of lansoprazole for combatting diseases caused by the genus <i>Campylobacter</i>
5,026,560	06/25/08	Formulation (spherical granules)
5,045,321	09/03/08	Formulation (spherical granules or tablets stabilized with inorganic salt)
5,093,132	09/03/08	Formulation stabilized with inorganic salt

EXCLUSIVITY SUMMARY FOR NDA # 20-406 SUPPL # —

Trade Name PREVACID Generic Name LAN SOPRAZOLE

Applicant Name TAP PHARMACEUTICALS HFD # 180

Approval Date If Known

PART I IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

a) Is it an original NDA? YES / X / NO / /

b) Is it an effectiveness supplement? YES / / NO / X /

If yes, what type? (SE1, SE2, etc.)

c) Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

YES / X / NO / /

If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

NA

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

NA

d) Did the applicant request exclusivity?

YES /___/ NO /X/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength and route of administration, previously been approved by FDA for the same use?

YES /___/ NO /X/

If yes, NDA # _____. Drug Name _____.

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /X/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product.

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /X/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____
NDA# _____
NDA# _____

2. Combination product.

If the product contains more than one active moiety(as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /X/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____
NDA# _____
NDA# _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

(a) In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

(b) Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion?

YES /___/ NO /___/

If yes, explain: _____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval:

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a) For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon:

b) For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2' YES /___/ NO /___/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

c) If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a) For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1		!	
IND #	YES /___/	!	NO /___/ Explain: _____
		!	_____
Investigation #2		!	
IND #	YES /___/	!	NO /___/ Explain: _____

(b) For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1		!	
YES /___/ Explain	_____	!	NO /___/ Explain _____
	_____	!	_____
	_____	!	_____
Investigation #2		!	
YES /___/ Explain	_____	!	NO /___/ Explain _____
	_____	!	_____
	_____	!	_____

(c) Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES / /

NO / /

If yes, explain: _____

Maria R. Walsh

Signature
Title: CSO

1-9-95

Date

Stephen R. Kidd MD

Signature of Office/
Division Director

1/30/95

Date

cc: Original NDA

Division File

HFD-84



November 12, 1993

Page 2

Finally, TAP Pharmaceuticals certifies that we did not and will not use in any capacity the services of any person debarred under subsections (a) or (b) [section 306 (a) or (b)], in connection with this application.

Please direct any questions you may have on this application to my attention.

Sincerely,

A handwritten signature in cursive script that reads "Judy Decker Wargel".

Judy Decker Wargel

Regulatory Products Manager

Phone: (708) 317-5781

Fax: (708) 317-5795

JDW/cjs

lense/ndenov12

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

NDA # 20-406 Trade (generic) names PREVACID (OMEPRazole) CAPSULES

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

- ☐ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.
- ☐ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.
 - ☐ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.
 - ☐ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)
- ☐ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).
 - ☐ a. The applicant has committed to doing such studies as will be required.
 - ☐ (1) Studies are ongoing.
 - ☐ (2) Protocols have been submitted and approved.
 - ☐ (3) Protocols have been submitted and are under review.
 - ☐ (4) If no protocol has been submitted, on the next page explain the status of discussions.
 - ☐ b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.
- ☒ 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

Explain, as necessary, the foregoing items:

Maria Walsh CSO
Signature of Preparer

Date 1-9-95

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

AP Action memos

M E M O R A N D U M

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

DATE: April 27, 1995
FROM: Director, Division of Gastrointestinal and Coagulation
Drug Products, HFD-180
SUBJECT: Approval Action for Lansoprazole, NDA 20-406
TO: Director, Office of Drug Evaluation I, HFD-100

The proposed labeling needs to be considered prior to signing the approval letter. We met with the firm after the approvable letter, and worked out all of their concerns. In the package under "FDA Draft 2" the result of that interaction can be found.

The most important changes are to allow retreatment of erosive esophagitis, and to provide results of the studies in "poorly responsive" patients in this clinical trials section. We would be glad to discuss the rationale for these and other changes as you wish.

Overall we think the labeling adequately incorporates the changes made in the draft sent with the approvable letter. Therefore, a letter approving the application is transmitted for your consideration.

SF

Stephen Fredd, M.D.

CC:
NDA 20-406
HFD-180
HFD-180/HGallo-Torres
HFD-131/CSO/MWalsh
HFD-180/SFredd:4/27/95
f/t deg: 4/27/95/5/1/95
MEMO\NDA20406.5SF

MEMORANDUM

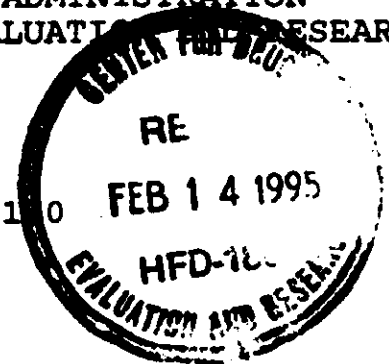
DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: FEB 14 1995

FROM: Director, Office of Drug Evaluation I, HFD-100

SUBJECT: Lansoprazole

TO: Dr. Stephen Fredd, HFD-180



Most aspects of lansoprazole seem clear at this point. It is effective in all of the usual proton pump situations but for now after healing of erosive esophagitis is not approvable because of safety concerns, although effectiveness in that setting is overwhelmingly demonstrated, and the gastric ulcer claim was withdrawn.

There are just a few areas needing discussion:

I. Letter

- A. The first question regarding maintenance asks for analyses to decide whether L therapy should be given to all patients after healing or only to those who started with at least grade 3/4 erosive disease; grade 2 patients would be maintained only if they recurred.

This is an interesting question but what analyses you have in mind is not clear. One could perhaps ask about recurrences in the placebo groups in maintenance studies. If baseline grade 2 patients recur at grades 3/4, would that be a reason to treat from the start, even if the acute episode was no worse than grade? In any case, we should be clear as to what we are expecting. It seems to me to be at least as much a matter of judgement as of data or analyses. I presume this discussion will also be relevant to labeling of omeprazole and the H₂-antagonists.

- B. What is the concern reflected in the request for PK information? There are no active metabolites and the 2 fold increase in T 1/2 and AUC seen in the elderly and 4 fold increase of both in cirrhotics have led to no changes in dosing. Given our indifference to changes of this considerable size (dosage adjustments in cirrhotics need only be "considered"), why are we asking about Asians, patients with alcoholic liver impairment (doesn't cirrhosis cover that?) and cholestasis (which patients are those, exactly??)

II. Conclusions/Labeling

A. Dose

It is very clear in several cases that 15-60 mg of L give equivalent results. This is true for esophagitis maintenance, a claim we are not accepting at this time, and for DU and GU. The proposed dose for erosive esophagitis, however, is 30 mg. As a short term dose, there is certainly no safety reason not to use this dose, but we need to be clear on the evidence. Unfortunately, there is only one trial in erosive esophagitis (87-092) that explored a lower dose and Dr. Gallo Torres has pointed out that Study 87-092 included no truly severe patients. Studies that did (88-269, 89-349, 89-393) only used the 30 mg dose, which was regularly superior to 150 mg b.i.d. ranitidine.

Dr. Gallo-Torres concluded from study 89-092 that 15 mg was as effective as 30 mg, a reasonable conclusion, but there was a trend in that study toward earlier healing with the higher doses (64, 73, 76% at 4 weeks on 15, 30, 60 mg respectively) and, in fact, the 60 mg was nominally significantly superior to 15 mg at 4 weeks and the higher doses had a slight advantage at 6 and 8 weeks.

All in all, therefore, I agree with the choice of 30 mg as acute treatment for erosive esophagitis, even though 15 mg is also a clearly effective dose.

B. GU Claim

There is clearly one satisfactory supportive study (M87-091) but the second poses some problems.

I would note only a few points:

1. The healing rates are very high, probably higher, even in an active control trial, than we ever see on placebo.
2. The need for an evaluable patients analysis in this active control trial is perhaps debatable. I have pointed out that in active control equivalence trials an ITT analysis is not conservative (noise obscures differences) but that is not true where a difference is shown.
3. The size of the therapeutic gain of L over famotidine (MOR p. 266) is not really pertinent, as this study is trying to show superiority to a highly active agent; any gain is impressive and, in any event would be clear evidence of effectiveness.

4. I would like to go over the other deficiencies (endoscopy, etc). If all readings were blind and all analyses blind to treatment assignment, they do not seem obviously fatal defects. One problem, of course, is that there is no replication of the effectiveness of 15 mg, but I wonder whether the DU esophagitis and maintenance data have made it unnecessary to replicate that. It is obvious that 15 mg of L treats peptic disease.

C. Place of Lansoprazole

Dr. Gallo-Torres suggests (MOR p. 316) that L should be reserved, because of its tumorigenicity, for situations where other agents won't suffice. There is, of course, no evidence at all that there is any situation where omeprazole won't suffice, so that the reservation may be for a "null set" of situations. On the other hand, as Dr. Gallo-Torres says, concern is wholly with long-term use, so he has no problem with short-term treatments (up to the 12 weeks needed for esophagitis) so long as the drug is not used for maintenance.

All this seems technically correct, but the overall conclusion needs explanation. Why treat esophagitis with L (which can't be used in the absolutely essential maintenance phase) when you could use omeprazole in both? Who would start, then switch, and to what end? Are we really encouraging maintenance outside the PI here? Is the proposed limited use realistic? For DU, I suppose, there will soon be a real (no maintenance post-H pylori treatment), role for short-term use of acid-reducers, so I have less trouble with that claim. I am not trying to settle this issue here, but we need to explain our reasoning clearly. Part of the explanation, of course, is that we really are not worried about the non-gastric carcinogenicity and the gastric concerns are common to all PPI's.

Philosophy aside, we need clear explanations of why we are recommending approval of L for healing and symptom relief in erosive esophagitis when Dr. Gallo-Torres argued against this. (I believe the reason is in part that there turned out to be adequate data on patients with severe disease and that 30 mg had no visible adverse effects, but I see no resolution of his concern regarding long-term use.)

- D. Labeling - See attached for comments, changes, questions.


Robert Temple, M.D.

M E M O R A N D U M

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

DATE: January 30, 1995

FROM: Director, Division of Gastrointestinal and Coagulation
Drug Products, HFD-180

SUBJECT: Approvable Action for Lansoprazole Delayed Release
Capsules, NDA 20-406.

TO: Director, Office of Drug Evaluation I, HFD-100

Lansoprazole is a proton pump inhibitor, formulated for oral use in delayed release capsules for the treatment of acute duodenal ulcer, acute erosive esophagitis, hypersecretory states such as Zollinger-Ellison syndrome, and

Like omeprazole, lansoprazole produced carcinoids in the preclinical carcinogenicity studies. Additionally, Leydig cell tumors were produced in Sprague-Dawley rats, a strain where such findings are rare. These changes were postulated to be due to an effect on testosterone levels in these animals that would not be found in man, but support for this hypothesis needs further review. Also the finding of retinal atrophy in long-term treatment of albino rats is thought to be due to a taurine deficiency. While humans get taurine from oral intake, rats require endogenous hepatic synthesis. The sponsor suggests that lansoprazole inhibits the enzyme critical to this synthesis, and they have performed some studies, recently provided to the division, to support this mechanism. Careful study of humans on long-term lansoprazole administration has found no evidence of drug-induced ophthalmologic toxicity. The interesting observation of retinal atrophy in rats would, therefore, not represent a risk to humans, if we concur with their analyses.

We are recommending that the short-term uses for duodenal ulcer and erosive esophagitis and the treatment of hypersecretory states be approved at this time, while we obtain and review more data on the long-term indication for

Regarding the long-term claim, the advisory committee recommended that we review further data, and consider limitations on that claim before proceeding. I have included the transcript of that meeting to give you adequate background information for your decision. The letter details the areas to be submitted and/or reviewed.

NDA 20-406
Page 2

Where the drugs are comparable, the labeling proposed for lansoprazole is similar to the omeprazole labeling. The approvable omeprazole labeling (with deletion of the boxed warning) is enclosed to provide an appropriate reference.

We hope you will sign the letter at your earliest convenience, and we would be pleased to discuss the application with you, if desired.


Stephen Fredd, M.D.

CC:
NDA 20-406
HFD-180
HFD-180/HGallo-Torres
HFD-180/JChoudary
HFD-181/CSO/MWalsh
HFD-180/SFredd: 1/30/95
f/t deg: 1/30/95
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SEP 19 1994

MEDICAL OFFICER'S REVIEW
NDA 20-406
PREVACID® (Lansoprazole) capsules

Reflux Esophagitis.

Pathological Hypersecretory Conditions, including
Zollinger-Ellison syndrome

Duodenal Ulcer,

Submitted by:

TAP Pharmaceuticals, Inc.

Reviewer:
Hugo E. Gallo-Torres, M.D., Ph.D.
HFD-180

**DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW**

NDA: 20-406

Date Submitted: November 17, 1993

Date Filed: January 7, 1994
(For all indications requested, except

Sponsor (Manufacturer): TAP Pharmaceuticals, Inc.

Drug: PREVACID® (Lansoprazole) capsules

Route of Administration: Oral

Pharmacological Category: Antisecretory/Antiulcer
Inhibitor of the (H⁺, K⁺)-ATPase enzyme (proton pump) of the parietal cell in the gastric mucosa

Proposed Indications: Pathological Hypersecretory conditions, including Z-E syndrome.

First Draft to Supervisor: June 1, 1994

Date Review Completed: September 15, 1994

Material Reviewed: This submission consists of 639 volumes, numbered 2.1 to 2.639. Materials in volumes pertaining to the clinical reports (Section 8.0, Vol. 2.127 to 2.392), Case Report Tabulations (Section 11, Vol. 2.487 to 2.587) and pertinent CRFs (Section 12.0, Vol. 2.588 to 2.635) were reviewed in detail here. Other sections such as Animal Pharmacology/Toxicology, Human Pharmacokinetics (Section 6.0, Vol. 2.911 to 2.125) and Statistical Methods (Section 10.0, Vol. 2.393 to 2.486) were perused but not reviewed in detail.

This review consists of nine sections.

The first (Section A) deals with General Information. Included are data on pharmacologically-related drugs, non-clinical pharmacology, toxicology and ADME in animals, Human Metabolism and Biopharmaceutics, PKs in Special Populations, drug-drug interactions, PDs (gastric acid secretion) and other pharmacologic effects.

In the next six sections, separate reviews for each indication are given: RE (Section B), D), S-T Tx of DU (Section E),

At the end of each of these six sections, a summary of Efficacy Data per indication is given, as part of the Integrated Summary of Efficacy.

The reviewer's overall Summary and Conclusions (on Safety) are given in Section H. The Safety Update is reviewed under separate document.

Recommendations for Regulatory Action can be found at the end of this MO Review (Section I).

MOR of NDA 20-406
PREVACID Capsules
(Lansoprazole)

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CSO Reviews

Draft LBLg

Division of Gastrointestinal & Coagulation Drug Products

CONSUMER SAFETY OFFICER REVIEW

Application Number: NDA 20-406

Name of Drug: Prevacid (lansoprazole) Capsules

Sponsor: TAP Pharmaceuticals

Material Reviewed

Submission Date(s): November 12, 1993

Receipt Date(s): November 15, 1993

Background and Summary Description: Draft bottle labels (30 and 100 count for both the 15 mg and 30 mg dose), blister packages (15 and 30 mg dose), and carton label for the 15 mg blister packages were submitted in the original NDA.

Review

The draft labels were reviewed for conformity with the labeling regulations as set forth in 21 CFR 201, specifically, 21 CFR 201.1, 201.5, 201.10, 201.15, 201.17, 201.18, 201.50, 201.51, 201.55, and 201.100.

All labeling requirements in the cited regulations were met except for 201.17. The carton label for the blister packages should indicate the placement of the expiration date.

Conclusion

Indication of the placement of the expiration date on the carton label for the blister packages will bring the labeling into conformity with 21 CFR 201.17. All other labeling requirements according to 21 CFR 201 were met.

Maria Walsh 1/12/95

Consumer Safety Officer

NDA 20-406

Page 2

CC:

Orig NDA 20-406

HFD-180

HFD-180/M.Walsh

HFD-180/S.Fredd

J.Gibbs

A.Shaw

R/D init: M.Walsh 1/10/95

A.Shaw 1/10/95

J.Gibbs 1/10/95

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