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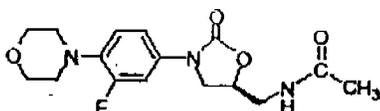
**21-130**

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**PHARMACOLOGY REVIEW**

OCT 12 1999

**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA**  
Division of Anti-infective Drug Products HFD-520**KEY WORDS:** Zyvox, Linezolid, PNU-100766, Mouse, Rat, Rabbit, Dog, Juvenile**Reviewer Name:** Kenneth Seethaler, R.Ph., Ph.D., D.A.B.T.**HFD#520****Date of submission:** May 3, 1999**Review completion date:** July 30, 1999**NDA number(s):** 21-130, 21-131, 21-132**Submission format:** Full reports**Scientific literature reviewed:** Yes ( ) No (x)**Information to sponsor:** Yes ( ) No (x)**Sponsor (or agent):** Pharmacia and Upjohn  
7000 Portage Road  
Kalamazoo, MI 49001**Contact person:** Peter DiRoma, Regulatory Manager  
Phone 616-833-8070**Drug:****Code name:** PNU-100766**Generic name:** Linezolid**Trade name:** Zyvox**Chemical name:** (S)-N-[[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]-methyl]-acetamide**Structure:**

Relevant INDs/NDAs/DMFs:

Drug Class: Oxazolidinone antibiotic

Indication: Treatment of Gram positive bacterial infections

This review covers three NDAs for linezolid.

NDA 21-130: Tablets, 400 and 600 mg

NDA 21-131: Intravenous Solution, 2 mg/mL

NDA 21-132: Powder for Oral Suspension, 100 mg/5 mL

The preclinical safety information was filed in NDA 21-130.

Note: The terms linezolid, PNU-100766, and U-100766 are used interchangeably throughout this review. Unless specified otherwise, the changes described in this review (increases, decreases, inhibitions, etc.) were statistically significant (when statistics were performed), and were considered to be treatment related, and biologically significant.

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## SAFETY PHARMACOLOGY

The following study was conducted to evaluate the cardiovascular and respiratory effects of linezolid in anesthetized beagle dogs. Four dogs (2 male, 2 female) received subcutaneous injections of morphine, followed by intravenous infusions of a mixture containing chloralose and urethane. After induction of anesthesia, the animals were also maintained on intravenous heparin and dextrose. The animals were prepared for recording of blood pressure, EKGs (lead II), and respiratory rates. Escalating doses of linezolid (3, 10, and 30 mg/kg) were infused intravenously.

Increases in heart rate and cardiac output (12-15%), and decreases in the P-R interval (4-8%) and the respiratory rate (20-30%) occurred after the completion of dosing. (TR No. 1470-95-017)

Note: It is the opinion of this reviewer that the data obtained in this study is of limited utility, because of the anesthesia, and the potential confounding effect of multiple drugs in the systemic circulation.

The effects of the compound on the enzyme monoamine oxidase and on cardiovascular responses to tyramine were also studied (TR No. 1425-96-012). When incubated *in vitro* with a human liver homogenate, U-100766 produced a reversible inhibition of monoamine oxidase. The inhibition was less than that produced by a positive control reference compound (1-deprenyl). In an experiment in anesthetized rats, the vasopressor and tachycardia responses to intravenous tyramine were potentiated in animals that had been pretreated with U-100766 (TR No. 7243-96-043). U-100766 also inhibited monoamine oxidase in rat brain (TR No. 7251-96-062).

Linezolid, at a dose of 125 mg/kg iv, prolonged thiopental-induced sleep time (loss of righting reflex) in female Sprague-Dawley rats (but not in males). TR No. 1470-96-007

Gastric emptying was studied in Sprague-Dawley rats. Based on a comparison of the weights of the stomach contents between control and treated groups, it was determined that PNU-100766 (62.5-100 mg/kg orally) caused nearly complete inhibition of gastric emptying (TR No. 1470-95-015).

In an experiment in pylorus-ligated Sprague-Dawley rats, a single dose of U-100766 (125 mg/kg iv) was associated with decreased gastric secretions (TR No. 1470-95-053). In another experiment in Sprague-Dawley rats, a single dose of U-100766 (125 mg/kg iv) had no effect on urine volume or sodium, potassium, or chloride excretion (TR No. 1470-95-054).

In other safety pharmacology studies, it was shown that linezolid, under the conditions tested, had no effect in the following experimental systems:

- Analgesia (Randall-Selitto method)
- Electroshock-induced convulsions
- Pentylenetetrazol-induced convulsions
- Isolated guinea-pig ileum
- Body temperatures
- Irwin behavioral screen

## PHARMACOKINETICS/TOXICOKINETICS

### A. PK Parameters

The following C<sub>max</sub> and AUC data was obtained from the intravenous one-month rat and dog studies:

Dose (mg/kg/day)	Day	Sex	C <sub>max</sub> Rat (µg/mL)	C <sub>max</sub> Dog (µg/mL)	AUC Rat (µg·h/mL)	AUC Dog (µg·h/mL)
10 <sup>A</sup>	1	M		6.28 ± 0.25		41.1 ± 8.2
		F		6.93 ± 0.24		41.2 ± 4.3
	28	M		10.4 ± 1.2		52.7 ± 7.1
		F		8.29 ± 1.59		55.6 ± 2.6
20 <sup>B</sup>	1	M	12.5 ± 0.4	14.3 ± 0.2	41.8 ± 5.3	112 ± 12
		F	12.3 ± 1.1	14.1 ± 2.6	48.9 ± 5.0	110 ± 27
	28	M	13.1 ± 0.4	19.7 ± 1.8	52.1 ± 4.0	128 ± 14
		F	13.8 ± 0.6	17.5 ± 1.3	58.4 ± 6.9	119 ± 16
40 <sup>C</sup>	1	M		35.6 ± 4.6		261 ± 14
		F		34.6 ± 6.9		284 ± 9
		M		42.3 ± 10.6		325 ± 39
		F		42.3 ± 5.5		384 ± 27
60 <sup>D</sup>	1	M	38.5 ± 1.9		150 ± 16	
		F	40.8 ± 1.0		201 ± 6	
	28	M	43.9 ± 3.6		199 ± 34	
		F	43.5 ± 2.5		201 ± 28	
200 <sup>E</sup>	1	M	141 ± 18		856 ± 113	
		F	161 ± 42		992 ± 64	
	28	M	150 ± 24		909 ± 73	
		F	185 ± 8		1410 ± 470	

<sup>A</sup> This dose level was a NOAEL for dogs.

<sup>B</sup> This dose level was a NOAEL for rats and was generally well tolerated in dogs.

<sup>C</sup> This dose level was associated with toxicity but no lethality in dogs.

<sup>D</sup> This dose level was generally well tolerated in rats.

<sup>E</sup> This dose level was associated with significant toxicity but no lethality in rats.

The following C<sub>max</sub> and AUC data was obtained from the oral one-month rat and dog studies:

Dose (mg/kg/day)	Day	Sex	C <sub>max</sub> Rat (µg/mL)	C <sub>max</sub> Dog (µg/mL)	AUC Rat (µg·h/mL)	AUC Dog (µg·h/mL)
20 <sup>A</sup>	1	M	7.85 ± 1.08	7.32 ± 1.42	37.0 ± 5.3	69.5 ± 4.5
		F	8.12 ± 1.56	6.09 ± 1.27	43.4 ± 3.3	70.0 ± 7.9
	28	M	7.55 ± 1.11	8.27 ± 0.89	47.9 ± 11.7	93.1 ± 8.9
		F	8.07 ± 1.71	7.89 ± 1.99	50.6 ± 10.1	92.9 ± 6.9
40 <sup>B</sup>	1	M		15.6 ± 3.7		167 ± 12
		F		16.0 ± 4.4		172 ± 26
	29	M		19.7 ± 2.7		238 ± 31
		F		19.7 ± 4.2		234 ± 30
50 <sup>C</sup>	1	M	17.7 ± 2.7		107 ± 14	
		F	15.5 ± 1.4		133 ± 6	
	28	M	15.6 ± 3.6		135 ± 32	
		F	16.5 ± 0.8		145 ± 3	
80 <sup>D</sup>	1	M		28.2 ± 2.3		342 ± 33
		F		30.1 ± 4.0		320 ± 45
	29	M		40.0 ± 15.0		637 ± 147
		F		34.2 ± 17.9		580 ± 192
125 <sup>E</sup>	1	M	36.0 ± 9.8		361 ± 26	
		F	33.7 ± 5.2		411 ± 11	
	28	M	36.4 ± 4.8		373 ± 37	
		F	32.7 ± 4.6		394 ± 25	

<sup>A</sup> This dose level was a NOAEL for both rats and dogs.

<sup>B</sup> This dose level was generally well tolerated in dogs.

<sup>C</sup> This dose level was generally well tolerated in rats but resulted in one death.

<sup>D</sup> This dose level was associated with significant toxicity but no lethality in dogs.

<sup>E</sup> This dose level was associated with significant toxicity but no lethality in rats.

The following C<sub>max</sub> and AUC data was obtained from the oral three-month rat and dog studies:

Dose (mg/kg/day)	Day	Sex	C <sub>max</sub> <sup>A</sup> Rat (µg/mL)	C <sub>max</sub> Dog (µg/mL)	AUC <sup>B</sup> Rat (µg·h/mL)	AUC Dog (µg·h/mL)
5	1	M		2.50 ± 0.46		17.4 ± 3.5
		F		2.22 ± 0.62		18.8 ± 4.4
	91	M		3.07 ± 0.36		24.2 ± 4.1
		F		2.25 ± 0.74		18.2 ± 5.2
10 <sup>C</sup>	1	M	2.98 ± 1.17	5.42 ± 0.79	11.2 ± 3.7	47.8 ± 1.9
		F	3.77 ± 0.62	5.50 ± 1.94	15.2 ± 1.8	41.8 ± 4.2
	91	M	4.24 ± 0.73	5.93 ± 0.69	21.4 ± 4.5	51.7 ± 4.3
		F	4.31 ± 0.63	5.11 ± 1.24	24.4 ± 2.8	61.0 ± 4.8
20 <sup>D</sup>	1	M		11.2 ± 0.8		93.6 ± 9.8
		F		10.1 ± 3.1		102 ± 17
	91	M		10.3 <sup>E</sup>		100 <sup>E</sup>
		F		10.5 ± 0.4		105 ± 10
40 <sup>F</sup>	1	M	11.5 ± 2.3	21.2 ± 6.6	65.4 ± 7.9	242 ± 30
		F	13.6 ± 2.4	23.3 ± 3.8	94.5 ± 10.8	212 ± 66
	91	M	18.3 ± 2.9	18.1 ± 4.2	119 ± 11	231 ± 52
		F	25.3 ± 7.3	17.4 ± 2.6	171 ± 29	158 ± 23
125 <sup>G</sup>	1	M	40.2 ± 8.9		337 ± 35	
		F	39.1 ± 11.5		352 ± 47	
	91	M	32.1 ± 6.4		285 ± 43	
		F	29.8 ± 15.6		279 ± 51	

- <sup>A</sup> C<sub>max</sub> represents the maximum plasma concentration of PNU-100766 after the PM dose.  
<sup>B</sup> AUC represents the area under the plasma concentration-time curve from zero to infinity on Day 1 in the rat study, from 0 to 24 hrs after the AM dose on Day 91 in the rat study, and from 0 to 24 hrs after the AM dose on Days 1 and 91 in the dog study.  
<sup>C</sup> This dose level was a NOAEL for rats.  
<sup>D</sup> This dose level was a NOAEL for dogs.  
<sup>E</sup> N = 2  
<sup>F</sup> Because of clinical toxicity, this dose level was reduced to 30 mg/kg/day on Study Day 37. This dose level was associated with significant toxicity but no lethality in dogs.  
<sup>G</sup> Because of clinical toxicity, this dose level was reduced to 80 mg/kg/day on Study Day 40. This dose level was associated with significant toxicity but no lethality in rats.

## B. Absorption

Linezolid is rapidly and extensively absorbed after oral administration to rats and dogs, with oral bioavailabilities of 75-95%, based on a comparison of the AUCs following oral and intravenous administration.

### C. Distribution

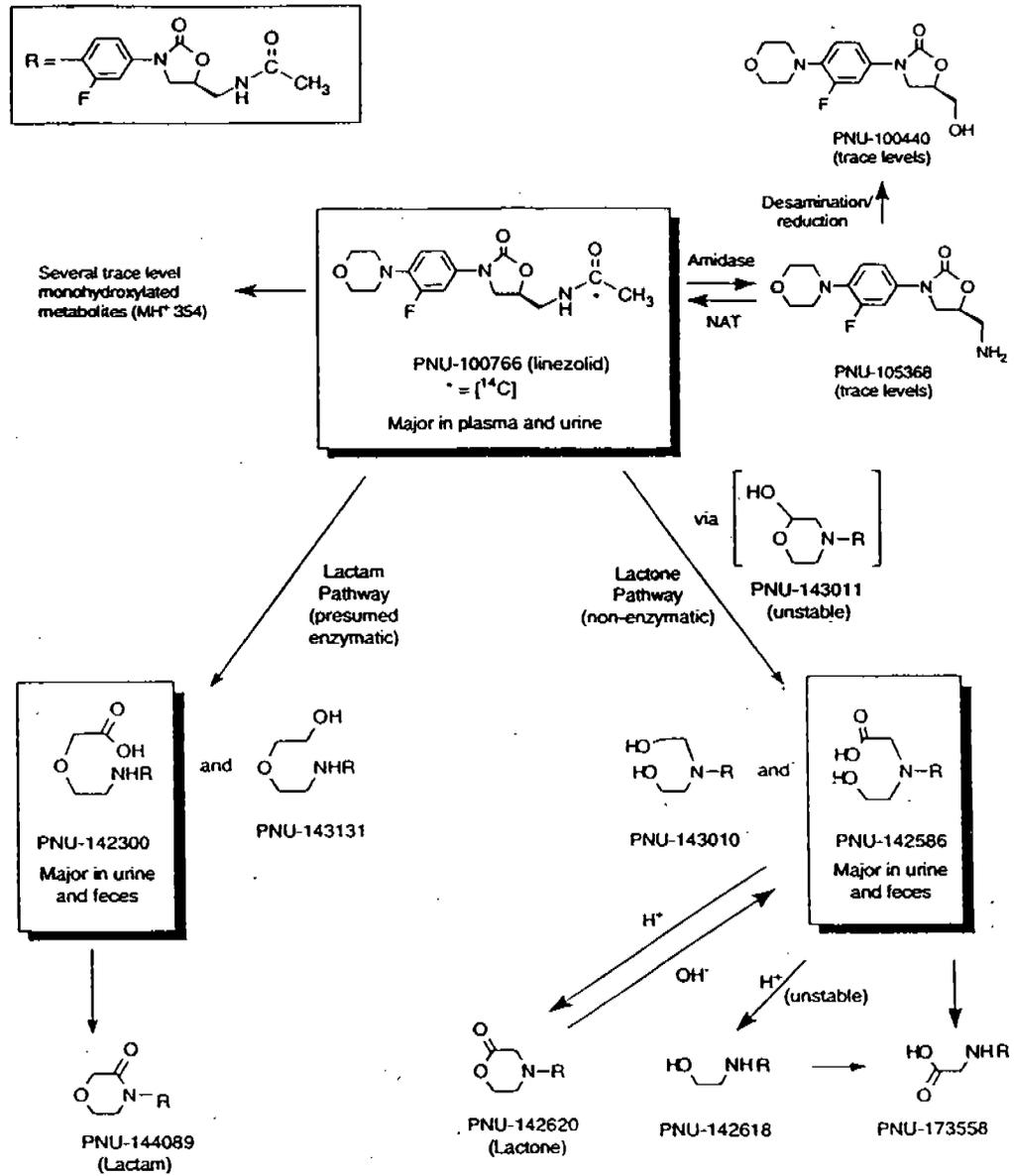
Linezolid is very well distributed to extravascular sites, with a volume of distribution approximately equal to total body water.

The following list shows the concentration of radioactivity in various tissues of Sprague-Dawley rats after a single intravenous dose (10 mg/kg) of C<sup>14</sup>-linezolid. The values listed are microgram equivalents of linezolid per gram of tissue. Each value is the mean (n=3) obtained at 0.3 hours after dosing.

<u>Tissue</u>	<u>Males</u>	<u>Females</u>
Blood	10.5	9.7
Plasma	11.3	10.7
Brain	2.0	1.7
Pituitary gland	9.6	9.3
Eyeball	4.4	4.2
Harderian gland	13.9	13.9
Thyroid	9.1	9.6
Thymus	9.2	8.6
Lung	10.4	10.0
Heart	11.0	10.3
Liver	19.8	17.7
Kidney	18.6	17.3
Adrenal	18.9	21.3
Spleen	9.6	9.0
Pancreas	8.8	8.3
White fat	1.7	1.6
Brown fat	7.8	8.0
Skin	7.8	7.1
Muscle	9.1	8.4
Bone marrow	9.4	8.3
Bone mineral	1.7	1.3
Mesenteric lymph node	9.2	8.1
Stomach	10.1	13.1
Small intestine	26.7	41.9
Large intestine	9.4	11.3
Bladder	11.6	11.3
Testis	4.2	-
Ovary	-	8.8
Uterus	-	9.6
Carcass	3.2	2.1

## D. Metabolism

The metabolic pathways for linezolid are shown below:



(NAT = N-acetyltransferase)

### E. Excretion

Linezolid is excreted mainly as parent drug and two carboxylic acid metabolites. Linezolid undergoes renal tubular reabsorption, but is excreted predominantly by the kidney.

Approximately 50-80% of the radioactivity from a C<sup>14</sup>-labeled dose of linezolid, could be detected in the urine. Fecal radioactivity accounted for 20-30% of the dose. In a study in bile duct-cannulated rats, approximately 15% of the administered radioactivity was excreted in the bile.

### F. Other Studies

The ability of PNU-100766 to induce liver microsomal enzymes was investigated. Liver specimens were obtained at necropsy from rats that had been dosed orally for 90 days, with PNU-100766 at levels of either 10, 40, or 80 mg/kg/day. Possible induction effects were evaluated by measuring the activities of five cytochrome P450 isoforms, i.e. CYP1A, CYP2B, CYP2E1, CYP3A, and CYP4A using the following enzyme systems:

1. 7-ethoxyresorufin-O-dealkylase
2. 7-pentoxyresorufin-O-dealkylase
3. 4-nitrophenylhydroxylase
4. testosterone-6 $\beta$ -hydroxylase
5. lauric acid-12-hydroxylase

The following five known inducers were used as positive controls respectively:

1.  $\beta$ -naphthoflavone
2. phenobarbital
3. isoniazid
4. pregnenolone-16 $\alpha$ -carbonitrile
5. clofibric acid

All of the positive controls were strong inducers of their respective marker enzymes. PNU-100766 had little or no effect on any of the enzymes (Study 95456).

In other studies, it was shown that linezolid has limited protein binding (less than 35%).

Linezolid crosses the placenta, and, in lactating rats, is secreted in breast milk.

Note: Some additional pharmacokinetic and systemic exposure data is presented in the "TOXICOLOGY" section of this review (juvenile studies, reproductive studies, acute and chronic studies).

## TOXICOLOGY

Note: All of the changes described in this review were statistically significant and considered to be treatment-related. All pivotal safety studies were conducted according to GLPs.

### A. Acute/Single-Dose Studies

#### Acute Oral Toxicity Study in Rats (TR No. 1470-95-004)

An aqueous suspension of PNU-100766 containing Avicel, Tween 80, and sodium acetate was administered orally to four groups of Sprague-Dawley rats (5/sex/group) at doses of either 0, 1000, 3000, or 5000 mg/kg/day. Additionally 2/sex/group were given the same doses for toxicokinetic study. The dose was divided into two equal doses 8 hours apart.

Clinical signs attributed to the treatment with U-100766 included alopecia, decreased activity, ataxia, and unkempt appearance. There were no deaths in the male animals; four of the five high dose females died between days 2 and day 12 without any additional clinical signs. Suppressed body weight gains of both sexes in the 3000 mg/kg/day group and males in the 5000 mg/kg/day group during the first week of the study returned to normal in surviving rats. Grossly, notable changes in the dead animals were limited to the stomach: dark spots on the mucosa and the presence of a white material, probably the drug. In the surviving rats, main gross necropsy findings were enlarged cecum and alopecia.

Toxicokinetic analysis indicated that average  $C_{max}$  values up to 8 hours after the morning doses of 500, 1500 and 2500 mg/kg were 152, 174 and 208  $\mu\text{g/mL}$  in males and 154, 293 and 239  $\mu\text{g/mL}$  in females, respectively.

The minimum lethal doses of PNU-100766 were 5000 mg/kg for female rats, and greater than 5000 mg/kg for the males.

#### Acute Intravenous Toxicity Study in Rats (TR No. 1470-95-032)

Four groups of Sprague-Dawley rats (3/sex/group) were given two intravenous injections of PNU-100766, 6 hours apart in one day. Doses of PNU-100766 in vehicle (0.01 M sodium citrate and HCl (q.s.) buffered solution (pH 4.8) containing 4.5% dextrose] were 0, 100, 200 and 400 mg/kg/day (0, 50, 100 and 200 mg/kg/dose) and drug concentrations in the vehicle were 0, 2, 4 and 8 mg of U-100766 per mL of the vehicle.

No systemic or local adverse drug effects were observed in any of the rats treated with PNU-100766. The no-observed-adverse-effect-level (NOAEL) of PNU-100766 was greater than 400 mg/kg.

**Acute Oral Toxicity Study in Dogs**  
(TR No. 1470-96-013)

PNU-100766 was administered orally, in gelatin capsules, to three groups of two male beagle dogs, in two equally divided doses, separated by an eight hour interval. The total doses were either 500, 1000, or 2000 mg/kg, after which the animals were observed for 14 days.

There were no deaths. Vomiting, tremors, and decreased activity were the primary clinical observations. Cmax values were 187, 206, and 269 mcg/ml after total doses of 500, 1000, and 2000 mg/kg respectively.

Note: No acute intravenous toxicity study in dogs was reported.

**B. Subchronic/Repeat-Dose Studies**

More than a dozen subchronic toxicity studies were reported in this NDA. Two-week and one-month studies were conducted in young adult rats and dogs, by both the oral and intravenous routes. Oral studies were conducted in juvenile rats, and a one-week oral study was conducted in rabbits.

**One-Month Oral Toxicity Study with a Recovery Phase in Rats**  
(TR No. 1470-95-025)

An aqueous suspension of PNU-100766 containing Avicel and Tween 80 was administered orally to Sprague-Dawley rats (approximately six weeks of age at the start of dosing) according to the following study design:

<u>Groups</u>	<u>Daily Dose</u> (mg/kg/day)	<u>Number of Rats</u>	
		Male	Female
Control	0 (vehicle)	15	15
Low Dose U-100766	20	10	10
Mid Dose U-100766	50	15	15
High Dose U-100766	125	15	15

Additionally, 3/sex/group rats were dosed 20, 50, or 125 mg/kg/day for toxicokinetic study, and were necropsied at dose termination. One-half of the daily dose was administered orally (gastric intubation needle) in the AM and the other one-half in the PM approximately 8 hours apart for 28 or 29 consecutive days. The first 10 rats/sex/group were necropsied at the termination of dosing; the remaining 5 rats/sex/group were necropsied following the 4-week recovery phase.

The most common treatment-related clinical signs were distended abdomen and salivation. Body weights decreased in males and females of the 125 mg/kg/day; food and water consumption decreased in high dose females only.

Hematology: Red blood cell and reticulocyte counts were decreased in both sexes and segmented neutrophils, monocyte and platelet counts, and hemoglobin and hematocrit levels were decreased in high dose females. Low- and mid-dose rats were not affected. An increased myeloid/erythroid cell ratio (M/E) in the bone marrow was seen in 2/10 males and 2/10 females of 125 mg/kg/day dose groups.

Blood Chemistry: Globulin and triglyceride levels were decreased and BUN levels were increased in both sexes. In females, glucose levels were increased and levels of total protein, aspartate aminotransferase and creatine kinase were decreased. The A/G ratio was slightly increased in high dose males.

Gross Observations: An enlarged cecum was seen in all dose-groups. A diffusely dark liver occurred in the mid and high-dose groups. Decreased adipose tissue, and large adrenals were noted in the high-dose group.

Organ Weights: At 125 mg/kg/day, absolute and relative adrenal weights were increased in females. Decreases in other organ weights (heart, kidneys, lungs and spleen) were noted in females and were considered to be secondary to suppressed body weight gain.

Histopathology: At 125 mg/kg/day: Mild hypocellularity of the bone marrow with a minimal decrease in hematopoiesis of the erythrocyte and megakaryocyte series was seen in 3/10 males, and 8/10 females. In the intestine, drug-related effects included a minimal to mild decrease in mucin in goblet cells and minimal epithelial cell necrosis and mucosal hemorrhage. One female exhibited mild ulceration of the duodenal mucosa. Minimal to mild lymphoid atrophy was found in the mesenteric and mandibular lymph nodes in both sexes. In the spleen, decreased hematopoiesis of the megakaryocyte series was seen. Decreased vacuolation was noted in portal hepatocytes in both sexes.

At 50 mg/kg/day: Minimal changes seen at a low incidence included bone marrow hypocellularity, lymphoid atrophy of lymph nodes, decreased mucin in intestinal goblet cells and necrosis of the intestinal epithelial mucosa.

At the end of 4-week recovery period, the only notable change was distension of the cecum in 1/5 females of the 50 mg/kg/day level and 2/5 females at the 125 mg/kg/day dose level. Hypocellularity of the bone marrow was completely reversed within 4 weeks of cessation of treatment.

The NOAEL was 20 mg/kg/day, and the 50 mg/kg/day dose level was well tolerated with only mild effects.

#### **One-Month Intravenous Toxicity Study with a Recovery Phase in Rats (TR No. 1470-95-042)**

PNU-100766 dissolved in 0.01 M sodium citrate, pH 4.8, containing 4.5% dextrose was administered to Sprague-Dawley rats by i.v. bolus injection twice daily for 28 days to males and 29 days to females at dose levels of 0 (vehicle), 20, 60 or 200 mg/kg/day (0, 10, 30, 100

mg/kg/dose in 0, 0.5, 1.5 or 5 mg/mL dosing solutions) at dose volumes of 20 mL/kg/dose (40 mL/kg/day). A 4-week recovery phase was included for the 0, 60 and 200 mg/kg/day dose levels. Groups of 15/sex/group were used; of these 10/sex/group were necropsied at dose termination. The remaining 5/sex/group were necropsied following the 4-week recovery phase.

PNU-100766 administered i.v. at dose levels of 20, 60 or 200 mg/kg/day was well tolerated. There were no moribund animals or deaths at any dose level during the study.

200 mg/kg/day: Treatment-related clinical observations at this dose level included salivation, and abdominal distension resulting from cecal enlargement. Decreased body weight gain and food consumption, were noted in both sexes. Reticulocyte, red blood cells and segmented neutrophil counts and hemoglobin and hematocrit levels were decreased in both sexes and the M/E ratio was increased. Histopathology changes included hypocellularity of the bone marrow and decreased hematopoiesis of the erythrocyte and megakaryocyte series in bone marrow and spleen.

Recovery from all toxic effects, including the bone marrow changes, was complete or nearly complete at the end of the 4-week recovery period.

60 mg/kg/day: At this dose level treatment-related findings were limited to salivation in one male and one female rat, decreased segmented neutrophil counts in females and a low incidence of bone marrow hypocellularity in both sexes. None of these changes were seen at the end of the recovery period.

The NOAEL in this study, was 20 mg/kg/day.

#### **One-Month Oral Toxicity Study in Juvenile Rats (Study 97-151)**

Non-treated, timed-pregnant Sprague-Dawley rats were maintained in nesting cages and were allowed to deliver litters normally. The pups from 78 litters were culled to 6/litter, and assigned to one of four groups. An aqueous suspension of PNU-100766 was prepared containing 5% Tween 80 and 1% Avicel (microcrystalline cellulose). The pH was adjusted to 4.5, and also contained 50 mM of sodium acetate. The suspension was administered orally via gastric intubation starting when the pups were 6-7 days of age. The groups received either 0, 10, 25, or 63 mg/kg/day for one month. There were 20 pups/sex/group in the study, of which 15 were sacrificed at the end of dosing, and 5 were maintained on a treatment-free recovery period of six weeks. Additional pups were also dosed, and bled at various times for pharmacokinetic measurements.

The pups were weaned at 16 days of age, and were then observed until the scheduled sacrifice. Evaluations for treatment-related effects were based on observations, body weights, food consumption, hematology, coagulation, serum chemistry, urinalysis, ophthalmic examinations, gross pathology, organ weights, microscopic histopathology, and limited electron microscopy.

There were 14 deaths during the study but they were thought to be not related to treatment because most were due to either cannibalism or dosing difficulties (crystals of drug were found in the lungs at necropsy). The effects that were considered to be treatment-related occurred mainly in the high-dose group, and consisted of the following: alopecia and thinning of the hair coat, slight but significant decreases in white blood cells (neutrophils, monocytes), increased serum aspartate aminotransferase, decreased testes weights, and increases in the numbers of large and multinucleated cells in the lumina of the seminiferous tubules of the testis. All of the changes were reversible in the recovery period. Systemic exposure to the test substance was demonstrated by the plasma drug concentrations shown in the following table:

Dose	Cmax	Cmax	Cmax	Cmax	AUC	AUC	AUC	AUC
mg/kg	mg/l	mg/l	mg/l	mg/l	Units:	mcg x	hours	/ ml
	Day 1	Day 1	Day 30	Day 30	Day 1	Day 1	Day 30	Day 30
	M	F	M	F	M	F	M	F
10	5.0	4.4	2.9	3.8	36.6	39.5	11.1	14.7
25	14.5	13.7	10.1	10.4	151.8	138.5	47.4	48.5
63	39.4	38.2	20.6	23.4	489.4	432.4	109.9	146.7

#### **One-Week Oral Toxicity Study in Rabbits (Study 96478)**

An aqueous suspension of PNU-100766 was administered orally by gastric intubation to groups of New Zealand albino rabbits (4 males/group) at doses of either 0, 25, 50, or 100 mg/kg/day for one week. Evaluations were based on clinical observations, body weights, hematology, serum chemistry, gross pathology, organ weights, and microscopic histopathology.

**Results:** A mortality rate of 50% (2/4) occurred in the high-dose group (100 mg/kg). The other signs of toxicity in this group were a reduced rate of body weight gain, diarrhea, and changes in organ weights. Adrenal weights (absolute and relative) were increased, while thymus, spleen, and liver weights (absolute) were decreased. Necrotizing enterocolitis of the ileum, cecum, and colon was seen microscopically in the two animals that died. Other microscopic changes seen in the high-dose group were hypertrophy of the adrenal cortex (2/3), lymphoid atrophy of the spleen and thymus (3/3), and atrophy of all cell types in the bone marrow (2/3). (One animal was not examined microscopically).

The effects seen at 25 and 50 mg/kg were non-remarkable.

#### **One-Month Oral Toxicity Study with a Recovery Phase in Dogs (TR No. 1470-95-016)**

Groups of 4/sex/group beagle dogs were administered PNU-100766 as bulk drug in gelatin capsules twice daily at dose levels of 0 (empty capsules), 20, 40 or 80 mg/kg/day (0, 10, 20 or 40 mg/kg/dose). An additional 2 dogs/sex/group were given 0, 40 or 80 mg/kg/day and allowed a 42 day recovery period before the final necropsy on day 72.

205  
No deaths occurred during the course of the study. The 80 mg/kg/day dose level was poorly tolerated; mild adverse effects were seen at 40 mg/kg/day; NOAEL was 20 mg/kg/day.

80 mg/kg/day: Adverse clinical findings included anorexia, marked body weight loss, emaciation, vomiting, and passing of mucous stools, with evidence of toxicity seen clinically from day 8. Decreased heart rate and prolonged QT interval values were present in EKGs of both males and females. The primary histopathologic changes included general bone marrow atrophy and atrophy of the thymus and lymph nodes, which was reflected in decreased numbers of circulating white blood cells, neutrophils, monocytes and platelets. Increased alanine aminotransferase levels were noted in one-half of the dogs during the last 2 weeks of dosing period or the first 2 weeks of the recovery phase, but no histopathological evidence of hepatocellular degeneration or necrosis was seen.

At the end of recovery period, no changes in clinical observations, EKGs, laboratory parameters, thymus, lymph nodes or intestine were observed. Slight changes were still evident in some dogs in the bone marrow, kidneys, testes and prostate, although all changes showed evidence of reversibility.

40 mg/kg/day: This dose level produced mild adverse drug effects. All dogs remained clinically normal throughout the study. The primary hematologic changes included slight decreases in circulating white blood cell, neutrophil and platelet counts. Other findings included increased urine specific gravity, acidified urine, decreased prostate weight, bone marrow atrophy, increased numbers of megakaryocytes with multiple separate nuclei, mild atrophy of the prostate, testis and epididymis, increased relative mean heart weights of female dogs, and decreased mucin in the goblet cells in the small and large of both sexes.

All dogs at this level recovered rapidly and completely during the recovery period, with most laboratory parameters within normal limits by day 12.

20 mg/kg/day was considered to be the NOAEL.

#### **One-Month Intravenous Toxicity Study in Dogs (TR No. 1470-95-052)**

For dosing, U-100766 was dissolved in a sterile isotonic solution containing sodium citrate and dextrose at a pH of 4.8. The dosing solution was administered intravenously to beagle dogs (6/sex/group) twice daily six hours apart. The doses were 0, 5, 10, or 20 mg/kg twice daily for one month (0, 10, 20, or 40 mg/kg/day). Evaluations for treatment-related effects were based on observations, body weights, food consumption, ophthalmic examinations, electrocardiograms, hematology, coagulation, serum chemistry, urinalysis, organ weights, gross pathology, bone marrow myeloid/erythroid ratios, and microscopic histopathology. After one month of dosing, two dogs per sex per group were maintained on a treatment-free recovery phase for six weeks.

There were no deaths in the study. One or more of the following effects were seen in high-dose animals: bone marrow hypoplasia, decreased levels of circulating erythrocytes, leucocytes

(neutrophils and monocytes), and platelets, and vacuolation of the proximal tubular epithelium of the kidney. The effects were reversible during the recovery period. In the mid-dose group, slight and reversible decreases in circulating leucocytes and platelets occurred, along with hypoplasia of erythroid elements in bone marrow, and vacuolation of renal proximal tubular epithelium. There was no evidence of vascular irritation in the study.

The NOAEL was 10 mg/kg/day.

### C. Three-Month Studies

#### Three-Month Oral Toxicity Study in Rats (Study 95-456)

An aqueous suspension of PNU-100766 was administered orally, by gastric intubation, to Sprague-Dawley rats (25/sex/group). The doses were 0, 5, 20, or 62.5 mg/kg twice daily (0, 10, 40, or 125 mg/kg/day). Because of excessive toxicity in the high-dose group, the total daily dose in this group was lowered from 125 to 80 mg/kg/day, on day 40 of the study. Evaluations for treatment-related effects were based on observations, body weight, food and water consumption, ophthalmic examinations, hematology, serum chemistry, urinalysis, gross pathology, organ weights, and microscopic histopathology. At the end of dosing, 20 animals per sex per group were sacrificed, and the remaining 5 rats/sex/group were placed on a recovery period of one month.

There were no deaths in the study. In the early weeks of the study, the signs of toxicity seen in the high-dose group, were thought to be severe enough to require lowering the dose in this group. The signs included salivation, alopecia, distended abdomen, unkempt appearance, and decreased food consumption and body weight. Starting on study day 40, the total daily dose was lowered to 80 mg/kg/day, which was better-tolerated.

The following effects occurred in the 80 mg/kg/day group: decreases in food consumption, body weight, circulating erythrocytes, reticulocytes, neutrophils, fibrinogen, albumin, globulin, total protein, and triglycerides; there were increases in mean red blood cell volume, mean cell hemoglobin, and serum alkaline phosphatase. There were also microscopic changes in the spleens and epididymides. The numbers of megakaryocytes in the cross-section of the spleen were decreased in all treated groups, as compared to controls. Inflammatory cells were observed in the body of the epididymis, and the epithelial cells lining the ductus epididymis were hypertrophied. Increased numbers of desquamated epithelial cells were observed in the lumen of the epididymis. Some of these changes were also present to a lesser extent, in the 40 mg/kg/day group. The NOAEL was 10 mg/kg/day.

Some, but not all of the findings, were reversible during the recovery period. The decreases in erythrocytes, fibrinogen, globulin, and total protein were still present at the end of the recovery period, as were the microscopic changes in the epididymis.

**Three-Month Oral Toxicity Study in Dogs**  
(TR No. 7226-97-007)

A three month oral toxicity study was conducted in beagle dogs. Linezolid was administered as compressed tablets in gelatin capsules at doses of 0, 5, 10, 20, or 40 mg/kg/day. On study day 37, the dose in the high-dose group had to be lowered to 30 mg/kg/day. Evaluations for treatment-related effects were based on a standard battery of toxicological measurements, including ophthalmic examinations, and electrocardiograms.

There were no deaths in the study. Decreased food consumption and body weight, and decreases in red blood cell counts, hematocrit, hemoglobin, and reticulocytes occurred in the high-dose group, during the time that the animals were dosed at 40 mg/kg/day. The toxicity was reversible when the dose was lowered to 30 mg/kg/day. The NOAEL was 20 mg/kg/day.

The pharmacokinetic parameters determined in the study are presented in the following table.

Parameter	6 mg/kg/day		10 mg/kg/day		20 mg/kg/day		40 or 30 mg/kg/day†	
	Male	Female	Male	Female	Male	Female	Male	Female
Day 1								
C <sub>max</sub> -AM (µg/mL)	2.42 ± 0.51	2.50 ± 0.29	5.54 ± 0.09	4.97 ± 0.54	10.7 ± 0.5	11.4 ± 0.6	18.7 ± 1.1	19.4 ± 2.7
t <sub>max</sub> -AM (h)	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.3 ± 0.6	1.0 ± 0.0	1.0 ± 0.0	1.7 ± 0.6	1.0 ± 0.0
C <sub>max</sub> -PM (µg/mL)	2.50 ± 0.46	2.22 ± 0.82	5.42 ± 0.79	5.50 ± 1.94	11.2 ± 0.8	10.1 ± 3.1	21.2 ± 6.0	23.3 ± 3.8
t <sub>max</sub> -PM (h)	1.3 ± 0.5	1.5 ± 0.6	1.7 ± 0.6	2.0 ± 1.7	1.0 ± 0.0	1.7 ± 0.6	2.3 ± 1.5	1.3 ± 0.6
C <sub>min</sub> (µg/mL)	0.0264 ± 0.0146	0.0222 ± 0.0083	0.137 ± 0.007	0.0769 ± 0.0255	0.176 ± 0.129	0.275 ± 0.145	1.15 ± 0.41	0.638 ± 0.713
AUC(0,24) (µg·h/mL)	17.4 ± 3.5	18.8 ± 4.4	47.8 ± 1.9	41.8 ± 4.2	93.8 ± 9.8	102 ± 17	242 ± 30	212 ± 66
AUC(0,∞) (µg·h/mL)	17.5 ± 3.5	16.9 ± 4.4	48.3 ± 1.9	42.0 ± 4.2	94.8 ± 10.3	104 ± 18	245 ± 33	214 ± 70
t <sub>1/2</sub> (h)	2.28 ± 0.23	2.14 ± 0.12	2.82 ± 0.05	2.39 ± 0.06	2.38 ± 0.41	2.57 ± 0.2	3.00 ± 0.33	2.57 ± 0.82
Day 91								
C <sub>max</sub> -AM (µg/mL)	2.84 ± 0.55	2.14 ± 0.42	5.59 ± 0.84	6.82 ± 0.91	11.6†	10.4 ± 1.5	18.2 ± 1.1	16.4 ± 1.9
t <sub>max</sub> -AM (h)	1.3 ± 0.5	1.3 ± 0.5	1.3 ± 0.6	1.3 ± 0.6	1.0†	1.3 ± 0.6	1.0 ± 0.0	1.0 ± 0.0
C <sub>max</sub> -PM (µg/mL)	3.07 ± 0.36	3.25 ± 0.74	5.93 ± 0.69	6.11 ± 1.24	10.3†	10.5 ± 0.4	18.1 ± 4.2	17.4 ± 2.6
t <sub>max</sub> -PM (h)	1.3 ± 0.5	1.5 ± 0.6	1.0 ± 0.0	2.7 ± 1.2	1.5†	1.3 ± 0.6	3.0 ± 1.7	1.3 ± 0.6
C <sub>min</sub> (µg/mL)	0.0839 ± 0.0431	0.0298 ± 0.0138	0.181 ± 0.050	0.367 ± 0.013	0.478†	0.425 ± 0.194	3.06 ± 1.87	0.466 ± 0.205
AUC(0,24) (µg·h/mL)	24.2 ± 4.1	18.2 ± 5.2	51.7 ± 4.3	51.0 ± 4.8	100†	105 ± 10	231 ± 52	158 ± 23

C<sub>max</sub>-AM represents the maximum concentration of U-100766 between the AM and PM doses. t<sub>max</sub> represents the time after the AM or PM dose. C<sub>max</sub>-PM represents the maximum concentration of U-100766 after the PM dose.  
C<sub>min</sub> represents the concentration of U-100766 at 24 h after the AM dose. Thus, the values of C<sub>min</sub> on days 14 and 44 equal the concentrations at zero time on days 15 and 45, respectively.  
AUC(0,24) represents the area under the concentration-time curve from zero to 24 h after the AM dose.  
AUC(0,∞) represents the area under the concentration-time curve from zero to infinity.  
t<sub>1/2</sub> represents the terminal half-life after the PM dose.  
†The dose level of 40 mg/kg/day was changed to 30 mg/kg/day on day 37.  
†n=2: Because the pharmacokinetic parameters for #42 were excluded from calculation of mean value. The reason was described in a separate report [7] which was included in the raw data file.

**D. Reproductive Toxicology Studies**

**Teratology Study in Mice**  
(Study 95-225)

A suspension of PNU-100766 was administered orally by gastric intubation to four groups of pregnant CD-1 mice (25 mice/group) during days 6-16 of gestation. The dose levels were 0, 50, 150, or 450 mg/kg/day. On gestation day 18, the dams were sacrificed and the fetuses removed by cesarean section. The fetuses were weighed, sexed, and examined for external and visceral or skeletal abnormalities, using standard stains and methodology. Some additional pregnant animals were dosed, and used for pharmacokinetic measurements.

Maternal and fetal toxicity occurred in the high-dose group. The dams had an unkempt appearance and a reduced rate of body weight gain as compared to controls. There was an increase in the number of resorptions and post-implantation loss, and there was complete litter resorption in eight of the dams in this group. There was a significant decrease in the mean weight of the viable fetuses in this group. Skeletal examinations revealed a significant increase in costal cartilage fusion, sometimes associated with abnormalities of the sternbrae, in the fetuses from the high-dose group.

The effects seen in the 50 and 150 mg/kg groups were similar to those observed in the control group. Adequate exposure to the test substance was demonstrated by mean maximum plasma concentrations of 36, 71, and 163 mcg/ml in the low, mid, and high-dose groups respectively.

### **Teratology Study in Rats (Study 95-207)**

This was a GLP study conducted by the sponsor in [redacted] as part of a combined Segment I/II/III study. A suspension of PNU-100766 was administered orally by gastric intubation to four groups of pregnant Sprague-Dawley rats (24 rats/group) during days 6-17 of gestation. The dose levels were 0, 2.5, 15, or 50 mg/kg/day. On gestation day 20, the dams were sacrificed and the fetuses removed by cesarean section. The fetuses were weighed, sexed, and examined for external and visceral or skeletal abnormalities, using standard stains and methodology. Study animals were bled at various times for pharmacokinetic measurements.

There were slight but statistically significant decreases in maternal body weights, and fetal weights in the high-dose group. Some instances of unossified sternbrae occurred in the mid and high-dose groups, but there were no visceral or skeletal abnormalities that could be attributed to treatment. Plasma drug concentrations measured 0.8, 7.8, and 24.1 mcg/ml on gestation day 6, and 1.1, 6.6, and 19.7 mcg/ml on gestation day 15, in the low, mid, and high-dose groups respectively.

### **Fertility and Reproductive Toxicity Study in Rats**

This was the Segment I portion of Study 95-207. A suspension of PNU-100766 was administered orally by gastric intubation to Sprague-Dawley rats (24 male and female pairs per group). The doses were as described previously, i.e. 0, 2.5, 15, and 50 mg/kg/day. Males were dosed for at least four weeks before cohabitation; females were dosed for two weeks before cohabitation, and throughout mating, gestation, and postpartum. The females were allowed to deliver normally, and were sacrificed after their litters had been weaned.

There was no effect on copulation or mean precoital interval. In the mid and high-dose groups, there were very slight decreases in the fertility index (percentage of cohabitated pairs producing a pregnancy) and the conception index (percentage of copulated pairs producing a pregnancy). Only the decrease in the conception index in the mid-dose group showed statistical significance. Supplementary experiments were added to this study in order to determine which sex was affected. Treated males were mated with untreated females, and untreated males were mated

with treated females. Similar decreases in fertility were again seen in the pairs where the male had been treated, but were not seen in the pairs where the male was untreated.

In the main study, 12 males per group were sacrificed for sperm evaluation. Sperm motility was slightly decreased in the mid and high-dose groups. The decrease was statistically significant in the mid-dose group, but not in the high-dose group. Overall, the trend appeared to be treatment-related. Microscopic histopathology revealed the presence of abnormal epithelial cells in the lumens of the epididymides from some animals in the high-dose group. There were no changes in the seminiferous tubules.

### **Prenatal and Postnatal Developmental Toxicity Study in Rats**

This was the Segment III portion of Study 95-207. The functional and behavioral development, and sexual maturation of the offspring (F-1 generation) were evaluated. This included measurements of body weights, pinna detachment, auditory response, eye opening, locomotor reflexes, maze learning, balanopreputial separation, vaginal opening, and reproductive performance.

Pinna detachment occurred on postpartum day 3 in most pups from the control and low-dose groups, and on day 4 in most pups from the mid and high-dose groups. The auditory response was delayed one to two days in pups from the mid and high-dose groups. The differences were statistically significant only in the high-dose group. Locomotor reflexes (geotaxic orientation) were also delayed in these two groups of pups. No other developmental changes were seen.

### **Fertility Study in Juvenile Male Rats** (Study 97-055)

This was a GLP study conducted by the sponsor in [redacted] it was designed to evaluate reproductive performance in male Sprague-Dawley rats that had been exposed to PNU-100766 during the period of spermatogenesis. Non-treated, timed-pregnant females were maintained in nesting cages. Male pups were divided into four groups (30/group). Female pups were sacrificed. A suspension of PNU-100766 was administered orally by gastric intubation once daily for seven weeks, starting at seven days of age. Between 7-42 days of age, the doses were 0, 12.5, 25, or 50 mg/kg. From 43-55 days of age the doses were doubled to 0, 25, 50, or 100 mg/kg. Pups were weaned at 22 days of age, and examined for balanopreputial separation at 45-49 days of age. Serum testosterone levels were also measured. At two weeks, 10 weeks, and 15 weeks after the last dose, 20 of the 30 male pups in each group were mated with virgin females. The other 10 male pups from each group were sacrificed for sperm evaluation. Copulated females were sacrificed for uterine examination.

In the high-dose group, balanopreputial separation was delayed by one to two days. Testosterone levels appeared to be decreased in the high-dose group, but there so much variability in the values that the difference lacked statistical significance. There were no effects on sperm counts or sperm motility. No treatment-related effects on reproductive indices or uterine examination parameters occurred during the first mating period (two weeks after the end of dosing).

However, at the later mating periods, there was an unexplained, slight decrease in the fertility index.

### Reproductive Toxicity and Reversibility Study in Male Rats (Study 96-027)

This was a GLP study conducted by the sponsor in [redacted]. It was designed to determine if the effects on male fertility were reversible. A suspension of PNU-100766 was administered orally to three groups of mature (approximately 10 weeks of age) male Sprague-Dawley rats (24 rats/group). The doses were 0, 50, or 100 mg/kg/day for 65 days. The treated males were mated with five sets of untreated females, during weeks 4, 7, 10, 13, and 18 of the study, i.e. the 4<sup>th</sup> and 7<sup>th</sup> weeks of dosing and 1, 4, and 9 weeks after completion of dosing. Male fertility was evaluated by the precoital interval, copulation index, fertility index, and conception index. Blood was drawn from the males for measurement of serum testosterone levels, and for plasma drug concentration measurements. The males were sacrificed on study days 148-151. Sperm was collected from the cauda epididymis for determination of concentration, and from the vas deferens for determination of motility. Microscopic histopathology was performed on the reproductive organs (testes, epididymides, prostate, and seminal vesicles). The female animals were sacrificed on gestation day 13, and underwent uterine examination.

No reproductive effects were seen at a dose of 50 mg/kg in this study, although adverse reproductive effects had been seen in previous studies at 15 and 50 mg/kg (see above). In the 100 mg/kg group in this study, decreased fertility index and decreased conception index (both statistically significant) occurred at the mating during the 7<sup>th</sup> week of treatment, but not at the mating during the 4<sup>th</sup> week of treatment. The decreases in the fertility and conception indices persisted through the first two mating periods during the recovery phase, but had finally reversed during the last mating period after nine weeks of recovery.

✓ In the 100 mg/kg group, serum testosterone levels were significantly decreased (by about 40%) after four weeks of dosing. At the end of the recovery period, there were no effects on sperm numbers or motility, however the sperm had not been examined during the treatment period. In four of the animals in this group, a sperm granuloma within the epididymis was observed microscopically. There were no microscopic findings in the testes.

There were no effects on the numbers of corpora lutea, implantation sites, or resorption sites in females mated with males from the 50 mg/kg group. Because of the reduced fertility in the 100 mg/kg males, no useful information was obtained from uterine examination of the females mated to this group.

Systemic exposure was demonstrated by plasma drug concentrations of 21.4 and 34.3 mcg/ml after four weeks of dosing at 50 and 100 mg/kg respectively.

## **E. Genetic Toxicology Studies**

### **Bacterial Reverse Mutation (Ames) Assay** (TR No. 7228-94-145)

PNU-100766 was tested (6.8 to 550 µg/plate) for ability to induce reverse mutation in the S. typhimurium tester strains TA-97A, TA 98, TA-100, and T-1535 in E. coli strain WP2 uvrA. Mutation tests were carried out using a preincubation method(37 C, 20 minutes) with and without S-9 fraction.

Toxicity in the form of reduced colonies was observed in all strains at 550 µg/plate and in TA-97A, TA-100, and TA-1535 at 183 µg/plate. At no dose level was an appreciable increase in revertants per plate observed, while the positive controls worked properly in all cases except Experiment I with TA-100 with metabolic activation, and those data and the without-activation TA-100 data from Experiment I were not used. The investigators concluded that U-100766 failed to demonstrate any tendency to cause mutations in bacteria and was judged to non-mutagenic under the conditions of this test.

### **AS52/XPRT Mammalian Cell Mutation Assay** (SR a0028298)

PNU-100766 was evaluated for the potential to induce mutations at the xanthine-guanine phosphoribosyl transferase locus in a AS52 Chinese hamster ovary cell line. PNU-100766 was dissolved in dimethylsulfoxide, and tested in concentrations ranging from 0-3600 mcg/mL, with or without a metabolic activation system, obtained from the livers of rats that had been induced with                      Dimethylnitrosamine with S9, and ethyl methanesulfonate without S9, were used as positive controls.

PNU-100766 was non-mutagenic in this assay system. The responses in the solvent control, and in both positive control groups, served to validate the assay.

### **Chromosomal Aberration Study** (TR No. 7228-97-003)

A chromosomal aberration study was conducted in vitro, in human blood peripheral lymphocytes. Blood was obtained from two healthy human donors (one male and one female). A culture of lymphocytes was prepared in McCoy's medium. After a range-finding cytotoxicity experiment, linezolid was incubated at concentrations of up to 2000 mcg/ml, both with and without the standard S-9 metabolic activation system. Mitomycin C, without metabolic activation, and cyclophosphamide with metabolic activation, were used as positive controls. Linezolid did not induce chromosome aberrations in this assay. The negative and positive controls both gave the appropriate responses.

**Mouse Micronucleus Test**  
(TR No. 7228-95-083)

PNU-100766 was dissolved in dimethylsulfoxide and administered orally (4 ml/kg) to CD-1 albino mice (15/sex/group) at doses of 0, 1000, 2500, or 5000 mg/kg (single dose). A positive control (triethylenemelamine) was carried through the assay. At 24, 48, and 72 hours after dosing, five animals per sex per group were sacrificed, and bone marrow was collected from the femur. The bone marrow was placed on a slide, stained, and prepared for reading the number of polychromatic erythrocytes, normochromatic erythrocytes, and micronucleated polychromatic erythrocytes.

There was no increase in the numbers of micronucleated polychromatic erythrocytes in PNU-100766-treated groups. The solvent control and the positive control gave expected results.

**Evaluation of U-100766 in the In Vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Primary Hepatocytes**  
(TR No. 7228-94-144)

PNU-100766 was tested in the unscheduled DNA synthesis (UDS) assay. Two experiments were conducted, each using rat primary hepatocyte cultures prepared by collagenase perfusion of a male            rat liver. In each experiment, the negative (solvent) control was L15 culture medium, and positive control was 2-acetylaminofluorene (2-AAF). For each dose, duplicate cultures were treated in the presence of [<sup>3</sup>H]thymidine for 18-20 hours. The cultures were fixed, washed, mounted on microscope slides, and evaluated for UDS by autoradiography.

Slides were evaluated for UDS at concentrations ranging from 3-1000 mcg/mL. All net grains (NG) counts for cultures treated with PNU-100766 were less than zero, and therefore indicative of a negative response.

**F. Special Toxicology Studies**

**Compatibility of Drug Solutions with Human Blood and Plasma**  
(TR No. 1470-95-036)

A sterile solution containing U-100766 (2 mg/ml), dextrose, and sodium citrate was prepared and adjusted to a pH of 4.8 with hydrochloric acid. Human blood was obtained from two healthy volunteers. The drug solution and corresponding placebo were mixed and incubated with both whole blood and with plasma, in a range of concentrations calculated to span the concentrations that will occur in the clinical studies. The hemolytic potential was assessed by the amount of hemoglobin release. The solutions were also analyzed spectrophotometrically for precipitates of drug/plasma proteins.

The drug solutions did not induce hemolysis or precipitation.

**PNU-100766: Acute Eye Irritation Study in Albino Rabbits**  
(TR No. 7228-94-130)

U-100766 pulverized into a fine powder and sprinkled onto the corneas and into conjunctival sacs of the eyes of 2 male albino rabbits. The right eye of each rabbit was rinsed with sterile water within 30 seconds post-instillation while the left eye was unrinsed.

In one rabbit, a dose of 20 mg powder/eye for 5 consecutive days caused slight conjunctival redness to the rinsed and unrinsed eyes at 1 hour after the third instillation only with a total irritancy score of 2 out of possible 110. The rinsed and unrinsed eyes appeared normal after the other 4 instillations and thereafter for the remainder of the 14-day study period.

In another rabbit, a single dose of 100 mg powder/eye for 1 day caused slight conjunctival redness, slight conjunctival swelling, and slight ocular discharge to the unrinsed eye at 1 hour after the instillation, with a total irritancy score of 8/110. Only a slight redness was observed at 24 hours after the instillation with a total irritancy score of 2/110. By day 2 post-instillation, the unrinsed eye appeared normal.

The results of above study indicated that U-100766 bulk drug powder was minimally irritating to rabbit eyes.

**Acute Dermal Irritation Study in Albino Rabbits**  
(TR No. 7228-94-129)

PNU-100766 pulverized into a fine powder, wetted with a few drops of sterile water to form a paste and applied to the intact and abraded skin sites of 2 male albino rabbits. The test material was kept in semi-occlusive contact with the skin sites for approximately 24 hours after each application.

In one rabbit, a single application of 100 mg/site for 5 consecutive days did not cause any dermal irritation to the intact skin site throughout the 14-day study period. The abraded skin site had slight erythema of the scratch marks at 24 hours after the first application, but appeared normal after each of the 4 remaining applications and thereafter, for the remainder of the study period.

In another rabbit, a single application at the dose of 500 mg/site for 1 day did not cause any dermal irritation to the intact or abraded skin sites throughout the 14-day study period.

The results of above study indicated that PNU-100766 bulk drug powder did not cause any irritation to the intact skin site after a single high dose or repeated low dose contacts, but was slightly irritating to the abraded skin following the first low dose contact but was nonirritating after the subsequent 4 low dose contacts.

## OVERALL SUMMARY AND EVALUATION:

The hematopoietic system, gastrointestinal system, and male reproductive organs were the primary target organs for linezolid toxicity in the preclinical studies. Decreases in erythrocyte, reticulocyte, neutrophil, monocyte, and platelet counts occurred in rat and dog studies. An increased myeloid/erythroid ratio, and bone marrow hypocellularity was observed microscopically. Decreases in megakaryocytes occurred in spleen. The hematopoietic effects occurred at doses as low as 40 mg/kg/day in dogs, and 80 mg/kg/day in rats. In studies with a recovery period, the effects on the hematopoietic system were shown to be reversible.

Gastrointestinal signs included decreased food consumption and diarrhea in rats and rabbits, and anorexia, vomiting, and mucous stools in dogs, along with histological changes in the large and small intestines of all three species.

Decreases in serum testosterone, testes weights, and sperm motility occurred in rats. Epididymal epithelial cell hypertrophy was also seen in rats. The effects on male reproduction were reversible. Female reproduction was not affected.

Linezolid was not genotoxic. It has not been tested for carcinogenicity.

## RECOMMENDATIONS:

In the subchronic/chronic animal studies, the NOAELs were generally in the range of 10-20 mg/kg/day, while signs of toxicity (e.g. bone marrow effects) started to appear at doses of 40 mg/kg and higher. The recommended human dose for linezolid (adults and pediatrics) is up to 600 mg twice daily, which is approximately 20 mg/kg/day, or about 750 mg/m<sup>2</sup>/day.

Despite this narrow safety margin, linezolid is considered to have a favorable benefit/risk ratio, mainly because of its efficacy in treating life-threatening infections, and because, in most cases, the toxic effects were found to be reversible.

From the standpoint of pharmacology/toxicology, approval of these three NDAs is recommended.

The following labeling is proposed:

## PRECAUTIONS

### **Carcinogenesis, Mutagenesis, Impairment of Fertility**

Although lifetime studies in animals have not been conducted to evaluate the carcinogenic potential of linezolid, no mutagenic or clastogenic potential was found in a battery of tests, including the Ames and AS52 assays, an in vitro unscheduled DNA synthesis (UDS) assay, an in vitro chromosome aberration assay in human lymphocytes, and an in vivo mouse micronucleus assay.

Linezolid did not affect the fertility or reproductive performance of adult female rats, [redacted] it reversibly decreased fertility in adult male rats when given at doses  $\geq 50$  mg/kg/day [redacted] with exposures approximately equal to or greater than the expected human exposure level (exposure comparisons are based on AUC, [redacted]). Epithelial cell hypertrophy in the epididymis may have contributed to the decreased fertility by affecting sperm maturation. Similar epididymal changes were not seen in dogs. [redacted]

[redacted] Although the concentrations of sperm in the testes were in the normal range, the concentrations in the cauda epididymis were decreased, and sperm from the vas deferens had decreased motility.

Mildly decreased fertility occurred in juvenile male rats treated with linezolid through most of their period of sexual development (50 mg/kg/day from days 7 to 36 of age, and 100 mg/kg/day from days 37 to 55 of age, with exposures ranging from 0.4-fold to 1.2-fold that expected in humans based on a comparison of AUCs). No histopathological evidence of adverse effects was observed in the male reproductive tract.

#### Pregnancy

**Teratogenic Effects. Pregnancy Category C:** Linezolid was not teratogenic in mice or rats at exposure levels 4-fold (in mice) or equivalent to (in rats) the expected human exposure level, based on AUCs. There are no adequate and well-controlled studies in pregnant women. ZYVOX should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

#### Non-teratogenic Effects

In mice, embryo and fetal toxicity was seen only at doses that caused maternal toxicity (clinical signs and reduced body weight gain). A dose of 450 mg/kg/day (4-fold the estimated human exposure level) correlated with increased postimplantational embryo death, including total litter loss; decreased fetal body weights; and an [redacted] increased incidence of costal cartilage fusion. In rats, mild fetal toxicity was observed at 15 and 50 mg/kg/day (exposure levels 0.13- to 0.64-fold the estimated human exposure, respectively). The effects consisted of decreased fetal body weights and reduced ossification of sternbrae, a finding often seen in association with decreased fetal body weights. Slight maternal toxicity, in the form of reduced body weight gain, was seen at 50 mg/kg/day.

When female rats were treated with 50 mg/kg/day of linezolid during pregnancy and lactation, survival of pups was decreased on postnatal days 1 to 4 [redacted]

[redacted] Pups permitted to mature to reproductive age, when mated, showed [redacted] increase in preimplantation loss [redacted]

### Nursing Mothers

Linezolid is excreted in the milk of lactating rats. Concentrations in milk were similar to those in maternal plasma. It is not known whether linezolid is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when ZYVOX is administered to a nursing woman.

### OVERDOSAGE

Supportive care is advised, with maintenance of glomerular filtration. Approximately 30% of a dose of linezolid is removed during a 3 [ ] hour hemodialysis session. Data are not available for removal of linezolid with peritoneal dialysis or hemoperfusion. Clinical signs of acute toxicity in animals were decreased activity and ataxia in rats and vomiting and tremors in dogs treated with [ ] mg/kg/day and 2000 mg/kg/day, respectively.

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cc: Original NDA 21-130  
HFD-104  
HFD-340  
HFD-520  
HFD-520/Pharm/K. Seethaler  
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