CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-130/S-003
21-131/S-003
21-132/S-003

PHARMACOLOGY REVIEW

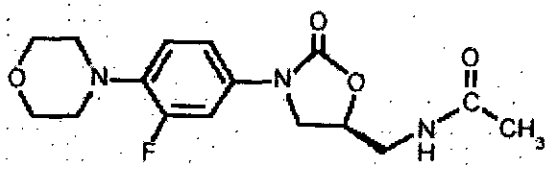
PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA numbers: 21,130 (tablets); 21,131 (IV); 21,132 (oral suspension)
Review number: 1
Sequence number/date/type of submission: SE5-003; 6/24/02; Pediatric Supplement
Information to sponsor: Yes (X) No ( ) (labeling negotiations ongoing)
Sponsor and/or agent: Pharmacia and Upjohn; Kalamazoo, MI
Manufacturer for drug substance: Oral dosage forms manufactured by sponsor; IV dosage form manufactured by Fresenius Kabi Norge AS, Halden, Norway

Reviewer name: Amy L. Ellis
Division name: Anti-Infective Drug Products
HFD #: 520
Review completion date: 12/13/02

Drug:

Trade name: Zyvox™
Generic name: linezolid
Code name: PNU-100766
Chemical name: (S)-N-[[3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide
Molecular formula/molecular weight: $C_{16}H_{20}FN_3O_4$/337.35
Structure:



Relevant INDs/NDAs/DMFs: INDs 49,195 and 55,618

Drug class: Oxazolidinone antimicrobial

Adult Indications: Vancomycin-resistant *Enterococcus faecium* infections, nosocomial pneumonia, complicated and uncomplicated skin and skin structure infections, community acquired pneumonia

Pediatric Indication: Uncomplicated skin and skin structure infections

Clinical formulations:

ZYVOX™ I.V. Injection is supplied as a ready-to-use sterile isotonic solution for intravenous infusion. Each mL contains 2 mg of linezolid. Inactive ingredients are sodium citrate, citric acid, and dextrose in an aqueous vehicle for intravenous administration.

ZYVOX™ Tablets for oral administration contain 400 mg or 600 mg linezolid as film-coated compressed tablets. Inactive ingredients are corn starch, microcrystalline cellulose, hydroxypropylcellulose, sodium starch glycolate, magnesium stearate, hydroxypropyl methylcellulose, polyethylene glycol, titanium dioxide, and carnauba wax.

ZYVOX™ for Oral Suspension is supplied as an orange-flavored granule/powder for constitution into a suspension for oral administration. Following constitution, each 5 mL contains 100 mg of linezolid. Inactive ingredients are sucrose, citric acid, sodium citrate, microcrystalline cellulose and carboxymethylcellulose sodium, aspartame, xanthan gum, mannitol, sodium benzoate, colloidal silicon dioxide, sodium chloride, and flavors.

Routes of administration: IV or oral, depending on formulation.

Proposed use: Treatment of infections listed above under *Indications*.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. Recommendation on Approvability: Approval, with appropriate warnings and caveats in the label.

B. Recommendation for Nonclinical Studies: None

C. [redacted]

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings: Target organs of toxicity for linezolid include the bone marrow, lymphoid tissues (thymus, lymph nodes, spleen, etc.), and liver. Extramedullary hematopoiesis in the spleen and liver was significantly reduced following linezolid exposure. Lymphoid depletion and reduction of extramedullary hematopoiesis were especially striking in juvenile animals. The margin of safety for linezolid toxicity between animals and humans is not large. Due to the variable human pharmacokinetics of this drug, Cmax and AUC values observed in the clinic (especially in pediatric subjects) are similar to values obtained in animals that showed signs of linezolid toxicity. Additionally, spermatogenesis in rats was adversely affected by this drug, but this effect appeared reversible. Fertility in adult female rats was not reduced by linezolid treatment. Linezolid did not appear to be teratogenic in mice or rats at exposure levels approximately 10-fold or equivalent to human exposure, respectively, but it was fetotoxic in these species. Linezolid was neither mutagenic nor clastogenic in a battery of *in vitro* and *in vivo* assays.

B. Pharmacologic Activity: Linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, interfering with the translation process in susceptible bacteria.

C. Nonclinical Safety Issues Relevant to Clinical Use: Myelosuppression has been observed not only in animal studies, but in adult humans. This toxic effect is predicted to occur in pediatric patients. Decreased extramedullary hematopoiesis and lymphoid depletion of thymus, spleen and lymph nodes observed in adult and juvenile animal is of particular concern to pediatric patients. In general, the severity of linezolid toxicity appeared greater in young animals than adults; this is a potential

concern for pediatric patients as well, particularly because lymphoid depletion is of special concern in the young and the human pediatric NDA database is small.

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence (TL)- _____

Concurrence (Deputy Div Dir)- _____

Non-Concurrence - _____
(see memo attached)

C. cc: list:

PM/Duvall-Miller
MO/Nambiar
MO/Thompson
Micro/Marsik
Stat/Brittain
Biopharm/Zheng

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Linezolid binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, interfering with the translation process in susceptible bacteria.

II. SAFETY PHARMACOLOGY:

No new safety pharmacology studies were submitted to this NDA and none were requested. According to the review of the original NDA for linezolid, the compound is a reversible inhibitor of monoamine oxidase *in vitro*. *In vivo*, linezolid pretreatment potentiated tyramine-induced tachycardia and blood pressure increases in anesthetized rats. Linezolid was also associated with delayed gastric emptying. Other standard safety pharmacology tests (e.g., cardiovascular, Irwin screen, electroshock- and pentylenetetrazol-induced convulsions) did not demonstrate drug-induced effects.

III. PHARMACOKINETICS/TOXICOKINETICS:

No new PK studies were submitted to this NDA and none were requested. There are some toxicokinetic investigations linked to specific studies in this NDA and these will be reviewed in conjunction with the appropriate toxicology study. According to the review of the original NDA for linezolid, the drug is rapidly absorbed in both rats and dogs, with oral bioavailability of 75-95%. Linezolid appears to be widely distributed to the tissues and its volume of distribution is approximately equal to total body water. The highest tissue levels of radiolabeled compound detected in rats 0.3 hours after IV dosing were found in the liver, kidneys, adrenals, and small intestine (all exceeded the plasma concentration). Linezolid excretion in rats was predominantly renal (parent drug and carboxylic acid metabolites), with about 20-30% excreted in feces. It is excreted in the breast milk of lactating rats and was shown to cross the placenta in rats. Protein binding of linezolid is relatively low (<35%).

IV. GENERAL TOXICOLOGY:

Target organs of toxicity for linezolid in adult and juvenile rats and dogs include the bone marrow, lymphoid tissues (thymus, lymph nodes, spleen, etc.), and liver. Extramedullary hematopoiesis in the spleen and liver is reduced following linezolid exposure. Additionally, spermatogenesis is also adversely affected by this drug. Lymphoid depletion and reduction of extramedullary hematopoiesis are especially striking in juvenile animals and are of greater concern in juveniles than adults. In young animals, extramedullary hematopoiesis is more active than in adults and the lymphoid tissues (particularly thymus and spleen) are a more important source of white blood cells than bone marrow in young animals.

Several repeat-dose toxicity studies were submitted in the original NDAs and in the INDs for linezolid. Those that were included in this NDA amendment were all performed in juvenile animals. Some repeat-dose toxicity studies conducted in juvenile animals will be reviewed under

the Reproductive and Developmental Toxicology section as their endpoints focused on reproductive organs and other organ systems were not examined microscopically.

Additionally, batches of linezolid containing the impurity PNU-177636 (present at 0.4%) and PNU-142063 A (present at 1-2%) were tested in toxicity studies in rats, using an oral and an IV route of administration, respectively. These impurities did not alter the toxicity profile of linezolid or change the pharmacokinetics of the parent drug.

Fifty-three Day Oral Dose Toxicity Study When Dose Administration was Initiated in Juvenile Male Sprague-Dawley Rats (Study No. 97-108; Report No. a0015316)

R.K. Jensen, G.W. Peng, R.A. Jolly (P&U, Kalamazoo, MI)

Report dated 9/21/98, not GLP

Animals: Male Sprague-Dawley rats (Crl:CD(SD)BR; from untreated time-mated F0 females), 6 days of age on the first day of dosing, 35 per treatment group; litter size was adjusted on Postnatal Day (PND) 1 so that each contained 6 male pups only. Litters were again culled on PND 4 so that each contained 5 male pups only.

Diet: Juvenile rats were kept with their respective dams for *ad libitum* nursing during the period of lactation. PMI Certified Rodent Diet #5002 (pelleted, and in meal form from PND 14-35) and deionized, chlorinated tap water were provided *ad libitum*.

Drug Dose and Route of Administration: Linezolid (Lot No. (D)5014-TJF-864) was suspended in aqueous 1% Avicel RC-591, 5% Tween 80, and 50 mM sodium acetate (pH 4.5). Vehicle or 100 mg/kg of linezolid was administered orally once daily from PND 5-58 at a dose volume of 8 ml/kg.

Length and Conduct of Study: The pups received linezolid (Lot No. (D2)28596-DMK-79) or vehicle once daily from PND 6-59.

Dams were observed once daily, except on Gestation Day (GD) 21, when they were observed several times throughout the day for signs of parturition. The day when parturition was complete was considered PND 0. Pups were observed daily during the dosing period. Litters were culled on PND 1 to contain 6 males each, then again on PND 4 to contain 5 males each. On PND 17, pups were weaned from the dams. Pups were weighed on PND 1, then daily during dosing.

Three to four pups were sacrificed about 3-4 hours after drug was given on dosing days 7, 10, 11, 17, 29, and 53. During the time between days 7-17 when the most severe signs of clinical toxicity were being manifested, the drug-treated animals were chosen for sacrifice based on their condition (i.e., pups that appeared sick or moribund were chosen for sacrifice). On day 10, 7 from the linezolid group were sacrificed. Blood samples for linezolid levels were drawn at this time. Plasma specimens were frozen and sent to [redacted] for analysis using a validated HPLC assay. On day 7, tissues collected for microscopic evaluation included testes, epididymides, and prostate. These tissues were weighed before processing. Samples of skin and jejunum were collected for analysis of Proliferating Cell Nuclear Antigen (PCNA) using Western blotting techniques. Microscopic examination of skin samples included measurement of

hair follicle length. Additional tissues collected on the other sacrifice days included duodenum, jejunum, ileum, cecum, colon, liver, kidneys, heart, spleen, lungs, sternum, and gross lesions. The report states that prostate weights on days 7-14 were not accurate; microscopic analysis showed that additional tissue was present. In the control group, 14 rats were not sacrificed, but removed from the study alive and used for other purposes.

Results: The mortality in this study was greater than expected. Thirteen drug-treated pups were found dead (4 on day 9, 4 on day 10, 2 on day 13, 2 on day 14, and 1 on day 20).

Clinical signs indicating severe toxicity included decreased activity and cool to the touch. Hair loss was observed in most of the drug-treated rats beginning on days 9-12 of treatment. Regrowth of hair in the surviving animals was seen beginning on days 26-27 of treatment.

Decreased body weight gain in the linezolid-treated rats was observed from dosing days 5-35. The animals gained weight at a rate similar to controls (or greater than controls in some animals) after this time. On days 9-17 of dosing, mean body weight in the linezolid group was about half of control.

PCNA was not detected in the jejunum of either control or drug-treated rats. In control rats, PCNA was readily detected in the skin on days 7 and 10, was present at a low level on day 17, and could not be detected thereafter. On days 7 and 10, PCNA levels were visibly lower in the skin samples from drug-treated rats than controls. The more dramatic decrease was on day 10 and it correlated with the time that hair loss began to be observed in this group. Microscopic evaluation of skin from rats that died or were sacrificed at around this time showed histologic correlates that accounted for the hair loss, including decreased hair follicle length, and follicle cells in the catagen phase of the cycle. In rats that were sacrificed on day 29 or beyond, microscopic evaluation of the skin were consistent with the regrowth of hair observed in these animals (longer hair follicles, follicle cells in anagen phase). The adverse effects on skin and hair were believed to be secondary to (or at least exacerbated by) poor nutrition in the drug-treated rats because they began to reverse in surviving animals that were continuing to be treated with drug. It is noted, however, that hair loss in juvenile rats treated with linezolid has been observed in animals that did not show signs of malnourishment or severe toxicity.

At gross necropsy, small thymus and spleen were observed in most rats that died or were sacrificed from days 9-14. Many of these rats had an empty GI tract or had red or discolored GI contents. The rats that survived until day 53 had cecal enlargement.

The only tissues weighed were testes, epididymides, and prostate. The small numbers of rats sacrificed on each study day and the variability of the organ weight data made its interpretation problematic.

Microscopic evaluation of the testes revealed minimal to marked degeneration or necrosis of individual cells in rats that died or were sacrificed between dosing days 9-29. However, no differences in the testes were observed between linezolid-treated and control rats when animals were sacrificed on dosing day 53. No changes in epididymides or prostate glands were observed in any of the rats. Bone marrow hypocellularity (moderate to marked, 15/15) was seen in the sternum of drug-treated rats sacrificed between days 9-11. Extramedullary hematopoiesis in spleen and liver was not observed in these rats, in contrast to control animals which all exhibited minimal to mild extramedullary hematopoiesis in the liver and marked extramedullary hematopoiesis in the spleen. Lymphoid atrophy of the spleen was observed as well (4/15 mild, the remaining 11/15 moderate to marked). Two of 3 drug-treated animals sacrificed on day 17 had sternal bone marrow with normal cellularity, but with a greater percentage of immature cells, indicating recovery in this tissue (the other animal in this group exhibited mild bone marrow

hypocellularity). These animals also showed recovery of extramedullary hematopoiesis in the liver and spleen and they did not exhibit lymphoid atrophy. Microscopic changes in the liver seen in pups from the linezolid group sacrificed between dosing days 9-11 did not appear to be directly drug-related. Decreased amounts of cortical and trabecular bone in the sternum (mild to moderate, 15/15) were observed in the drug-treated pups sacrificed between days 9-11. This has been observed in other juvenile rat studies, and although it may be related to delayed growth due to malnourishment, a direct effect of linezolid cannot be ruled out. Hypoplasia/atrophy of the GI tract mucosa (mild to moderate, 11/15) and decreased intraepithelial lipid content (moderate to marked, 11/15) in the mucosa of the small intestine were seen in linezolid-treated rats sacrificed between dosing days 9-11; these may have been associated with malnourishment.

The mean plasma concentration of linezolid 3-4 hours after drug administration changed over the course of the study. The highest concentrations were found on the days when the most severe toxicity and highest mortality occurred.

Mean Plasma Linezolid Concentrations in Rats Receiving 100 mg/kg/day

Day	Mean Plasma Level ± SD (μg/ml)	N
7	57.8 ± 18.7	3
10	90.6 ± 22.3	7
11	93.2	1
17	35.0 ± 5.96	3
29	17.8 ± 3.43	4
53	40.6 ± 5.22	4

When rat pups were given daily oral doses of 100 mg/kg linezolid from PND 6-59, significant mortality was observed from dosing days 9-14. The animals that were moribund sacrificed at this time exhibited bone marrow hypocellularity, reduced extramedullary hematopoiesis in the spleen and liver, and lymphoid atrophy of the spleen. Animals that were sacrificed at the end of the dosing period did not demonstrate these microscopic changes. Focal degeneration or necrosis of testicular cells was also observed in the animals that were sacrificed before the end of the dosing period, but not in the animals sacrificed after receiving drug for the entire study. Data from other studies conducted in juvenile rats confirm that linezolid treatment does not appear to be associated with testicular changes. Finally, it is noted that adult rats of this strain have been given doses of up to 125 mg/kg (Cmax and AUC of approximately 35 μg/ml and 385 μg·hr/ml) of linezolid for one month without mortality. These adult rats did lose significant amounts weight during the study, and microscopic examination showed mild bone marrow hypocellularity with a minimal decrease of hematopoiesis (erythrocyte and megakaryocyte series), minimal to mild lymphoid atrophy of mesenteric and mandibular lymph nodes, and decreased hematopoiesis in the spleen (megakaryocyte series). The microscopic changes observed in the juvenile animals that were sacrificed early in the current study were generally more severe than those observed in the adults.

V. GENETIC TOXICOLOGY:

No new genetic toxicology studies were submitted with the parent compound, linezolid, tested alone, and none were necessary. This portion of the drug labeling will remain unchanged from a scientific standpoint, though some grammatical changes will be recommended. Linezolid was negative for both mutagenic and clastogenic activity in the following *in vitro* assays: Ames bacterial reversion, CHO cell mutation, unscheduled DNA synthesis, and chromosomal aberration in human lymphocytes. Additionally, linezolid did not induce micronucleus formation in mouse bone marrow cells *in vivo*.

The Ames assay and a chromosome aberration test in human peripheral lymphocytes were conducted with linezolid mixtures containing one of the following impurities or degradation products: PNU-141960 (present at 3%), PNU-177636 (present at 3%), PNU-142063 A (present at 20%), and PNU-140155 (present at 5%). The compounds were not genotoxic under the conditions of these studies. In addition, 2 carboxylic acid metabolites of linezolid, PHA-142300A and PHA-142586A (found in mice, rats, dogs, and humans) were both tested in the Ames assay and were negative. None of these studies had been requested by the Division.

VI. CARCINOGENICITY:

Carcinogenicity studies have not been performed with linezolid.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Linezolid did not affect fertility or reproductive performance in adult female rats. It caused altered spermatogenesis in adult male rats, leading to reversible reductions in fertility (manifested by reduced pregnancy rate and increased preimplantation loss in their mates). The affected sperm were not viable and had mitochondrial abnormalities. Juvenile male rats also experienced reduced fertility upon sexual maturity (manifested by increased preimplantation loss in their mates) when treated with linezolid for relatively long periods (e.g., PND 7-55). When juvenile males were exposed for shorter periods (GD 6 through PND 5; PND 5-21; PND 22-35), decreased fertility was not observed. However, those treated from PND 22-35 had sperm with reduced motility and had an increase in the percentage of abnormal sperm though the magnitude of these effects was apparently not great enough to affect the animals' fertility. These effects on spermatogenesis were reversible.

Linezolid was not teratogenic in mice or rats, even at doses associated with maternal toxicity. Embryo and fetal toxicities (postimplantation loss, decreased fetal body weights, increased incidence of costal cartilage fusion) were observed in mice, but only at maternally toxic doses. Fetal toxicity (decreased fetal body weights, reduced ossification) was also observed in rats, at doses that were not maternally toxic or induced only slight maternal toxicity. Fetal exposure of rats during late pregnancy and lactation was associated with a slight reduction in neonatal survival (which may not have been drug-related) and with a small increase in preimplantation loss when F1 pups were mated to one another.

PNU-100766: An Oral Study of Fertility and Early Embryonic Development to Implantation in the Female Rat (Report No. SR a0066816; P&U Study No. 1998-0244; Contractor Study No. WIL-351001)

Report author E. Daniel is employed by P&U, Kalamazoo, MI; the study was conducted at ——— according to U.S., Japanese, and OECD GLP.

Report dated 1/7/00

Animals: Crl:CD(SD)IGS BR (Sprague-Dawley) rats, 25 dams per group (females were obtained from the breeder, then mated 1:1 at the laboratory with resident males of the same strain), 172-292 g at the initiation of dosing. An additional 10 nonpregnant females per group were used for toxicokinetics.

Diet: PMI Certified Rodent LabDiet® 5002 and water purified by reverse osmosis were available *ad libitum*.

Drug Dose and Route of Administration: Linezolid (Lot No. (D2)1500-5148-JLH-48M) was suspended in 1% Avicel containing 5% Tween 80 and 50 mM sodium acetate (pH 4.5). Dams received 0 (vehicle), 15, 50, or 100 mg/kg single daily oral doses of linezolid for 14 days prior to cohabitation through Gestation Day (GD) 7 at a dose volume of 10 ml/kg.

Length and Conduct of Study: Drug was administered for 14 days prior to cohabitation through GD 7. Vaginal smears to monitor the estrous cycle were taken for 14 days prior to administration of drug and during the 14 day dosing period prior to cohabitation. Dams were observed twice daily for viability and detailed clinical observations were recorded daily. These dams were also observed for clinical signs of toxicity about one hour after dosing. Body weights and food consumption were measured/recorded twice weekly starting with the initiation of estrous monitoring and on GD 0, 3, 7, 10, 13, and 15. The day that a female rat showed evidence of mating (vaginal plug or presence of sperm in a vaginal smear) was considered GD 0. If no evidence of copulation was observed after 10 days of cohabitation, the female rat was paired with a second male.

Dams were sacrificed on GD 15 and the numbers of corpora lutea, live/dead embryos, resorbed embryos, and implantation sites were determined. Uteri that appeared nonpregnant were stained with ammonium sulfide to confirm the lack of implantation sites. A gross necropsy was conducted on each dam. The ovaries, brain, and pituitary gland of each dam were weighed.

Blood samples for toxicokinetics were drawn on day 14 of dosing 0, 0.5, 1, 4, 10, and 24 hours after administration of drug. The toxicokinetic portion of the study was conducted twice, using 5 nonpregnant animals per group each time. The first group of samples showed signs of coagulation and an unacceptably high level of hemolysis, so the toxicokinetic study was repeated. Plasma samples were frozen and sent to P&U for HPLC analysis using UV detection (limit of quantification ———

Results: All of the dams survived until scheduled sacrifice. Treatment-related clinical signs included soft stools in all linezolid groups and diarrhea in the mid and high dose groups. Clinical

signs observed only the high dose group included alopecia, yellow matting at the urogenital area, decreased defecation when handled, and salivation.

Prior to gestation, mean body weight gain during days 17-21 of dosing was statistically significantly ($p<0.01$) reduced compared to control in the mid and high dose linezolid group. However, weight gain did not significantly differ from control at other intervals except that during days 14-17 of dosing, the high dose group gained significantly ($p<0.05$) more weight than controls. On day 0 of gestation, mean body weights did not differ significantly between the control and linezolid groups. From days 0-7 of gestation, both the mid and high dose linezolid-treated rats gained significantly less weight than controls ($p<0.05$ and $p<0.01$, respectively; control mean body weight gain was 33 g compared to 26 and 19 g in the mid and high dose groups). From days 7-15 of gestation (after drug treatment ended), the mid and high dose animals gained more weight than controls, though only the high dose mean differed significantly from controls ($p<0.05$). During the premating period, food consumption in the linezolid groups was reduced compared to control at several intervals. The reductions were not large, though they did reach statistical significance, and occurred more frequently in the mid and high dose groups. During GD 0-7, food consumption was reduced only in the high dose group compared to control ($p<0.01$, 22 vs. 18 g/rat/day). From GD 7-15, food consumption was similar among all groups, though consumption in the high dose group actually exceeded that in the control group from GD 10-15.

There appeared to be no drug-related effects on female reproductive performance. Linezolid exposure did not affect the estrous cycle. The mating index in all groups was 100%. Group mean precoital intervals were similar and the fertility indices for all groups ranged from 92-100% (with 92% being the control value).

Numbers of corpora lutea, live/dead embryos, and early resorptions did not differ significantly among the treatment groups. Pre- and postimplantation losses were similar between control and drug-treated dams. No significant difference in mean organ weights (brain, ovaries, pituitary) among the groups was observed.

The vast majority of samples from control rats contained no detectable levels of drug. A few control samples had peaks at known retention time for linezolid, but the investigators did not believe that the animals had been dosed inadvertently because the levels were extremely low and the timing of their collection was not consistent with the animal having been accidentally dosed. Despite the sample hemolysis in the first trial, the toxicokinetic values between the 2 trials were similar.

Toxicokinetics of Linezolid in Female Rats (mean ± SD)

	15 mg/kg		50 mg/kg		100 mg/kg	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Cmax (µg/ml)	7.5 ± 1.4	6.8 ± 2.9	19.9 ± 8.1	20.1 ± 2.3	34.7 ± 10.3	37.5 ± 5.4
Tmax (h)	1 ± 0	1 ± 0	0.9 ± 0.2	0.8 ± 0.3	2.8 ± 1.6	3.3 ± 1.5
$T_{1/2}$ (h)	3.5 ± 2.1	2.5 ± 0.9	2.9 ± 1.1	2.3 ± 0.3	4.7 ± 2.6	3.5 ± 2.5
$AUC_{0\text{-}24\ h}$ (µg·h/ml)	41 ± 12	33 ± 5	220 ± 40	166 ± 35	417 ± 59	410 ± 48

When linezolid was administered to female rats orally at doses up to 100 mg/kg/day starting 14 days before cohabitation and continuing through GD 7, no adverse effects on female

reproductive parameters and embryo survival were observed. At 100 mg/kg, clear evidence of maternal toxicity (clinical signs, reductions in body weight gain and food consumption) was observed. The NOAEL for maternal toxicity was 15 mg/kg.

PNU-100766: Ten-Week Oral Dose Toxicity and Fertility Study with a Reversibility Phase of up to 14 Weeks in Adult Male Sprague-Dawley Rats (Report No. SR a0018276; Study No. 97-070)

R.K. Jensen, G.W. Peng (P&U, Kalamazoo, MI)

Report dated 2/11/99 with amendment dated 3/27/02, U.S. GLP (except for sperm analysis)

Animals: Male and female Sprague-Dawley (Crl:CD(SD)BR) rats, only males were drug-treated; 9-10 weeks of age, 295-361 on the first day of dosing, 30 male rats per treatment group

Diet: PMI Certified Rodent Diet #5002 and tap water (deionized and chlorinated) were available *ad libitum*.

Drug Dose and Route of Administration: Linezolid (Lot No. (D)5014-TJF-864) was suspended in Avicel RC-591 (1% microcrystalline cellulose and carboxymethylcellulose, 5% polysorbate 80 and 50 mM acetate buffer in purified water, pH 4.5). Drug (12.5 mg/ml suspension) or vehicle was administered orally once daily to male rats for 10 weeks at doses of 0 or 100 mg/kg (dose volume of 8 ml/kg).

Length and Conduct of Study: Adult male rats received daily oral doses of vehicle or 100 mg/kg linezolid for 10 weeks. Animals were observed daily for changes in appearance and behavior from study days 1-74, then weekly thereafter. Body weights were measured prior to the initiation of drug administration, then weekly and at the time of sacrifice.

After 57 days of dosing, the first 20 rats per group were cohabitated 1:1 for up to 14 days with virgin females of the same species. The day that evidence of mating was detected (copulatory plug or presence of sperm in a vaginal smear) was considered Gestation Day (GD) 0. Females were sacrificed on GD 13 or 13 days after separation from the male if no evidence of mating was detected (sacrifice could happen earlier if it was obvious that the female was pregnant). The uterus of each female was examined for implantation sites (using ammonium sulfide staining if needed) and the numbers of live and dead embryos was recorded. The number of corpora lutea was also determined.

After 10 weeks (70-71 days) of treatment, the last 10 males in each group were sacrificed. Blood samples for measuring the levels of testosterone and luteinizing hormone (LH) were taken. Animals underwent gross necropsy and the testes, epididymides, and prostate were weighed. The right testis and epididymis and the prostate were examined using light microscopy, as were any gross lesions. Portions of the corpus of the left epididymis were also examined via electron microscopy for the first 3 rats in the interim sacrifice group. Sperm were collected from the left vas deferens for determination of motility/viability. Sperm head counts were determined from the left testis and left cauda epididymis.

On study day 141 (after a 10 week drug-free period), the first 20 rats in each group were again cohabitated 1:1 with virgin females of the same strain. Procedures for cohabitation and

examination of the females and embryos were the same as for the first mating. The males were sacrificed on or about study days 168-169. Sacrifice and tissue collection procedures and measurements were the same as for the interim sacrifice group, above.

Blood samples for toxicokinetics were taken on day 45 of dosing from the first 5 rats in the 100 mg/kg linezolid dose group at 1, 3, 7, 12, and 24 hours after drug administration. Plasma samples were frozen and sent to ——— for measurement of linezolid concentration using a validated HPLC method (lower limit of quantification of ——— µg/ml).

Results: Soft stools and/or diarrhea were observed in most drug treated rats during the second week of dosing. Salivation was occasionally observed, but believed to be related to the poor palatability of the drug. No other clinical signs associated with drug treatment were seen.

Slight decreases in mean body weight gain were observed in the drug-treated males compared to controls during the first 3 weeks of the study, but the decrease was only statistically significant during the first week. Mean body weights never differed significantly between the control and linezolid groups.

At the first cohabitation, the mean precoital interval did not differ between the treatment groups and the copulation rate was 100% for both. However, the fertility and conception indices were dramatically reduced in the 100 mg/kg linezolid groups. In control animals, these indices were both 80% (16/20 cohabitated females pregnant) and the linezolid group, they were 15% (3/20 cohabitated females pregnant). Of the 3 females that were pregnant, one had complete embryo loss. Due to the contribution of this animal, both pre- and post-implantation losses were increased in the 100 mg/kg linezolid group compared to control. The complete embryo loss was also associated with a reduction in the mean numbers of implantation sites and live embryos in the drug-treatment group.

This decrease in fertility was reversed by the time of the second cohabitation, which followed a 10 week drug-free period. In the control group, the copulation, fertility, and conception indices were 95%, 95%, and 100%, and in the linezolid group, these indices were 100%, 95%, and 95%, respectively. In both groups, 19/20 cohabitated females were pregnant. The mean numbers of implantation sites, live/dead embryos, and pre- and postimplantation losses were similar in both the control and linezolid groups.

Data from the interim sacrifice of the first 10 males per group show that a decrease in viable sperm accounts for the reduced fertility observed after 10 weeks of drug treatment. Sperm motility was 0% in the drug treated rats vs. 79% in the control group. The heads and/or necks of most sperm from the linezolid-treated rats were detached from the tails. Mean sperm head counts (both absolute and relative to weight of tissue) in the cauda epididymis was reduced in the linezolid group, though mean absolute and relative testicular sperm head counts were similar between the control and drug-treated groups. A small decrease (not statistically significant) in the mean serum testosterone level in the drug-treated males is unlikely to be biologically significant (1.44 ± 0.74 vs. 1.15 ± 0.73 ng/ml), particularly because it was not accompanied by a significant change in LH (0.40 ± 0.16 vs. 0.33 ± 0.09 ng/ml). Gross necropsy revealed enlarged cecae in the drug-treated rats. Neither absolute nor relative testicular or prostate weights differed significantly between control and drug-treated rats. Epididymal weights were slightly decreased in the linezolid group, but the reduction was not statistically significant. The epididymal weight reduction may have been related to the lower mean sperm head count in this tissue. One linezolid-treated rat had a sperm granuloma on the cauda epididymis. Moderate hypertrophy of

the principal and clear cells that lined the tubules of the corpora epididymis was observed in 10/10 rats, as was minimal to mild degeneration/necrosis of individual epididymal cells (in either corpus or cauda). Two of 10 rats had mild or marked increases in the number of abnormal germinal cells in the epididymal lumen (they also had degeneration of individual spermatocytes or spermatids in the testes) and 3/10 animals had no sperm or a decreased number of sperm in the epididymides. Moderately inhibited or delayed spermiation was observed in the testes of 5/10 linezolid-treated rats and 1/10 controls. Electron microscopy of the epididymal corpus (performed in 3 rats) revealed only an apparent increase in the number of spherical, membrane-bound lysosomes in the supranuclear position.

When the cohabitated males were sacrificed, no differences in sperm motility or head counts were observed between the control and linezolid groups. Mean serum testosterone and LH levels did not differ between the groups. Absolute and relative weights of testes, prostate, and epididymides did not significantly differ between control and linezolid-treated rats. One linezolid treated rat had a sperm granuloma. There were no other microscopic changes in the epididymides and testes that appeared treatment-related.

After 45 daily 100 mg/kg doses of linezolid, Cmax was 44.5 $\pm$ 7.8 µg/ml and $AUC_{0\text{-}24\ h}$ was 444.1 $\pm$ 35.1 µg·h/ml. Tmax was 1.8 $\pm$ 1.1 h and half life of the drug was 2.5 $\pm$ 0.8 h.

Eight to 10 weeks of daily 10 mg/kg oral linezolid dosing was associated with significantly decreased fertility in adult male rats. This appeared to be related to altered spermatogenesis and was reversed after a 10-14 week drug-free period.

PNU-100766: A 14-Day Reproductive Toxicity Study (oral) in Juvenile (PND 22 through PND 35) Male Rats with Recovery (Report No. SR a0095038; Study No. 2000-0012

M.E. McNerney (P&U, Kalamazoo, MI)

Report dated 5/10/02, Not GLP, no QA statement

Animals: Male juvenile Sprague-Dawley (Crl:CD[SD]IGS BR) rats, 22 days of age on the first day of dosing, 70 rats per treatment group

Diet: PMI Certified Rodent Diet #5002 and water (deionized via reverse osmosis, then filtered and chlorinated) were available *ad libitum*.

Drug Dose and Route of Administration: Linezolid (Lot No. not provided in report) was suspended in Avicel RC-591 (1% microcrystalline cellulose and carboxymethylcellulose, 5% polysorbate 80 and 50 mM acetate buffer in purified water, pH 4.5). Drug (10 mg/ml suspension) or vehicle was administered orally once daily for 2 weeks at doses of 0 or 100 mg/kg (dose volume of 10 ml/kg). Dose selection was based on results from previous multiple dose juvenile and adult rat studies where 100 mg/kg doses were well tolerated, but 125 mg/kg doses were not.

Length and Conduct of Study: The rats received drug daily from days 22-35 of age. Ten per dose group per time point were sacrificed without cohabitation one day, 5 weeks, and 10 weeks after the final dose of drug was given. Twenty per dose group were cohabitated with untreated

virgin female rats of the same strain prior to their sacrifice 5-7 weeks or 10-12 weeks after drug treatment ended.

Clinical signs were recorded daily during the dosing and recovery periods. Food consumption was measured daily during dosing. Body weights were recorded daily during dosing and twice weekly during recovery. The rats were observed daily to see whether balanopreputial separation had occurred starting on Postnatal Day 40 until that developmental landmark was achieved.

Males assigned to the cohabitation groups were placed 1:1 with females confirmed to have regular estrous cycles. The animals were paired until evidence of copulation was observed (vaginal plug or presence of sperm in vaginal lavage) or for up to 14 days. Females that showed evidence of copulation were sacrificed on presumed Day 13 of gestation. Those that did not appear to have copulated were sacrificed 13 days after the end of cohabitation, unless signs of pregnancy necessitated their earlier sacrifice. Any uteri that appeared nonpregnant were stained with ammonium sulfide so that any implantation sites could be visualized. The numbers of corpora lutea, live and dead embryos in each female were counted. Males were sacrificed either after positive evidence of copulation was observed or at the end of the breeding period.

The males that were sacrificed at the end of the dosing period (no recovery) were perfused with McDowell-Trump's fixative and their right and left testes and epididymides removed and placed in the same fixative until processed for histopathologic analysis. When the remaining rats were necropsied, a segment of the left vas deferens from each animal was removed and used to examine sperm motility and morphology. At least 200 sperm/rat were analyzed using a Hamilton-Thorne IVOS CASA system. The left cauda epididymus of each rat was removed, weighed to the nearest 0.001 g and frozen until analysis. These samples were thawed and homogenized, with a sample of the homogenate used to measure caudal epididymal sperm concentration (in millions of sperm per gram cauda weight). After weighing, the left testes of each animal was perforated and centrifuged so that interstitial fluid could be collected and its testosterone level measured. Following the removal of the left vas deferens, epididymus, and testis, all rats were perfused with McDowell-Trump's fixative. The right testis and epididymus of each rat were removed and immersed in McDowell-Trump's fixative. These tissues underwent microscopic analysis.

Blood samples for the determination of plasma linezolid levels were collected from 4-5 animals per time point on the last day of dosing immediately prior to administration of drug, then 0.5, 1, 2, 4, 8, and 24 hours afterward.

Results: One linezolid-treated rat assigned to the 10 week recovery/cohabitation group died on day 11 of dosing due to an intubation accident. This rat was replaced by one originally assigned for immediate sacrifice following the completion of dosing.

Treatment-related clinical signs of toxicity were not observed in any of the rats. Mean body weight was statistically significantly ($p<0.05$) less in the linezolid group than in the controls beginning on day 7 of dosing and persisting until about the eleventh day of recovery, but the differences were relatively small, not exceeding 4.7% at any time. Group mean daily food consumption was also significantly ($p<0.01$) reduced in the drug-treated rats compared to controls. It is noted that the reduction was small, not exceeding 10% and usually around 4-7%. The reduction in food consumption was only apparent during the first week of dosing.

Toxicokinetic sampling on day 14 indicated a mean Cmax of 31.9 ± 3.55 µg/ml at 4 hours, with an earlier peak of 31.6 ± 3.62 µg/ml at 1 hour. The mean $AUC_{0\text{-}24\ hr}$ in the rats was 340.6 µg·hr/ml.

The time until balanopreputial separation was not influenced by linezolid treatment. This developmental landmark was achieved by Postnatal Day 45 in all rats except for one drug-treated animal. Balanopreputial separation was observed in this rat on Postnatal Day 47.

When the treated males were cohabitated with untreated females 5 weeks after the end of treatment, 19/20 control pairs (95% copulation index) and 17/20 linezolid pairs (85% copulation index) showed positive evidence of copulation. The mean precoital interval (approximately 3 days) was similar between the groups. One male from each of the treatment groups did not produce a pregnancy in the female with which it copulated, thus, the conception index (# pairs with pregnant female/# pairs with confirmed copulation) was almost the same (about 94-95%) for both groups. The fertility indices (# pairs with pregnant female/# pairs cohabitated) in the control and linezolid groups were 90% and 80%, respectively. The investigator noted that the copulation and fertility indices were slightly lower for the linezolid-treated males than controls, but indicated that both values were within the historical control range. Gestation data from the female rats paired with the treated males did not demonstrate any linezolid-related effects. Mean pre- and postimplantation losses were similar between control and drug-treated rats and the numbers/dam of corpora lutea, implantation sites, and live embryos were similar between groups.

No differences in the mean copulation, fertility, or conception indices or in precoital interval were observed between control and linezolid-treated males who were cohabitated with untreated females 10 weeks after treatment. Mean postimplantation loss was similar between control and drug-treated rats and the numbers/dam of corpora lutea, implantation sites, and live embryos were similar between groups. However, mean preimplantation loss was elevated in the dams paired with linezolid-treated males compared to controls (12.1 ± 27.8% vs. 1.11 ± 2.57%). The number of females that experienced preimplantation loss was also higher in the linezolid pairs than controls (7/18 vs. 3/18). Two linezolid pairs experienced more preimplantation loss than the others. In one case, only one live embryo was observed *in utero*, no evidence of postimplantation loss was seen, and the dam had 9 or 11 corpora lutea. The other pairing did not produce a pregnancy despite copulation. The investigators believed that this suggested subfertility in these drug-treated males and the FDA reviewer agrees that this is a reasonable hypothesis.

The mean testosterone level in testicular interstitial fluid was elevated approximately 2-fold in linezolid-treated rats sacrificed 5 weeks after treatment, though the coefficient of variation was quite high (about 95%). A similar peak was observed in a previous study and the investigators thought it was possibly a response to the presence of abnormal sperm. Mean testosterone levels in males sacrificed 6 weeks after the end of dosing or beyond did not differ appreciably between control and linezolid-treated rats.

Neither absolute nor relative mean testicular or mean epididymal weights appeared to have been affected by linezolid treatment, regardless of the time of sacrifice. One or two individuals in both drug-treated or control groups had values (high or low) outside of a 2 standard deviation range around the means.

Sperm motility in the rats sacrificed exactly 5 weeks after the final dose of linezolid was significantly reduced compared to controls (91.6 ± 3.06% vs. 53.7 ± 28.6% motility, $p<0.01$). The percentage of sperm with normal morphology was also decreased in the rats sacrificed at the 5 week time point compared to controls (96.1 ± 2.0% vs. 64.2 ± 24% normal, $p<0.01$).

Abnormalities noted included separation of (or missing) head and flagellum (seen in both control and drug-treated rats), and degenerate midpieces containing abnormal flagella, with or without normal heads (seen only in drug-treated rats). Previous studies have also demonstrated linezolid-related adverse effects on sperm. The current study again illustrated the importance of timing sperm collection to capture linezolid-induced effects, as significant differences were not seen in the rats that were cohabitated 5 weeks after the last drug dose, but not sacrificed until mating was completed. Additionally, no significant difference in mean sperm parameters was observed in the rats sacrificed 10 weeks after the end of dosing (or longer than 10 weeks in the case of the cohabitated males). The mean caudal epididymal sperm concentrations did not differ significantly between control and drug-treated groups, but there were 3 individual drug-treated animals all sacrificed at different times (5-12 weeks after treatment) that had sperm concentrations which were less than half of any group's mean 0-297 million sperm/g epididymus).

Gross necropsy revealed unilateral agenesis of the left testis and epididymus in one control male and unilateral small testis and epididymus in a second control male. Small testes were observed in 3 drug-treated rats; two bilateral and one unilateral. Bilateral small epididymides were also seen in one of the drug-treated rats with bilateral small testes. Microscopic examination revealed a higher incidence and severity of testicular lesions in linezolid-treated rats than controls.

Most rats from both treatment groups had no microscopic changes in the testes (64/70 controls; 57/70 linezolid). The only histological change that was of potential concern was severe diffuse degeneration of seminiferous tubules in 4 rats from the linezolid group. Two of these rats were only unilaterally affected, making the degeneration unlikely to have been drug-related in these cases. Of these 2 animals, one was sacrificed without cohabitation 5 weeks after the end of dosing, so both testes were examined microscopically. The other animal was in the 10 week cohabitation group. It was fertile and sperm parameters in the testis that was not available for microscopic examination were normal. However, the 2 remaining rats appeared to have been bilaterally affected. Both were assigned to the 10 week cohabitation group, so only one testis from each animal was available for microscopic examination, but the sperm parameter data from these rats showed that the contralateral testes had severely reduced numbers of sperm in the head and body of the epididymal lumen. Neither of these rats was fertile; one was infertile, the other subfertile (its mate had 9 corpora lutea and only 1 implantation site).

Clinical signs of toxicity were not observed in juvenile male rats treated from days 22-35 of age with oral doses of 100 mg/kg linezolid. Mean sperm motility was reduced in drug-treated rats sacrificed exactly 5 weeks after the end of dosing and the mean percentage of abnormal sperm observed in these animals was increased. However, reductions in fertility (as indicated by copulation, fertility, or conception indices, precoital interval, or pre- or post-implantation losses) were not observed in the rats in the 5 week recovery group that were cohabitated with untreated females, nor were the sperm parameters in these animals different from control when they were sacrificed at the end of the mating period. Mean sperm motility and morphology data in the rats sacrificed 10 weeks after treatment did not differ from the control group. However, increased mean preimplantation loss was observed in the rats in the 10 week cohabitation group. This increased loss was primarily attributable to 2 rats. Microscopic examination revealed severe diffuse degeneration of the seminiferous tubules in the right testes of these animals and dramatic decreases in sperm motility, normal sperm morphology, and number of sperm in the left testes. The fact that these animals were affected bilaterally (in contrast to 2 other drug-treated animals with similar microscopic findings that appeared to have been affected only unilaterally)

heightened the concern that their testicular failure was related to linezolid treatment. However, other studies conducted in juvenile rats have not demonstrated testicular failure following similar linezolid doses and bilateral testicular failure was observed in a control animal in one of these studies. Thus, it appears unlikely that linezolid treatment was the cause of testicular failure in these rats.

VIII. SPECIAL TOXICOLOGY STUDIES:

No special toxicology studies were submitted with this NDA supplement and none were required.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions:

Linezolid is an efficacious antimicrobial with significant toxicities. These include suppression of bone marrow, decreased extramedullary hematopoiesis in liver and spleen, and lymphoid depletion of thymus, spleen, and lymph nodes in adult and juvenile rats and dogs. Bone marrow suppression has been observed in adult humans, and it may also occur in pediatric patients. Lymphoid depletion is of special concern in the very young because the thymus and spleen are a more important source of white blood cells than bone marrow in this population.

General Toxicology Issues:

For a comprehensive discussion of linezolid toxicity in adult animals, the reader is referred to the original submission of NDA 21,131 and to IND 49,195 (including amendments). The following summary will focus specifically on issues pertaining to the toxicity of linezolid in juvenile animals.

The target organs of toxicity are similar in juvenile and adult rats and dogs and include bone marrow, lymphoid tissues (thymus, lymph nodes, spleen, etc.), and liver. Extramedullary hematopoiesis in the spleen and liver was markedly reduced following linezolid exposure.

In a study conducted in 3 week old beagle dogs, both clinical signs of linezolid toxicity and histopathological changes including bone marrow hypoplasia and lymphoid depletion appeared to be more severe in juvenile dogs than adult animals. In this juvenile dog study, the NOAEL was 50 mg/kg/day (given as a single daily oral dose for 28 days). However, at 100 mg/kg/day, growth retardation and reduced hematopoiesis in bone marrow, spleen and liver were seen in almost all of the dogs as was significant depletion of lymphoid tissues (where the majority of lymphocytes are being formed at this stage of development). Body weight gain in these animals was severely suppressed and a lack of rebound growth during a 4 week recovery period suggested that the growth retardation could be permanent. The mean relative weights (organ:body weight ratio) of spleen and thymus were reduced in the high dose dogs compared to controls when animals were sacrificed at the end of treatment. Following the recovery period, the mean relative thymus weight was still reduced in high dose males compared to control. The organ appeared histologically normal in both high dose recovery males and females. Additionally, the undecalcified bone marrow specimens from the high dose dogs also appeared histologically normal following recovery. While these observations suggest that the bone marrow and lymphoid tissues of the dogs treated with 100 mg/kg/day of linezolid for 28 days were able to recover at least partially after a one month drug-free period, it is not possible to ascertain from this study whether these animals would be immunologically compromised

because of the lymphoid depletion that occurred during a critical period of development. Although it is likely that reduced nursing and food intake contributed to growth retardation (though these could not be measured under the conditions of this study), a direct effect of linezolid could not be ruled out. The pharmacology reviewer does not believe that it would be correct to assume that linezolid did not have direct toxic effects on bone marrow and lymphoid tissues, particularly in light of data from adult animals (see below) that had bone marrow hypocellularity and lymphoid depletion in the absence of severe clinical signs. The margin of safety, based on AUC comparisons, is small. The NOAEL in the current juvenile dog study provided an exposure only about 30% greater than that seen in humans at the highest recommended dose of linezolid. A dose of linezolid that was associated with significant toxicity in the juvenile beagles provided an AUC that was about 2.6 times higher than that seen in humans given the highest recommended dose (10 mg/kg q8h up to 600 mg q8h in pediatric patients up to age 11, excluding premature infants, and 600 mg q12h in adolescents and adults). It is important to consider that the AUCs in human subjects are highly variable and some patients may have AUCs that are close to those observed in animals that demonstrated significant toxicity. In adult dogs given linezolid orally bid for one month, mild bone marrow atrophy was observed at low incidence beginning at 40 mg/kg/day. This dose was tolerated with only mild clinical signs of toxicity. A greater incidence and severity of bone marrow hypoplasia was seen when the dose was doubled. Adult dogs that received 80 mg/kg/day oral doses of linezolid demonstrated clinical signs of significant toxicity and had exposure levels similar to the juveniles that received the 100 mg/kg dose. However, adult dogs that received 40 mg/kg/day IV doses of linezolid (given bid) did not experience severe clinical signs and also had exposure levels similar to the high dose juvenile dogs. These animals had bone marrow hypoplasia and decreased numbers of RBCs, WBCs, and platelets in peripheral blood.

Juvenile rats also appeared to be more sensitive to bone marrow suppression and lymphoid depletion than adults. They were also more sensitive to linezolid-induced alopecia. It is important to consider that the same mg/kg dose of drug in adult and juvenile rats may produce much higher exposure levels in young animals, possibly because renal excretion is the major pathway of linezolid elimination and it is not as efficient in young rats as it is in older juveniles and adults. However, this did not appear to account entirely for the generally greater incidence and severity of bone marrow suppression and lymphoid depletion in the juvenile rats. Additionally, individual animals appeared to be more sensitive than others to linezolid-induced toxicity and mortality. In an experiment where juvenile rats were treated with 100 mg/kg/day of linezolid for several weeks starting on PND 6, most that did not survive until scheduled sacrifice either died or were sacrificed in moribund condition during the second week of dosing. These animals all exhibited moderate to marked bone marrow hypocellularity and most also exhibited moderate to marked lymphoid atrophy of the spleen. If the animals survived until their scheduled sacrifice on day 53 of dosing, these changes were not observed despite continued drug treatment. In some studies with juvenile rats of the Crl:CD(SD) IGS BR strain given doses of linezolid $\geq$50 mg/kg, acute endocardial thrombosis and coagulative necrosis of hepatocytes were observed in animals that did not survive until scheduled sacrifice. These effects did not appear to be directly related to linezolid, but to starvation and dehydration which led to blood stasis in the heart, promoting formation of thrombi and poor perfusion to the liver. However, both lymphoid depletion and bone marrow suppression were observed in some animals of this strain that received a lower 20 mg/kg dose of linezolid (Cmax 11 μg/ml; AUC 110 μg·h/ml). While it is true that these findings occurred in rats that did not survive until their scheduled sacrifice, these

individuals did not exhibit dramatic body weight loss or other signs of severe clinical toxicity prior to death.

Recommendations:

I recommend approval of this NDA supplement with the caveat that appropriate warnings and precautions concerning its use in pediatric patients should be included in the label.

Labeling with basis for findings:

The following statement should appear in the *Warnings* section as a separate paragraph, immediately following the first bolded paragraph:

"In adult and juvenile dogs and rats, myelosuppression, reduced extramedullary hematopoiesis in spleen and liver, and lymphoid depletion of thymus, lymph nodes, and spleen were observed (see **ANIMAL PHARMACOLOGY**)."

The basis for this statement is discussed in part IX. Detailed Conclusions and Recommendations, under General Toxicology Issues.

The *Carcinogenesis, Mutagenesis, Impairment of Fertility* and *Pregnancy* sections should read as follows:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Lifetime studies in animals have not been conducted to evaluate the carcinogenic potential of linezolid. Neither mutagenic nor clastogenic potential was found in a battery of tests including: assays for mutagenicity (Ames bacterial reversion and CHO cell mutation), 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. It reversibly decreased fertility and reproductive performance in adult male rats when given at doses ≥ 50 mg/kg/day, with exposures approximately equal to or greater than the expected human exposure level (exposure comparisons are based on AUCs). The reversible fertility effects were mediated through altered spermatogenesis. Affected spermatids contained abnormally formed and oriented mitochondria and were non-viable. Epithelial cell hypertrophy and hyperplasia in the epididymis was observed in conjunction with decreased fertility. Similar epididymal changes were not seen in dogs.

In sexually mature male rats exposed to drug as juveniles, mildly decreased fertility was observed following treatment 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 up to 1.7-fold greater than mean AUCs observed in pediatric patients aged 3 months to 11 years. Decreased fertility was not observed with shorter treatment periods, corresponding to exposure in utero through the early neonatal period (gestation day 6 through postnatal day 5), neonatal exposure (postnatal days 5 to 21), or to juvenile exposure (postnatal days 22 to 35). Reversible reductions in sperm motility and altered sperm morphology were observed in rats treated from postnatal day 22 to 35.

Pregnancy

Teratogenic Effects. Pregnancy Category C: Linezolid was not teratogenic in mice or rats at exposure levels 6.5-fold (in mice) or equivalent to (in rats) the expected human exposure level, based on AUCs. However, embryo and fetal toxicities were seen (see **Non-teratogenic Effects**). 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 toxicities were seen only at doses that caused maternal toxicity (clinical signs and reduced body weight gain). A dose of 450 mg/kg/day (6.5-fold the estimated human exposure level based on AUCs) correlated with increased postimplantational embryo death, including total litter loss, decreased fetal body weights, and an increased incidence of costal cartilage fusion.

In rats, mild fetal toxicity was observed at 15 and 50 mg/kg/day (exposure levels 0.22-fold to approximately equivalent to the estimated human exposure, respectively based on AUCs). The effects consisted of decreased fetal body weights and reduced ossification of sternebrae, 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 (approximately equivalent to the estimated human exposure based on AUCs) of linezolid during pregnancy and lactation, survival of pups was decreased on postnatal days 1 to 4. Male and female pups permitted to mature to reproductive age, when mated, showed an increase in preimplantation loss.

The changes in these sections of the label are primarily editorial. Some additional data from studies in juvenile rats have been added to the last paragraph of the *Carcinogenesis, Mutagenesis, Impairment of Fertility* section. Additionally, the sponsor was asked to correct some dose comparisons in the *Pregnancy* section and to use the same values consistently throughout the section. They have done so, and their changes were found acceptable by the Division.

The most recent proposal for the *Animal Pharmacology* section is as follows:

Target organs of linezolid toxicity were similar in juvenile and adult rats and dogs. Dose- and time-dependent myelosuppression, as evidenced by bone marrow hypocellularity/decreased hematopoiesis, decreased extramedullary hematopoiesis in spleen and liver, and decreased levels of circulating erythrocytes, leukocytes, and platelets, have been seen in animal studies. Lymphoid depletion occurred in thymus, lymph nodes and spleen. Generally, the lymphoid findings were associated with anorexia and suppression of body weight gain, which may have contributed to the observed effects. These effects were observed at exposure levels that are comparable to those observed in some human subjects. The hematopoietic and lymphoid effects were reversible, although in some studies, reversal was incomplete within the duration of the recovery period.

This section has been expanded from the current linezolid label to include data from juvenile animal studies, particularly a recent study conducted in 3 week old dogs (reviewed in IND

49,195-297; -299; -302). The data from the adult animals is primarily from the original linezolid NDA. There was some juvenile rat data in the original NDA and a number of other studies were submitted in amendments to IND 49,195.

X. APPENDIX/ATTACHMENTS:

Addendum to review: No

Other relevant materials (Studies not reviewed, appended consults, etc.): Nothing to report.

Any compliance issues: None known.

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

/s/

Amy Ellis
12/18/02 11:24:13 AM
PHARMACOLOGIST
Approval of supplement recommended, with appropriate warnings and caveats in label.
Terry- You signed the paper copy of this review on 12/18/02.

Terry Peters
12/18/02 01:17:34 PM
PHARMACOLOGIST

Lillian Gavrilovich
12/18/02 05:17:28 PM
MEDICAL OFFICER

Review and Evaluation of Pharmacology and Toxicology Data
Division of Anti-Infective Drug Products, HFD-520

IND#: 49,195-304; -308 and NDAs 21,130; 21,131; 21, 132 (SE5-003)

Date CDER Received/Type of Submission: IND 49,195-304 (IT, 11/20/02) and -308 (IT, 12/19/02); all NDAs (BP, 12/9/02; BM, 12/19/02)
Reviewer: Amy L. Ellis, Ph.D.
Dates Assigned: IND: 11/25/02, 12/24/02; NDA: 12/20/02, 1/2/03
Number of Volumes: IND: 1 (304), 3 (308); NDA is electronic
Date Review Started: 12/13/02
Date 1ST Draft Completed: 1/6/03
Scientific Literature Reviewed: not necessary

APPEARS THIS WAY
ON ORIGINAL

KEY WORDS: linezolid, juvenile dog, final report

Sponsor: Pharmacia & Upjohn
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Kalamazoo, MI 49001-0199
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Review Contains Information to be Communicated to Sponsor: No

Submission Contains Any Integrated Tox Study Summaries in Lieu of Final Reports: Amendment 308 and the electronic NDA contain the final report of the pivotal juvenile dog study (2002-0197). The electronic NDA also contains a statement regarding changes between the latest draft received by the division and the final report of this study.

Drug Information:

Class:	Oxazolidinone antimicrobial
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

Relevant INDs/NDAs/DMFs: IND 55,618

Studies reviewed within these submissions:

PNU-100766: 4-Week Oral Toxicity Study With a 4-Week Recovery in Juvenile Beagle Dogs (Study No. 02-044; P&U Study No. 2002-0197)

PNU-100766: 3-Week Oral Toxicity Study in Juvenile Beagle Dogs (Study No. 02-034; P&U Study No. 2002-0196)

Studies not reviewed:

PHA-142300A: Evaluation in the Preincubation Mutagenesis Assay in Bacteria (Ames Assay) (P&U Study No. 2001-0420; Report No. SR a0100419)

PHA-142586A: Evaluation in the Preincubation Mutagenesis Assay in Bacteria (Ames Assay) (P&U Study No. 2001-0419; Report No. SR a0100431)

Both of these reports found in IND 49,195-308 were submitted previously to the pediatric supplement SE5-003 of NDAs 21,130; 21,131, and 21,132. Two carboxylic acid metabolites of linezolid, PHA-142300A and PHA-142586A (found in mice, rats, dogs, and humans) were tested in the Ames assay and neither appeared mutagenic.

REVIEWS:

PNU-100766: 4-Week Oral Toxicity Study With a 4-Week Recovery in Juvenile Beagle Dogs (— Study No. 02-044; P&U Study No. 2002-0197; Report No. d0489278)

Final report dated 11/25/02; signed GLP statement present dated 11/22/02 (excludes analysis of ECG data which was conducted by board-certified veterinary cardiologist, but not under GLP regulations); signed QA statement present.

This is the final report of a study that was submitted in draft form under IND 49,195 and reviewed under amendments 297, 299, and 302. The sponsor has submitted a statement that the only significant addition to the final report are the data tables providing the measurements of heart rates and ECG parameters and an additional statement taken from the report of the veterinary cardiologist who evaluated these data. The previous draft contained only a signed statement from the veterinary cardiologist. Other than the addition of these data, the sponsor's statement indicates that minor typographical edits were the only changes made between the latest draft received by the division and the final report of the study.

Thus, the information and conclusions drawn from the data in the pivotal 4 week juvenile dog study remain unchanged, as does the pharmacologist's review of these data. No addendum to the previous review is needed.

PNU-100766: 3-Week Oral Toxicity Study in Juvenile Beagle Dogs (— Study No. 02-034; P&U Study No. 2002-0196)

Report dated 11/5/02, dose-setting study (not GLP)

Summary: This was the dose-setting study for the pivotal juvenile dog study. Groups of 3 dogs/sex (3 week old beagles, 1.06-1.82 kg) were given 0 (vehicle), 60, or 120 mg/kg/day (bid

oral doses given approximately 8 hours apart) of linezolid (Batch No. (D2) 1500-5150-JLH-49M) suspended in 1% Avicel (microcrystalline cellulose and carboxymethylcellulose), 5% polysorbate 80 and 50 mM acetate buffer in purified water. The animals received drug twice daily for 3 weeks. Pups were housed with their dams and could suckle *ad libitum*; they were also given [redacted] wetted with reconstituted milk powder twice daily and had access to water *ad libitum*.

ECGs were taken twice prior to dosing and on days 1, 12, and 19 of dosing and analyzed by a board certified veterinary cardiologist. Blood samples for hematology and serum chemistry were drawn prior to dosing, on day 11, and prior to euthanasia/necropsy. Blood samples for toxicokinetics were taken on the days that the first and final doses of linezolid were administered: immediately before dosing and 1, 2, 4, 8, 10, 12, 16, and 24 hours after the first daily dose was given. Plasma levels of linezolid were determined at [redacted] using an HPLC method. A complete necropsy was performed on all animals and a list of tissues collected and examined microscopically is appended to this review.

Two male and 1 female dogs from the high dose group were sacrificed in moribund condition on days 15, 18, and 17 of dosing, respectively. All were lethargic, dehydrated, weak, and had pale gums or skin. One of the males and the female had pneumonia with alveolar inflammation and large numbers of Gram negative rods. The female also showed evidence of drug aspiration, so the pneumonia may have been related to a dosing error. The investigators believed that the animals' debilitated state made them more vulnerable to pneumonia. The other male sacrificed in moribund condition had acute pulmonary hemorrhage and drug solution was present in its lungs; thus, its moribundity was attributed to a dosing accident.

Clinical signs of toxicity observed in the high dose animals included reduced activity, quiet, decreased appetite (not observed to nurse or eat much food), weakness, lethargy and dehydration. One low dose female (animal 12) also showed several of these signs. Body weight gain in the high dose dogs and the low dose female that showed signs of toxicity was dramatically reduced compared to controls.

Linezolid treatment did not appear to have any effects on the ECG (report contains statement from board certified veterinary cardiologist).

Mean RBC counts, hemoglobin, and hematocrit were modestly reduced in high dose dogs beginning on day 11 of dosing and slightly reduced in the low dose female (animal 12) at the end of dosing. The number and percentage of reticulocytes were reduced in some high dose dogs and in the low dose female. Serum chemistry evaluation revealed mildly increased cholesterol levels in the high dose dogs at the end of dosing, as well as 2 low dose animals. Slightly reduced levels of inorganic phosphorous were observed in most drug-treated dogs, but no microscopic tissue changes (e.g., in bone or kidneys) were observed that accounted for the reduction. The pharmacology reviewer does not believe that the serum chemistry changes appear biologically significant.

Absolute thymus weights and thymus to brain or body weight ratios were reduced significantly compared to controls (e.g., 67-93%) in the high dose animals and in the low dose female that showed clinical signs of toxicity. The low thymus weights correlated with lymphoid depletion or lymphoid necrosis.

Drug-related microscopic changes were observed in lymphoid tissues, bone marrow, and liver. Minimal to moderate atrophy of the erythroid series was seen in undecalcified femoral bone marrow and in marrow from the sternum of high dose dogs (3/3 males, 1/3 females). Two

of these dogs also showed bone marrow atrophy of the myeloid series. Both of these dogs had pneumonia, and the investigators felt that this condition confounded the observation. They believed that the myeloid atrophy may have been due to mobilization of these cells from the bone marrow as part of the severe inflammatory process and not directly related to linezolid toxicity. Mild to marked lymphoid depletion of the thymus was observed in 3/3 high dose males, 2/3 high dose females and 1/3 low dose females (animal 12). Lymphoid necrosis was also observed in one of the high dose males. Minimal to moderate lymphoid depletion was also observed in other lymphoid tissues of some of these dogs, including the spleen, mesenteric and/or mandibular lymph nodes, and Peyer's patches in the small intestine. The report attributed the lymphoid depletion mainly to stress, but did not rule out the possibility that there was a drug-related component. The reviewer believes that the depletion is more likely to be drug-related than secondary to stress based on observations from other animal studies and the fact that it is not unusual for a compound that causes bone marrow depletion or atrophy to also be associated with lymphoid depletion. Minimal to mild hyaline change was seen in the cytoplasm of hepatocytes from 2/3 high dose males, 2/3 high dose females, and 1/3 low dose females; this change has been observed in linezolid toxicity studies with adult animals.

Linezolid did not appear to accumulate to any great extent in the dogs over the course of the dosing period and there did not appear to be any significant gender differences in pharmacokinetic parameters. Drug half life was about 2-2.5 hours at the low dose and 2.5-4 hours at the high dose. On the first day of dosing, mean Cmax and AUG ($\pm$ SD) in the 60 and 120 mg/kg dose groups were 15.8 $\pm$ 2.33 µg/ml and 165 $\pm$ 12.6 µg·h/ml at the low dose and 37.1 $\pm$ 5.30 µg/ml and 460 $\pm$ 29 µg·h/ml at the high dose. On the last day, these values were 25.7 $\pm$ 4.65 µg/ml and 209 $\pm$ 46.2 µg·h/ml at the low dose and 37.9 $\pm$ 8.49 µg/ml and 315 $\pm$ 89.9 µg·h/ml at the high dose. The toxicokinetic report states that very low plasma levels of linezolid were measured in control animals (< 0.03 µg/ml). The investigators theorize that the fact that pups from different dose groups were present in each litter may have resulted in low level contamination.

In general, the dogs tolerated the 60 mg/kg dose, but the higher dose was associated with significant toxicity. The investigators believed that the 120 mg/kg dose exceeded the MTD for linezolid in these dogs and would be too high for a 4-week study. Thus, the high dose for the pivotal 4-week juvenile dog study was set at 100 mg/kg/day, with lower doses of 25 and 50 mg/kg/day. These dose levels appear to have been appropriate choices based on the results of the current study.

OVERALL SUMMARY AND EVALUATION:

The final report for pivotal juvenile dog study 2002-0197 has been submitted by the sponsor. It contained some additional ECG data, but there were no changes to the report to alter the conclusions that were reached by the FDA pharmacology reviewer upon evaluation of the drafts.

Dose-setting data for this study were also submitted. Similar toxic effects of linezolid (bone marrow suppression and lymphoid depletion) were observed in both the dose-setting and pivotal toxicity studies in juvenile dogs. The doses chosen for the pivotal study appear to have been appropriate.

RECOMMENDATIONS: There are none.

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APPEARS THIS WAY
ON ORIGINAL

Histopathology Inventory for IND #49,195

Study	2002-0196
Species	Beagle Dog
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur- undecalcified)	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	
Liver	X*
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum (w/ marrow)	X
Stomach	X
Testes	X*
Thymus	X*
Tibia (proximal w/ joint surface)	X
Thyroid	X*
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

* organ weight obtained

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/s/

Amy Ellis
1/14/03 11:12:47 AM
PHARMACOLOGIST

No changes between draft and final reports of pivotal juvenile dog study that altered conclusions reached by the pharmacology reviewer upon evaulation of the drafts. Doses chosen for the pivotal study appear to have been appropriate.
Terry- You signed the paper copy of this review on 1/13/03.

Terry Peters
1/14/03 11:34:36 AM
PHARMACOLOGIST

Lillian Gavrilovich
1/14/03 12:02:23 PM
MEDICAL OFFICER