Appendix A. Preclinical Summary

Nonclinical Pharmacology, ADME, and Toxicology Summary

Table of Contents

1 SAFETY PHARMACOLOGY

1.1 General Introduction

Safety pharmacology studies were conducted to assess the potential effects of linezolid on the cardiovascular, central nervous, and gastrointestinal systems. All safety pharmacology studies conducted are listed in Table 3.

1.2 Cardiovascular Effects

Intravenous 10 or 30 mg/kg doses of linezolid in anesthetized dogs did not produce significant cardiovascular or respiratory changes. Analysis of ECG data from the repeated-dose toxicity studies in dogs showed that linezolid had no effect on the QTc interval.

1.3 Gastrointestinal and Renal Effects

No biologically relevant effects on intestinal contraction were observed in studies of isolated guinea pig ileum. In oral dosing studies, gastrointestinal effects in rats were limited to a marked reduction in gastric emptying at doses of 62.5 and 100 mg/kg. When linezolid was administered intravenously, reduced gastric secretion and gastric emptying were noted at a dose of 125 mg/kg. The effect of linezolid on gastric emptying may have contributed to inappetence observed at high doses in the repeated-dose toxicology studies. Intravenous doses of up to 125 mg/kg had no effect on gastrointestinal propulsion. No effects on urine volume or urinary excretion of sodium, potassium, or chloride were seen with intravenous doses of up to 125 mg/kg; however, increases in water consumption were observed in female rats with 30 and 125 mg/kg intravenous doses.

1.4 Neuropharmacological Effects

No biologically relevant effects were noted in the functional observational battery up to a single oral dose of 100 mg/kg in rats. At single intravenous doses of 125 mg/kg, moderate decreases in activity parameters (horizontal, vertical, and stereotypic) and urine and fecal output in female rats were noted 5 minutes postdose, and an increase in urine pools in females was seen 3 hours postdose. Rats recovered from the effects by 3 hours postdose.

Single intravenous doses of up to 125 mg/kg did not affect maximal electroshock-induced or pentylenetetrazol-induced convulsions or analgesic activity in rats, while doses of 30 and 125 mg/kg potentiated thiopental-induced hypnosis in females only.

2 ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION (ADME)

2.1 General Introduction

Nonclinical ADME studies were performed in mice, rats, rabbits and dogs, following single- or multiple-dose regimens administered via oral and intravenous routes. Various in vitro metabolism studies using rat and human tissues and cDNA-expressed human cytochrome P450 enzymes were conducted to elucidate the non-renal component of linezolid clearance.

2.2 Analytical Methods

Linezolid was assayed by HPLC-UV and HPLC-MS-MS methods. Optimal HPLC-UV conditions attained lower limits of quantitation of 0.05-0.005 µg/mL. Linezolid was radiolabeled at the carbonyl carbon of the acetamide substituent. Despite a small amount of acetamide hydrolysis (<5% of dose as $^{14}CO_2$), the acetamide radiolabel accurately depicts the disposition of linezolid-related materials. The chiral center is both chemically and metabolically stable.

2.3 Pharmacokinetics After a Single Dose

The overall clearance rate of linezolid is determined mainly by extensive renal tubular reabsorption of parent drug and slow oxidative metabolism to ionic metabolites. The elimination kinetics of intravenous linezolid were biexponential (See pharmacokinetic summary in Table 1). The volume of distribution at steady state (Vss) was similar to total body water, accurately predicting good tissue penetration. Compared to hepatic blood flow rates, the total plasma clearance (CL) of linezolid was low to moderate. After intravenous doses, CL decreased as the dose increased in mice, rats, and dogs. Absorption of linezolid was rapid and complete. Maximum plasma concentrations (Cmax) generally increased less than dose proportionally in dogs after oral administration. Across a wide single oral dose range, there were no major gender differences in linezolid toxicokinetics in rats or dogs.

In anesthetized male rats, linezolid had a renal clearance of 0.94 mL/min/kg. This was 8% of the glomerular filtration rate, indicating an extensive net renal tubular reabsorption of parent drug and, by inference, a significant metabolism component in the total clearance (10.5 mL/min/kg). Linezolid is neutral (pKa =1.7) within the physiological pH range. As such, renal tubular reabsorption is independent of urine pH and is a significant contributor to linezolid's low to moderate total clearance rate in all species.

Drug-related radioactivity was near-quantitatively recovered within 48 hours in all species. The mean total recovery was 95.7% of dose in mice, 98.8% in rats, 98.9% in dogs, and 92.3% to 95.0% in humans. Radioactivity was predominately excreted in urine in mice (53% to 59% of dose), rats (73% of dose), and humans (80% to 85% of dose), and radioactivity was excreted equally between urine and feces in dogs.

2.4 Pharmacokinetics After Repeated Dosing

The pharmacokinetics of linezolid in rats and dogs were assessed in all key repeated-dose toxicology studies. A summary of steady state linezolid AUC data from rat and dog studies is presented in comparison to human linezolid AUC data in Figures 1 and 2.

In general, repeated-dose pharmacokinetic parameters determined for linezolid were consistent across intravenous and oral dosing routes and across species. AUC generally increased greater than in proportion to dose in mice, rats, rabbits, and dogs. Since this was observed in both intravenous and oral studies, clearance processes were somewhat saturable with increasing dose. In rats in the 30-day and 3-month oral dose toxicity studies over the dose range 5 to 63 mg/kg, given twice daily (total dose, 10 to 125 mg/kg/day), the mean AUC was approximately 21 to 394 μg·h/mL. In the 30-day and 3-month oral dose toxicity studies in dogs over the dose range 2.5 to 40 mg/kg, given twice daily (total dose, 5 to 80 mg/kg/day), the AUC was approximately 18 to 637 μg·h/mL. In the 30-day and 3-month oral dose toxicity studies, mean Cmax values of up to 40 and 42 μg/mL were observed in rats and dogs, respectively. In comparison, human subjects that received 750 to 1250 mg/day repeated oral doses had a steady state AUC and Cmax (end of dosing) of approximately 83 to 147 μg·h/mL and 13 to 19 μg/mL, respectively. The steady-state AUC and Cmax in human subjects administered repeated intravenous doses of 1000 or 1250 mg/day were approximately 81 to 94 μg·h/mL and 14 to 16 μg/mL, respectively.

There was no consistent or significant increase in clearance over the duration of each study, indicating that induction of the enzymes responsible for metabolism did not occur. As such, in 14-day to 3-month studies, the AUC at steady state was similar to, or not more than, two times the AUC on day 1.

There were no significant gender effects on the repeated-dose pharmacokinetics of linezolid in dogs. Female rat AUCs were 10% to 20% higher than in males. The pharmacokinetics of linezolid in pregnant and nonpregnant rats were similar.

Linezolid was well absorbed in juvenile rats. The AUC was higher than in adult rats, but decreased as the studies progressed and the animals matured. However, Cmax did not consistently decrease with dose day. The dose day effects on AUC were consistent with the maturation of renal and hepatic drug clearance mechanisms.

The pharmacokinetic parameters determined for linezolid after repeated oral or intravenous dosing indicated that the mice, adult and juvenile rats, rabbits, and dogs in the toxicology studies were exposed to linezolid over a wide range of Cmax and AUC values. At an equivalent milligram per kilogram dose level, the AUC data indicated that human exposure to linezolid was higher than in the animal species in the order:
human > dog > rat > mouse > rabbit. However, systemic linezolid steady-state mean AUCs achieved in higher dose animal toxicology studies exceeded the highest steady-state mean AUCs in humans by a factor of approximately 10 in rats and 4 to 6 in dogs.

2.5 Distribution

In male rats, an oral dose of [^{14}C]linezolid was widely distributed, with levels similar to blood in most soft tissues. Relative to blood, lower levels were observed in brain, eye, white

fat, bone mineral, and testis at 0.3 hours. The tissues containing radioactivity concentrations higher than the blood at 0.3 hours after oral dosing were liver, kidney, adrenal gland, and gastrointestinal tract. Overall distribution was similar in male and female rats.

Autoradiograms revealed extensive distribution to lungs, skin and other soft tissues. This unusually good tissue penetration supports the clinical indications (Figure 3).

Radioactivity related to linezolid was excreted in milk by lactating rats. Milk contained primarily linezolid. These data indicate that linezolid and its metabolites are excreted in the milk of lactating animals and that nursing infants of mothers dosed with linezolid may be exposed to linezolid and its metabolites.

Radioactivity related to linezolid crossed the placenta in pregnant rats, resulting in fetal exposure that was comparable to maternal blood exposure. These data indicate that fetal exposure to linezolid or its metabolites may occur in pregnant women dosed with linezolid.

Protein binding of linezolid in rat, dog, and human plasma was 32%, 24%, and 31%, respectively.

2.6 Biotransformation

In plasma, parent drug was the major radioactive component in all species. This observation is consistent with slow systemic formation and relatively rapid renal clearance of the carboxylic acid metabolites. The plasma metabolite profile was reasonably consistent across species, although mice, rats, and dogs circulated relatively more PNU-142300 than humans.

All major (>0.5 % of dose excreted - 5 metabolites) and most minor human metabolites (<0.5 % of dose excreted - up to approximately 15 metabolites) have been synthesized and were also observed and quantified in mice, rats, and dogs. A scheme summarizing the major metabolic pathways of linezolid is shown in Figure 4. The major metabolites, PNU-142586 and PNU-142300, did not have antibacterial activity.

There are three main pathways in the clearance of linezolid; excretion of intact linezolid in urine, and two distinct non renal pathways that form metabolites PNU-142300 and PNU-142586, respectively (Figure 4). Both major metabolites are derived from morpholine ring oxidation and are structurally similar to metabolites of the morpholine-containing drugs moclobemide and timolol. Of these two major non renal elimination pathways, PNU-142300 represents a relatively constant and low 9% to11 % of dose.

In vitro studies on the possible roles of the major oxidases involved in drug metabolism (P450, FMO and MAO), showed that formation of the major metabolite, PNU-142586, is not mediated by major human isoforms of these enzymes. In vitro studies using human liver microsomes also showed that the formation of PNU-142586 is initiated by a chemical oxidation that is a non-enzymatic (non catalytic) process. This is in accord with weak antioxidant character of the morpholine group. The enzymes or oxidants that contribute to PNU-142586 formation in vivo have not been elucidated.

Linezolid was not a substrate or inhibitor of any of the major human CYP450 isoforms and did not induce rat CYP450 isoforms. Therefore, clinically significant drug interactions

mediated through inhibition or induction of major human isoforms of CYP P450 are not expected.

2.7 Species Comparison

The ADME data collected to date on linezolid in rats, dogs, and humans were relatively consistent across species and support the use of the rat and dog as the principal nonclinical safety species. Systemic clearance was low to moderate, in part as a result of renal tubular reabsorption of parent drug and relatively slow oxidative biotransformation. Linezolid plasma AUC generally increased greater than in proportion to dose in mice, rats, and dogs. The Cmax of linezolid tended to increase less than dose proportionally. There were no significant gender differences in linezolid pharmacokinetics in dogs; however, female rats sometimes had slightly higher AUC than males. Linezolid was rapidly and extensively absorbed after oral dosing with oral bioavailability of greater than 95% in rats, dogs, and humans. Protein binding of linezolid was low (<35%) in animal and human plasma. Linezolid was well distributed to extravascular sites with volumes of distribution on the order of total body water in animals and humans. Linezolid circulated mainly as parent drug in all species examined. Mice, rats, and dogs, like humans, were all exposed to relatively lower plasma concentrations of the two major human metabolites, PNU-142586 and PNU-142300. Renal excretion of linezolid and its metabolites was the major excretion route in mice, rats, and humans, and was approximately equivalent to fecal elimination in dogs. Linezolid and the inactive carboxylic acid metabolites, PNU-142586 and PNU-142300, were the major excretion products in mice, rats, dogs, and humans, although humans generally produced relatively more PNU-142586 than the preclinical species. A wide range of AUC and Cmax values for linezolid were achieved in toxicology studies and exposure of animal species to linezolid has far exceeded the exposures measured in humans.

3 TOXICOLOGY

3.1 General Introduction

The toxicity of linezolid was evaluated in acute oral and intravenous toxicity studies in rats and an acute oral toxicity study in dogs, repeated-dose oral toxicity studies up to 3 months in duration in rats and dogs, a 4-week oral toxicity study in juvenile rats, repeated-dose intravenous toxicity studies up to 1 month in duration in rats and dogs, developmental and reproductive toxicity studies in mice and adult and juvenile rats, mutagenic potential studies in vitro and in vivo, and special toxicology studies (handler safety [ocular and dermal irritation] studies and MAO inhibition studies). All toxicology studies conducted are listed in Table 3.

3.2 Acute Toxicity

3.2.1 Rat

When the acute oral toxicity of linezolid was evaluated in rats given two equally divided doses of drug on one day, the minimum lethal dose was between 1000 and 3000 mg/kg; 2/5 males and 5/5 females in the 3000 mg/kg group and all 10 rats in the 5000 mg/kg group died. Clinical signs in surviving and moribund animals included decreased activity, salivation, alopecia, and body weight loss. No mortality or clinical signs were observed in the 1000 mg/kg dose group. No toxic signs or adverse effects were seen in acute IV toxicity studies when rats were administered doses of up to 400 mg/kg.

3.2.2 Dog

In male dogs given two equally divided doses of linezolid orally on one day, the minimum lethal dose was greater than 2000 mg/kg/day. Vomiting, tremors, and decreased activity were the primary clinical observations. No symptoms were observed 24 hours after the evening dose. Food consumption and body weight gains in dogs given 500 and 2000 mg/kg/day were suppressed slightly in the early phase of the observation period and returned to normal thereafter.

3.3 Repeated-Dose Toxicity

Studies performed to assess the toxicity of linezolid after repeated dosing are listed in Table 3. The primary target organs of toxicity were the hematopoietic and gastrointestinal systems in rats and dogs, and the reproductive system in rats. The no-observed-adverse-effect levels (NOAELs) were 10 mg/kg/day in the 3-month oral rat study, 20 mg/kg/day in the 1-month oral rat study, and 20 mg/kg/day in the 1- and 3-month oral dog studies. For the evaluation of potential human risk, Table 2 compares linezolid exposure (AUC) at the NOAELs and lowest-observed-adverse-effect levels (LOAELs) in the pivotal repeated-dose toxicity studies with the estimated human exposure.

3.3.1 Hematopoietic Effects

Linezolid produced myelosuppression in rats and dogs that was time- and dose-dependent, and reversible. Findings included mild bone marrow hypocellularity and moderate decreases in red blood cell, white blood cell, and platelet counts. There was no evidence of bone marrow aplasia in any study. No hematopoietic effects were observed in dogs dosed for 5 days with intravenous doses of up to 150 mg/kg/day. A 1-month recovery period was sufficient for the reversal of myelosuppression in most studies, and in the case of the 3-month dog study, reversal of effects was observed during the dosing phase of the study when the dose was reduced from 40 to 30 mg/kg/day.

Evaluation of data from Phase III comparator-controlled clinical studies revealed that linezolid did not produce serious hematologic effects at the clinically recommended doses of up to and including 600 mg twice daily in studies up to 28 days in duration. That analysis included data for 2046 patients who received at least one dose of linezolid and 2001 patients

who received a comparator antimicrobial agent. The percentages of substantially abnormal hematology values were similar between the linezolid groups and the comparator groups, and there was no evidence of clinically significant hematopoietic suppression.

3.3.2 Gastrointestinal Effects

Gastrointestinal effects were observed in rats and dogs that were likely primarily related to antibiotic-induced alterations in intestinal microflora. Findings in rats included decreased food consumption and diarrhea, which resulted in decreased weight gain, and histological changes in the large and small intestines (atrophy of intestinal mucosa and necrosis of epithelial cells in the intestinal crypts) in the 2-week study at high doses of 200 and 1000 mg/kg/day. In the longer-term definitive studies in rats, treatment-related decreases in body weight gain and food consumption were not accompanied by microscopic findings. Reduced gastric emptying, noted in the safety pharmacology studies in rats, may have been a contributing factor to the inappetence. In dogs, anorexia, vomiting, and mucous stools accompanied weight loss. Effects reversed with cessation of treatment.

Digestive complaints, such as nausea and diarrhea, were the most frequently reported adverse events in linezolid-treated patients in the comparator-controlled Phase III clinical studies. The overall incidence of digestive complaints was similar between the linezolid and comparator groups. Almost all antimicrobial agents produce gastrointestinal side effects, including nausea, vomiting, diarrhea, and intestinal cramping, which have been attributed to a combination of direct effects on the intestinal epithelium and indirect effects resulting from alterations of the normal microbial flora.

3.3.3 Effects on Reproductive Organs

Epididymal epithelial cell hypertrophy was seen in the 3-month rat study. Hypertrophy of the epididymal epithelium is one possible cause of the reversible infertility observed in adult rats in the reproductive function studies (see section 3.4.1). While the 1-month recovery period was insufficient for the epididymal hypertrophy to reverse in the 3-month study, findings in a subsequent study of adult male rat fertility and reversibility demonstrated that the epididymal effects reversed with a longer recovery period. In that study, both decreased fertility and epididymal changes reversed with a 14-week recovery period.

In the 1-month oral dog study, animals were 6 months old when dosing was initiated. Microscopic findings and organ weight changes in prostate, testes, and epididymides were observed at all dose levels; all changes were reversible. The microscopic changes were similar to those which occur as a function of normal sexual maturation; there was no histological evidence of tissue damage. Similar effects were not observed in the 1-month intravenous or 3-month oral dog studies, which used dogs 7 to 10 months old at dose initiation and achieved similar drug exposures. There was no evidence of epididymal epithelial hypertrophy in any dog study.

3.3.4 Liver Effects

Changes in liver weights were reported in rats in the two 14-day studies at dose levels of 200 or 250 mg/kg/day. The findings were inconsistent, with decreased weights in one study and increased weights in the other. Dogs exhibited elevated alanine aminotransferase (ALT) levels without gross or microscopic changes at oral doses of 80 mg/kg/day and above, while no changes in liver enzymes were seen with intravenous doses of up to 120 mg/kg/day. In addition, a slight, transient elevation in ALT was noted in one dog in the 2000 mg/kg dose group in the acute toxicty study. Thus, there is evidence for hepatic effects in animals at high dose levels. However, no histological evidence of liver damage was observed in any study, and the ALT increases were reversible.

Review of the comparator-controlled Phase III clinical studies has shown little evidence of significant hepatic effects with linezolid therapy at doses up to 600 mg twice daily given for up to 28 days. There were few adverse events involving liver function abnormalities. These episodes were usually of mild to moderate intensity, were self-limited, and did not lead to discontinuation of study medication. Additionally, evaluation of hepatic laboratory parameters showed that hepatic chemistry values, [ALT, aspartate aminotransferase (AST)] were comparable for patients taking linezolid or a comparator (oxacillin, clarithromycin, vancomycin, cefpodoxime, and ceftriaxone). The percentage of patients with at least one substantially abnormal AST/ALT value (twice baseline or twice the upper limit of normal values) was not different between linezolid treatment and all comparators. Thus, based on adverse events data and safety laboratory monitoring, linezolid at clinical doses up to and including 600 mg twice daily is not considered to present a significant risk for hepatic dysfunction.

3.3.5 Other Effects

Alopecia, observed at high dose levels, was minimal and patchy in adult rats and was reversible. Treatment-related alopecia has not been reported in linezolid clinical trials.

At high dose levels, adverse effects considered to be secondary to anorexia and body weight loss included elevated blood urea nitrogen levels observed in some dog studies, an absence of spermatids in the epididymis in rats in the 14-day study, and, in both species, enlarged adrenal glands with cortical hypertrophy, hypocellularity of the bone marrow, reduced weights of a number of organs, and atrophy of the lymphoid tissues. These changes reversed with cessation of treatment.

Findings in rats related to the antibacterial activity of linezolid included enlarged ceca that resulted in abdominal distension, decreased levels of total protein and globulin, an increased albumin/globulin ratio, and decreased white blood cell counts. Similar effects have been reported with other antibacterials in animal toxicity studies and have been attributed to alterations in the intestinal microflora and/or to decreased leukocyte and globulin production (especially of gamma globulin) with decreased antigenic stimulation secondary to a decreased body burden of bacteria.

3.3.6 Effects in Juvenile Rats

Juvenile Sprague-Dawley rats 6 to 7 days old at dose initiation were administered linezolid orally for 1 month or 53 days. The adverse effects observed in juveniles were qualitatively similar to those observed in adults, and were reversible. The hematopoietic, gastrointestinal, and reproductive systems were the primary target organs in juveniles. Hair loss was more extensive and occurred at a higher incidence than in adults. Evidence of delayed testicular maturation was observed during the dosing period in the 1-month study, in which large and/or large multinucleate cells were noted in the seminiferous tubules of the testes. There was no evidence of testicular degeneration at any dose level. The NOAEL for linezolid in juvenile rats was 25 mg/kg/day in the 1-month study. Systemic exposure was highest on day 1, with AUC values in juvenile rats equal to or greater than values previously observed in adult rats treated orally at double the daily dose levels. By day 30, AUC values had decreased 3- to 4-fold. C_{max} values showed a similar trend. Decreases in these parameters were considered to result from maturation of renal and hepatic drug clearance mechanisms. In contrast to rats, the average clearance values in human pediatric patients were higher than adults. A therapeutic dose of 10 mg/kg produced exposures similar to adults at sampling times up to 1 hour, and exposures lower than adults at later times. Thus, the animal data do not accurately reflect an increased risk of adverse effects when linezolid is used in a pediatric population.

3.4 Reproduction Studies

3.4.1 Fertility and General Reproductive Performance

Linezolid did not affect fertility or reproductive performance in adult female rats; the NOAEL for female F_0 reproductive toxicity was 100 mg/kg/day.

Linezolid decreased fertility in sexually mature male rats when given at doses ≥50 mg/kg/day for 4 to 10 weeks with exposures approximately equal to or greater than the expected human exposure level (Table 2). Epithelial cell hypertrophy in the epididymis may have contributed to the decreased fertility by affecting sperm maturation. Light microscopic examination of the testes did not show overt drug-induced effects, although an effect on spermatogenesis cannot be excluded. Although the concentrations of sperm in the testes were in the normal range, concentrations in the cauda epididymidis were decreased and sperm from the vas deferens had decreased motility. Mildly decreased testosterone levels were observed, but these decreases were not considered of sufficient magnitude to be responsible for the observed effects. Additionally, supplementation with testosterone did not prevent the adverse fertility effects, epididymal hypertrophy, or decreased sperm motility. The fertility effects seen after 10 weeks of dosing at 100 mg/kg/day reversed after a 14-week recovery phase. The NOAEL for the epididymal effect was 10 mg/kg/day in the 3-month general toxicity study, and the NOAEL for male fertility effects was 15 mg/kg/day with a threshold dose for effects at about 50 mg/kg/day. A number of commonly used antibacterials, including some aminoglycosides, cephalosporins, and quinolones, are known to adversely affect spermatogenesis and to produce testicular injury in rats. However, the relevance of these findings to humans

remains unclear because antibacterial therapy has not been shown to directly affect fertility in humans with the exception of sulfasalazine, which causes reversible oligospermia.

Linezolid administered at an estimated maximum tolerated dose level to juvenile rats 7 through 55 days of age produced a subtle decrement in fertility with some evidence of decreased copulation indices (sperm in a vaginal smear or a copulatory plug in the vagina) and increased preimplantation losses at exposures 0.4-fold to 1.2-fold that expected in humans (Table 2). These changes were first apparent 10 weeks after the cessation of dosing and had not reversed at 15 weeks postdosing, the last interval evaluated. There were no treatment-related changes in motility (viability) of sperm from the vas deferens or sperm head counts [absolute or relative (per gram of tissue)] in the testes or cauda epididymides at the interim or final sacrifices and no evidence of epididymal hypertrophy. This study differed from the adult rat fertility studies in that the animals were treated for most of the period of sexual development. Thus, the duration of exposure in the juvenile rats is biologically analogous, in terms of sexual maturation and spermatogenesis, to approximately 3 to 4 years of continuous dosing in adolescent male humans. The 25/50 mg/kg/day dose level was the no-effect level for these findings in juvenile rats (the 25 mg/kg/day dose level was increased to 50 mg/kg/day beginning on day 37 to compensate for clearance increases with age). Commonly used members of almost every class of antibacterial agents have been shown to delay or inhibit spermatogenesis in young rats, including erythromycin, tylosin, gentamicin, neomycin, tetracycline, penicillin G, and several cephalosporins. Extrapolation of these findings to humans is difficult, because of substantial differences in the initiation of spermatogenesis and in the spermatogenic cycle between rats and humans.

3.4.2 Embryotoxicity

In a developmental toxicity study in mice, the NOAEL for maternal, embryo, and fetal toxicity was 150 mg/kg/day. Embryotoxic and fetotoxic effects occurred only at a maternotoxic dose level. At a dose of 450 mg/kg/day (4-fold the estimated human exposure level, Table 2), decreased body weight gain and clinical signs in dams correlated with embryotoxicity (increased postimplantation embryo death including total litter loss in 8 of 24 gravid mice) and fetal toxicity (decreased body weights with an increased incidence of costal cartilage fusion) with no treatment-related malformations. The increased incidence of costal cartilage fusion consisted of an exacerbation of the normal genetic predisposition to sternal variations in this strain of mice. There was no evidence of teratogenicity at any dose level.

Rat fetuses in utero were exposed to linezolid concentrations similar to those in maternal plasma. In an embryo-fetal development study in rats, linezolid was not teratogenic. Evidence of mild fetal toxicity was observed at the two highest dose levels, 15 and 50 mg/kg/day (exposure levels 0.13- to 0.64-fold the estimated human exposure level, respectively, Table 2). The effects consisted of decreased fetal body weights (8% and 15% for the 15 and 50 mg/kg/day dose groups, respectively) 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 the 50 mg/kg/day dose level. The NOAEL for embryo-fetal development was 2.5 mg/kg/day, based on the mild fetal toxicity at the 15 and 50 mg/kg/day dose levels.

Other antibacterials have been shown to cause embryo-fetal toxicity in animal models. Representatives of the tetracycline (oxytetracycline) and quinolone (ofloxacin) classes of antibacterials cause effects on embryo-fetal development in rodents similar to those of linezolid, albeit at higher doses relative to the human dose. Vancomycin hydrochloride caused decreased fetal body weights in rabbits when given during the period of organogenesis. Such findings have not prevented general use of these antibacterials in women of childbearing potential if the potential benefit justifies the potential risk to the fetus.

3.4.3 Peri-Postnatal Toxicity

In two perinatal and postnatal development studies conducted in rats, F_1 pups were potentially exposed in utero or via milk but were not dosed directly. Separate drug distribution studies showed drug levels to be similar in reproductive organs and blood, and linezolid and its metabolites to be secreted in milk. In the first study, slightly increased postimplantation loss with a corresponding decreased number of live pups at birth occurred in both the F_1 and F_2 pups of the 50 mg/kg/day dose group. Survival of pups of the 50 mg/kg/day dose group was decreased on postpartum days 1 through 4. Body weights were decreased in pups of the 15 and 50 mg/kg/day dose groups and developmental delays were present in pups of the 50 mg/kg/day dose group; these findings were reversible. The NOAEL for the F_1 generation was 2.5 mg/kg/day. In a recently completed study, decreases in F_1 body weights and a slight reduction in F_1 fertility were noted in the 50 mg/kg/day group. Pups that were permitted to mature to reproductive age, when mated, showed evidence of a dose-related increase in preimplantation loss at maternal doses ≥2.5 mg/kg/day, with exposures below those expected in humans. Decreases in viable F_2 fetuses, implantation sites, gravid uterus weights, and gestation body weights (days 16-20) were noted in the 50 mg/kg/day F_1 group. Based on increased preimplantation loss observed at the 2.5 mg/kg/day dose level, no NOAEL for F_1 reproductive toxicity was identified in the ongoing perinatal and postnatal study.

3.5 Mutagenic Potential

Linezolid was nonmutagenic and nonclastogenic in all genotoxicity assays, including those designed to measure chemically induced gene mutation in bacterial and mammalian cells (the Ames and AS52 assays, respectively) and those designed to measure chromosome aberrations in human lymphocytes in vitro and micronuclei in mouse bone marrow cells in vivo. In addition, linezolid did not induce unscheduled DNA synthesis (UDS) in vitro, a measure of DNA repair following chemically induced DNA damage.

3.6 Carcinogenic Potential

Linezolid is intended for use in therapy 30 days or less in duration and has shown no evidence of mutagenicity or clastogenicity. Therefore, carcinogenicity bioassay studies have not been conducted.

3.7 Other Information

3.7.1 Local Tolerance

Separate local tolerance studies were not conducted with intravenous linezolid. However, there was no evidence that linezolid produced vascular irritation in the intravenous 14-day or 1-month rat toxicity studies or in the 5-day, 14-day, or 1-month intravenous toxicity studies in dogs.

3.7.2 Handler Safety Studies

In ocular and dermal irritation studies in rabbits, linezolid caused minimal and transient irritation when administered as a single dose of 100 mg/eye and was slightly irritating to abraded skin when applied at a dose of 100 mg/site/day for 5 days.

3.7.3 Monoamine Oxidase Inhibition Studies

Oxazolidinones as a compound class were shown to be inhibitors of human monoamine oxidase (MAO). Therefore, a series of in vitro experiments were conducted using rat and human tissues to assess the ability of linezolid to inhibit MAO-A and MAO-B. Unlike the well-known MAO inhibitors clorgyline and selegiline, linezolid was shown to be a weak and reversible MAO inhibitor with Ki values of 56 and 0.71 µM for the inhibition of human MAO-A and MAO-B, respectively. In contrast, the irreversible selective MAO-A inhibitor, clorgyline, had MAO-A and MAO-B Ki values of 0.0013 and 0.71 µM, respectively, while the irreversible selective MAO-B inhibitor, selegiline, had MAO-A and MAO-B Ki values of 2 and 0.004 µM, respectively. The Ki values of the major linezolid metabolites, PNU-142586 and PNU-142300, and minor metabolite PNU-105368, were approximately 147, 1100, and 133 µM, respectively. These Ki values are many times higher than plasma levels of these metabolites in humans.

A rodent model was developed to test the in vivo effects of MAO inhibition by linezolid. The methodology progressed from intravenous administration of crystalline tyramine in anesthetized rats to oral administration of large doses of crystalline tyramine in conscious, fasted rats. Oral doses of 50 mg/kg linezolid (producing plasma levels of approximately 20 µg/mL) were required to potentiate the vasopressor effects of high doses of orally administered tyramine. The tyramine potentiation effects of linezolid were similar after 1, 3, 5, or 14 days of oral dosing, were eliminated when tyramine was dosed orally in food, and, unlike irreversible inhibitors, were eliminated when dosing was terminated.

Although the vasopressor effects of high intravenous and oral doses of tyramine were potentiated by high plasma concentrations of linezolid in rats, efforts to develop a similar rat model using catecholamines found in over-the-counter cold remedies were unsuccessful. Administration of oral pseudoephedrine and phenylpropanolamine at doses 3-fold those recommended for clinical use to conscious, linezolid-pretreated dogs did not produce a clinically relevant vasopressor response. An additional series of experiments showed linezolid to be a weak inhibitor of serotonin and dopamine turnover in conscious rats after acute or repeated administration. However, the magnitude of the changes with

high doses of linezolid was small when compared to irreversible MAO inhibitors such as clorgyline. Finally, experiments were conducted to characterize the physiologic and behavioral effects of linezolid in a rabbit model of the serotonin syndrome. In those experiments, 50 and 150 mg/kg doses of linezolid did not have the same liability as irreversible inhibitors such as clorgyline to induce the serotonin syndrome.

In summary, nonclinical studies showed linezolid to be a weak and reversible MAO inhibitor.

4 SUMMARY

Linezolid was shown to have superior pharmacokinetic characteristics, and the nonclinical species were extensively exposed to linezolid and its metabolites. The pharmacokinetic behavior of linezolid was similar across nonclinical species, and, overall, mice, rats, and dogs were considered to be good models of the behavior of linezolid in humans. The dose-limiting toxicities of linezolid were well defined. In repeated-dose toxicity studies reversible hematopoietic and gastrointestinal effects were observed in rats and dogs, and reversible reproductive effects were observed in male rats. Linezolid and its metabolites were excreted in the milk of lactating rats and crossed the placenta in pregnant rats. No evidence of teratogenicity was seen, but effects on male fertility, mild fetal toxicity, and reproductive effects on the F1 generation were observed in rats. Linezolid showed no evidence of genotoxicity. Additionally, linezolid was shown to be a weak, reversible monoamine oxidase (MAO) inhibitor.