National Institute of
Environmental Health Sciences

NIEHS Technical Report on the Reproductive, Developmental, and General Toxicity Study of 3'-Azido-3'-deoxythymidine (AZT), Trimethoprim (TMP)/ Sulfamethoxazole (SMX), and Folinic Acid Combinations Adminstered by Gavage to Swiss (CD-1®) Mice

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Administered by Gavage to
Swiss (CD-1®) Mice

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FOREWORD

Infection with human immunodeficiency virus (HIV) causes immunosuppression and leads to acquired immunodeficiency syndrome (AIDS) with a broad spectrum of opportunistic infections. Prophylaxis and treatment of AIDS are generally combination therapies of antiretroviral agents with antimicrobial drugs specific for the opportunistic infections. The National Institute of Environmental Health Sciences (NIEHS), under the AIDS research program, is evaluating AIDS therapeutics for reproductive, developmental, and general toxicity in rodents. These evaluations may include single therapeutic agents or combination therapies when the toxic potential of these agents in animal models is not available or is incomplete.

CONTRIBUTORS

This report on the reproductive, developmental, and general toxicity studies of 3'-azido-3'-deoxythymidine (AZT), trimethoprim/sulfamethoxazole (TMP/SMX), and folinic acid combinations is based primarily on studies that began in May 1994, and ended in July 1994, at Southern Research Institute, Birmingham, AL.

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PEER REVIEW

The draft report on the reproductive, developmental, and general toxicity studies of 3'-azido-3'-deoxythymidine (AZT), trimethoprim/sulfamethoxazole (TMP/SMX), and folinic acid combinations was evaluated by peer reviewers. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these studies are appropriate and ensure that this reproductive, developmental, and general toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were reviewed prior to the finalization of this document. Changes were made such that the concerns of the reviewers were addressed to the extent possible.

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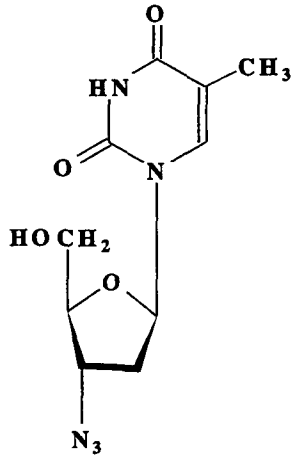
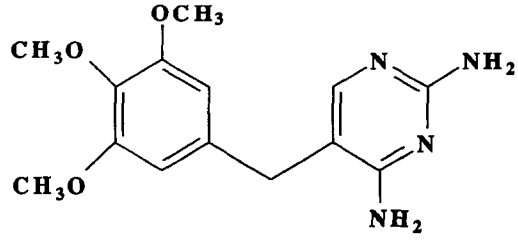
ABSTRACT

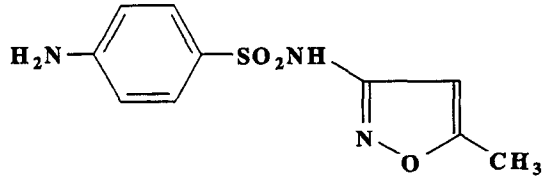
3′-Azido-3′-deoxythymidine (AZT), Trimethoprim (TMP)/Sulfamethoxazole (SMX), and Folinic Acid Combinations



AZT
Molecular Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24
CAS No.: 30516-87-1



TMP
Molecular Formula: $C_{14}H_{18}N_4O_3$
Molecular Weight: 290.32
CAS No.: 8064-90-2



SMX
Molecular Formula: $C_{10}H_{11}N_3O_3S$
Molecular Weight: 253.31
CAS No.: 8064-90-2

The toxicity of combinations of AZT (200 or 400 mg/kg), TMP/SMX (1,000, 2,000, or 3,000 mg/kg), and folinic acid (10 mg/kg) was evaluated in Swiss (CD-1®) mice treated by oral gavage. The doses of AZT are equivalent to two and four times the therapeutic dose in humans (based on body surface area); doses of TMP/SMX are one, two, and three times the therapeutic dose for toxoplasmosis in mice. The dose of folinic acid is 100 times the nutritional requirement in mice. Male mice (10 per group) were dosed from day 5 until

the day prior to sacrifice on day 25 or 26. Females were divided into two groups designated female-A mice and female-B mice. The female-A mice (20 per group) were dosed from day 0 to sacrifice. They were cohabited with treated males on days 9 to 13 to test for effects on mating behavior, fertilization, and implantation, and caesarean sections were performed on days 28 to 32. The females designated as female-B mice (20 per group) were cohabited with untreated males on days 0 to 4. Sperm-positive female-B mice were dosed during organogenesis on days 6 to 15 of presumed gestation and sacrificed on day 4 of lactation.

Summarized in Table 1 are the significant effects of treatment with AZT, TMP/SMX, and/or folinic acid. The most significant effect of AZT alone in adult mice was a mild dose-related anemia in males and females. Combination therapy with AZT and TMP/SMX resulted in hematopoietic toxicity of far greater severity than that following treatment with either test compound alone. Male and female-A mice that received combination therapy developed severe anemia, reticulocytopenia, and thrombocytosis accompanied by cellular depletion of bone marrow and diminished splenic hematopoiesis. Mortality occurred in female-A mice that were treated with AZT and TMP/SMX combinations. Female-B mice that received combination therapy had a mild regenerative anemia with reticulocytosis. Female-A and female-B mice that received AZT and TMP/SMX with or without folinic acid showed marked declines in mean body weight. In general, other manifestations of toxicity included pallor, piloerection, labored breathing, diminished motor activity, and emaciation. Thyroid gland hyperplasia was observed in all groups treated with TMP/SMX alone or in combination with AZT. Combination therapy as well as therapy with TMP/SMX alone also resulted in hepatocyte hypertrophy.

The most striking effect on reproductive and developmental parameters was the presence of cleft palate in 100% of the litters of female-B mice treated with greater than 2,000 mg TMP/SMX per kg body weight either alone or in combination with AZT. In female-A and female-B mice, AZT alone produced decreases in uterine weights, live litter sizes, pup weights, and fetal weights and increased pup deaths and resorptions. TMP/SMX produced mortality in female-A and female-B litters; reduced the number of litters, litter size, and pup weight; and increased the number of pups dying on days 1 to 4. In males, TMP/SMX decreased testicular weight, spermatid heads per testis, and average spermatid count; treatment with either AZT or TMP/SMX reduced sperm motility.

With the exception of thyroid gland hyperplasia and the development of cleft palates, the combination of AZT and TMP/SMX resulted in toxicity of greater severity than that subsequent to the administration of either compound alone. Supplementation with folinic acid failed to significantly ameliorate any toxic effect of AZT and/or TMP/SMX.

TABLE 1
Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice[a]

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Hematology			
AZT alone	Mild anemia Mild leukopenia Mild thrombocytosis	Mild anemia Mild leukopenia Mild thrombocytosis	No significant alterations
TMP/SMX alone	Mild anemia Mild monocytosis	Mild anemia Mild thrombocytosis	No significant alterations
Folinic acid alone	No significant alterations	No significant alterations	No significant alterations
AZT + folinic acid	Mild anemia Mild leukopenia Mild thrombocytosis	Mild anemia Mild leukopenia Mild thrombocytosis	No significant alterations
TMP/SMX + folinic acid	Mild anemia Mild monocytosis	Mild anemia Mild thrombocytosis	No significant alterations
AZT + TMP/SMX	Severe anemia Mild leukopenia Marked reticulocytopenia Marked thrombocytosis	Severe anemia Mild leukopenia Marked reticulocytopenia	Mild anemia Mild leukocytosis Mild thrombocytosis Reticulocytosis
AZT + TMP/SMX + folinic acid	Severe anemia Mild leukopenia Marked reticulocytopenia Marked thrombocytosis	Severe anemia Mild leukopenia Marked reticulocytopenia	Mild anemia Mild leukocytosis Reticulocytosis
Histopathology, Spleen			
AZT alone	Slight increase in hematopoiesis	Slight decline in hematopoiesis	Slight increase in hematopoiesis
TMP/SMX alone	Slight increase in hematopoiesis	Slight decline in hematopoiesis	No significant alterations
Folinic acid alone	No significant alterations	No significant alterations	No significant alterations
AZT + folinic acid	Slight increase in hematopoiesis	Slight decline in hematopoiesis	Slight increase in hematopoiesis
TMP/SMX + folinic acid	Slight increase in hematopoiesis	Slight increase in hematopoiesis	No significant alterations
AZT + TMP/SMX	Prominent decline in hematopoiesis	Prominent decline in hematopoiesis	Prominent increase in hematopoiesis
AZT + TMP/SMX + folinic acid	Prominent decline in hematopoiesis	Prominent decline in hematopoiesis	Prominent increase in hematopoiesis

TABLE 1
Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Histopathology, Thymus			
AZT alone	Slight atrophy	No significant alterations	No significant alterations
TMP/SMX alone	Slight atrophy	Slight atrophy	No significant alterations
Folinic acid alone	No significant alterations	No significant alterations	No significant alterations
AZT + folinic acid	Slight atrophy	No significant alterations	No significant alterations
TMP/SMX + folinic acid	Slight atrophy	Slight atrophy	No significant alterations
AZT + TMP/SMX	Prominent atrophy	Prominent atrophy	Prominent atrophy in the 400 + 3,000 + 0 group
AZT + TMP/SMX + folinic acid	Prominent atrophy	Prominent atrophy	No significant alterations[b]
Histopathology, Thyroid Gland			
AZT alone	No significant alterations	No significant alterations	No significant alterations
TMP/SMX alone	Severe hyperplasia	Severe hyperplasia	Mild hyperplasia
Folinic acid alone	No significant alterations	No significant alterations	No significant alterations
AZT + folinic acid	No significant alterations	No significant alterations	No significant alterations
TMP/SMX + folinic acid	Severe hyperplasia	Severe hyperplasia	Mild hyperplasia
AZT + TMP/SMX	Severe hyperplasia	Severe hyperplasia	Mild hyperplasia
AZT + TMP/SMX + folinic acid	Severe hyperplasia	Severe hyperplasia	Mild hyperplasia

TABLE 1
Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Treatment Regimen	Male Mice[c]	
	Bone Marrow	Liver
Histopathology, Bone Marrow and Liver		
AZT alone	Mild bone marrow depletion	Slight hepatocyte hypertrophy
TMP/SMX alone	Minimal bone marrow depletion	Prominent hepatocyte hypertrophy
Folinic acid alone	No significant alterations	No significant alterations
AZT + folinic acid	Minimal bone marrow depletion	No significant alterations (folinic acid diminished AZT effect)
TMP/SMX + folinic acid	Minimal bone marrow depletion	Prominent hepatocyte hypertrophy
AZT + TMP/SMX	Mild bone marrow depletion	Mild hepatocyte hypertrophy (AZT diminished the TMP/SMX effect)
AZT + TMP/SMX + folinic acid	Moderate bone marrow depletion	Minimal hepatocyte hypertrophy (AZT diminished the TMP/SMX effect)
Reproductive/Developmental		
	Litter Size	**External Abnormalities**
AZT alone	Diminished	No significant alterations
TMP/SMX alone	No significant alterations	Cleft palate
Folinic acid alone	No significant alterations	No significant alterations
AZT + folinic acid	Diminished	No significant alterations
TMP/SMX + folinic acid	No significant alterations	Cleft palate
AZT + TMP/SMX	Absence of litters	Cleft palate
AZT + TMP/SMX + folinic acid	Absence of litters	Cleft palate

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] The AZT + TMP/SMX combination dose that showed prominant atrophy was not evaluated with folinic acid
[c] Bone marrow and liver not routinely examined in female mice

INTRODUCTION

AIDS is a lethal multi-system disease that has become a major public health problem since its recognition in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegal *et al.*, 1981). The etiological agent of AIDS is a retrovirus now referred to as HIV (Coffin, 1986). To date, the most effective single agent in the treatment of HIV has been the first dideoxynucleoside analogue used in clinical trials, zidovudine (3′-azido-3′-deoxythymidine, AZT, Retrovir, azidothymidine, compound S, BW A509U, CAS No. 30516-87-1), commonly referred to as AZT (Vince *et al.*, 1988; Amin, 1989).

AZT therapy produces numerous beneficial effects in AIDS patients, including decreases in morbidity and increases in lifespan (Amin, 1989; Jeffries, 1989). The most important adverse effects of AZT are anemia and granulocytopenia, which are believed to reflect bone marrow toxicity (Richman, 1988; Amin, 1989). Two types of anemia may occur with AZT therapy: macrocytic megaloblastic anemia and normocytic normochromic anemia.

Several subacute and subchronic rodent toxicity studies have demonstrated that the primary toxicity of AZT is myelosuppression. Male Swiss (CD-1®) mice were administered 100, 250, 500, or 1,000 mg AZT/kg body weight by gavage for 30 days (Mansuri *et al.*, 1990). No mortality or body weight effects were evident from AZT treatment. Erythropenia and increased mean cell volume were observed at all doses, and anemia was observed at the 1,000 mg/kg dose. Pathologic findings in the AZT-treated mice were consistent with the hematological results and included lymphoid depletion, reticuloendothelial hyperplasia in the spleen and thymus, and bone marrow hypocellularity.

In a 14-week subchronic study (NTP, 1998), B6C3F$_1$ mice were treated with 0, 25, 50, 100, 400, or 1,000 mg AZT/kg body weight in 0.5% methylcellulose by gavage twice daily. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in both males and females. Dose-related anemia was evident on days 23 and 93. To evaluate the ability of treated animals to reverse any compound-related effects when treatment is stopped, additional groups were administered 0, 50, 400, or 1,000 mg/kg AZT twice daily for 92 days and then held without additional treatment for 29 days. Improvement of hematology parameters indicated recovery of the bone marrow after treatment stopped. An apparently nontoxic, treatment-related

clinical finding that affected AZT-treated B6C3F$_1$ mice was a darkening of the skin on the tail, feet, and/or muzzle.

Oral bioavailability of AZT was determined in female B6C3F$_1$ mice by comparison of the area under the curve obtained from an oral dose to that of an intravenous dose at the same concentration (Trang *et al.*, 1993). Bioavailability was found to be 0.86, 0.78, and 0.97 for the 15, 30, and 60 mg/kg oral doses. The mean elimination half-life values ranged from 17.3 to 19.9 minutes for the three intravenous doses and from 16.5 to 21.9 minutes for the three oral doses. Based on these results, the internal dose of AZT was linear and dose proportional over the oral-dose concentration range administered.

Standard teratology studies of AZT have been performed in rats and rabbits (Ayers, 1988). Rats were dosed orally with 125 to 500 mg/kg on gestation days 6 to 15. No fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes post-dosing were 61 μg/g or 76 times the antiviral inhibitory dose for 50% of the viral population being tested (ID_{50}). Rabbits were dosed orally at 125 to 500 mg/kg on gestation days 6 to 18, and no fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes post-dosing were 40.2 μg/g, or 50 times the antiviral ID_{50}.

Female Wistar rats were dosed three times orally with 100 mg/kg AZT at 5-hour intervals on gestation day 10 for a total dose of 300 mg/kg (Greene *et al.*, 1990). No adverse effects on maternal body weight gain, feed consumption, fertility, and hematological parameters or growth and survival of offspring were observed. AZT concentration measurements 30 minutes after the last dose were 62.6 μg/mL in maternal plasma and 21.1 μg/g in fetal tissue.

Studies in C_3H/He mice concluded that AZT has a direct toxic effect on the developing mouse embryo (Toltzis *et al.*, 1991). Female mice were exposed to 0, 0.25, 0.5, or 2.5 mg AZT/mL drinking water for 8 weeks during mating and throughout gestation. All AZT groups had fewer pregnant mice per group, fewer pups per litter, and increased resorptions per mouse. Dose-related embryolethality was observed.

Since AIDS is a disease of immune suppression, the majority of AIDS patients actually die from characteristic opportunistic infections (Hardy, 1991; Harkins and Herriot, 1992), and the treatment of AIDS is increasingly one of combination therapy of anti-AIDS drugs and anti-infective drugs (Goldschmidt and Dong, 1992). The most common opportunistic infection and the leading cause of death in AIDS patients is *Pneumocystis carinii* pneumonia (PCP), which is found in 60% to 80% of AIDS patients (Harkins and Herriott, 1992).

Although drugs that are very active against pneumocystis are available, they are associated with a high incidence of adverse reactions in AIDS patients, requiring withdrawal of therapy in as many as 50% of cases. Trimethoprim (TMP) in combination with sulfamethoxazole (SMX), designated as cotrimoxazole (TMP/SMX), is an example of a PCP therapy that is highly effective. TMP is a potent and selective competitive inhibitor of microbial dihydrofolate reductase, the enzyme that reduces dihydrofolate to tetrahydrofolate. TMP is, therefore, a folic acid antagonist. The microbial dihydrofolate reductase enzyme system is 50,000 times more sensitive to TMP than the mammalian enzyme system.

In humans, TMP is absorbed almost completely after oral administration, and levels are detectable in the bloodstream after 5 minutes (Gleckman *et al.*, 1981; Salter, 1982). Peak serum concentrations of 2 to 4 μg/mL are achieved within 1 to 4 hours. The mean peak level of TMP is approximately 1.5 μg/mL. This concentration decreases to 0.75 to 1.0 μg/mL after 12 hours. The presence of food in the stomach does not appear to delay absorption. Effective levels of TMP persist in the blood for up to 24 hours after a therapeutic dose (100 mg TMP every 12 hours). Absorption in children does not appear to differ from that in adults. Steady-state serum concentrations occur within 72 hours of daily TMP administration (100 mg every 12 hours for 10 days). Approximately 85% to 90% of plasma TMP exists as the intact compound, 45% bound to protein (primarily albumin). TMP is absorbed completely from the gastrointestinal tract, resulting in minimal fecal excretion (Schwartz and Ziegler, 1969).

TMP is a weak base, with a pKa of 7.3, and is lipophilic (Salter, 1982). The compound is widely distributed in the body after oral administration; the volume of distribution of TMP is 14 to 21 L. This compound achieves therapeutic concentrations in body fluids (e.g., saliva, sputum, vaginal fluid, prostatic secretions, pleural effusions, aqueous humor, bile, and cerebrospinal fluid) and organs (e.g., skin, liver, lung, testis, and kidney). Amniotic fluid, cord blood, and fetal tissue concentrations approximate 50% to 75% of the maternal serum concentrations (Ylikorkala *et al.*, 1973; Reid *et al.*, 1975). TMP enters breast milk, but the significance of this observation remains undefined (Miller and Salter, 1973). Rieder (1973) has described the complex pharmacokinetic behavior of TMP in bile, where concentrations generally exceed those needed for therapeutic activity.

The elimination half-life value for TMP is 10 to 14 hours in adults and 2.5 to 6 hours in children with normal renal function (Gleckman *et al.*, 1981). Urinary concentrations of TMP vary considerably, ranging from 10.9 to 206 μg/mL (Gleckman *et al.*, 1979). Renal insufficiency prolongs the elimination half-life, reduces urinary concentrations, and augments the serum concentrations of intact TMP (Bergan *et al.*, 1979). The clearance of

TMP is not affected by the urine flow rate but is increased by lowering of the urine pH (Salter, 1982). Bergan and Brodwall (1972) showed that TMP is subject to passive nonionic tubular diffusion at pH values above 7.0.

Urinary excretion of TMP declines when the creatinine clearance is reduced below 30 mL/min (Sharpstone, 1969). However, even with advanced renal disease, urinary concentrations of TMP exceed the minimum inhibitory concentrations (MICs) for most urinary pathogens. Abnormal serum concentrations of TMP have not been experienced by patients with liver disease (Follath, 1979).

TMP can inhibit the elimination of creatinine by the kidney, thereby causing elevated serum creatinine concentrations (Berglund *et al.*, 1975). The compound also interferes with the chemical analysis of serum creatinine.

Five metabolites of TMP have been found in dogs, rats, and humans (Sigel *et al.*, 1973). Two of these metabolites are conjugated in both plasma and urine, whereas two others are conjugated and excreted in the urine, essentially in the form of glucuronides. Schwartz and Rieder (1970) reported that unchanged TMP accounted for 77.5% of compound-related radioactivity in 24-hour cumulative urine samples. Sigel and Brent (1973) reported a lower figure of 46%. The simultaneous administration of SMX does not alter the metabolic fate of TMP (Salter, 1982).

The concentration of unchanged TMP in the feces after daily doses of 0.5 or 1.0 g is usually about 1.0 μg/g feces (Romankiewicz, 1974). Studies with radiolabeled TMP indicate that less than 4% of the compound is found in human feces for at least 6 days after oral administration.

Sulfonamides, which are frequently used in combination with TMP, are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA) and hence prevent the normal utilization of PABA for the synthesis of folic acid (pteroylglutamic acid) (Woods, 1940). The term sulfonamide is used in this report as a generic name for derivatives of PABA (i.e., sulfanilamide and sulfamethoxazole). More specifically, sulfonamides are competitive inhibitors of dihydropteroate synthase, the microbial enzyme responsible for the incorporation of PABA into dihydropteroic acid, the immediate precursor of folic acid. Sensitive microorganisms are those that must synthesize their own folic acid; microorganisms that can utilize preformed folate are not affected. Bacteriostasis induced by sulfonamides is competitively counteracted by PABA. Sulfonamides do not affect mammalian cells by this mechanism, since mammalian cells require preformed folic acid and cannot synthesize it. Mammalian cells are, therefore, comparable to sulfonamide-insensitive microorganisms that utilize preformed folate.

SMX is a sulfonamide that is frequently used in combination with TMP. SMX is a weak acid, with a pKa of 6.0, and is fairly well distributed; however, after an orally administered dose as a 1:5 ratio of TMP/SMX, the concentration of active SMX in tissue fluids is relatively lower than that of TMP (Salter, 1982). The concentration of active SMX in aqueous humor (Salmon *et al.*, 1975), bile (Rieder *et al.*, 1974), cerebrospinal fluid (Nicholls, 1972), sputum (Hughes, 1979), and interstitial fluid (Chisholm *et al.*, 1973) is approximately 25% to 30% of the corresponding concentration in plasma. The lungs (Hausen *et al.*, 1973) and kidneys (Craig and Kunin, 1973) contain especially high concentrations of SMX. Since renal tissue is usually contaminated with urine, it contains levels similar to those in urine, normally about 30 times the level in plasma (Salter, 1982). Levels in bone are adequate for antibacterial activity. Levels of SMX, however, are generally lower in tissues than in plasma (Schwartz and Rieder, 1970; Craig and Kunin, 1973).

The percentages of protein-bound SMX at total plasma concentrations of 91 and 253 μg/mL were 67.8% and 59%, respectively (Salter, 1982). At similar concentrations of N^4-acetylsulfamethoxazole, 79.9% and 61% of the compound was bound. For pharmacokinetic calculations, 60% binding is a satisfactory approximation. The addition of SMX to plasma reduced the binding of TMP by 3% to 4%, but the protein binding of SMX was unchanged by TMP at therapeutically attainable concentrations of the two compounds (Schwartz and Ziegler, 1969).

The half-lives of TMP and SMX in plasma are not significantly different after single or multiple doses (Salter, 1982). The mean half-life of TMP is 10.1 $\pm$ 0.6 hours (mean $\pm$ standard error, n=36), and that of SMX is 11.4 $\pm$ 0.9 hours. The half-life of SMX in infants during the first 10 days of life is considerably longer than in adults. The half-life falls rapidly, however, being about 9 hours at 3 weeks of age and 4 to 5 hours at 1 year. It then increases toward the half-life characteristic for adults (approximately 10 to 12 hours).

SMX is primarily excreted by the kidneys, and high concentrations of the active compound are found in the urine (Schwartz and Rieder, 1970). It is excreted partly unchanged and partly as metabolites. About 60% of orally ingested SMX is excreted in the urine within 48 hours. Of the excreted compound, approximately half is N^4-acetylsulfamethoxazole, one-fifth is the N^4 conjugate, one-sixth is the unchanged parent compound, and one-tenth is another N^4 free compound (Bagdon, 1964).

Renal clearance of SMX increases with a rising flow rate; it is independent of urine pH when this value is below 7.0 but increases with a rising pH at values above 7.0 (Salter, 1982). In patients with renal impairment, the clearance of SMX is not significantly affected; however, the clearance of N^4-acetylsulfamethoxazole is prolonged in those with severe renal failure.

The clinical reasons for coadministration of TMP and SMX at a ratio of 1:5 are not entirely clear (Salter, 1982). However, Kremers *et al.* (1974) showed that in five healthy subjects receiving two tablets of TMP/SMX daily for 9 days, the ratio of the concentrations of TMP to that of active SMX in plasma was between 1:15 and 1:22. These plasma ratios are within the range in which the potentiation of antibacterial activity is optimal for most bacterial species (Bushby, 1973). Nevertheless, the 1:5 ratio is somewhat arbitrary, because the responses of individual organisms vary and the optimal ratio differs from species to species.

The pharmacokinetic profiles of TMP and SMX are closely but not perfectly matched to achieve a constant ratio of 1:20 in their concentrations in blood and tissues. The ratio in blood is often greater than 1:20 and that in tissues is frequently less. After a single oral dose of the combined preparation, TMP is absorbed more rapidly than SMX. The concurrent administration of the compounds appears to diminish the absorption of SMX. Peak blood levels of TMP usually occur within 2 hours in most patients, while peak concentrations of SMX occur within 4 hours after a single oral dose. As pointed out earlier, the half-lives of TMP and SMX are approximately 11 and 10 hours, respectively.

The clinical dose for treatment of PCP in AIDS patients is 20 mg TMP/kg body weight in combination with 100 mg SMX/kg body weight (Wofsy, 1987). The therapeutic dose for effective treatment of rats with experimental PCP is 50 mg/kg TMP administered in drinking water in combination with 250 mg/kg SMX (Hughes and Smith, 1984). Nguyen and Stadtsbaeder (1985) have reported that the therapeutic dose in mice for toxoplasmosis is 153 mg/kg TMP administered in drinking water in combination with 769 mg/kg SMX for 42 days. No toxic effects in the treated mice were reported.

In general, studies of chronic toxicity have not suggested many problems related to clinical use. One noteworthy point is that hypersensitivity reactions cannot be predicted in studies with animals. Prolonged administration (6 months) of relatively large doses of TMP (100 to 300 mg/kg) and SMX (100 to 1,200 mg/kg) to animals does affect blood-forming elements in bone marrow. This effect is probably caused by TMP (Salter, 1982).

Daily doses of 100 or 300 mg/kg TMP plus doses of 100 or 600 mg/kg SMX given by oral gavage to male and female rats for 6 months caused a decrease in body weight. Two of 20 rats in the low dose group and nine of 20 rats in the high dose group did not survive the 6-month study. Although the blood chemistry of the rats was normal, the white blood cell count, red blood cell count, and platelet count fell significantly (Salter, 1982).

In another study, Wistar rats and Patas monkeys received oral gavage doses of 300 mg/kg TMP plus 1,200 mg/kg SMX daily for 6 months. There was a decrease in white blood cell counts, but not platelets, at 1 month; by 6 months, bone marrow hypoplasia had occurred. Even severe changes were reversed when use of the test compounds was discontinued (Udall, 1969).

Of six monkeys that received 300 mg/kg TMP plus 600 mg/kg SMX, five had marked decreases in platelet count; in addition, four exhibited decreases in red blood cell and white blood cell counts. These animals also had an increased concentration of blood urea nitrogen, and one had an increased activity of aspartate aminotransferase in serum. Administration of folinic acid (2 mg/kg orally) reduced hematologic toxicity (Salter, 1982).

Swarm *et al.* (1973) reported that Charles River CD rats treated continuously with SMX or with TMP/SMX in the diet developed metastasizing thyroid carcinomas after 52 to 60 weeks. Treatment of rats with TMP for the same interval did not have this effect, nor did treatment of monkeys with TMP, SMX, or TMP/SMX for this period. Previous work (Udall, 1969) described a goitrogenic effect in rats with shorter-term administration (an effect already known to be caused by other sulfonamides) but no carcinomas (MacKenzie and MacKenzie, 1943). Since this pathologic effect is not seen in hypophysectomized animals, the thyroid hyperplasia appears to result from hyperproduction of thyroid-stimulating hormone secondary to long-term depression of thyroid function. This finding appears to be species specific and is unlikely to be of clinical importance in humans (Salter, 1982).

There is a paucity of studies reported on the reproductive toxicity of TMP in the mouse. Timmermans (1974) reported that eight daily doses of 100 mg/kg TMP (route not specified) in the rat resulted in incomplete or stoppage of spermatogonia divisions and perturbed meiosis in most spermatocytes. In addition, Kreutz (1981) reported that 750 to 1,500 mg/kg TMP given daily to pregnant rats from day 5 to day 13 of gestation resulted in toxicity in the mother as well as the fetus, including increased resorption rates, decreased fetal body weight, and skeletal anomalies that were "typical effects of folic acid antagonists."

The oral lethal dose causing 50% mortality (LD_{50}) values for TMP and SMX in mice is difficult to calculate because it is impossible to administer sufficient amounts of the two compounds to the animals (Salter, 1973). However, Yamamoto *et al.* (1973) calculated the LD_{50} to be 7,100 mg/kg TMP and 4,100 mg/kg SMX. The acute toxicity of these compounds is clearly very low (Salter, 1982). Reported mouse oral LD_{50} values for TMP/SMX (1:5) are 5,513 mg/kg (*Merck Index*, 1989) and 3,740 mg/kg (RTECS, 1989).

STUDY RATIONALE

It is well documented in the literature that AZT administration, folate deficiency, and vitamin B_{12} deficiency all result in the inhibition of DNA synthesis with similar morphological manifestations such as macrocytosis, anemia, and granulcytopenia, and it was previously mentioned that TMP/SMX is an antifolate drug (Walzer *et al.*, 1988). The changing etiology of macrocytosis in humans was discussed by Snower and Weil (1993). Whereas vitamin B_{12} and folic acid deficiency were previously quite significant in the etiology of macrocytosis, therapy with AZT is currently the leading cause of this condition in humans. Revell *et al.* (1991) demonstrated that absorption of folic acid was significantly impaired in HIV disease. Israel and Plaisance (1991) stated that bone marrow toxicity occurs more often and is enhanced in patients receiving AZT therapy if they have low serum vitamin B_{12} levels or low normal serum folic acid levels. Herzlich *et al.* (1990), studying human bone marrow cells *in vitro*, stated that some of what passes for "AZT damage" to bone marrow cells may in fact be coincident deficiency of vitamin B_{12} and that whether folate or vitamin B_{12} supplementation may partially overcome apparent "AZT inhibition" of DNA synthesis (hematologic toxicity) will require further study.

The objective of this study was to obtain controlled data on the general, reproductive, and developmental toxicity of AZT and TMP/SMX when administered in combination and to determine if supplementation with folinic acid would prevent or diminish toxicity. Folinic acid, also known as leucovorin, is a stable form of N^5-formyl-H_4 folate that can be used for administration of reduced folate (Stokstad and Koch, 1967). A study in rodents would provide useful information on the safety of the therapies (AZT, TMP/SMX, and folinic acid) used in AIDS treatment. The Swiss (CD-1®) mouse chosen for this study is one of the mouse models routinely used for evaluation of reproductive and developmental toxicity. The animal model used in this study was not folate deficient and lacks the variable of a viral infection that compromises the immune system.

Male and female Swiss (CD-1®) mice were treated orally with AZT (200 or 400 mg/kg) alone, with TMP/SMX (1,000, 2,000, or 3,000 mg/kg) alone, or with combinations of AZT and TMP/SMX. Selected AZT doses were two and four times the human therapeutic dose of 10 mg/kg per day (PDR, 1994; Freireich *et al.*, 1966), and TMP/SMX doses were one, two, and three times the therapeutic dose in mice for treatment of toxoplasmosis (Nguyen and Stadtsbaeder, 1985). Selected groups were supplemented with folinic acid (10 mg/kg), which is equivalent to 100 times the minimum daily requirement of folic acid for the mouse (NRC, 1978). Because folinic acid is an active form of folic acid, it was predicted that supplementation might decrease the toxicity associated with TMP and SMX, which inhibit the synthesis or action of folic acid. Adult mice were evaluated for clinical findings, mean body weights, sperm function, pathology, and clinical pathology parameters. Offspring were evaluated for viability, external abnormalities, and mean body weights.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3′-Azido-3′-deoxythymidine (AZT; lot 1401-R-9) was manufactured by Raylo Chemicals (Edmonton, Alberta) and supplied as a powder. Trimethoprim/sulfamethoxazole (TMP/SMX; lot 7493-150-01) was manufactured by Napp Chemicals (Lodi, NJ) and received as a 1:5 TMP:SMX mixture. Folinic acid pentahydrate, calcium salt (lot 11H0858) was obtained from Sigma Chemical Company (St. Louis, MO).

The purity of the AZT was determined to be 99.7% by high-performance liquid chromatography. Infrared and nuclear magnetic resonance spectrometry analyses performed on TMP and SMX produced spectra which were consistent with the structures of trimethoprim and sulfamethoxazole and agreed with the reference spectra. Purity of TMP and SMX as determined by HPLC was greater than 99%. The control vehicle used in this study was corn oil.

DOSE FORMULATIONS

The required amounts of the test articles were combined with the required amount of control vehicle. The dose formulation was then stirred until visually homogeneous.

The folinic acid used in this study was not identified at receipt as a pentahydrate. This form of the chemical contains approximately 12% water by weight. Therefore, the actual dose concentration of folinic acid administered was approximately 8.8 mg folinic acid/kg body weight, or 88 times the recommended daily requirement for mice (NRC, 1978).

Stability studies at refrigeration and ambient temperature for AZT/TMP/SMX formulations in corn oil indicated that the dose formulations were stable for 32 days when stored refrigerated. Dose formulations were stored under refrigeration, protected from light, and used within 7 days.

Samples at each dose concentration from the initial and final dose formulations were analyzed. Residual dose formulations taken from the animal room after dosing were also analyzed. Additionally, samples taken from

the top, middle, and bottom of the dose formulations at the highest (20.0 mg/mL) and the lowest (10.0 mg/mL) concentrations of AZT and the dose formulation with the highest concentration of AZT in combination with the highest concentration of TMP/SMX were analyzed in conjunction with the first analysis to confirm homogeneity. The homogeneity analyses (AZT only) resulted in standard deviations that ranged from 0.76% to 6.3%. Prior to dosing, the concentrations of AZT in the final mix were within 10% of theoretical values with the exception of two of the dose formulations at 20.0 mg/mL (400 mg/kg) that assayed at 71.5% and 85.5% of the target concentrations. Post-dosing results for these same dose formulations ranged from 105% to 118% of the target concentrations. The dose formulations were not assayed for TMP/SMX or folinic acid because of interference by the corn oil vehicle.

STUDY DESIGN

Swiss (CD-1®) mice were obtained from Charles River Laboratories, Raleigh, NC (Areas R10 and R16), and were approximately 15 weeks old when placed on study. The mice were housed five males or five females per cage during quarantine before randomization and were individually housed after randomization, except during cohabitation.

The mice were housed in polycarbonate cages with solid bottoms and sides. Average temperature in the animal rooms was 71.2° F, with a standard deviation of 0.6° F; average relative humidity was 54.4%, with a standard deviation of 6.3%.

At terminal sacrifice, blood samples were collected from five male and five female sentinel animals as part of the animal disease screening program. Results indicated that all animals were free of viral antibodies.

Folinic acid is the active form of folic acid used as an antidote to folic acid antagonists. Folic acid is a dietary essential required for DNA synthesis, chromosomal replication, and cell division. A deficiency in folic acid primarily affects cells with rapid turnover, such as the hematopoietic system cells. Folic acid deficiency is manifested morphologically by megaloblastic anemia and is also associated with fetal anomalies. TMP is a folic acid antagonist, and SMX prevents the synthesis of this vitamin by inhibiting the incorporation of para-aminobenzoic acid into the molecule. In addition, AZT therapy is associated with macrocytosis. Therefore, based on the known toxic effects of these compounds given alone, an additive or synergistic toxicity may occur with combination therapy. It was predicted that the supplementation of folinic acid to selected dose groups in

this study might decrease the hematopoietic and reproductive toxicity associated with these compounds that inhibit the synthesis or action of folic acid.

AIDS patients are receiving combination therapy with TMP/SMX and AZT as well as supplementation with folinic acid. Controlled laboratory data are required to evaluate the potential toxicity of this combination therapy. At present, there are no alternatives to whole animal models for this purpose. The Swiss (CD-1®) mouse chosen for this study is one of the mouse models routinely used for reproductive and developmental toxicity studies by the NIEHS.

The basic premise for dose concentration recommendations is that the high dose concentration should induce some measurable evidence of toxicity (e.g., anemia, weight loss, target organ toxicity). In a number of studies conducted with this protocol, AZT at the doses selected (200 and 400 mg/kg) caused resorptions and decreased litter sizes. The human dose is 10 mg/kg per day (PDR, 1994). The selected doses are 20 and 40 times the human doses, but on a body surface area basis, the doses are close to two and four times the therapeutic dose (Freireich *et al.*, 1966).

The clinical dose of TMP/SMX for *Pneumocystis carinii* pneumonia (PCP) therapy in AIDS patients is 120 (20 mg/kg TMP +100 mg/kg SMX) mg/kg per day (Wofsy, 1987). The equivalent mouse dose would be 1,500 mg/kg. The recommended dose for control of pneumocystis infection in severe combined immunodeficiency mice is 600 (100 mg/kg TMP + 500 mg/kg SMX) mg/kg per day, and 1,000 mg/kg is the therapeutic dose for treatment of toxoplasmosis in mice. The selected doses (1,000, 2,000, and 3,000 mg/kg TMP/SMX) are 1 to 1.6 times, 2 to 3.3 times, and 3 to 5 times the therapeutic dose for mice and 8.3 times, 16.6 times, and 25 times the human therapeutic dose. On a body surface area basis, the selected doses of TMP/SMX (1,000, 2,000, and 3,000 mg/kg) are close to 0.75 to 2 times the human therapeutic dose (Freireich *et al.*, 1966).

Limited information on the reproductive toxicity of TMP/SMX is available. "In rats, oral doses of 533 mg/kg sulfamethoxazole or 200 mg/kg trimethoprim produced teratologic effects manifested mainly as cleft palates. The highest dose which did not cause cleft palates in rats was 512 mg/kg sulfamethoxazole or 192 mg/kg trimethoprim when administered separately . . . cleft palates were observed in one litter out of nine when 355 mg/kg of sulfamethoxazole was used in combination with 88 mg/kg of trimethoprim" (PDR, 1994).

The dose selected for folinic acid in this study was 10 mg/kg, equivalent to 100 times the minimum daily requirement of folic acid for the mouse (NRC, 1978). However, the folic acid content of NIH-07 diet varies from 1.8 to 3.7 mg/kg (NTP, 1998), equivalent to 0.36 to 0.75 mg/kg body weight.

The recommended daily requirement for folic acid in humans is 200 μg per day (*Goodman and Gilman's*, 1990). The total dose of folinic acid administered following methotrexate therapy (36 mg/kg) is 150 mg (PDR, 1994). This dose, given over 2.5 days (60 mg per day), is equivalent to 300 times the daily requirement.

The dose of folinic acid is based on clinical and experimental reports in the literature. The clinical dose for AIDS patients is 5 mg per day (PDR, 1994), which is equivalent to 1 mg/kg in the mouse (Freireich *et al.*, 1966). The dose of folinic acid used in the rat to prevent cytopenias induced by TMP/SMX is 1 mg per day (5 mg/kg for a 200 g rat), which is equivalent to 10 mg/kg in the mouse.

The design of this study is a modification of a design published elsewhere (Harris *et al.*, 1992). A brief summary of the study design is provided in Table 2. The oral route of administration was selected because it is the route used in humans, and the study was conducted in Swiss (CD-1®) mice because this strain is routinely used for reproductive and developmental toxicity evaluations. Combinations of AZT (200 or 400 mg/kg), TMP/SMX (1,000, 2,000, or 3,000 mg/kg), and/or folinic acid (10 mg/kg) were administered by gavage as a single formulation in corn oil. Total daily doses of 20 mL/kg were divided into two equal doses of 10 mL/kg given approximately 6 hours apart. Mice were divided into three groups as follows:

Male Mice: Ten males were assigned to each dose group. Prior to dosing, male mice were cohabited with female-B mice on study days 0 to 4. Males were dosed from study day 5 to the day prior to sacrifice. Males were cohabited with female-A mice on study days 9 to 13 to identify any effects of treatment on mating behavior. On study day 25 or 26, all male mice were weighed, and blood samples were obtained from the retroorbital sinus for hematology and clinical chemistry evaluations. The males were euthanized with CO_2, necropsy was conducted, and the testes and epididymides were collected and prepared for evaluation of sperm parameters as described in the Sperm Function Evaluation section.

Female-A Mice: Twenty females were assigned to each dose group. Female-A mice were dosed from study day 0 to the day prior to sacrifice. Males were cohabited with female-A mice on study days 9 to 13 to identify any effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. During the cohabitation period, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually, and that day was

designated as day 0 of gestation. At the end of the cohabitation period, all animals were housed individually. Prior to parturition on day 18 of presumed gestation (study days 28 to 32), all female-A mice were weighed, and blood samples were taken from the retroorbital sinus for hematology and clinical chemistry evaluations. The female-A mice were then euthanized with CO_2, and necropsy and caesarean section evaluations were conducted. Live fetuses were removed, weighed, anesthetized on ice, and preserved in Bouin's fixative. The uteri of all sperm-negative females were examined for evidence of unsuccessful pregnancy and then press-plated between two heavy plates of glass to visualize implantation sites. Additional endpoints for all female-A mice included gravid uterine weight and number of implantation sites, resorptions, corpora lutea, and dead and live fetuses.

Female-B Mice: Twenty females were assigned to each dose group. Prior to dosing, female-B mice were cohabited with males on study days 0 to 4. During the cohabitation period, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually, and that day was designated as day 0 of gestation. At the end of the cohabitation period, sperm-negative female-B mice were euthanized with CO_2 and discarded without necropsy; all other animals were housed individually. Sperm-positive female-B mice were assigned evenly across dose groups prior to gestation day 6. Female-B mice were subsequently dosed during gestation days 6 to 15, during the fetal organogenesis period, to identify effects on fetal development. Residual effects on parturition and the beginning of lactation were also evaluated. After gestation day 16, the bedding material and feeders were no longer changed. From gestation day 17 until the litters were delivered, female-B mice were observed twice daily for evidence of labor or delivery. The day of completed delivery was determined to the nearest day and designated as postnatal day 0. On postnatal days 0 and 1, dam body weights were recorded along with the number of live and dead pups, the number of male and female pups, the incidence of any gross malformations, and live pup weights. Dead pups were discarded. On postnatal day 4, all female-B mice, including any that did not deliver, were weighed, and blood samples were collected from the retroorbital sinus for hematology and clinical chemistry evaluations. These mice were then euthanized with CO_2, and complete necropsies were performed. The uterus was removed and press-plated. All pups were weighed and given a thorough external examination for lesions and malformations, and the gender was recorded. The pups were then euthanized with CO_2 and preserved in Bouin's fixative.

Clinical Pathology

All blood samples were collected from the retroorbital sinus under CO_2:O_2 anesthesia. Blood for hematology analyses was collected in a tube containing EDTA, and blood for clinical chemistry analyses was collected in

a tube without anticoagulant. Animals were selected in random order for blood collection, and samples were analyzed in the order collected.

Erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin concentration; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; and leukocyte differentials were determined in whole blood using a Technicon H·1™ automated hematology analyzer. Reticulocyte counts were conducted using a Coulter Model Elite® Flow Cytometer. Blood smears were prepared to manually verify reticulocyte counts, leukocyte differentials, and morphologies if necessary.

Blood urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were determined using the Roche Cobas Fara® automated analyzer. Priority for clinical chemistry tests was assigned in the order listed above.

Sperm Function Evaluation

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis). Modified Tyrode's buffer was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered neutral saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemocytometer. To quantify spermatogenesis, a testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Histopathology

A gross necropsy was performed on all animals (except sperm-negative female-B mice), and histopathology was conducted on the tissues listed in Table 2. The tissues were fixed in formalin, trimmed to a maximum thickness of 0.3 cm for processing, embedded in paraffin, sectioned to a thickness of 4 to 6 microns, stained with hematoxylin and eosin, and examined by light microscopy.

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Study Laboratory
Southern Research Institute, Birmingham, AL

Strain and Species
Swiss (CD-1®) mice

Animal Source
Charles River Laboratories, Raleigh, NC

Time Held Before Studies
24 days

Average Age When Studies Began
102 days

Date of First Dose
Male mice 1 or 8 June 1994
Female-A mice 27 May or 3 June 1994
Female-B mice 2-6 or 9-13 June 1994

Duration of Dosing
Male mice day 5 to day prior to sacrifice
Female-A mice day 0 to day prior to sacrifice
Female-B mice gestation days 6 to 15

Days of Cohabitation
Male mice and female-A mice days 9 to 13
Male mice and female-B mice days 0 to 4
When possible, 1 male and 2 females within the same dose group were housed together by consecutive animal number

Date of Last Dose
Male mice 26 June or 3 July 1994
Female-A mice 23-27 June or 30 June-4 July 1994
Female-B mice 11-15 or 18-22 June 1994

Necropsy Dates
Male mice days 25 to 26
Female-A mice days 28 to 32
Female-B mice days 24 to 28 of presumed gestation (sperm-positive, not delivered), postnatal day 4 (sperm-positive, delivered)

Average Age at Necropsy
Male mice 127-128 days
Female-A mice 130-134 days
Female-B mice 126-130 days

Size of Study Groups
Male mice 10 per dose group
Female-A mice 20 per dose group
Female-B mice 20 per dose group

Method of Animal Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights

Animals per Cage
One animal per cage, except during cohabitation

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Method of Animal Identification
Tail tattoo

Diet
NIH-07 pelleted feed, available *ad libitum*

Water Distribution
Tap water (Birmingham, AL), available *ad libitum*

Cages
Polycarbonate cages with solid bottoms and sides

Bedding
Heat-treated hardwood chips (Sani-Chips®, P J Murphy Forest Products Corporation, Montville, NJ)

Cage Filters
Reemay® spun-bonded polyester (Andico, Birmingham, AL)

Racks
Stainless steel (Lab Products, Maywood, NJ)

Animal Room Environment
Temperature 71 2° ± 0 6° F
Relative humidity 54 4% ± 6 3%
Fluorescent light 12 hours fluorescent light/day
Room air minimum of 10 changes/hour

Doses
Daily doses in corn oil gavage
0 mg AZT + 0 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
0 mg AZT + 0 mg TMP/SMX + 10 mg folinic acid per kg body weight per day
200 mg AZT + 0 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 0 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 0 mg TMP/SMX + 10 mg folinic acid per kg body weight per day
0 mg AZT + 1,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
200 mg AZT + 1,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 1,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
0 mg AZT + 2,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
0 mg AZT + 2,000 mg TMP/SMX + 10 mg folinic acid per kg body weight per day
200 mg AZT + 2,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 2,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 2,000 mg TMP/SMX + 10 mg folinic acid per kg body weight per day
0 mg AZT + 3,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
0 mg AZT + 3,000 mg TMP/SMX + 10 mg folinic acid per kg body weight per day
200 mg AZT + 3,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day
400 mg AZT + 3,000 mg TMP/SMX + 0 mg folinic acid per kg body weight per day

Type and Frequency of Observation
Mortality/moribundity twice daily
Clinical findings once daily
Vaginal plugs days 10 to 14 for female-A mice, days 1 to 5 for female-B mice
Body weights days 3, 5, 9, 13, 17, 21, 23, and sacrifice for male mice, days 0, 4, 12, 16, 20, 23, 26, and sacrifice for female-A mice, gestation days 0, 8, 12, 15, and postnatal days 0, 1, and 4 for female-B mice, postnatal days 0, 1, and 4 for pups

Method of Sacrifice
CO_2 O_2 asphyxiation

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Necropsy Complete necropsies were performed on all breeder animals except sperm-negative female-B mice
Histopathology Complete histopathology was performed on all mice In addition to gross lesions and associated lymph nodes, the following tissues were examined: bone marrow, brain, heart, kidneys, liver, thyroid gland, lungs and bronchi, spleen, testes with epididymis and tunica vaginalis, and thymus (male mice), bone marrow, brain, femur, kidneys, liver, lungs, spleen, thymus, and thyroid gland (female mice) Pups were examined externally only
Clinical Pathology Hematology and clinical chemistry evaluations were conducted on all animals at terminal sacrifice
Sperm Function Evaluation Conducted on all males at terminal sacrifice

STATISTICAL METHODS

Paternal and maternal body weight evaluations were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance (Snedecor and Cochran, 1967). If Bartlett's test was not significant ($P > 0.05$) and the analysis of variance was significant ($P \leq 0.05$), then Dunnett's test (Dunnett, 1955) was used to identify the statistical significance of individual groups. If Bartlett's test was significant ($P \leq 0.05$), the Kruskal-Wallis test (Hollander and Wolfe, 1973) was used; in cases in which the Kruskal-Wallis test was significant ($P \leq 0.05$), Dunn's (1964) method of multiple comparisons was used to identify the statistical significance of individual groups. These methods were also used to analyze fetal body weight and pup body weight (per litter) as well as all other evaluations involving continuous data. For F_0 generation sires and dams, the analysis of covariance (Snedecor and Cochran, 1967) was used to evaluate mean body weight changes and mean maternal body weight changes. Observations for delivered and dead conceptuses of the female-A dams and for fetuses from female-A dams caesarean-sectioned on an estimated day 14 of gestation were excluded from fetal body weight summaries and statistical analyses.

Group means and standard deviations were calculated for hematology and clinical chemistry parameters and for final mean body and epididymis weights. Epididymis/body weight ratios were also calculated. Final mean body weights of males and females and mean epididymis weights and epididymis/body weight ratios of males for each dosed group were compared to those of the control group by a two-tailed Student's *t*-test. The standard deviations used in the *t*-tests were obtained by pooling the individual values for the control and dosed groups. Hematology and clinical chemistry data were evaluated using Dunnett's test.

All target organ (bone marrow, liver, spleen, thymus, and thyroid) endpoints were analyzed for test compound interaction using a three-way analysis of variance; male reproductive endpoints were analyzed for compound interaction using a two-way analysis of variance. If a significant interaction was observed for target organs, mean values were plotted to graphically display the interaction between the compounds. If no interaction was detected for target organs or reproductive parameters, averages were taken at each dose concentration of a compound over all levels of the other test compounds. Control and dosed group means were compared using either Williams' (1971, 1972) or Dunnett's (1955) multiple comparison procedures. The choice between the two tests was based on the evidence of a dose-related trend in the data as assessed by Jonckheere's (1954) test. Williams' test was applied if there was an indication of trend ($P < 0.01$), and Dunnett's test was used in the absence of a trend. In selected cases in which interaction was found to be significant, the multiple comparison tests described above were used to detect treatment-related effects.

Proportion data (e.g., clinical findings and incidences of pregnancy, resorption, death, and total resorption) for mice presumed pregnant were analyzed using the Cochran-Armitage test for a linear trend in proportions (Snedecor and Cochran, 1967) and Fisher's exact test (Siegel, 1956).

RESULTS

SURVIVAL AND CLINICAL FINDINGS

Male Mice

Seven males died before the end of the study (Table 3). With the exception of three accidental gavage deaths, these deaths are potentially treatment related. Two deaths in the 400 + 3,000 + 0 mg/kg group were preceded by several days of body weight loss and are, therefore, likely to be related to treatment with 3′-azido-3′-deoxythymidine (AZT) or trimethoprim/sulfamethoxazole (TMP/SMX). Statistically significant ($P \leq 0.05$) incidences of pallor and piloerection occurred in the 200 + 3,000 + 0 mg/kg and 400 + 3,000 + 0 mg/kg groups. A significant incidence of piloerection was also noted in the 400 + 2,000 + 10 mg/kg and 0 + 3,000+ 0 mg/kg groups. Other less significant findings generally occurred in the mice that died and occurred only on the day of death.

TABLE 3
Early Deaths of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Number of Deaths	Day of Death	Type of Death	Clinical Findings
0 + 2,000 + 0	1	16	Found dead	None
400 + 2,000 + 10	1	22	Gavage accident	Piloerection, pallor, head swelling
0 + 3,000 + 0	1	10	Moribund	Decreased motor activity, labored breathing, abdominal distension
0 + 3,000 + 0	1	14	Gavage accident	Labored breathing, pallor, piloerection
400 + 3,000 + 0	1	8	Gavage accident	None
400 + 3,000 + 0	1	14	Found dead	None
400 + 3,000 + 0	1	21	Moribund	Pallor, piloerection, labored breathing

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)

Female-A Mice

Among female-A mice, there were 73 early deaths (Table 4), occurring primarily in the groups administered 2,000 or 3,000 mg TMP/SMX per kg body weight in conjunction with 200 or 400 mg AZT/kg body weight. Only one of these deaths was a gavage accident. Folinic acid had no obvious impact on mortality or clinical findings in the female-A groups.

Female-A mice exhibited a higher frequency of clinical findings compared to male and female-B mice. This is likely due to a higher total dose in female-A mice resulting from increased duration of treatment as well as the increased maternal body weight that occurred late in gestation. In addition to pallor and piloerection, which were significantly increased in most groups administered AZT and TMP/SMX, significant occurrences of labored breathing, decreased motor activity, and emaciation were noted.

Female-B Mice

There were nine early deaths in the female-B group (Table 5), including one gavage accident. Most deaths occurred in mice administered 3,000 mg TMP/SMX per kg body weight, regardless of the dose of AZT.

The only significant incidences of clinical findings (pallor and piloerection) occurred in the 400 + 3,000 + 0 mg/kg group. As discussed earlier, the apparent difference in toxicity for the female-A mice and female-B mice results from a higher total dosage administered to the female-A mice late in gestation due to increased maternal weights and the fact that female-A mice were dosed for a longer period of time.

TABLE 4
Early Deaths of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Number of Deaths	Day of Death	Type of Death	Clinical Findings
400 + 0 + 10	1	17	Moribund	Piloerection, pallor, paralysis
0 + 2,000 + 10	1	3	Gavage accident	Decreased motor activity, labored breathing
0 + 2,000 + 10	2	15, 17	Moribund	Mass, pallor, decreased motor activity, cold to touch, tremors
200 + 2,000 + 0	7	17 - 31	Moribund	Labored breathing, pallor, decreased motor activity, hunched, cold to touch, emaciation, piloerection
400 + 2,000 + 0	8	21 - 28	Moribund	Decreased motor activity, labored breathing, pallor, cold to touch, hunched, piloerection
400 + 2,000 + 10	10	17 - 31	Moribund	Decreased motor activity, labored breathing, pallor, piloerection, cold to touch, emaciated, hunched
400 + 2,000 + 10	1	30	Found dead	Piloerection, pallor
0 + 3,000 + 0	2	3, 29	Found dead	Piloerection, pallor
0 + 3,000 + 10	3	4 - 30	Found dead	Piloerection, pallor
0 + 3,000 + 10	1	16	Moribund	Piloerection, pallor, decreased motor activity, labored breathing
200 + 3,000 + 0	2	16, 29	Found dead	Piloerection, pallor
200 +3,000 +0	16	17 - 30	Moribund	Piloerection, pallor, decreased motor activity, emaciation, hunched, labored breathing
400 + 3,000 + 0	2	14, 5	Found dead	Piloerection, pallor
400 + 3,000 + 0	17	13 - 30	Moribund	Piloerection, pallor, labored breathing, emaciation, decreased motor activity

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)

TABLE 5
Early Deaths of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Number of Deaths	Day of Presumed Gestation	Type of Death	Clinical Findings
0 + 2,000 + 0	1	16	Gavage accident	Decreased motor activity, cold to touch, labored breathing
0 + 3,000 + 0	1	15	Found dead	None
0 + 3,000 + 10	2	3, 14	Found dead	None
0 + 3,000 + 10	1	14	Moribund	Labored breathing, pallor, piloerection
200 + 3,000 + 0	1	19	Found dead	None
400 + 3,000 + 0	2	14, 16	Moribund	Labored breathing, pallor, piloerection
400 + 3,000 + 0	1	16	Found dead	Piloerection, pallor

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)

BODY AND ORGAN WEIGHTS

Male Mice

Administration of AZT, TMP/SMX, or folinic acid alone or in combination did not affect mean body weights or body weight gains during the study (Figure 1). No biologically important differences occurred. The average body weight gain for the 0 + 3,000 + 10 mg/kg group was significantly increased ($P \leq 0.05$) for days 5 to 21; this was not considered biologically important, as the difference from the control value was small.

The right epididymis weights and epididymis-to-body-weight ratios were comparable among dose groups.

Female-A Mice

Mean body weights and body weight gains were generally comparable among all groups for days 0 to 16 (Figure 2). The 200 + 3,000 + 0 mg/kg and 400 + 3,000 + 0 mg/kg groups did exhibit significant decreases in mean body weight gains during this interval.

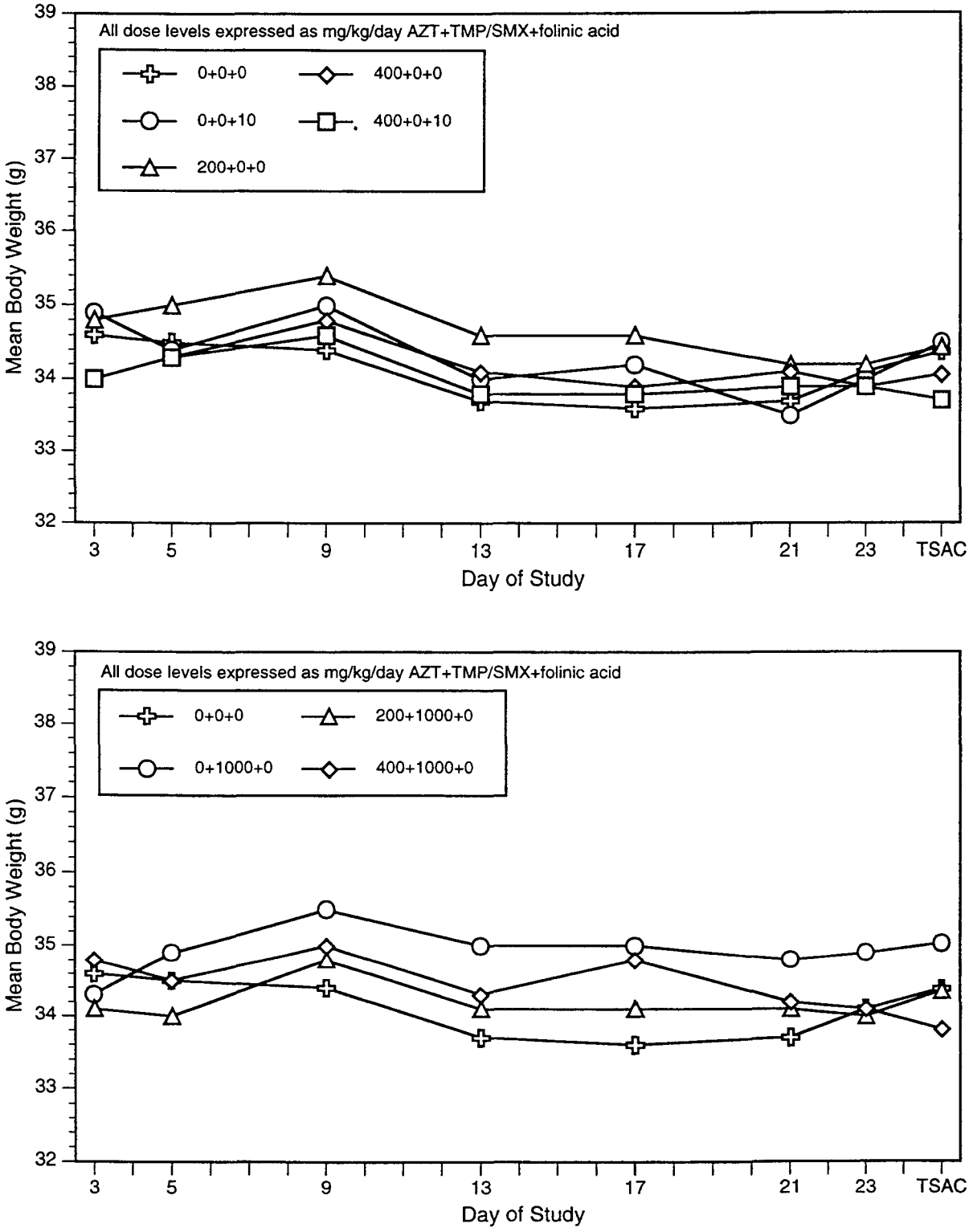


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (TSAC=terminal sacrifice)

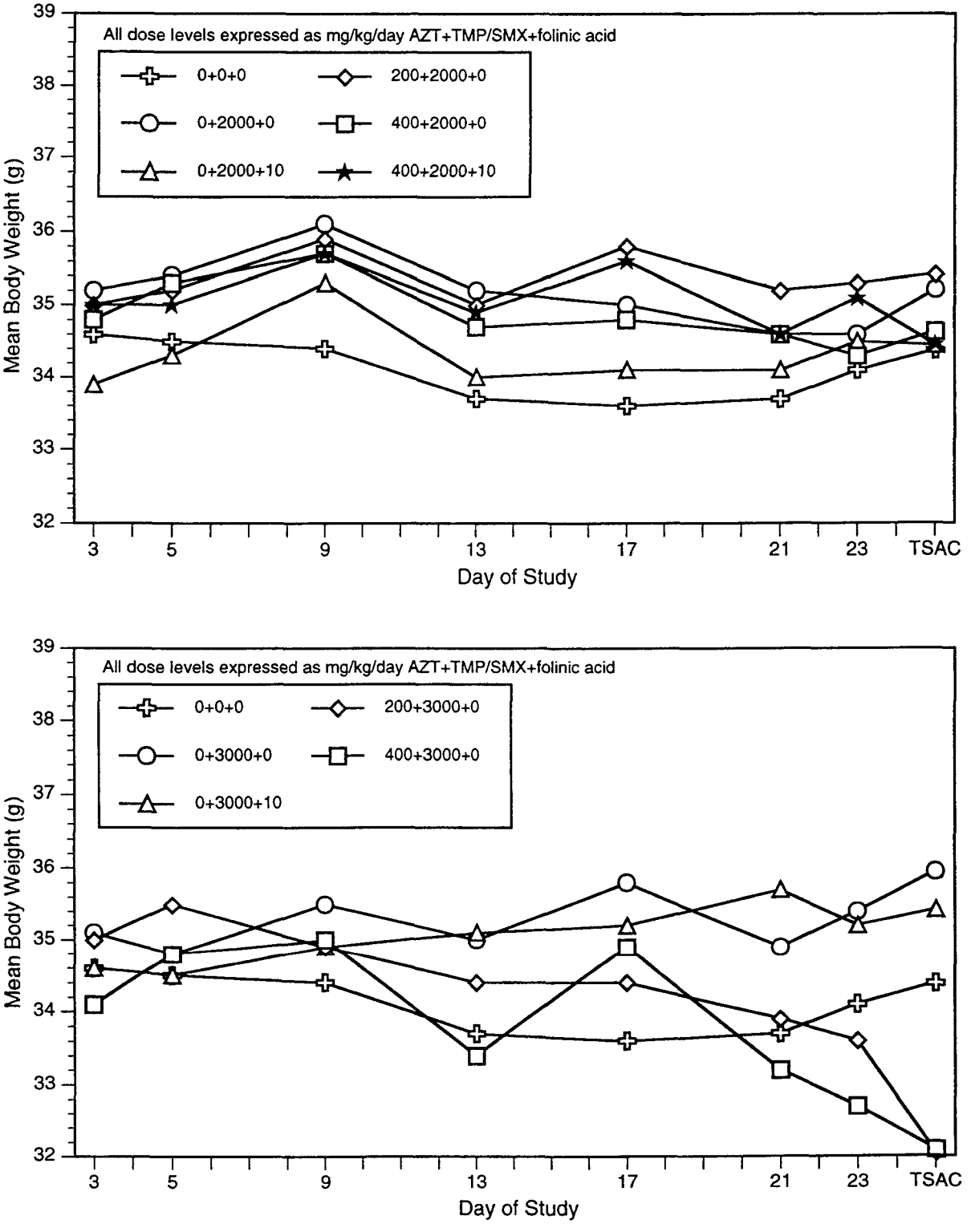


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (TSAC=terminal sacrifice)

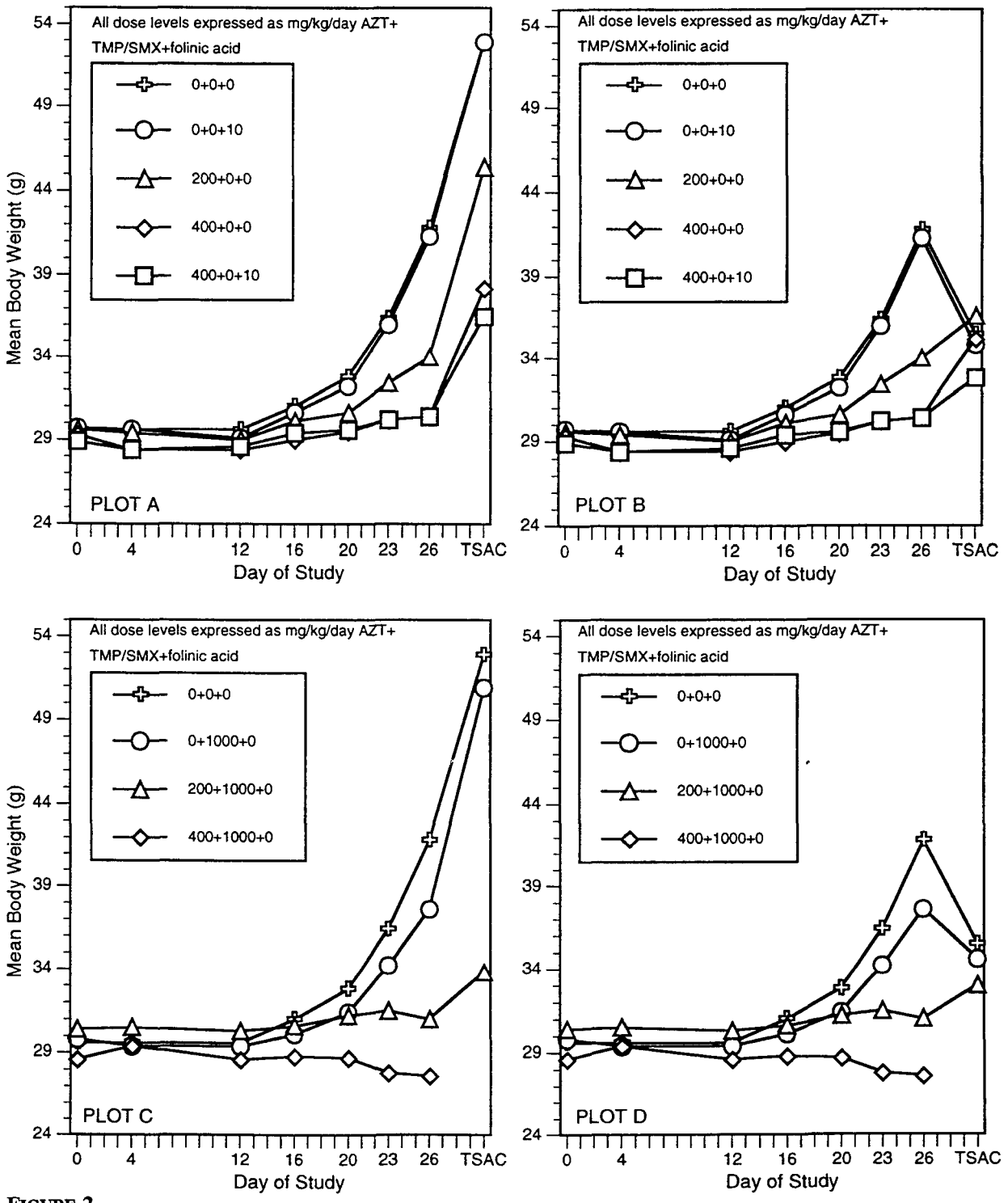


FIGURE 2

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (Plots A and C show body weights from day 0 through terminal sacrifice [TSAC]. Plots B and D show the identical data, except that body weights at TSAC are after gravid uterine weights have been subtracted. Group final mean body weights and corrected body weights include only values for dams that were actually pregnant and were sacrificed and caesarean-sectioned as scheduled on presumed day 18 of gestation.)

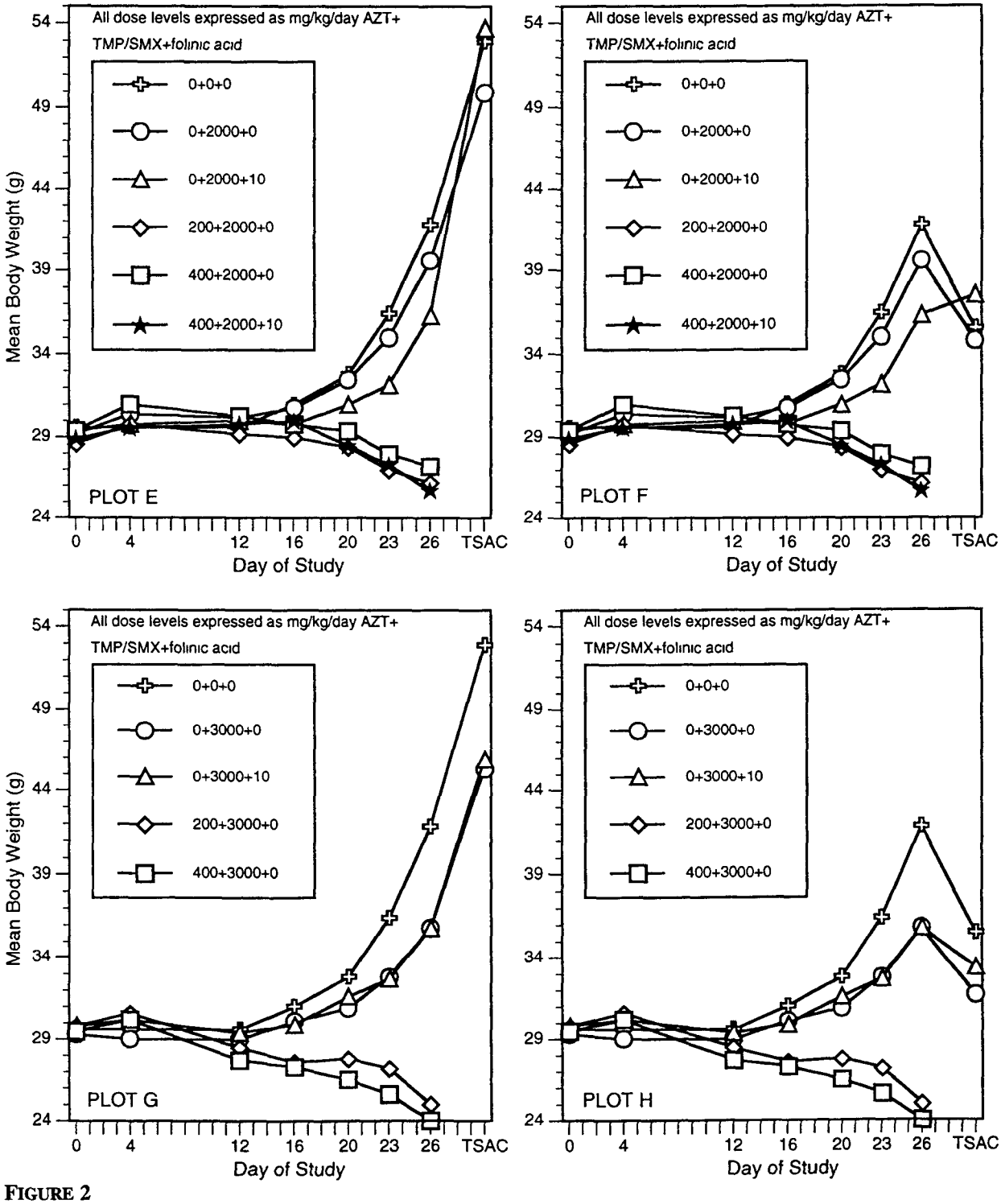


FIGURE 2

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (Plots E and G show body weights from day 0 through terminal sacrifice [TSAC]. Plots F and H show the identical data, except that body weights at TSAC are after gravid uterine weights have been subtracted. Group final mean body weights and corrected body weights include only values for dams that were actually pregnant and were sacrificed and caesarean-sectioned as scheduled on presumed day 18 of gestation.)

During study days 20 to 26, significant decreases in mean body weights and/or body weight gains occurred for the following groups: 200 + 0 + 0 mg/kg, 400 + 0 + 0 mg/kg, 400 + 0 + 10 mg/kg, 200 + 1,000 + 0 mg/kg, 400 + 1,000 + 0 mg/kg, 200 + 2,000 + 0 mg/kg, 400 + 2,000 + 0 mg/kg, 400 + 2,000 + 10 mg/kg, 200 + 3,000 + 0 mg/kg, and 400 + 3,000 + 0 mg/kg. These decreases in mean body weight represented resorption of conceptuses and changes in gravid uterine weight.

Gravid uterine weights were significantly ($P \leq 0.05$) reduced by administration of AZT (200 or 400 mg/kg) with or without folinic acid (Table 6). Final mean body weights, gravid uterine weights, or corrected mean body weights were not evaluated for the following groups due to the lack of viable fetuses: 400 + 1,000 + 0 mg/kg, 200 + 2,000 + 0 mg/kg, 400 + 2,000 + 0 mg/kg, 400 + 2,000 + 10 mg/kg, 200 + 3,000 + 0 mg/kg, and 400 + 3,000 + 0 mg/kg. Final mean body weights, gravid uterine weights, and corrected maternal mean body weights for those groups with viable fetuses were comparable to control values or significantly decreased

TABLE 6
The Effect of Oral Administration of AZT, TMP/SMX, and Folinic Acid on Final Mean Body Weights (FMBW) and Gravid Uterine Weights (GUW) of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study in Swiss (CD-1®) Mice

Dose[a]	Number	Mean Body Weights		
		FMBW	GUW	CBW[b]
0 + 0 + 0	8	52 9 ± 5 5	17 4 ± 4 9	35 5 ± 4 0
0 + 0 + 10	14	52 9 ± 5 7	18 1 ± 4 1	34 8 ± 2 5
200 + 0 + 0	9	45 4 ± 8 8	8 8 ± 6 7	36 6 ± 3 8
400 + 0 + 0	4	38 2 ± 3 1*	3 0 ± 1 9▲	35 2 ± 2 2
400 + 0 + 10	6	36 5 ± 5 2*	3 7 ± 3 6*	32 8 ± 3 7
0 + 1,000 + 0	10	50 9 ± 6 7	16 3 ± 4 8	34 6 ± 3 0
200 + 1,000 + 0	6	33 8 ± 6 7*	0 8 ± 0 5*	33 0 ± 6 4
0 + 2,000 + 0	13	49 9 ± 3 6	15 1 ± 2 1	34 8 ± 2 3
0 + 2,000 + 10	7	53 7 ± 2 7	16 2 ± 1 0	37 5 ± 2 3
0 + 3,000 + 0	10	45 3 ± 4 7*	13 6 ± 2 7	31 7 ± 3 4
0 + 3,000 + 10	9	45 9 ± 5 8	12 4 ± 3 9	33 4 ± 4 1

* Significantly different from the control group ($P \leq 0$ 05) by Dunnett's or Dunn's test
▲ Not statistically significant, probably due to small sample size
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day), data include only groups with viable fetuses
[b] Corrected body weight = final mean body weight minus gravid uterine weight

($P \leq 0.05$) compared to the control group. Final mean body weights were significantly decreased ($P \leq 0.05$) in the following groups: 400 + 0 + 0 mg/kg, 400 + 0 + 10 mg/kg, 200 + 1,000 + 0 mg/kg, and 0 + 3,000 + 0 mg/kg. Gravid uterine weights were significantly decreased in the 400 + 0 + 10 mg/kg and the 200 + 1,000 + 0 mg/kg groups.

Female-B Mice

Mean body weights on day 15 of gestation were significantly decreased ($P \leq 0.05$) in the following groups: 200 + 2,000 + 0 mg/kg, 400 + 2,000 + 0 mg/kg, 400 + 2,000 + 10 mg/kg, 200 + 3,000 + 0 mg/kg, and 400 + 3,000 + 0 mg/kg. Mean body weights for days 0, 8, and 12 for all groups and day 15 for groups other than those listed above were not different from those of the control group (Figure 3).

Mean body weight gains for all groups for days 0 to 8 were comparable to control values. Mean body weight gains during the intervals day 8 to 12 and/or day 12 to 15 were decreased in the following groups: 400 + 0 + 10 mg/kg, 200 + 1,000 + 0 mg/kg, 400 + 1,000 + 0 mg/kg, 200 + 2,000 + 0 mg/kg, 400 + 2,000 + 0 mg/kg, 400 + 2,000 + 10 mg/kg, 200 + 3,000 + 0 mg/kg, and 400 + 3,000 + 0 mg/kg. For days 0 to 15 of gestation, the following groups had decreased mean body weight gains: 400 + 0 + 10 mg/kg, 400 + 1,000 + 0 mg/kg, 200 + 2,000 + 0 mg/kg, 400 + 2,000 + 0 mg/kg, 400 + 2,000 + 10 mg/kg, 200 + 3,000 + 0 mg/kg, and 400 + 3,000 + 0 mg/kg.

For groups with viable pups, mean pup weights on day 0 of lactation were decreased in the following groups: 400 + 0 + 0 mg/kg, 400 + 0 + 10 mg/kg, 0 + 2,000 + 0 mg/kg, 0 + 2,000 + 10 mg/kg, 0 + 3,000 + 0 mg/kg, and 0 + 3,000 + 10 mg/kg. For pups that survived until day 4 of lactation, pup weights were comparable among the groups, with the exception of the 400 + 0 + 10 mg/kg group, for which mean pup weight was decreased ($P \leq 0.05$) compared to pups in the control group.

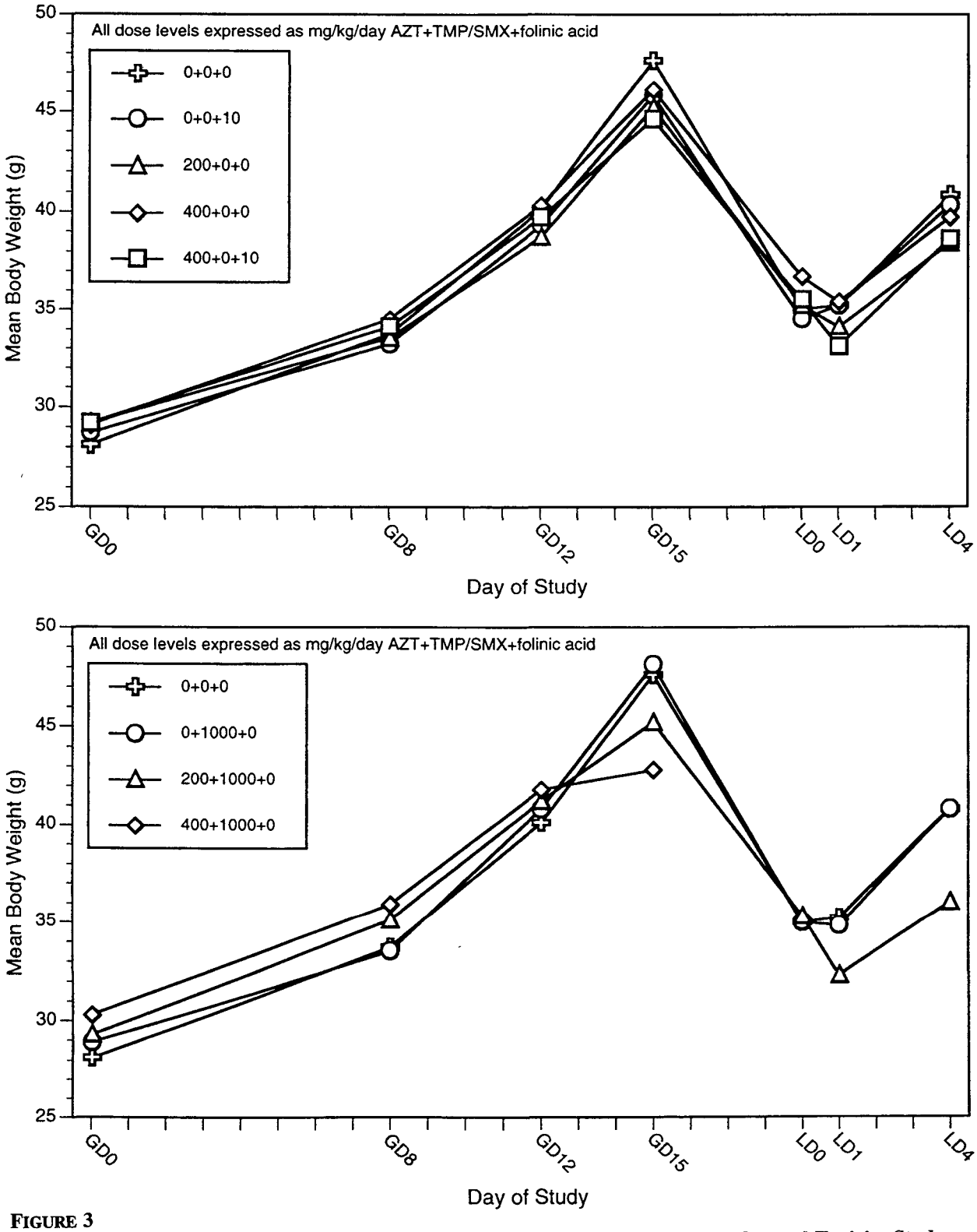


FIGURE 3
Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (GD=gestation day; LD=lactation day)
Note: Data exclude values for dams that had no live or surviving pups at weighing.

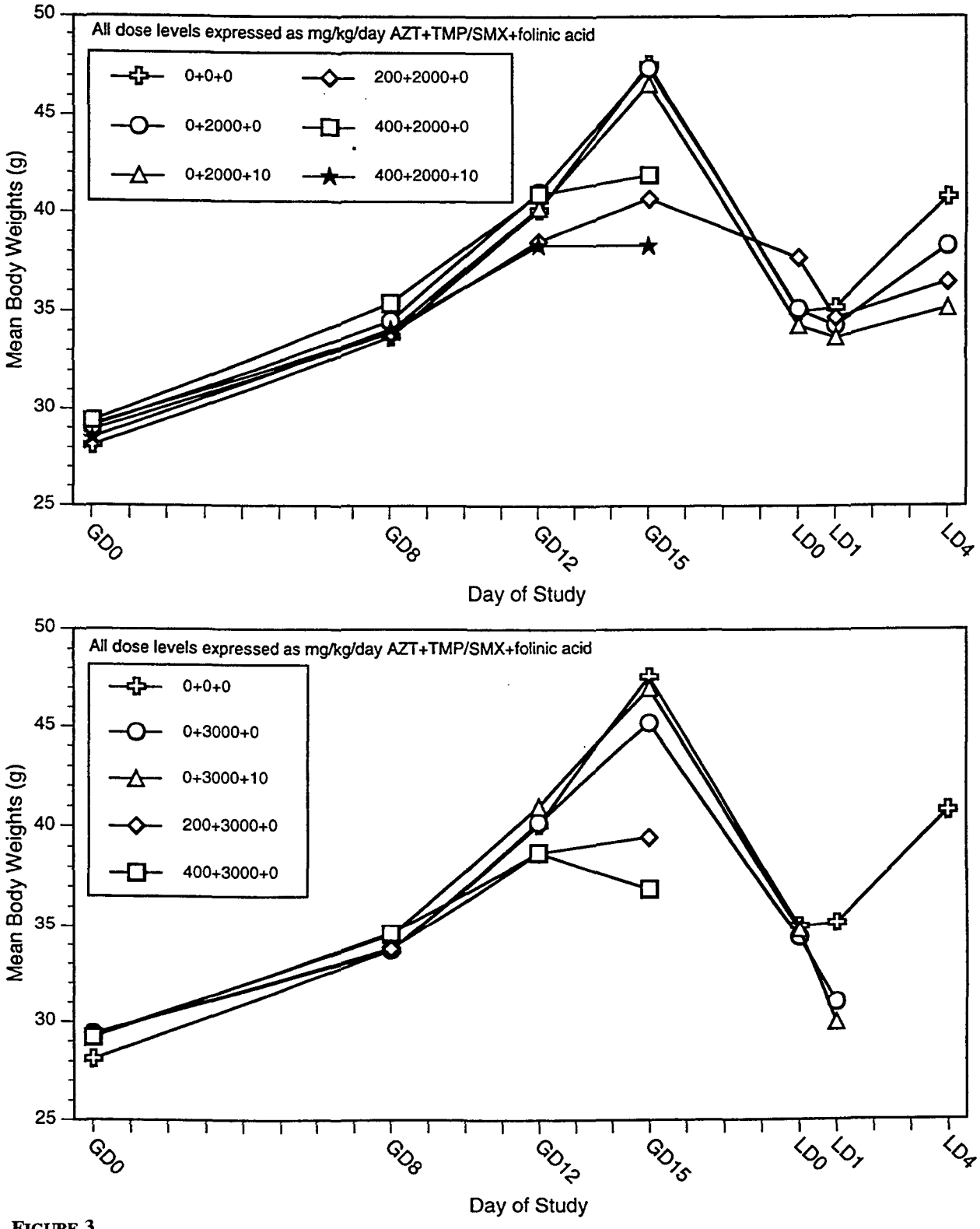


FIGURE 3
Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (GD=gestation day; LD=lactation day)
Note: Data exclude values for dams that had no live or surviving pups at weighing.

CLINICAL PATHOLOGY

Hematology

Although difficulties arise when comparing results of males and females or of groups dosed differently, it appears that the duration of dosing had a major impact on the severity of the hematology results. The severity was greatest in the female-A groups dosed for approximately 30 days. Alterations were not as prominent in the males dosed for approximately 20 days, and the female-B groups dosed for approximately 10 days had rather mild alterations. Specific details for males and females are discussed in the following sections.

Male Mice

In general, male mice administered AZT alone developed a slight anemia, neutropenia, and thrombocytosis. TMP/SMX alone resulted in a mild anemia only in the 3,000 mg/kg group and a treatment-related monocytosis. Administration of folinic acid alone resulted in no biologically significant alterations.

Combination therapy with AZT and TMP/SMX resulted in a severe anemia, a marked reticulocytopenia, a prominent leukopenia, and a significant thrombocytosis. The severity of the anemia was far greater than that observed subsequent to treatment with either compound alone. An unexpected decrease in mean cell volume (MCV) values and an elevation in mean cell hemoglobin concentration (MCHC) values accompanied the anemia.

With the exception of a marginal decrease in the severity of the AZT-induced anemia and thrombocytosis, administration of folinic acid had no major protective impact on any of the hematology parameters evaluated.

AZT Alone

The most significant alteration detected subsequent to the administration of AZT alone to male mice was a dose-related anemia. Respective mean erythrocyte (RBC) counts (Figure 4 and Table A1) of male mice receiving 200 or 400 mg AZT/kg body weight were approximately 20% ($7.98 \times 10^6/\mu L$; $P \leq 0.01$) and 32% ($6.79 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count ($9.96 \times 10^6/\mu L$) of the control group. Reduced hemoglobin (Hgb) and hematocrit (Hct) values paralleled the dose-related declines in RBC counts. Increases in MCV and mean cell hemoglobin (MCH) values also accompanied the dose-related anemia. Significant alterations were not present in the MCHC values or reticulocyte counts (Figure 5 and Table A1).

Although not statistically significant, the leukocyte (WBC) count (Figure 6 and Table A1) of male mice administered AZT alone was lower than that of the control group. Evaluation of the corresponding differential

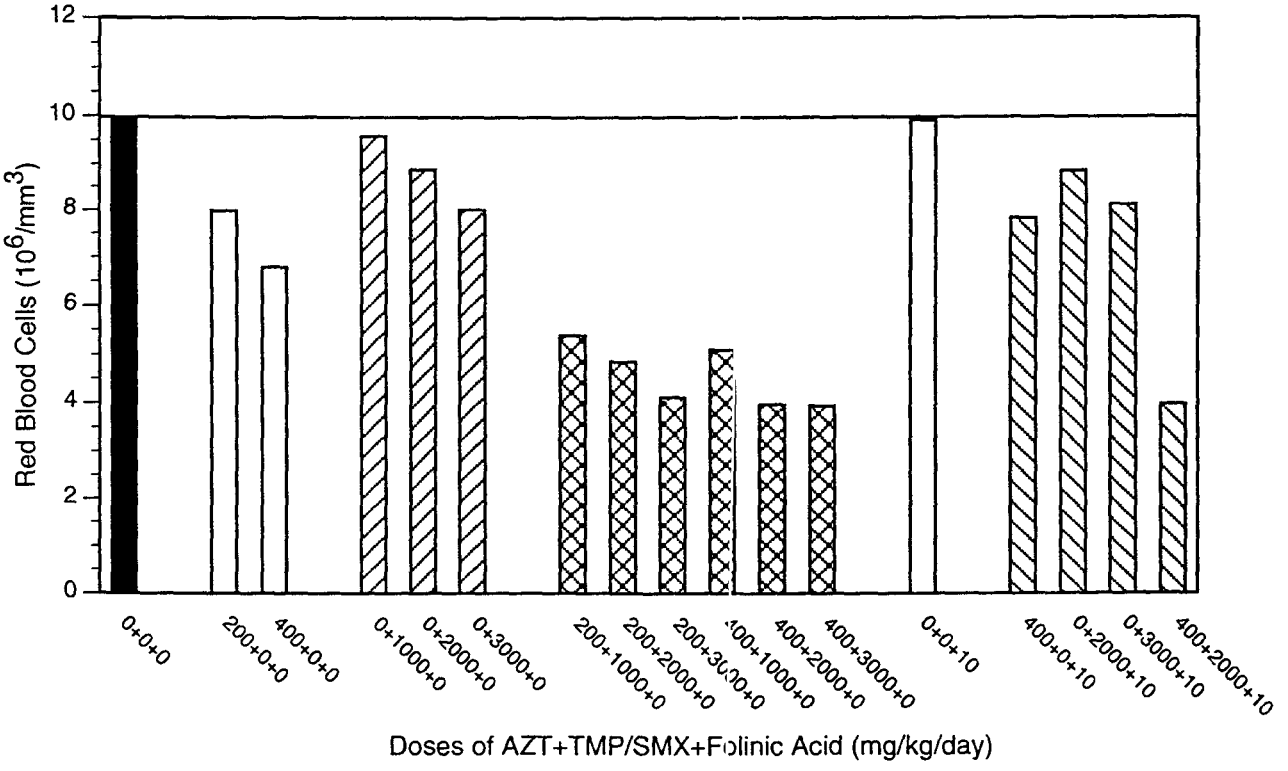


FIGURE 4
Mean Erythrocyte Values for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

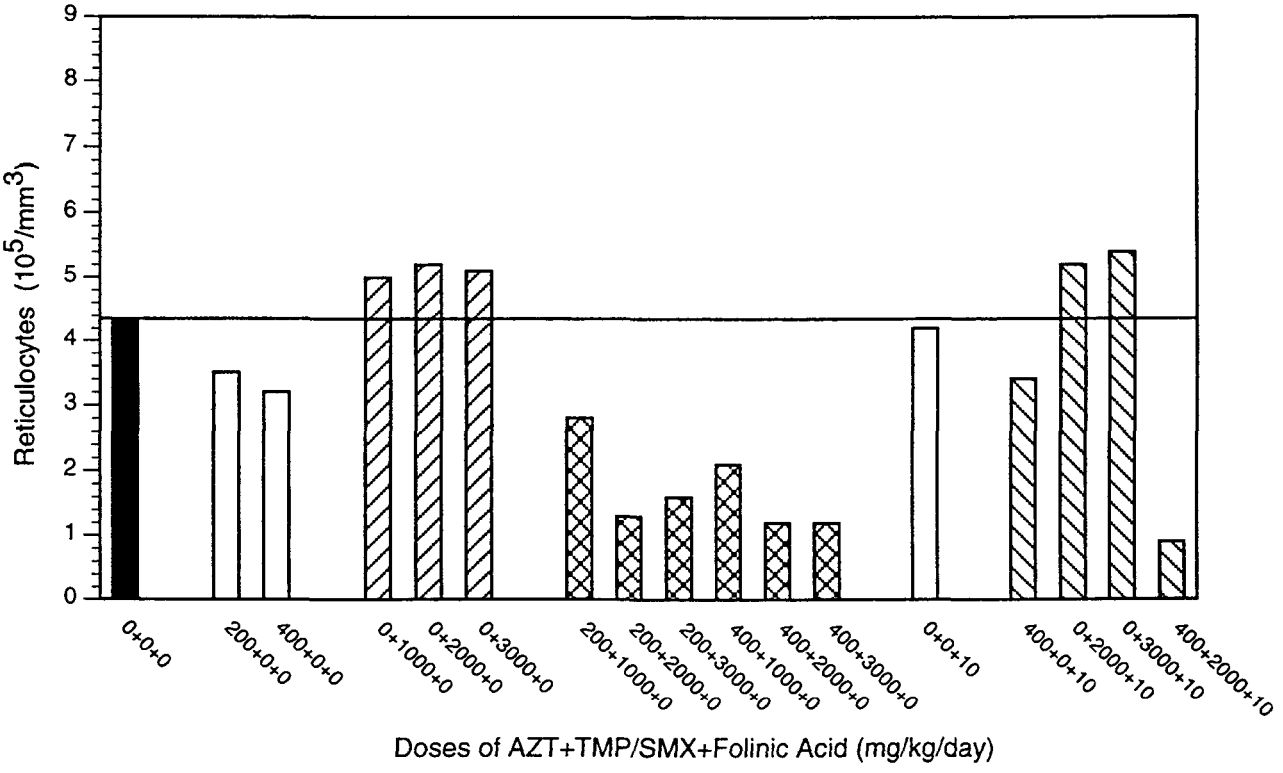


FIGURE 5
Mean Reticulocyte Values for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

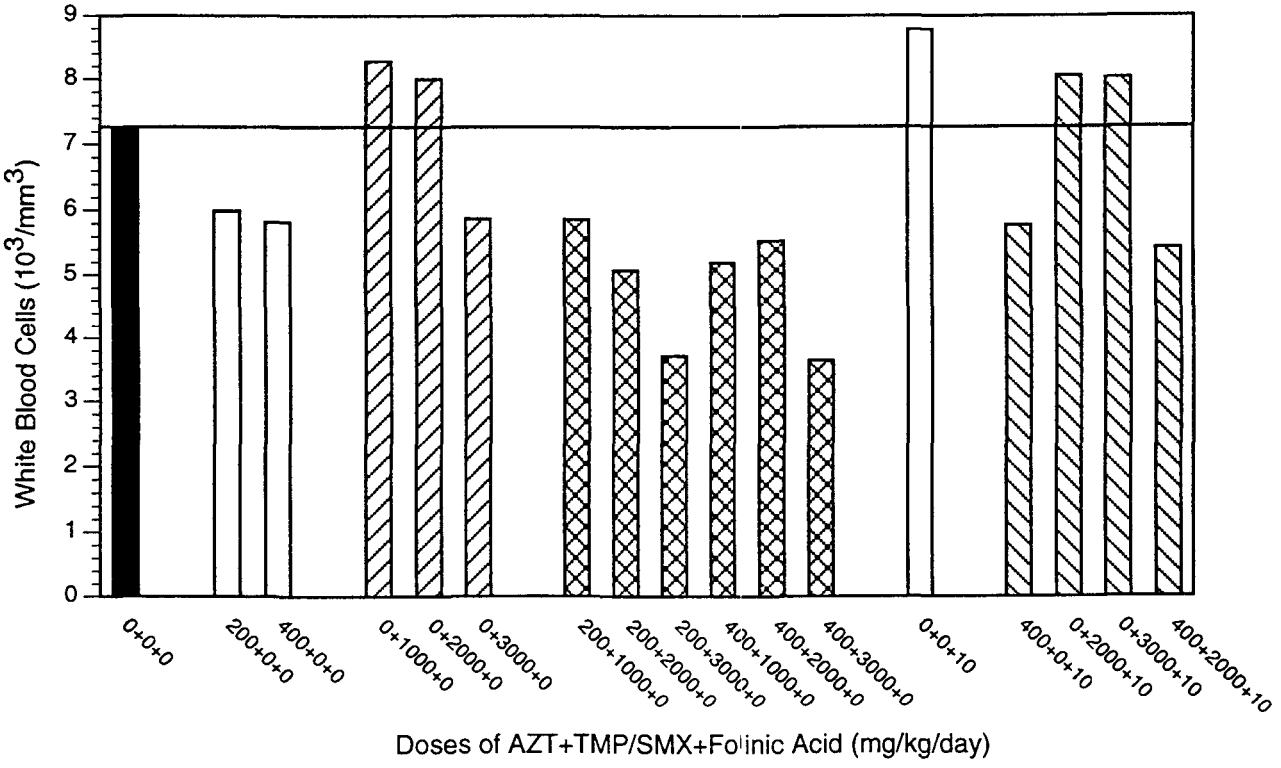


FIGURE 6
Mean Leukocyte Values for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

data revealed that the marginal leukopenia was predominately neutrophilic. Respective mean segmented neutrophil counts for males receiving 200 or 400 mg/kg AZT were approximately 50% ($0.61 \times 10^3/\mu L$; $P \leq 0.05$) and 38% ($0.76 \times 10^3/\mu L$) lower than the mean segmented neutrophil count ($1.22 \times 10^3/\mu L$) of the control group. Significant alterations were not encountered in the other leukocyte differential parameters.

A treatment-related elevation in platelet count (Figure 7 and Table A1) was also evident in male mice administered AZT alone. The mean platelet count of male mice receiving 400 mg/kg AZT alone was approximately 40% ($1,517 \times 10^3/\mu L$; $P \leq 0.05$) higher than the mean platelet count ($1,094 \times 10^3/\mu L$) of the control group.

TMP/SMX Alone

A mild anemia was evident in male mice administered 3,000 mg TMP/SMX per kg body weight. The mean RBC count (Figure 4 and Table A1) of male mice administered 3,000 mg/kg was approximately 20% ($8.01 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count ($9.96 \times 10^6/\mu L$) of the control group. Reduced Hgb and Hct values paralleled the decline in RBC counts. Statistically significant alterations were not evident in MCV, MCH, or MCHC values. Significant alterations were not detected in any of the hematology parameters evaluated in male mice receiving 1,000 or 2,000 mg/kg, and reticulocyte counts (Figure 5 and Table A1) were not altered in any of the treatment groups.

A mild elevation in platelet count (Figure 7 and Table A1) was detected in male mice administered 3,000 mg/kg. Although not statistically significant, the mean platelet count of male mice administered 3,000 mg/kg was approximately 30% ($1,397 \times 10^3/\mu L$) higher than the mean platelet count ($1,094 \times 10^3/\mu L$) of the control group. Significant alterations did not occur in the mean platelet count of male mice administered 1,000 or 2,000 mg/kg.

Significant alterations were not evident in the WBC counts (Figure 6 and Table A1) of male mice administered TMP/SMX. Evaluation of the corresponding differential data, however, revealed a significant elevation in the number of monocytes of the 3,000 mg/kg group. Male mice administered 3,000 mg/kg had a mean monocyte count approximately 90% ($0.29 \times 10^3/\mu L$; $P \leq 0.05$) higher than the mean monocyte count ($0.15 \times 10^3/\mu L$) of the control group. Biologically significant alterations were not detected in the other differential parameters.

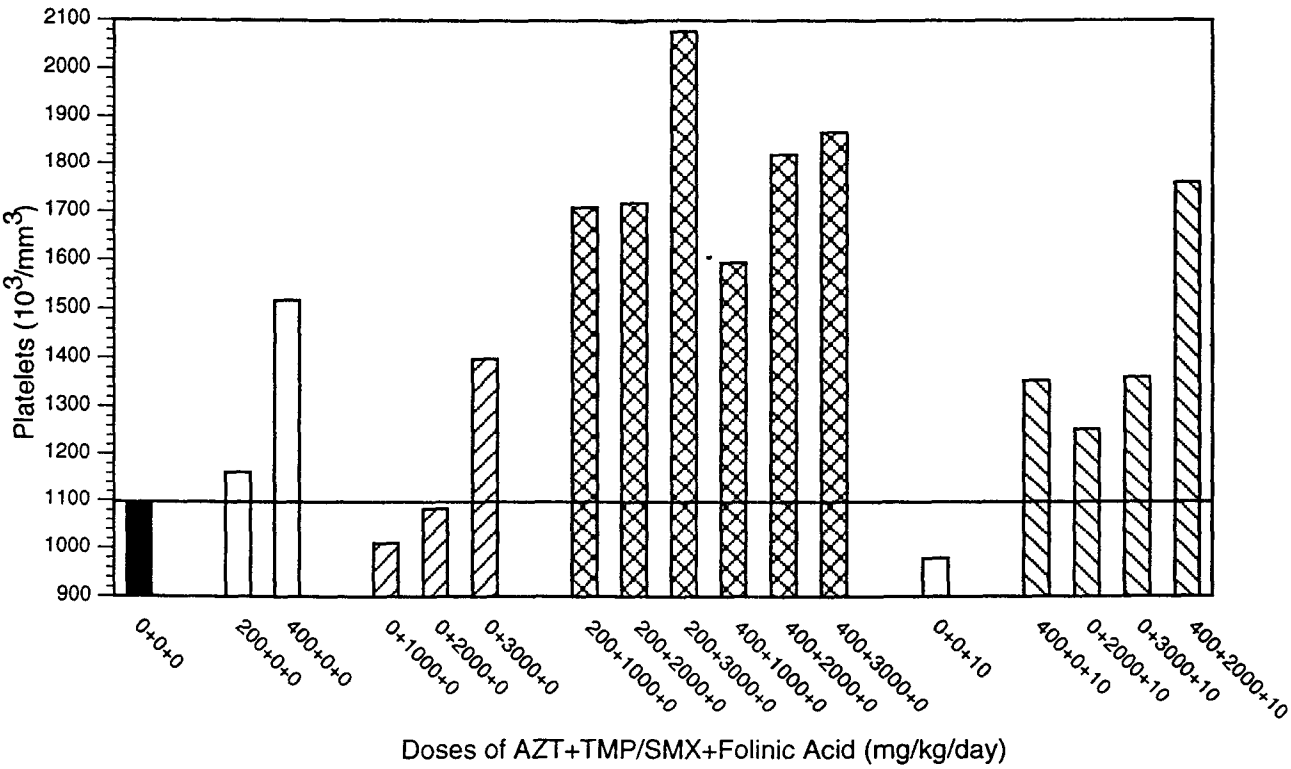


FIGURE 7
Mean Platelet Values for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

Folinic Acid Alone

Administration of 10 mg folinic acid/kg body weight alone to male mice did not result in any biologically significant alterations in an any of the hematological parameters evaluated during this study.

AZT and TMP/SMX Combinations

A distinct treatment-related anemia resulted from the administration of AZT and TMP/SMX combinations, and the severity of the anemia was far greater than that due to either compound alone. Respective mean RBC counts (Figure 4 and Table A1) in male mice receiving 200 mg AZT/kg body weight + 1,000, 2,000, or 3,000 mg TMP/SMX per kg body weight were approximately 46% ($5.39 \times 10^6/\mu L$; $P \leq 0.01$), 51% ($4.85 \times 10^6/\mu L$; $P \leq 0.01$), or 59% ($4.12 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count ($9.96 \times 10^6/\mu L$) of the control group. Administration of 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX resulted in respective RBC counts approximately 49% ($5.09 \times 10^6/\mu L$; $P \leq 0.01$), 60% ($3.97 \times 10 /\mu L$; $P \leq 0.01$), or 60% ($3.95 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count of the control group. Reduced Hgb and Hct values paralleled the treatment-related declines in RBC counts. Significant declines in MCV values and elevations in MCHC values occurred in some of the treatment groups that had severe anemia.

Although significant alterations in reticulocyte counts (Figure 5 and Table A1) were not observed in male mice administered AZT alone or TMP/SMX alone, a marked reticulocytopenia was evident subsequent to combination therapy. Respective mean reticulocyte counts obtained from male mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 35% ($2.8 \times 10^5/\mu L$), 70% ($1.3 \times 10^5/\mu L$; $P \leq 0.01$), or 63% ($1.6 \times 10^5/\mu L$; $P \leq 0.01$) lower than the mean reticulocyte count ($4.3 \times 10^5/\mu L$) of the control group. Respective mean reticulocyte counts detected in male mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 51% ($2.1 \times 10^5/\mu L$; $P \leq 0.01$), 72% ($1.2 \times 10^5/mm^3$; $P \leq 0.01$), or 72% ($1.2 \times 10^5/mm^3$; $P \leq 0.01$) lower than the mean reticulocyte count of the control group.

Although not statistically significant, a mild decrease in mean WBC counts (Figure 6 and Table A1) was evident in male mice receiving 200 or 400 mg/kg AZT + 1,000 or 2,000 mg/kg TMP/SMX. A distinct leukopenia ($P \leq 0.01$) occurred in male mice receiving the AZT and 3,000 mg/kg TMP/SMX combination. Respective mean WBC counts detected in male mice receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX were approximately 49% ($3.71 \times 10^3/\mu L$) or 50% ($3.64 \times 10^3/\mu L$) lower than the mean WBC count ($7.25 \times 10^3/\mu L$) of the control group. Evaluation of the corresponding differential data revealed statistically significant declines in segmented neutrophil counts of groups receiving AZT + 1,000 or 2,000 mg/kg TMP/SMX and a statistically significant lymphopenia (Table A1) in males receiving AZT + 3,000 mg/kg TMP/SMX. Respective mean

segmented neutrophil counts for male mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 62% ($0.46 \times 10^3/\mu L$; $P \leq 0.01$), 58% ($0.51 \times 10^3/\mu L$; $P \leq 0.05$), or 43% ($0.69 \times 10^3/\mu L$) lower than the mean segmented neutrophil count ($1.22 \times 10^3/\mu L$) of the control group. Respective mean segmented neutrophil counts for male mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 63% ($0.45 \times 10^3/\mu L$; $P \leq 0.01$), 69% ($0.38 \times 10^3/\mu L$; $P \leq 0.01$), or 40% ($0.73 \times 10^3/\mu L$) lower than the mean segmented neutrophil count ($1.22 \times 10^3/\mu L$) of the control group. Mean lymphocyte counts of male mice receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX were approximately 50% ($2.81 \times 10^3/\mu L$; $P \leq 0.05$) or 51% ($2.79 \times 10^3/\mu L$; $P \leq 0.05$) lower than the mean lymphocyte count ($5.65 \times 10^3/\mu L$) of the control group. A statistically significant eosinopenia was also evident in male mice receiving AZT and 3,000 mg/kg TMP/SMX. Respective mean eosinophil counts in male mice receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX were approximately 72% ($0.05 \times 10^3/\mu L$; $P \leq 0.05$) or 89% ($0.02 \times 10^3/\mu L$; $P \leq 0.01$) lower than the mean eosinophil count ($0.18 \times 10^3/\mu L$) of the control group. Statistically significant alterations were not evident in any of the other differential parameters.

A marked treatment-related thrombocytosis occurred in male mice receiving AZT and TMP/SMX combinations. Respective mean platelet counts (Figure 7 and Table A1) of male mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 60% ($1,708 \times 10^3/\mu L$; $P \leq 0.01$), 60% ($1,717 \times 10^3/\mu L$; $P \leq 0.01$), or 90% ($2,077 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean platelet count ($1,094 \times 10^3/\mu L$) of the control group. Respective mean platelet counts of male mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 50% ($1,595 \times 10^3/\mu L$; $P \leq 0.01$), 70% ($1,818 \times 10^3/\mu L$; $P \leq 0.01$), or 70% ($1,866 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean platelet count ($1,094 \times 10^3/\mu L$) of the control group.

AZT and Folinic Acid Combination

Administration of 10 mg/kg folinic acid in conjunction with 400 mg/kg AZT resulted in a marginal decrease in the severity of the anemia when compared to male mice administered 400 mg/kg AZT alone. The RBC count (Figure 4 and Table A1) of males administered 400 mg/kg AZT alone was approximately 32% ($6.79 \times 10^6/\mu L$) lower than the control group ($9.96 \times 10^6/\mu L$). The RBC count of the group receiving AZT and 10 mg/kg folinic acid was approximately 21% ($7.84 \times 10^6/\mu L$) lower than that of the control group.

Alterations in MCV and MCH values were not as pronounced in male mice receiving AZT + folinic acid as they were in mice administered AZT alone.

A significant thrombocytosis did not occur in male mice receiving AZT + folinic acid, whereas a significant elevation in platelet count (Figure 7 and Table A1) was evident in mice administered 400 mg/kg AZT alone. The platelet count of the group receiving combination therapy (AZT + folinic acid) was approximately 20% ($1,353 \times 10^3/\mu L$) higher than in the control group ($1,094 \times 10^3/\mu L$), whereas mice administered 400 mg/kg AZT alone had a mean platelet count approximately 40% ($1,517 \times 10^3/\mu L$; $P \leq 0.05$) higher than the control group ($1,094 \times 10^3/\mu L$).

Biologically significant differences were not observed in the reticulocyte count (Figure 5 and Table A1), WBC count (Figure 6 and Table A1), or differential values when mice receiving AZT + folinic acid were compared to mice administered AZT alone. The statistically significant decline in the mean segmented neutrophil count observed in mice receiving 400 mg/kg AZT + 10 mg/kg folinic acid was similar to the decline observed in mice administered 200 mg/kg AZT alone.

TMP/SMX and Folinic Acid Combinations

Administration of 10 mg/kg folinic acid in conjunction with 2,000 or 3,000 mg/kg TMP/SMX had no significant impact on any of the RBC parameters when compared to the values obtained subsequent to the administration of TMP/SMX alone (Figure 4 and Table A1). Significant differences in reticulocyte (Figure 5), total WBC (Figure 6), and platelet counts (Figure 7) likewise did not occur. Augmentation of the previously described monocytosis, however, did occur with folinic acid supplementation (Table A1). The significance of these elevated monocyte counts is not clear. No other biologically significant alterations were observed when mice receiving TMP/SMX were supplemented with folinic acid.

AZT, TMP/SMX, and Folinic Acid Combination

Administration of 10 mg/kg folinic acid in conjunction with AZT and TMP/SMX resulted in no significant differences in any of the parameters evaluated when compared to the group receiving 400 mg/kg AZT + 2,000 mg/kg TMP/SMX. This group of mice had a marked anemia (Figure 4 and Table A1) accompanied by an elevated MCHC. Reticulocytopenia and thrombocytosis (Figures 5 and 7) were also evident. Although a statistically significant decline in the WBC count was not evident (Figure 6), evaluation of the corresponding differential data revealed a significant granulocytopenia (Table A1).

Female-A Mice

In general, the anemia, neutropenia, and thrombocytosis induced by AZT was slightly more severe in female-A mice than in males. TMP/SMX alone resulted in a slight anemia, thrombocytosis, and monocytosis with trends

similar to those observed in male mice. No biologically significant alterations occurred subsequent to folinic acid administration alone.

Combination therapy with AZT and TMP/SMX resulted in a severe anemia, a marked reticulocytopenia, a prominent leukopenia, and a significant thrombocytosis. As with the males, the severity of the anemia was far greater than that observed subsequent to administration of either compound alone. Low MCV values and elevated MCHC values accompanied the anemia.

Administration of folinic acid in combination with AZT and TMP/SMX resulted in no protective effects.

AZT Alone

Administration of AZT alone to female-A mice resulted in a dose-related anemia ($P \leq 0.01$), and the severity of the anemia was slightly greater than in males. Respective mean RBC counts (Figure 8 and Table A2) of female-A mice receiving 200 or 400 mg/kg AZT were approximately 28% ($6.26 \times 10^6/\mu L$) or 39% ($5.27 \times 10^6/\mu L$) lower than the mean RBC count ($8.64 \times 10^6/\mu L$) in the control group. Reduced Hgb and Hct values paralleled the dose-related declines in RBC counts. Elevations in MCV and MCH values also accompanied the dose-related anemia. Significant alterations in MCHC values were not observed, and reticulocyte counts (Figure 9 and Table A2) were within the normal range.

Administration of AZT alone to female-A mice also resulted in a compound-related leukopenia. Respective mean WBC counts (Figure 10 and Table A2) in female-A mice receiving 200 or 400 mg/kg AZT were approximately 32% ($4.57 \times 10^3/\mu L$; $P \leq 0.01$) or 25% ($5.00 \times 10^3/\mu L$; $P \leq 0.05$) lower than the mean WBC count ($6.69 \times 10^3/\mu L$) of the control group. Evaluation of the corresponding differential data revealed significant declines in segmented neutrophils ($P \leq 0.01$) and monocytes ($P \leq 0.01$). Respective mean segmented neutrophil counts for the female-A mice receiving 200 or 400 mg/kg AZT were approximately 54% ($0.72 \times 10^3/\mu L$) or 68% ($0.50 \times 10^3/\mu L$) lower than the mean segmented neutrophil count ($1.56 \times 10^3/\mu L$) of the control group. Respective mean monocyte counts for the same treatment groups were approximately 47% ($0.09 \times 10^3/\mu L$) or 53% ($0.08 \times 10^3/\mu L$) lower than the mean monocyte count ($0.17 \times 10^3/\mu L$) of the control group. Statistically significant alterations in basophil counts were not considered to be biologically significant, and no other treatment-related alterations were evident in the other differential parameters evaluated.

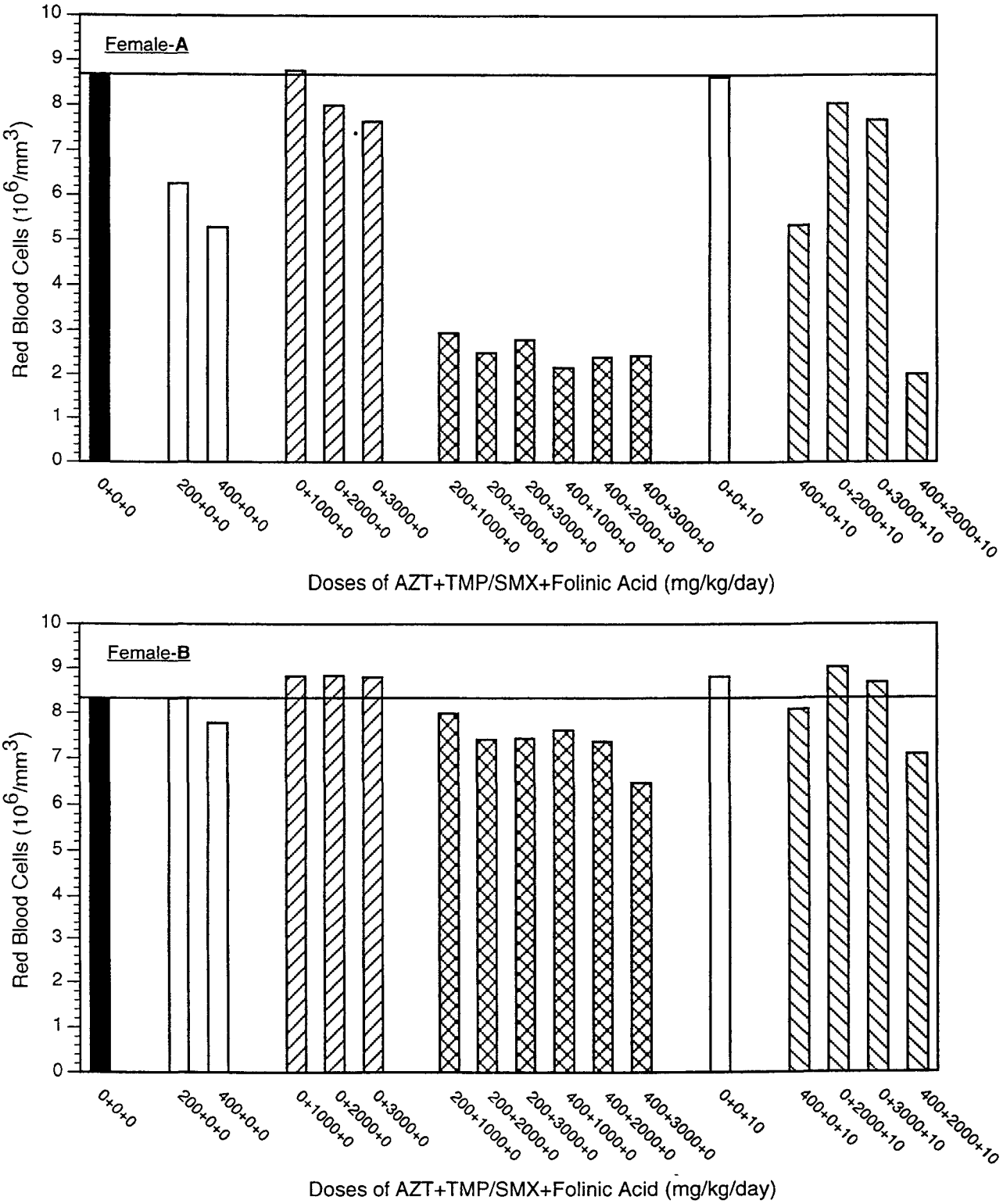


FIGURE 8
Mean Red Blood Cell Values for Female Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

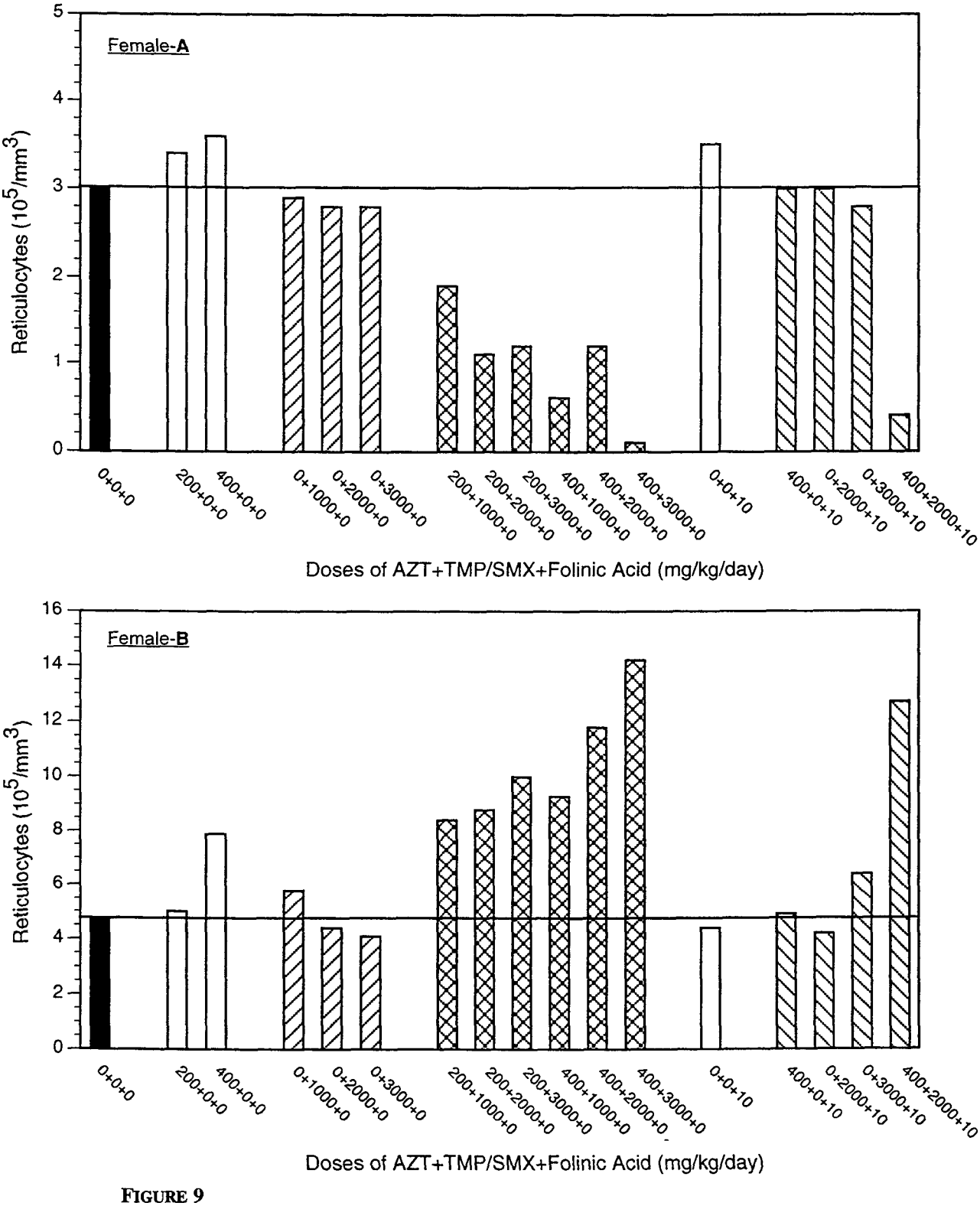


FIGURE 9
Mean Reticulocyte Values for Female Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

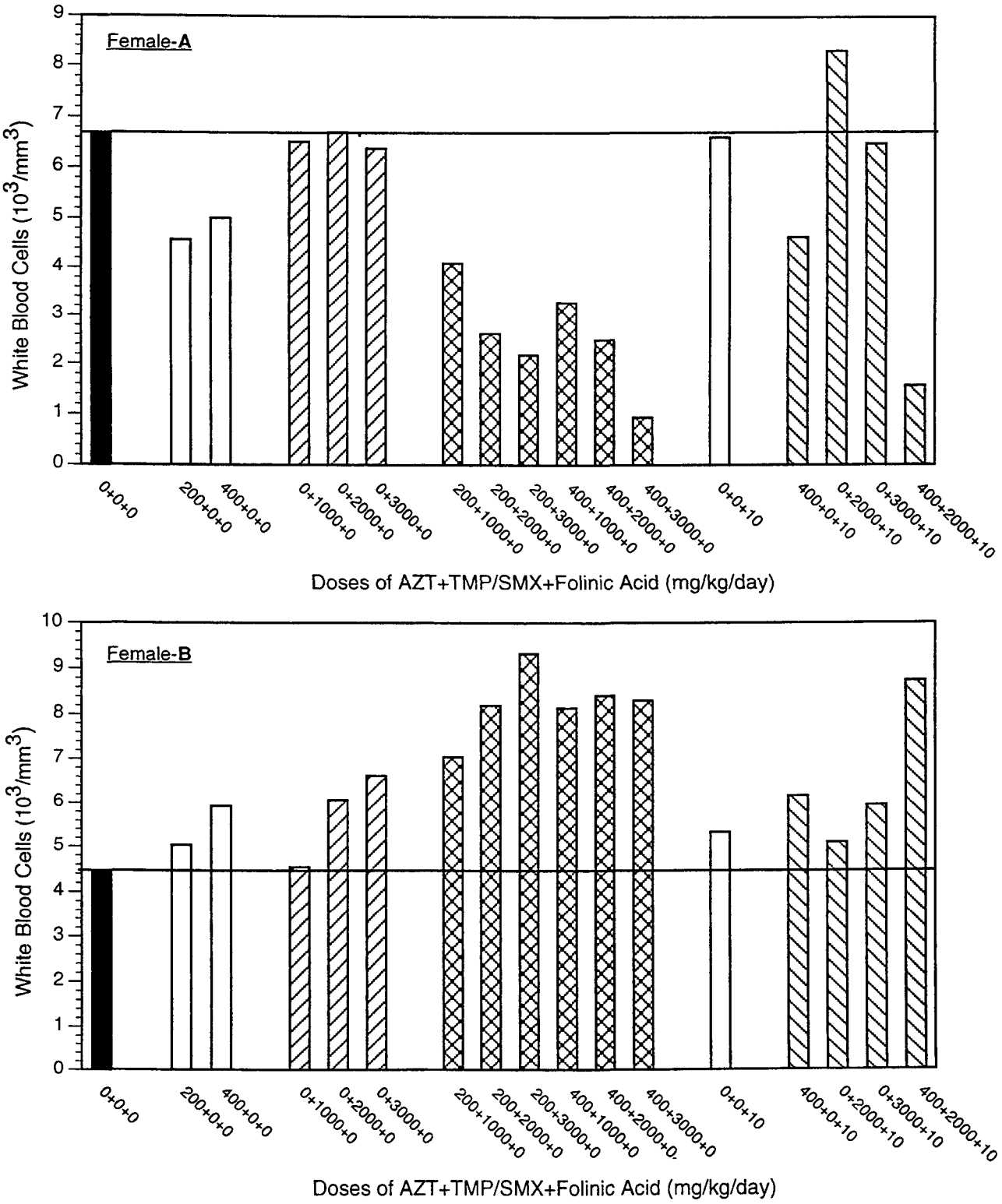


FIGURE 10
Mean Leukocyte Values for Female Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

A distinct dose-related thrombocytosis was evident in female-A mice administered AZT alone. Respective mean platelet counts (Figure 11 and Table A2) for female-A mice receiving 200 or 400 mg/kg AZT alone were approximately 20% (1,325 $\times$ $10^3/\mu L$) or 60% (1,819 $\times$ $10^3/\mu L$; $P \leq 0.01$) higher than the mean platelet count (1,149 $\times$ $10^3/\mu L$) in the control group.

No other biologically significant alterations were observed in any of the other hematology parameters evaluated in female-A mice administered AZT alone.

TMP/SMX Alone

A mild anemia ($P \leq 0.01$) occurred in the female-A mice administered the highest dose of TMP/SMX alone. The mean RBC count (Figure 8 and Table A2) in the female-A mice administered 3,000 mg/kg was approximately 12% (7.64 $\times$ $10^6/\mu L$) lower than the mean RBC count (8.64 $\times$ $10^6/\mu L$) of the control group. Reduced Hgb and Hct values paralleled the decline in the mean RBC counts. Statistically significant alterations were not observed in MCV, MCH, or MCHC values. Significant alterations were not observed in any of the RBC parameters in female-A mice receiving 1,000 or 2,000 mg/kg TMP/SMX.

Significant alterations were not evident in the total WBC count (Figure 10 and Table A2) of female-A mice administered TMP/SMX alone. Evaluation of the corresponding differential data, however, revealed a treatment-related elevation in mean monocyte counts of the two highest dose groups ($P \leq 0.01$). Respective mean monocyte counts of female-A mice receiving 2,000 or 3,000 mg/kg TMP/SMX were approximately 60% (0.27 $\times$ $10^3/\mu L$) and 70% (0.28 $\times$ $10^3/\mu L$) higher than that (0.17 $\times$ $10^3/\mu L$) of the control group. The mean eosinophil count of the 3,000 mg/kg TMP/SMX group was approximately 64% (0.05 $\times$ $10^3/\mu L$; $P \leq 0.05$) lower than the mean (0.14 $\times$ $10^3/\mu L$) of the control group. Significant alterations were not evident in the other differential parameters evaluated.

Folinic Acid Alone

Administration of 10 mg/kg folinic acid to female-A mice did not result in any biologically significant alterations in any of the hematological parameters evaluated during this study.

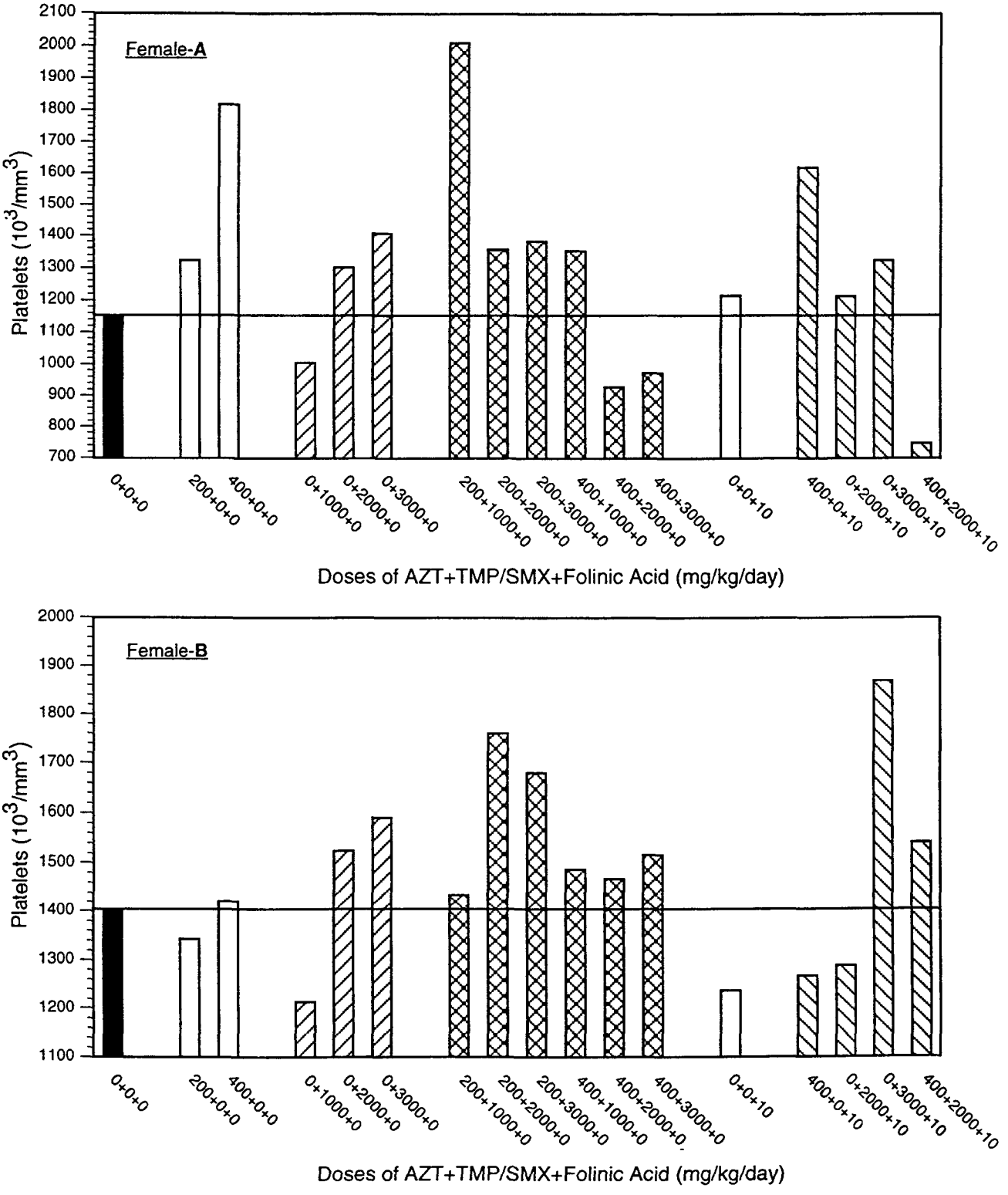


FIGURE 11
Mean Platelet Values for Female Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

AZT and TMP/SMX Combinations

A distinct treatment-related anemia ($P \leq 0.01$) resulted from the administration of AZT and TMP/SMX combinations, and the severity of the anemia was far greater than that observed subsequent to the administration of either compound alone. Respective mean RBC counts (Figure 8 and Table A2) in female-A mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 66% ($2.94 \times 10^6/\mu L$), 71% ($2.49 \times 10^6/\mu L$), or 68% ($2.79 \times 10^6/\mu L$) lower than the mean RBC count ($8.64 \times 10^6/\mu L$) of the control group. Respective mean RBC counts of female-A mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 75% ($2.16 \times 10^6/\mu L$), 72% ($2.40 \times 10^6/\mu L$), or 72% ($2.43 \times 10^6/\mu L$) lower than the mean RBC count in the control group. It should be noted that the groups receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX had, respectively, four and two survivors available for hematology evaluations at the end of the study. The anemia was accompanied by reduced Hgb and Hct values, significant treatment-related declines in MCV and MCH values, and consistent elevations in MCHC values (Table A2), suggestive of a hemolytic process.

A distinct treatment-related reticulocytopenia (Figure 9 and Table A2) occurred in mice receiving the AZT and TMP/SMX combinations. Respective mean reticulocyte values in female-A mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 37% ($1.9 \times 10^5/\mu L$), 63% ($1.1 \times 10^5/\mu L$; $P \leq 0.01$), or 60% ($1.2 \times 10^5/\mu L$) lower than the mean reticulocyte count ($3.0 \times 10^5/\mu L$) of the control group. Respective mean reticulocyte counts for mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 80% ($0.6 \times 10^5/\mu L$; $P \leq 0.01$), 60% ($1.2 \times 10^5/\mu L$; $P \leq 0.01$), or 97% ($0.1 \times 10^5/\mu L$; $P \leq 0.01$) lower than the mean reticulocyte count of the control group.

A statistically significant thrombocytosis was evident only in female-A mice receiving 200 mg/kg AZT + 1,000 mg/kg TMP/SMX. The platelet count (Figure 11 and Table A2) of this treatment group was approximately 80% ($2,010 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean ($1,149 \times 10^3/\mu L$) of the control group.

A prominent treatment-related leukopenia ($P \leq 0.01$) resulted from combination therapy with AZT and TMP/SMX. Respective mean WBC counts (Figure 10 and Table A2) of the female-A mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 39% ($4.09 \times 10^3/\mu L$), 61% ($2.62 \times 10^3/\mu L$), or 67% ($2.18 \times 10^3/\mu L$) lower than the mean WBC count ($6.69 \times 10^3/\mu L$) of the control group. Respective mean WBC counts in female-A mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 51% ($3.26 \times 10^3/\mu L$), 63% ($2.49 \times 10^3/\mu L$), or 86% ($0.94 \times 10^3/\mu L$) lower than the mean WBC count of the control group. Evaluation of the corresponding differential data revealed that granulocytes, as well as mononuclear cells, participated in the leukopenia, with

granulocytopenia being especially prominent. Respective mean segmented neutrophil counts for female-A mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 86% ($0.22 \times 10^3/\mu L$; $P \leq 0.01$), 94% ($0.09 \times 10^3/\mu L$; $P \leq 0.01$), or 88% ($0.18 \times 10^3/\mu L$; $P \leq 0.05$) lower than the mean segmented neutrophil count ($1.56 \times 10^3/\mu L$) of the control group. Respective mean segmented neutrophil counts of groups receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 95% ($0.08 \times 10^3/\mu L$; $P \leq 0.01$), 94% ($0.10 \times 10^3/\mu L$; $P \leq 0.05$), or 99% ($0.02 \times 10^3/\mu L$) lower than the mean segmented neutrophil count of the control group.

The concurrent lymphopenia in mice receiving AZT and TMP/SMX combinations was not as severe as the decreases in neutrophils. Respective mean lymphocyte counts (Table A2) for female-A mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were 23% ($3.70 \times 10^3/\mu L$), 48% ($2.48 \times 10^3/\mu L$; $P \leq 0.01$), or 59% ($1.94 \times 10^3/\mu L$; $P \leq 0.05$) lower than the mean lymphocyte count ($4.79 \times 10^3/\mu L$) of the control group. For the groups receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX, respective mean lymphocyte counts were approximately 35% ($3.13 \times 10^3/\mu L$; $P \leq 0.01$), 51% ($2.33 \times 10^3/\mu L$; $P \leq 0.01$), or 81% ($0.91 \times 10^3/\mu L$; $P \leq 0.05$) lower than the mean lymphocyte count of the control group. Reduced monocyte counts (Table A2) were evident in all of the combination treatment groups. Monocyte counts ranged from 65% ($0.06 \times 10^3/\mu L$; $P \leq 0.01$) lower than the control group ($0.17 \times 10^3/\mu L$) in the lowest AZT and TMP/SMX dose group to 0% in the group receiving the highest AZT and TMP/SMX combination. A similar reduction was evident in the eosinophil counts (Table A2), with a range from 36% ($0.09 \times 10^3/\mu L$) lower than the control group ($0.14 \times 10^3/\mu L$) in the low combination group to 0% in the group receiving the highest doses of AZT and TMP/SMX.

AZT and Folinic Acid Combination

Administration of 10 mg/kg folinic acid in conjunction with 400 mg/kg AZT had no significant impact on any of the hematological alterations caused by 400 mg/kg AZT alone. Alterations in RBC, Hgb, Hct, WBC, MCV, MCH, platelet, segmented neutrophil, and monocyte values were similar in female-A mice receiving AZT alone and AZT + folinic acid.

TMP/SMX and Folinic Acid Combinations

Administration of 10 mg/kg folinic acid in conjunction with 2,000 or 3,000 mg/kg TMP/SMX had no biologically significant impact on any of the hematological alterations induced by the administration of TMP/SMX alone. Mild declines in RBC, Hgb, and Hct values and significant elevations in monocyte counts occurred with remarkable similarity in female-A mice administered TMP/SMX alone and in groups receiving TMP/SMX + folinic acid.

AZT, TMP/SMX, and Folinic Acid Combination

Administration of 10 mg/kg folinic acid to female-A mice receiving the AZT and TMP/SMX combinations did not prevent the anemia, leukopenia, or reticulocytopenia (Figures 8, 9, and 10 and Table A2) induced by the administration of AZT and TMP/SMX. In fact, mice receiving 400 mg/kg AZT + 2,000 mg/kg TMP/SMX + 10 mg/kg folinic acid had a lower RBC count than any other group. The mean RBC count of this group was approximately 77% ($2.00 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count ($8.64 \times 10^6/\mu L$) of the control group. No protection was evident and no biologically significant differences occurred subsequent to the addition of folinic acid.

Female-B Mice

In general, hematological alterations in female-B mice were not as prominent as previously described for female-A mice. Administration of AZT alone did not result in overt anemia. A marginal decline in the RBC count did occur in female-B mice administered 400 mg/kg AZT alone, and elevated MCV and MCH values were evident. TMP/SMX administered alone produced no alterations, and changes did not occur subsequent to the administration of folinic acid alone.

Combination therapy with AZT and TMP/SMX resulted in anemia in the highest dose group, and in contrast to the hematological findings described in male and female-A mice, significant elevations in MCV values were evident. Treatment-related elevations in lymphocyte and monocyte counts were also evident, and MCHC values tended to be lower.

Administration of 10 mg/kg folinic acid appeared to inhibit the reticulocytosis induced by AZT alone but had no significant impact on other hematological parameters. Folinic acid administered in combination with TMP/SMX resulted in only a minor elevation in platelet counts. Folinic acid administration in conjunction with AZT + TMP/SMX produced only minor degrees of protection against the elevated MCV and MCH values. Anemia was not prevented.

AZT Alone

Hematological alterations encountered in female-B mice were considerably less severe than those previously described. Although overt anemia did not occur, a mild decrease in the RBC count (Figure 8 and Table A3) was evident in female mice receiving the highest dose of AZT. The mean RBC count of female-B mice receiving 400 mg/kg AZT was approximately 6% ($7.77 \times 10^6/\mu L$) lower than the mean ($8.31 \times 10^6/\mu L$) of the control group. Significant alterations in Hgb and Hct values were not present. Marginal elevations in MCV and MCH values accompanied the slight decline in the RBC count. The reticulocyte count (Figure 9 and

Table A3) of the 400 mg/kg group was approximately 68% ($7.9 \times 10^5/\mu L$; $P \leq 0.05$) higher than the mean reticulocyte count ($4.7 \times 10^5/\mu L$) of the control group. Significant alterations were not detected in MCHC values, platelet counts (Figure 11 and Table A3), WBC counts (Figure 10 and Table A3), or any of the differential parameters of female-B mice administered AZT alone.

TMP/SMX Alone

Administration of 1,000, 2,000, or 3,000 mg/kg TMP/SMX to female-B mice did not result in any biologically significant alterations in any of the hematological parameters evaluated during this study.

Folinic Acid Alone

Administration of 10 mg/kg folinic acid to female-B mice did not result in any biologically significant alterations in any of the hematological parameters evaluated during this study.

AZT and TMP/SMX Combinations

Administration of 400 mg/kg AZT and 3,000 mg/kg TMP/SMX to female-B mice resulted in a mild anemia. The mean RBC count (Figure 8 and Table A3) of female-B mice receiving 400 mg/kg AZT + 3,000 mg/kg TMP/SMX was approximately 22% ($6.48 \times 10^6/\mu L$; $P \leq 0.01$) lower than the mean RBC count ($8.31 \times 10^6/\mu L$) of the control group. Mild declines in Hgb and Hct values paralleled the diminished RBC counts. The administration of 200 or 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX resulted in a 4% to 11% increase in MCV and MCH values as compared to the controls.

Treatment-related increases in reticulocyte counts ($P \leq 0.01$) correlated with the elevations in MCV values. Respective mean reticulocyte counts (Figure 9 and Table A3) of female-B mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 80% ($8.4 \times 10^5/\mu L$), 90% ($8.8 \times 10^5/\mu L$), or 110% ($10.0 \times 10^5/\mu L$) higher than the mean reticulocyte count ($4.7 \times 10^5/\mu L$) of the control group. Respective mean reticulocyte counts of female-B mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 100% ($9.3 \times 10^5/\mu L$), 150% ($11.8 \times 10^5/\mu L$), or 200% ($14.2 \times 10^5/\mu L$) higher than the mean of the control group.

Treatment-related increases in total WBC counts (Figure 10 and Table A3) were especially prominent in female-B mice receiving combination therapy with AZT and TMP/SMX. Respective mean WBC counts in mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX were approximately 60% ($7.05 \times 10^3/\mu L$; $P \leq 0.05$), 80% ($8.20 \times 10^3/\mu L$; $P \leq 0.01$), or 110% ($9.32 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean WBC count ($4.47 \times 10^3/\mu L$) of the control group. Respective mean WBC counts in female-B mice

receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 80% ($8.14 \times 10^3/\mu L$; $P \leq 0.01$), 90% ($8.42 \times 10^3/\mu L$; $P \leq 0.01$), or 90% ($8.31 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean of the control group. Evaluation of the corresponding differential data revealed that the leukocytosis was predominately mononuclear in origin. Respective mean lymphocyte counts for female-B mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 70% ($5.29 \times 10^3/\mu L$; $P \leq 0.05$), 110% ($6.54 \times 10^3/\mu L$; $P \leq 0.01$), or 140% ($7.61 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean lymphocyte count ($3.19 \times 10^3/\mu L$) of the control group. Respective mean lymphocyte counts for female-B mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 100% ($6.40 \times 10^3/\mu L$; $P \leq 0.01$), 110% ($6.70 \times 10^3/\mu L$; $P \leq 0.01$), or 100% ($6.44 \times 10^3/\mu L$; $P \leq 0.01$) higher than the mean of the control group. Although not always statistically significant, marked elevations in monocyte counts were also evident. Respective mean monocyte counts for female-B mice receiving 200 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 110% ($0.23 \times 10^3/\mu L$; $P \leq 0.01$), 160% ($0.28 \times 10^3/\mu L$; $P \leq 0.05$), or 140% ($0.26 \times 10^3/\mu L$) higher than the mean ($0.11 \times 10^3/\mu L$) of the control group. Respective mean monocyte counts in female-B mice receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX levels were approximately 110% ($0.23 \times 10^3/\mu L$; $P \leq 0.01$), 100% (0.22×10 /μL), or 150% ($0.27 \times 10^3/\mu L$; $P \leq 0.05$) higher than the mean monocyte count of the control group. Biologically significant alterations were not observed in the other differential parameters.

AZT and Folinic Acid Combination

Administration of 10 mg/kg folinic acid to female-B mice in combination with 400 mg/kg AZT had no significant impact on the RBC alterations induced by the administration of 400 mg/kg AZT alone. Both treatment groups had statistically significant elevations in MCV and MCH values of a similar severity. Significant differences did occur, however, in reticulocyte counts (Figure 9 and Table A3). Mice administered AZT alone had a mild statistically significant elevation in the reticulocyte count, whereas mice receiving AZT and folinic acid had a reticulocyte count similar to that of the controls.

TMP/SMX and Folinic Acid Combinations

Administration of 10 mg/kg folinic acid in combination with 2,000 or 3,000 mg/kg TMP/SMX had no impact on RBC, WBC, or reticulocyte parameters. A mild elevation in the platelet count (Figure 11 and Table A3) was observed in mice receiving 3,000 mg/kg TMP/SMX + 10 mg/kg folinic acid. The mean platelet count of this treatment group was approximately 33% ($1,870 \times 10^3/\mu L$; $P \leq 0.05$) higher than the mean ($1,403 \times 10^3/\mu L$) of the control group.

AZT, TMP/SMX, and Folinic Acid Combination

Administration of 400 mg/kg AZT + 2,000 mg/kg TMP/SMX + 10 mg/kg folinic acid did not significantly alter the mild anemia, reticulocytosis, or lymphocytosis that occurred in mice administered only AZT and TMP/SMX. Although values were slightly elevated, statistically significant increases in MCV and MCH levels were not evident in mice receiving AZT + TMP/SMX + folinic acid. A statistically significant increase in these parameters did occur in female-B mice administered AZT and TMP/SMX alone. The differences in the MCV and MCH values between the two treatment groups was minor.

Clinical Chemistry

Male Mice

AZT Alone

Administration of 200 or 400 mg/kg produced no biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A1).

TMP/SMX Alone

Administration of 1,000 or 2,000 mg/kg TMP/SMX resulted in no biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A1). However, the mean alanine aminotransferase (ALT) activity for male mice receiving 3,000 mg/kg TMP/SMX was 100 IU/L as compared to 21 IU/L for the control group. This significant ($P \leq 0.01$) increase is suggestive of hepatotoxicity. The statistically significant decline in alkaline phosphatase (ALP) activity was not considered to be biologically significant.

Folinic Acid Alone

Administration of 10 mg/kg folinic acid resulted in no biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A1).

AZT and TMP/SMX Combinations

Biologically significant alterations in clinical chemistry parameters were limited to the two treatment groups receiving AZT in combination with 3,000 mg/kg TMP/SMX. Respective mean creatinine levels (Tables 7 and A1) for male mice receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX were 0.68 mg/dL ($P \leq 0.01$) or 0.61 mg/dL ($P \leq 0.01$) compared to the mean creatinine level (0.26 mg/dL) of the control group. Corresponding elevations in blood urea nitrogen (BUN) values (Table A1) were not evident. Although not statistically significant, minor elevations in ALT activity (Tables 7 and A1) in the same treatment groups were suggestive of hepatotoxicity. Respective mean ALT values in male mice receiving 200 or 400 mg/kg

TABLE 7
Summary of Principal Alterations in Clinical Chemistry Parameters for the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in in Swiss (CD-1®) Mice

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
AZT alone	—	—	—
TMP/SMX alone	ALT elevations	ALT elevations, mild increases in creatinine	—
Folinic acid alone	—	—	—
AZT + folinic acid	—	—	—
TMP/SMX + folinic acid	ALT elevations	Mild increases in creatinine	—
AZT + TMP/SMX	ALT elevations, mild increases in creatinine	Mild increases in creatinine	—
AZT + TMP/SMX + folinic acid	—	Mild increase in creatinine	—

AZT + 3,000 mg/kg TMP/SMX were 51 IU/L or 42 IU/L compared to the mean ALT value (21 IU/L) of the control group. Minor statistically significant declines in ALP activity were not considered to be biologically significant.

AZT and Folinic Acid Combination

Administration of 10 mg/kg folinic acid in combination with 400 mg/kg AZT resulted in no significant alterations in any of the clinical chemistry parameters (Tables 7 and A1) evaluated during this study.

TMP/SMX and Folinic Acid Combinations

Administration of 10 mg/kg folinic acid in combination with 2,000 mg/kg TMP/SMX resulted in no significant alterations. A minor elevation in ALT activity ($P \leq 0.05$) was observed in male mice receiving 3,000 mg/kg TMP/SMX + 10 mg/kg folinic acid (Tables 7 and A1). The mean ALT value of this group was 53 IU/L compared to the mean (21 IU/L) of the control group. This minor elevation was compatible with a minor degree of hepatotoxicity and was of a lesser severity than in mice administered 3,000 mg/kg TMP/SMX alone.

AZT, TMP/SMX, and Folinic Acid Combination

Male mice receiving 400 mg/kg AZT + 2,000 mg/kg TMP/SMX + 10 mg/kg folinic acid had no biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A1).

Female-A Mice

AZT Alone

Administration of 200 or 400 mg/kg AZT to female-A mice resulted in no biologically significant elevations in any of the clinical chemistry parameters evaluated. Minor statistically significant declines encountered in ALT and aspartate aminotransferase (AST) activities (Table A2) were considered to be biologically insignificant.

TMP/SMX Alone

Administration of 1,000 mg/kg TMP/SMX alone resulted in no significant elevations in any of the clinical chemistry parameters evaluated (Table A2), whereas 2,000 or 3,000 mg/kg TMP/SMX alone produced minor statistically significant elevations in creatinine concentrations and ALT activities (Tables 7 and A2). Respective mean creatinine concentrations for female-A mice receiving 2,000 or 3,000 mg/kg TMP/SMX were 0.53 mg/dL ($P \leq 0.01$) or 0.52 mg/dL ($P \leq 0.05$) compared to the mean creatinine concentration (0.37 mg/dL) of the control group. Corresponding elevations in BUN values did not occur. Respective mean ALT activities for female-A mice receiving 2,000 or 3,000 mg/kg TMP/SMX were 44 IU/L ($P \leq 0.01$) or 66 IU/L ($P \leq 0.01$), compared to the mean ALT activity (22 IU/L) of the control group. A minor statistically significant decline in AST activity of the 1,000 mg/kg TMP/SMX group was not considered to be biologically significant.

Folinic Acid Alone

Administration of 10 mg/kg folinic acid to female-A mice did not result in biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A2).

AZT and TMP/SMX Combinations

Administration of 200 or 400 mg/kg AZT + 1,000 mg/kg TMP/SMX did not result in biologically significant elevations in any of the clinical chemistry parameters evaluated (Table A2). Minor statistically significant declines in AST activity were considered to have no biological importance.

Minor elevations in BUN and serum creatinine values were evident in female-A mice receiving AZT and 2,000 mg/kg TMP/SMX. Respective mean creatinine levels of female-A mice receiving 200 or 400 mg/kg AZT + 2,000 mg/kg TMP/SMX were 0.56 mg/dL ($P \leq 0.01$) or 0.55 mg/dL ($P \leq 0.05$), compared to the mean (0.37 mg/dL) of the control group. Respective mean BUN values for the same treatment groups were

24.1 mg/dL or 25.4 mg/dL, compared to the mean BUN value (15.9 mg/dL) of the control group. Minor statistically significant declines in ALP activity observed in the same treatment groups were not considered to be biologically relevant. Statistically significant alterations in mice receiving AZT and 3,000 mg/kg TMP/SMX were considered to be irrelevant because of the limited number of survivors.

AZT and Folinic Acid Combination

Biologically significant alterations were not detected in any of the clinical chemistry parameters (Tables 7 and A2) evaluated in female-A mice receiving 400 mg/kg AZT + 10 mg/kg folinic acid. Statistically significant alterations in ALP and AST activities were not considered to be biologically significant.

TMP/SMX and Folinic Acid Combinations

Female-A mice receiving 3,000 mg/kg TMP/SMX + 10 mg/kg folinic acid had a minor significant elevation in the serum creatinine concentration ($P \leq 0.01$). The mean creatinine concentration was 0.56 mg/dL, compared to the mean (0.37 mg/dL) of the control group. A corresponding elevation in BUN was not evident (Tables 7 and A2).

AZT, TMP/SMX, and Folinic Acid Combination

Female-A mice receiving 400 mg/kg AZT + 2,000 mg/kg TMP/SMX + 10 mg/kg folinic acid had a minor elevation in the creatinine concentration ($P \leq 0.01$). Creatinine concentration of this group was 0.64 mg/dL, compared to the mean (0.37 mg/dL) of the control group. The corresponding BUN value was 24.0 mg/dL, compared to the mean (15.9 mg/dL) of the control group. A statistically significant decline in ALP activity was not considered to be biologically significant (Tables 7 and A2).

Female-B Mice

AZT Alone

Female-B mice receiving 200 or 400 mg/kg AZT had no biologically significant alterations in any of the clinical chemistry parameters evaluated during this study (Tables 7 and A3).

TMP/SMX Alone

Administration of 1,000, 2,000, or 3,000 mg/kg TMP/SMX alone resulted in no biologically significant alterations in any of the parameters evaluated (Table 7). Statistically significant declines in BUN values have no known biological relevance (Table A3).

Folinic Acid Alone

Female-B mice receiving 10 mg/kg folinic acid had no biologically significant alterations in any of the parameters evaluated (Tables 7 and A3).

AZT and TMP/SMX Combinations

Female-B mice receiving AZT and TMP/SMX combinations had no biologically significant alterations in any of the clinical chemistry parameters evaluated in this study (Tables 7 and A3). A statistically significant increase in the ALT activity of the group receiving 200 mg/kg AZT + 1,000 mg/kg TMP/SMX was not considered to be biologically significant because a dose-related pattern was not evident. Decreased BUN and creatinine values observed in many treatment groups were not considered to be biologically significant.

AZT and Folinic Acid Combination

Biologically significant alterations did not occur in any of the chemistry parameters evaluated in mice receiving 400 mg/kg AZT + 10 mg/kg folinic acid (Tables 7 and A3).

TMP/SMX and Folinic Acid Combinations

Female-B mice receiving combinations of TMP/SMX and folinic acid had no biologically significant alterations in clinical chemistry parameters (Tables 7 and A3). Lower BUN values of the group receiving 3,000 mg/kg TMP/SMX + 10 mg/kg folinic acid were considered to be within a normal range.

AZT, TMP/SMX, and Folinic Acid Combination

Administration of 400 mg/kg AZT + 2,000 mg/kg TMP/SMX + 10 mg/kg folinic acid did not result in any biologically significant alterations in any of the clinical chemistry parameters evaluated (Tables 7 and A3). Lower BUN and creatinine values have no known biological significance.

NECROPSY OBSERVATIONS

In general, there was good correlation between gross and microscopic lesions. The presence or increased incidence of gross lesions probably due to administration of one or more of the test chemicals was observed in the spleen, thymus, and thyroid gland. These lesions included enlarged or small spleen, small thymus, and enlarged thyroid gland.

Spleen

Gross lesions of the spleen were observed in a total of only 4 of the 170 male mice in the study. The incidences of gross splenic lesions in males were too low to be considered meaningful.

Among the female-B groups receiving AZT, there was a dose-related increase in the incidences of enlarged spleens. Among the female-A groups, very few mice (a total of 6 of 340 mice) showed splenic enlargement. Considering all mice in all groups together, a total of 57 mice showed splenic enlargement at necropsy, and of those 57 mice, 48 had been receiving AZT. Within the female-B groups, the highest incidence of splenic enlargement occurred in the groups receiving 400 mg/kg AZT + 2,000 or 3,000 mg/kg TMP/SMX. TMP/SMX given alone had little if any meaningful effect on the incidence of splenic enlargement. Folinic acid appeared to have no meaningful effect on the occurrence of splenic enlargement.

Small spleens were observed in some mice receiving TMP/SMX, with or without additional treatment of AZT. Almost all of the mice with small spleens were in the female-A group except one mouse in each of the two male groups receiving 200 or 400 mg/kg AZT + 3,000 mg/kg TMP/SMX. Treatment with AZT alone did not result in small spleens in any mouse group. There was no consistent effect of folinic acid on the occurrence of small spleens.

Thymus

Small thymuses were observed in mice in many of the treatment groups. Corresponding with the duration of treatment, small thymuses were most frequent among the female-A mice, less frequent among males, and least frequent among female-B mice. AZT given alone at either dose had no apparent effect on thymus size in any group. In every group of the female-A mice receiving TMP/SMX, small thymuses in some mice were observed. In the female-A group, administration of 200 or 400 mg/kg AZT + 2,000 or 3,000 mg/kg TMP/SMX caused increased incidences of small thymuses, as compared to mice dosed only with TMP/SMX at the respective doses. Among males, trends seen in response to treatment with the test compounds were similar to those seen in female-A mice, but the effects in males were generally less pronounced and less consistent. Small thymuses were observed among dosed female-B mice at incidences no greater than that observed in the control group.

Folinic acid administration had no consistent effect on the incidence of small thymuses.

Thyroid Gland

Thyroid glands of many mice receiving TMP/SMX were observed to be enlarged at necropsy (Plate 1). In the two mice not receiving TMP/SMX in which thyroid gland enlargement was reported at necropsy, no microscopic correlate (such as hyperplasia of the follicular epithelium) was found on microscopic examination of the thyroid gland. Between the 1,000 and 2,000 mg/kg TMP/SMX groups, there was a dose-related effect on the incidence of thyroid gland enlargement; the maximum effect on the incidence of thyroid gland enlargement was reached at 2,000 mg/kg TMP/SMX. There was no evidence that administration of either folinic acid or AZT modified the effect of TMP/SMX in causing thyroid gland enlargement, except where treatment with some of the higher-dose combinations of AZT + TMP/SMX apparently contributed to a reduced incidence of thyroid gland enlargement by causing death before thyroid gland enlargement had time to develop.

HISTOPATHOLOGIC OBSERVATIONS

Microscopic examinations of compound-related lesions are summarized in Table 8 and shown in Plates 1 through 13. Suspected or actual target organs for microscopic lesions caused by the test chemicals included the bone marrow, liver, spleen, thymus, and thyroid gland.

Among female mouse groups, histopathologic examination of the bone marrow and liver was done only in some females that died early. However, the bone marrow and liver were routinely examined in all male mice. Therefore, the histopathologic effects of compound treatment on bone marrow and liver were more readily assessed in males than in females. The spleen, thymus, and thyroid gland were routinely examined in all male and female groups, irrespective of the time of death.

Bone Marrow Lesions

Some cellular depletion (depletion of hematopoietic cells) of bone marrow was observed in all male groups receiving AZT and/or TMP/SMX (Table 9). Criteria for severity grades of bone marrow lesions are as follows:

Minimal severity - Depletion of approximately 5% or less of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region when available.

Mild severity - Depletion of approximately 6% to 20% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available.

Moderate severity - Depletion of approximately 21% to 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available.

PLATE 1
Thyroid glands of two female Swiss (CD-1®) mice. Enlarged thyroid gland at left is from a female-A mouse given 2,000 mg TMP/SMX per kg body weight per day for 5 weeks by gavage. Normal thyroid gland at right is from a control female. H&E; 3.35×

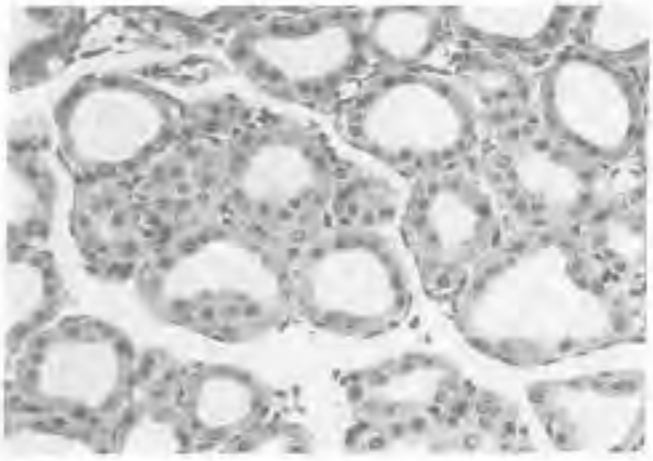


PLATE 2
Thyroid gland of a female-A Swiss (CD-1®) mouse given 2,000 mg TMP/SMX per kg body weight per day for 5 weeks by gavage showing diffuse hyperplasia of follicular epithelium. H&E; 220× (same animal as pictured at left in Plate 1)

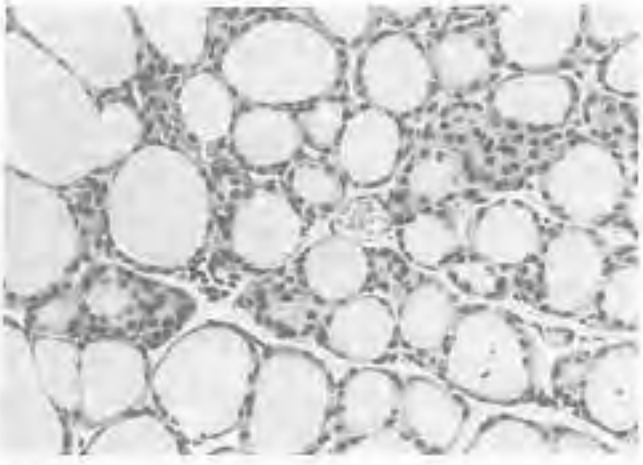


PLATE 3
Thyroid gland of a control female Swiss (CD-1®) mouse showing no lesions. H&E; 220× (same animal as pictured at right in Plate 1)

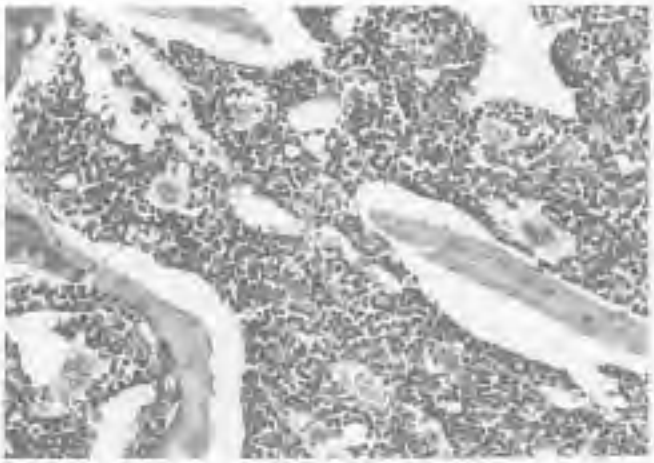


PLATE 4
Bone marrow of a control male Swiss (CD-1®) mouse showing no lesions. H&E; 220×

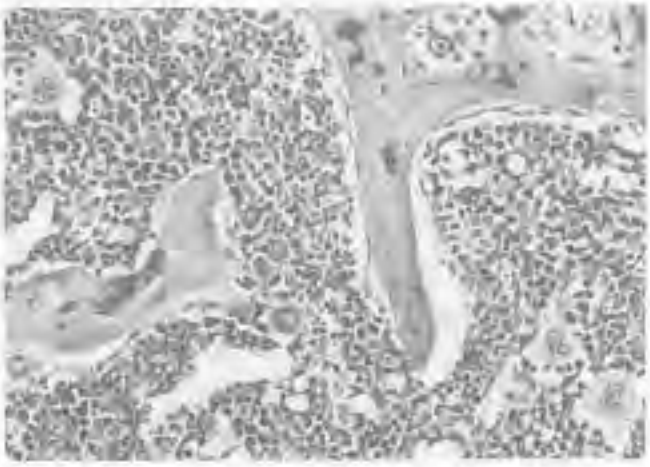


PLATE 5
Bone marrow of a male Swiss (CD-1®) mouse given 400 mg AZT/kg body weight per day for 3 weeks by gavage showing cellular depletion. H&E; 220×

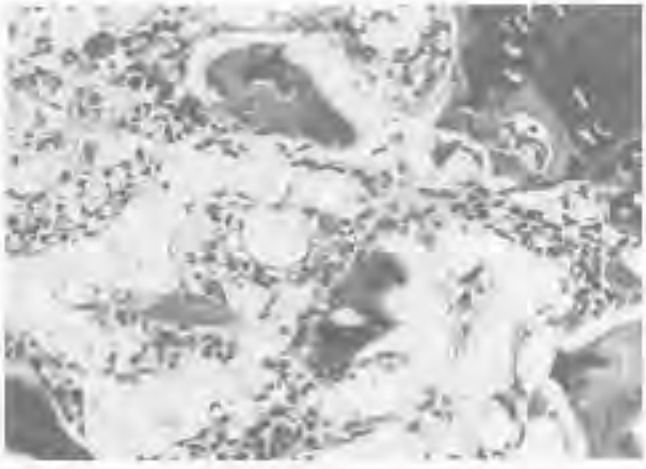


PLATE 6
Bone marrow of a male Swiss (CD-1®) mouse given 400 mg AZT and 3,000 mg TMP/SMX per kg body weight per day for 3 weeks by gavage showing cellular depletion (advanced). H&E; 220×

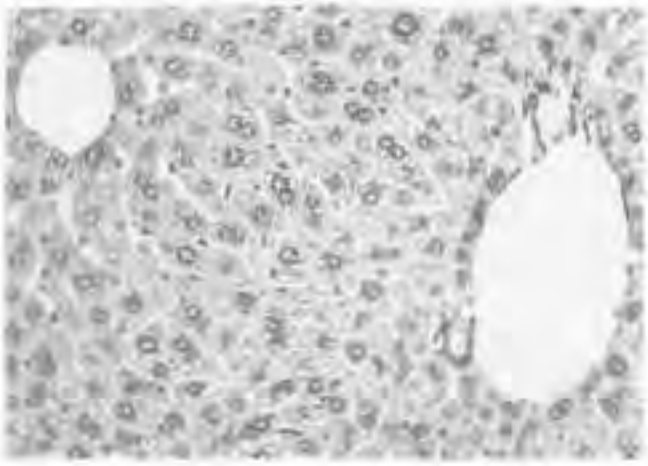


PLATE 7
Liver of a control male Swiss (CD-1®) mouse showing no lesions. H&E; 220×

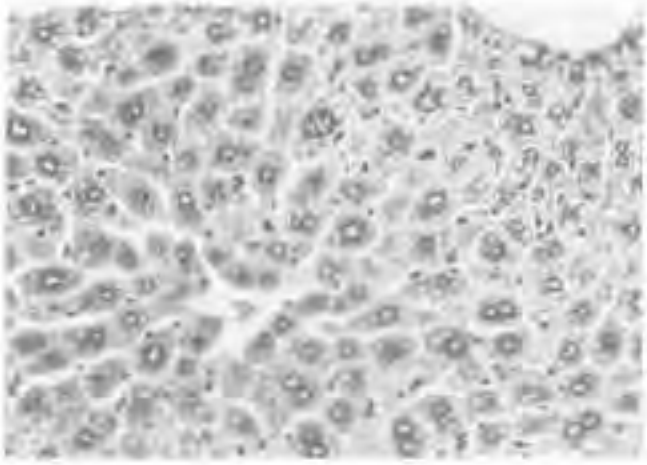


PLATE 8
Liver of a male Swiss (CD-1®) mouse given 400 mg AZT/kg body weight per day for 3 weeks by gavage showing centrilobular hypertrophy of hepatocytes. H&E; 220×

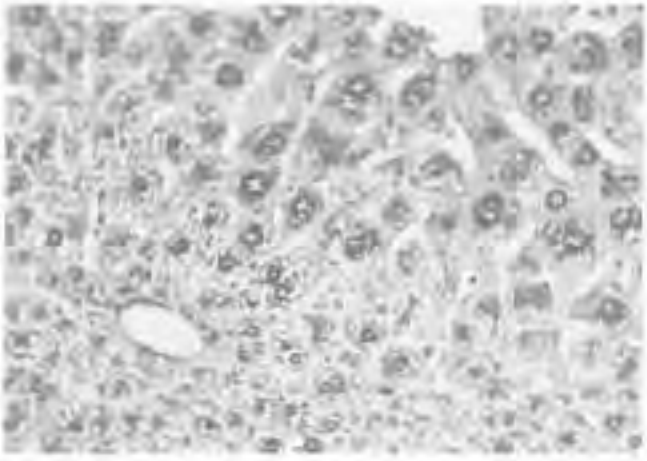


PLATE 9
Liver of a male Swiss (CD-1®) mouse given 400 mg AZT and 3,000 mg TMP/SMX per kg body weight per day for 3 weeks by gavage showing centrilobular hypertrophy of hepatocytes. H&E; 220×

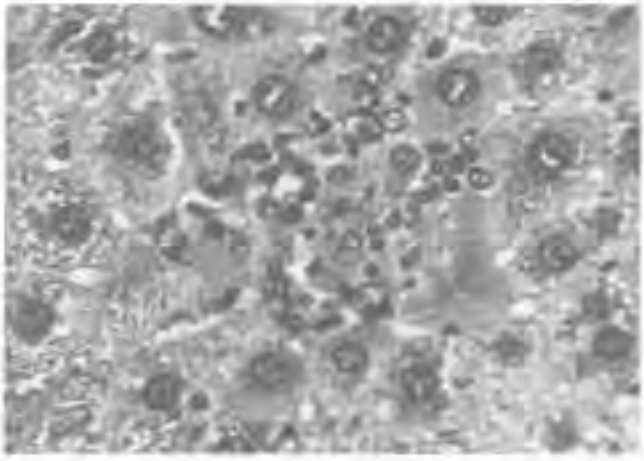


PLATE 10
Liver of a male Swiss (CD-1®) mouse given 3,000 mg TMP/SMX per kg body weight per day for 3 weeks by gavage showing focal necrosis of hepatocytes. H&E; 440×

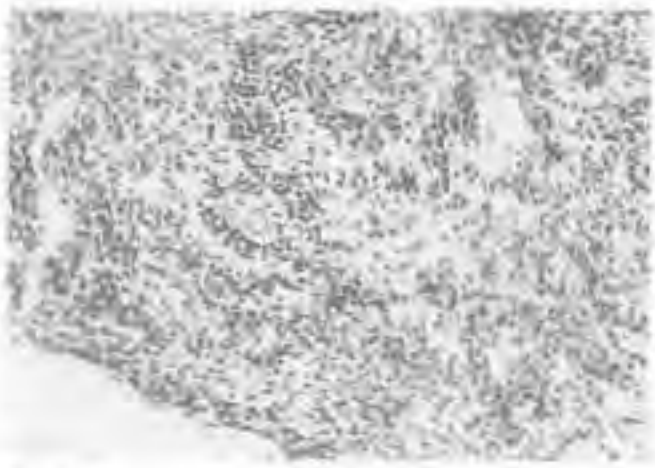


PLATE 11
Spleen of a control male Swiss (CD-1®) mouse showing no lesions. H&E; 220×.

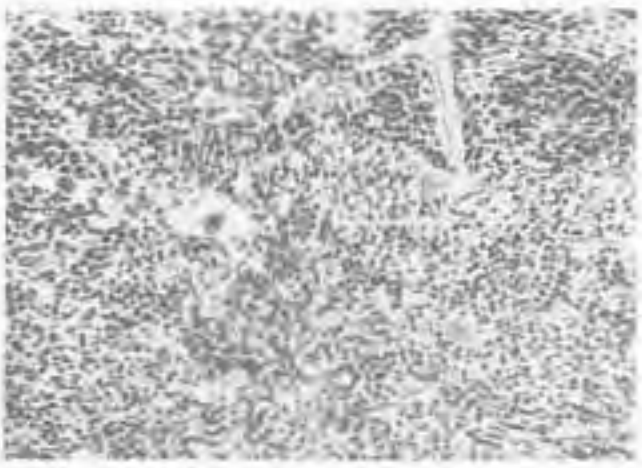


PLATE 12
Spleen of a male Swiss (CD-1®) mouse given 400 mg AZT/kg body weight per day for 3 weeks by gavage showing increased hematopoietic cell proliferation. H&E; 220×.

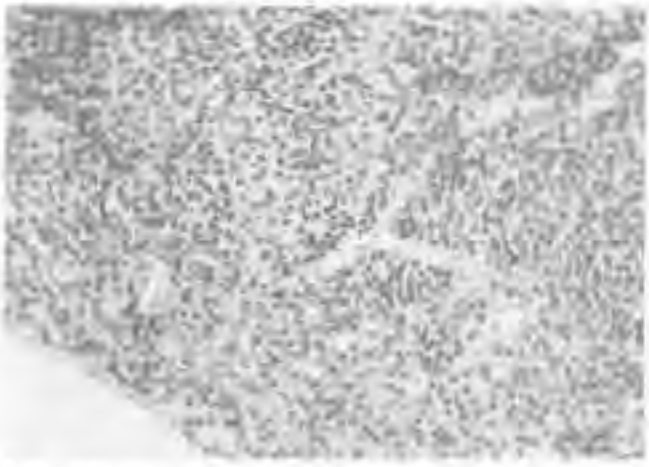


PLATE 13
Spleen of a male Swiss (CD-1®) mouse given 400 mg AZT and 3,000 mg TMP/SMX per kg body weight per day for 3 weeks by gavage showing decreased hematopoietic cell proliferation. H&E; 220×.

PLATE 14
Photograph of cleft palate (right) of a Swiss (CD-1®) pup given 400 mg AZT and 3,000 mg TMP/SMX and 10 mg folinic acid per kg body weight per day by gavage. Normal palate (left) of control pup shown for comparison.

TABLE 8
Summary of Compound-Related Lesions (Gross and Microscopic) in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Tissue	Magnification	Plate Number	Compound-Related Lesions
0 + 2,000 + 0, 0 + 0 + 0	Thyroid gland of both mice	Gross photograph (3 35×)	1	Thyroid of DF169 (on left) is enlarged; thyroid of VF91 (on right) is normal
0 + 2,000 + 0	Thyroid gland	220×	2	Hyperplasia, diffuse, follicle, epithelium
0 + 0 + 0	Thyroid gland	220×	3	None
0 + 0 + 0	Bone marrow	220×	4	None
400 + 0 + 0	Bone marrow	220×	5	Cellular depletion
400 + 3,000 + 0	Bone marrow	220×	6	Cellular depletion (advanced)
0 + 0 + 0	Liver	220×	7	None
400 + 0 + 0	Liver	220×	8	Hypertrophy, hepatocyte, centrilobular
400 + 3,000 + 0	Liver	220×	9	Hypertrophy, hepatocyte, centrilobular
0 + 3,000 + 0	Liver	440×	10	Necrosis, focal, hepatocyte
0 + 0 + 0	Spleen	220×	11	None
400 + 0 + 0	Spleen	220×	12	Increased hematopoietic cell proliferation
400 + 3,000 + 0	Spleen	220×	13	Decreased hematopoietic cell proliferation

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)

Marked severity - Depletion of more than 50% of the hematopoietic cells of the bone marrow, based on examination of the metaphyseal region where available.

Cellular depletion of bone marrow occurred in 100% of male mice receiving AZT, regardless of any other test compound administered along with AZT. The highest dose of 400 mg/kg AZT resulted in a greater mean severity of cellular depletion of bone marrow, as compared to the effect of the lowest dose of 200 mg/kg AZT.

In males, TMP/SMX caused dose-related increases in the incidence and severity of cellular depletion of bone marrow, and these increases were of lesser magnitude than those in male mice receiving only AZT. Treatment of males with both AZT and TMP/SMX had a much greater effect on the severity of cellular depletion of bone marrow as compared to the effect of AZT or TMP/SMX alone.

TABLE 9
Incidence and Mean Severity of Cellular Depletion of Bone Marrow in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Group Incidence	Mean Severity[b]
0 + 0 + 0	0/10	—
0 + 0 + 10	0/10	—
200 + 0 + 0	10/10	1.6
400 + 0 + 0	10/10	2.0
400 + 0 + 10	10/10	1.4
0 + 1,000 + 0	4/10	0.4
0 + 2,000 + 0	6/10	0.7
0 + 2,000 + 10	7/10	0.8
0 + 3,000 + 0	7/10	1.0
0 + 3,000 + 10	7/10	0.7
200 + 1,000 + 0	10/10	2.2
400 + 1,000 + 0	10/10	2.8
200 + 2,000 + 0	10/10	2.0
400 + 2,000 + 0	10/10	2.8
400 + 2,000 + 10	10/10	3.4
200 + 3,000 + 0	10/10	2.7
400 + 3,000 + 0	9/9	3.6

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of. 1=minimal, 2=mild, 3=moderate, 4=marked

Regression analysis of mean severity grades for bone marrow depletion in male mice indicated no interaction effect (Figure 12) between AZT and TMP/SMX. The dose response, as indicated by similar positive slopes (+0.004 to +0.006), appears to be the result of an additive effect of AZT and TMP/SMX.

In the female-A group, 400 mg/kg AZT + 3,000 mg/kg TMP/SMX caused marked severity (mean severity of 4.0) of cellular depletion of bone marrow. Treatment with folinic acid had no discernible histopathologic effect on bone marrow in males or females.

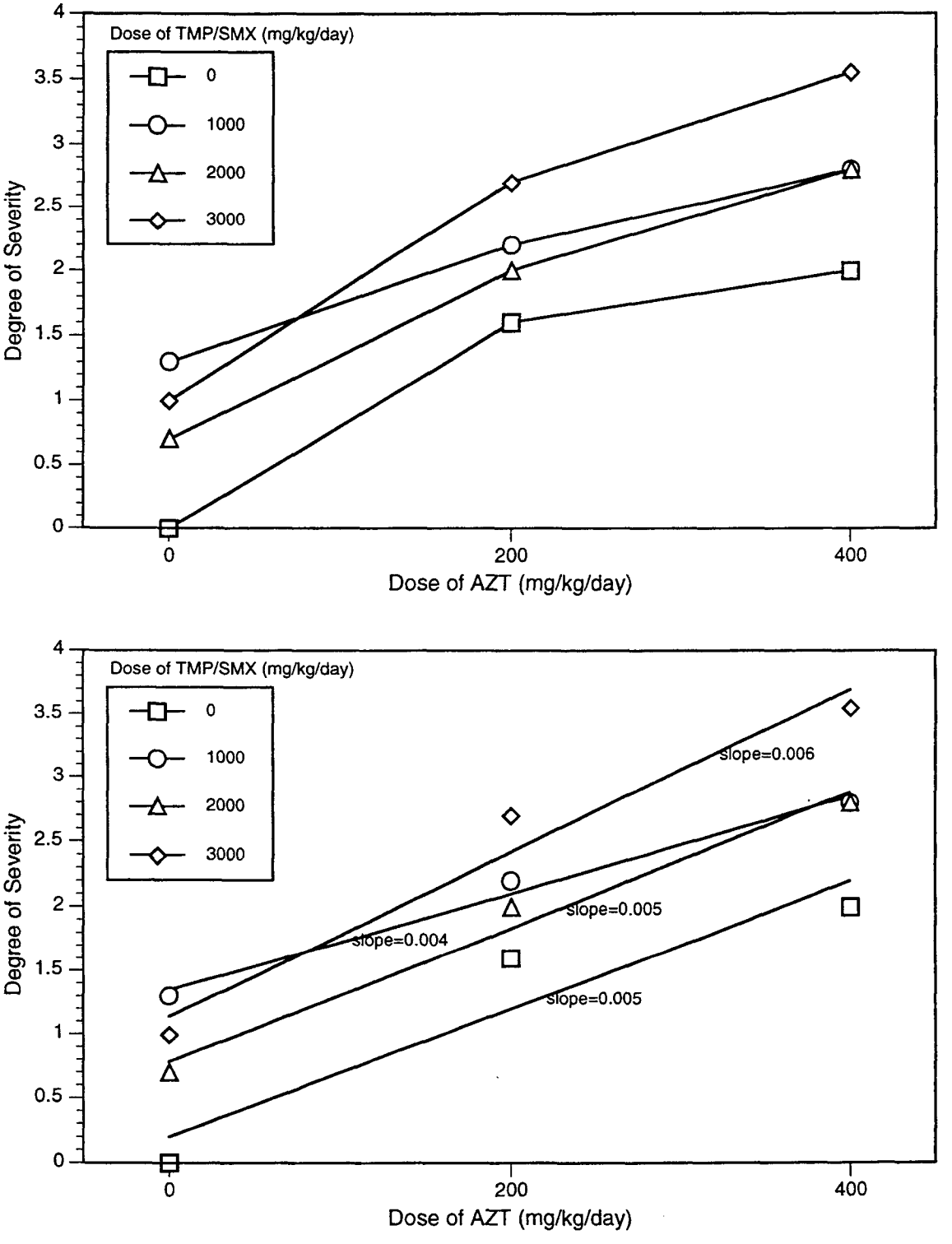


FIGURE 12
Severity of Cellular Depletion of Bone Marrow versus Dose for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

Liver Lesions

The principal test compound-related lesion of the liver was hypertrophy of hepatocytes in the centrilobular areas (Table 10). The diagnosis of hypertrophy was applied to livers in which most of the centrilobular hepatocytes were larger, with larger nuclei, as compared to hepatocytes in the periportal areas. Criteria for severity grades for hepatocyte hypertrophy were as follows:

Minimal severity - Average size of hepatocytes and hepatocyte nuclei are typically larger (estimated 1½ times average diameter) in centrilobular regions as compared to hepatocytes in other parts of the liver lobules. Staining intensity of hepatocytes is usually similar throughout liver lobule. Homogeneity of cytoplasm (degree of cytoplasmic vacuolization) of hepatocytes in centrilobular regions may differ in comparison with hepatocytes in other parts of the liver lobules.

Mild severity - Average size of hepatocytes and hepatocyte nuclei are typically larger (about 1½ to 2 times average diameter) in centrilobular regions as compared to hepatocytes in other parts of the liver lobules. Staining intensity of hepatocytes is usually similar throughout liver lobule. Homogeneity of cytoplasm (degree of cytoplasmic vacuolization) may differ in comparison with hepatocytes in other parts of the liver lobules.

Moderate severity - Average size of hepatocytes and hepatocyte nuclei are typically much larger (about 2 times average diameter or greater) in centrilobular regions as compared to hepatocytes in other parts of the liver lobules. Also, hepatocytes in centrilobular regions usually show distinct contrasts in homogeneity of cytoplasm (degree of cytoplasmic vacuolization) and/or staining intensity of cytoplasm as compared to hepatocytes elsewhere in liver lobules.

Relatively larger hepatocytes in the centrilobular areas, as compared to the periportal areas of the same liver, may be seen in toxicity studies in some normal control mice. This change was diagnosed in one of the control mice in this study. In all male groups receiving AZT and TMP/SMX, there were increased incidences and mean severity of hepatocyte hypertrophy in comparison with the control group, and those effects tended to be dose related. There was also a dose related increase in the incidence and mean severity of hepatocyte hypertrophy in male mice administered TMP/SMX alone.

Treatment with AZT alone also caused a dose-related increase in hepatocyte hypertrophy, which was of a lesser severity than that caused by treatment with TMP/SMX alone.

Male groups receiving various combinations of AZT and TMP/SMX had increased incidences and severity of hepatocyte hypertrophy when compared to the control group. Statistical analysis was performed on the mean severity grades (Table 11 and Figure 13) to determine if interaction occurred when the compounds were administered in combination.

TABLE 10
Incidence and Mean Severity of Hepatocyte Hypertrophy in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Group Incidence	Mean Severity[b]
0 + 0 + 0	1/10	0 1
0 + 0 + 10	1/10	0 1
200 + 0 + 0	3/10	0 4
400 + 0 + 0	6/10	1 0
400 + 0 + 10	4/10	0 7
0 + 1,000 + 0	4/10	0 8
0 + 2,000 + 0	8/10	1 2
0 + 2,000 + 10	8/10	1 7
0 + 3,000 + 0	9/10	2 5
0 + 3,000 + 10	10/10	2 3
200 + 1,000 + 0	4/10	0 6
400 + 1,000 + 0	5/10	1 2
200 + 2,000 + 0	10/10	2 4
400 + 2,000 + 0	10/10	2 0
400 + 2,000 + 10	7/10	1 1
200 + 3,000 + 0	10/10	2 0
400 + 3,000 + 0	8/10	2 0

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 11
Statistical Analysis of Mean Severity of Hepatocyte Hypertrophy in Male Mice Administered Combinations of AZT and TMP/SMX in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice[a]

AZT (mg/kg)	Number	TMP/SMX (mg/kg)			
		0	1,000	2,000	3,000
0	10	0.10 ± 0.10	0.80 ± 0.33	1.20 ± 0.29**	2.50 ± 0.31**
200	10	0.40 ± 0.22	0.70 ± 0.30	2.40 ± 0.22**	2.00 ± 0.00**
400	10	1.00 ± 0.30	1.20 ± 0.42	2.00 ± 0.15*	2.00 ± 0.37*

* Significantly different from the control group ($P \leq 0.05$) by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Mean severity is based on numerical scale of: 1=minimal, 2=mild, 3=moderate, 4=marked

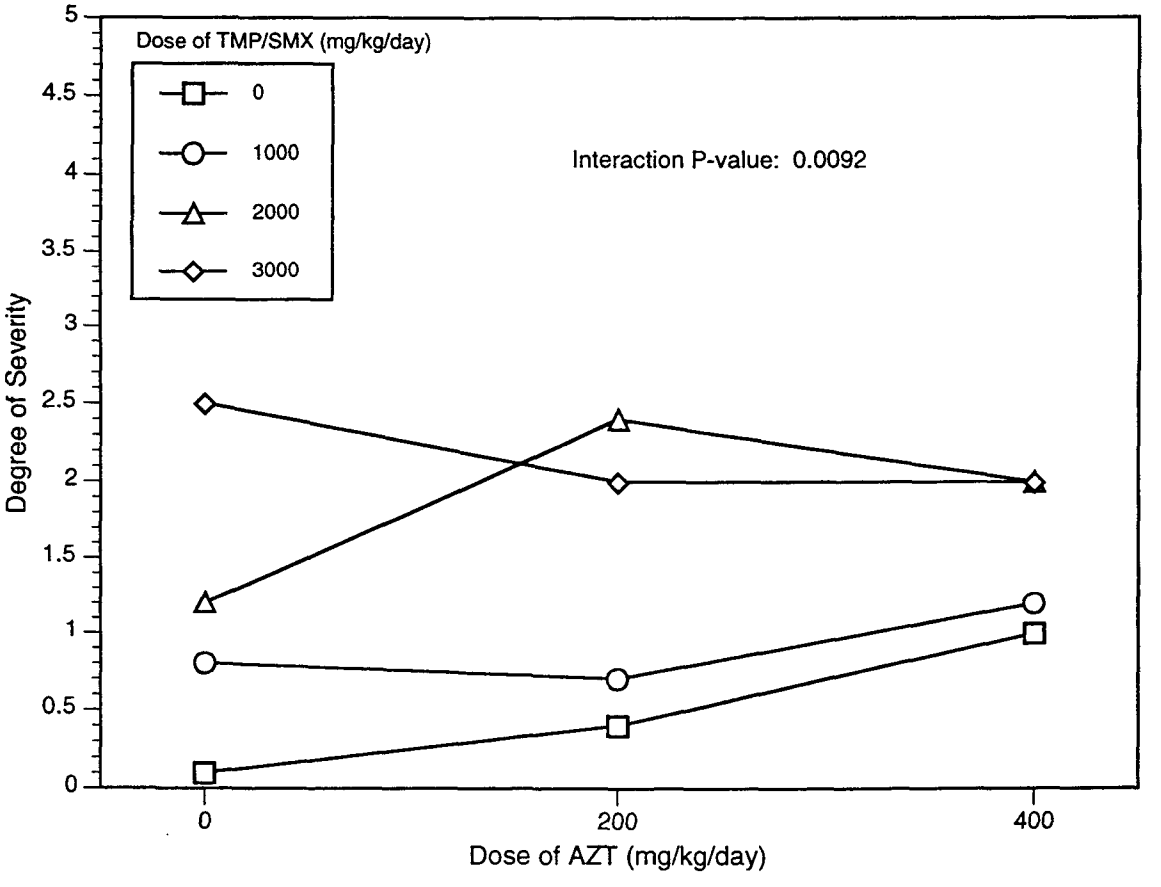


FIGURE 13
Severity of Hypertrophy of Centrilobular Hepatocytes versus Dose for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

Administration of AZT alone resulted in a minor degree of hepatocyte hypertrophy that was not statistically significant. Administration of 2,000 or 3,000 mg/kg TMP/SMX alone resulted in significant ($P \leq 0.01$) hepatocyte hypertrophy. When administered in combination, interaction occurred, as AZT appeared to diminish the effects of TMP/SMX, especially in groups receiving the highest combinations.

Administration of folinic acid appeared to offer some protection against the effect of AZT in the liver. The mean severity grade for hepatocyte hypertrophy was lowest in the group receiving 400 mg/kg AZT + 10 mg/kg folinic acid, and the interaction was statistically significant ($P=0.0314$) (Table 12).

Among female groups, data on microscopic examination of livers was insufficient to allow meaningful comparisons of the occurrence of hepatocyte hypertrophy.

Focal necrosis of hepatocytes generally occurred at a very low incidence in all male groups, including the control group. The initiating agent of the focal necrosis may have been some infectious microorganism of low pathogenicity.

In most instances, the liver lesion diagnosed as focal necrosis consisted of one to a very few hepatocytes undergoing nuclear pyknosis and fragmentation with shrinkage and hyalinization of cytoplasm and, as the necrotic process advanced, some fragmentation of affected hepatocytes. Typically, there was a localized infiltration of mixed inflammatory cells adjacent to the necrotic cell remnants. A minor portion of lesions diagnosed as focal necrosis consisted of more extensive focal areas of coagulative necrosis of hepatocytes, in

TABLE 12
Statistical Analysis of Mean Severity of Hepatocyte Hypertrophy in Male Mice Given AZT and Folinic Acid in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice[a]

AZT (mg/kg)	Folinic Acid (mg/kg)	
	0	10
0	1 15 ± 0 19 (40)	1.37 ± 0.23 (30)
400	1.55 ± 0 17 (40)	0.90 ± 0.20* (20)

* Significantly different from the control group ($P \leq 0$ 05) by Williams' or Dunnett's test
[a] Data are presented as mean ± standard error. Number of mice per group is given in parentheses Mean severity is based on numerical scale of· 1=minimal, 2=mild, 3=moderate, 4=marked

which major portions of affected liver lobules consisted of well-defined areas of hyalinized hepatocytes with nuclei in various stages of disintegration or with nuclei missing entirely. Often the borders of such lesions contained mixed inflammatory cells and fibroblasts. Criteria for severity grades were as follows:

Minimal severity - The presence of focal necrosis of hepatocytes in which the size or number of the necrotic foci is insufficient to warrant a severity grade of mild.

Mild severity - (a) One or more of the necrotic foci are of a diameter approximately equivalent to ½ or more of the diameter of a typical liver lobule, *or* (b) multiple necrotic foci (usually more than five) are present in the liver sections.

As compared to the control group, there appeared to be a slight increase in the incidence and severity of focal necrosis of hepatocytes in male mice in groups receiving 3,000 mg/kg TMP/SMX, including those groups also receiving AZT and/or folinic acid. Folinic acid caused an apparent reduction in the incidence or severity of liver necrosis caused by 3,000 mg/kg TMP/SMX.

Data on the occurrence of focal necrosis of hepatocytes in females were inconclusive.

Spleen Lesions

The spleen was examined routinely in males and females as a possible target organ. Hematopoietic cell proliferation was diagnosed in all spleens; some splenic hematopoiesis normally occurs in the spleen of normal mice. Comparison of the splenic lesion among groups was by necessity a comparison only of mean severity, inasmuch as the incidence was 100% within every group. Criteria for severity grades for hematopoietic cell proliferation follow:

Minimal severity - Approximately 15% or less of the red pulp is occupied by hematopoietic cells of the types normally found in bone marrow.

Mild severity - Approximately 16% to 50% of the red pulp is occupied by hematopoietic cells of the types normally found in bone marrow.

Moderate severity - Approximately 51% to 90% of the red pulp is occupied by hematopoietic cells of the types normally found in bone marrow.

Marked severity - Approximately 91% to 100% of the red pulp is occupied by hematopoietic cells of the types normally found in bone marrow.

As compared to the control group, TMP/SMX given alone at any dose concentration to males caused an increase in the mean severity of splenic hematopoiesis (Table 13). AZT given alone at the highest dose (400 mg/kg AZT) also caused an increase in splenic hematopoiesis. However, AZT administered to males in

TABLE 13
Severity of Splenic Hematopoiesis in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Mean Severity[b]
0 + 0 + 0	1.8
0 + 0 + 10	1.8
200 + 0 + 0	1.8
400 + 0 + 0	2.2
400 + 0 + 10	1.8
0 + 1,000 + 0	2.2
0 + 2,000 + 0	2.5
0 + 2,000 + 10	2.3
0 + 3,000 + 0	2.4
0 + 3,000 + 10	2.4
200 + 1,000 + 0	1.7
400 + 1,000 + 0	1.2
200 + 2,000 + 0	1.5
400 + 2,000 + 0	1.4
400 + 2,000 + 10	1.1
200 + 3,000 + 0	1.5
400 + 3,000 + 0	1.3

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of: 1=minimal, 2=mild, 3=moderate, 4=marked

any combination with TMP/SMX appeared to cause a slight reduction in the severity of splenic hematopoiesis in comparison with the control group.

Among female-B mice, in which dosing occurred for a period of 10 days and ceased about 9 days prior to sacrifice, the degree of severity of splenic hematopoiesis in comparison with the control group was greater in the group administered 400 mg/kg AZT alone and in the groups receiving 400 mg/kg AZT + 1,000, 2,000, or 3,000 mg/kg TMP/SMX.

Apparently, treatment of female-B mice with AZT at the highest dose, with or without simultaneous treatment with TMP/SMX, caused a slight increase in the severity of splenic hematopoiesis.

The results were different with female-A mice (Table 14), which were dosed continuously until one day prior to sacrifice, for a total dosing period of 27 to 31 consecutive days. Among female-A mice, in comparison with the control group, all groups receiving AZT and/or TMP/SMX had a reduced severity of splenic hematopoiesis. The greatest reductions were seen in female-A mice receiving the highest dose combinations of AZT with TMP/SMX.

TABLE 14
Mean Severity of Splenic Hematopoiesis in Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Mean Severity[b]
0 + 0 + 0	2.7
0 + 0 + 10	2.1
200 + 0 + 0	2.4
400 + 0 + 0	2.4
400 + 0 + 10	2.3
0 + 1,000 + 0	2.3
0 + 2,000 + 0	2.4
0 + 2,000 + 10	2.6
0 + 3,000 + 0	2.4
0 + 3,000 + 10	2 3
200 + 1,000 + 0	1.9
400 + 1,000 + 0	1 5
200 + 2,000 + 0	1 6
400 + 2,000 + 0	1.5
400 + 2,000 + 10	1 7
200 + 3,000 + 0	1 4
400 + 3,000 + 0	1 3

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of. 1=minimal, 2=mild, 3=moderate, 4=marked

A statistical analysis of mean severity grades for splenic hematopoiesis reveals prominent differences between female-A and female-B mice (Table 15). Although complicated by pregnancy, the differences were believed to reflect the duration of treatment with the test compounds Female-A mice were dosed continuously for approximately 30 days until the day prior to necropsy Female-B mice were dosed for approximately 10 days followed by a short recovery period prior to necropsy. Trends in hematopoietic cell activity in male mice dosed for approximately 20 days were similar to those in female-A mice dosed for approximately 30 days.

TABLE 15
Statistical Analysis of Mean Severity of Hematopoietic Cell Proliferation in the Spleen in Swiss (CD-1®) Mice Administered Combinations of AZT and TMP/SMX in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

AZT (mg/kg)	TMP/SMX (mg/kg) 0	1,000	2,000	3,000
Male Mice				
n	10	10	10	10
0	1 80 ± 0 20	2 20 ± 0 13	2 50 ± 0 17*	2 40 ± 0 16*
200	1 80 ± 0 25	1 70 ± 0 26	1 50 ± 0 17	1 50 ± 0 17
400	2 20 ± 0 20	1 20 ± 0 13**	1 40 ± 0 16**	1 30 ± 0 15**
Female-A Mice				
n	20	20	20	20
0	2 70 ± 0 11	2 30 ± 0 11*	2 40 ± 0 11	2 39 ± 0 12[b]
200	2 40 ± 0 15	1 90 ± 0 18*	1 60 ± 0 15**	1 40 ± 0 13**
400	2 35 ± 0 13	1 50 ± 0 15**	1 45 ± 0 15**	1 25 ± 0 10**
Female-B Mice				
n	14	15	16	14
0	2 79 ± 0 11	2 93 ± 0 15	2 27 ± 0 12*[c]	2 43 ± 0 14*
200	2 64 ± 0 13	3 13 ± 0 13*[b]	3 06 ± 0 11	2 79 ± 0 19
400	3 00 ± 0 00[b]	3 27 ± 0 15	2 88 ± 0 09	3 19 ± 0 23[b]

* Significantly different from the control group (P≤0 05) by Williams' or Dunnett s test
** P≤0 01
[a] Data are presented as mean ± standard error Mean severity is based on numerical scale of 1=minimal, 2=mild, 3=moderate, 4=marked
[b] n=16
[c] n=15

Regression analysis of mean severity grades for spleen hematopoietic activity in males (Figure 14) indicated a significant interaction between AZT and TMP/SMX. Administration of AZT alone resulted in increased hematopoietic activity. Although not statistically significant, the corresponding slope was positive (+0.001). When the compounds were administered in combination, statistically significant declines in hematopoietic activity were evident, and the corresponding negative slope (-0.003) indicated significant interaction between AZT and TMP/SMX.

Regression analysis of mean severity grades for hematopoietic activity in the spleen of female-A mice (Figure 15) was probably complicated somewhat by pregnancy; however, a compound interaction similar to that in males was evident.

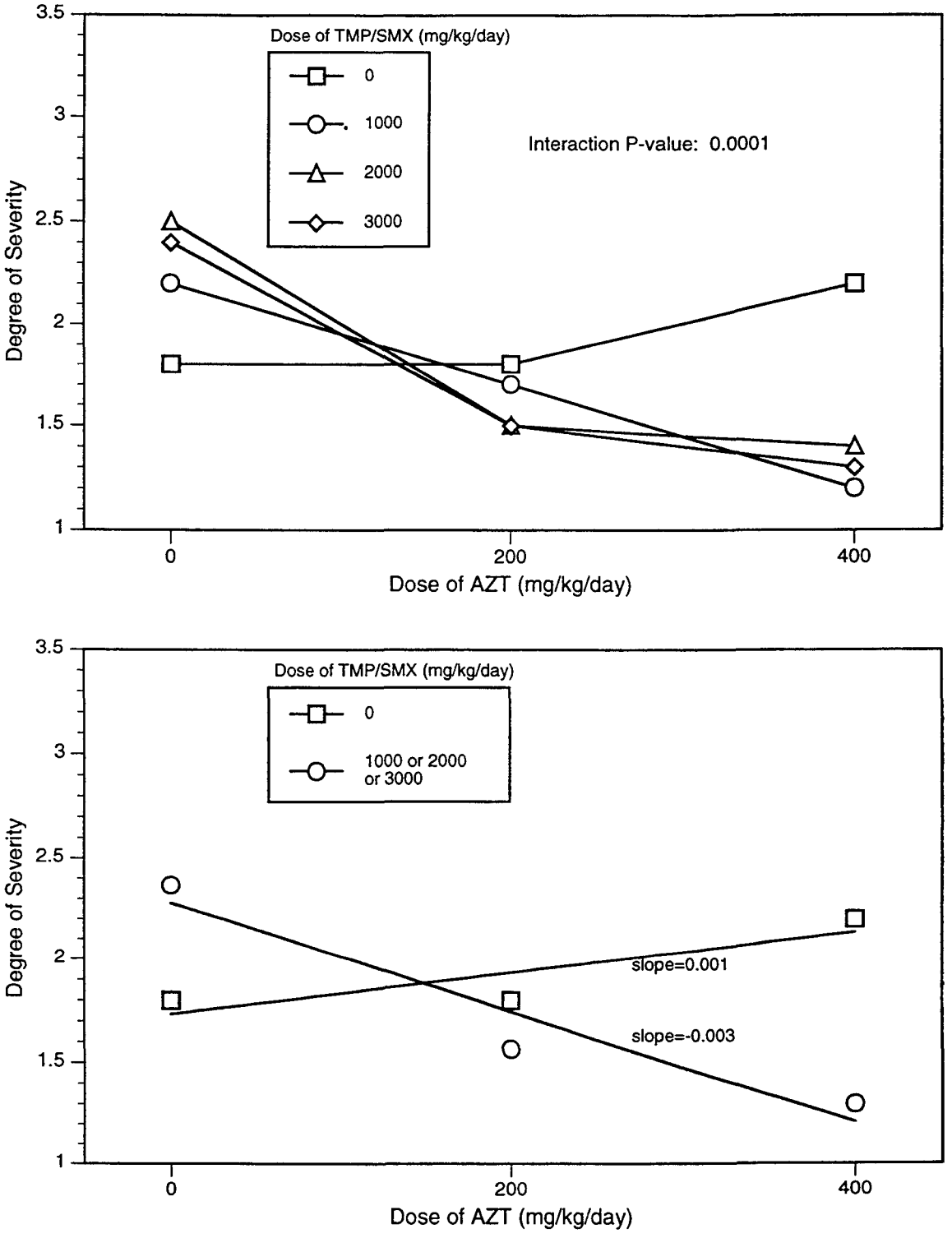


FIGURE 14
Severity of Splenic Hematopoiesis versus Dose for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

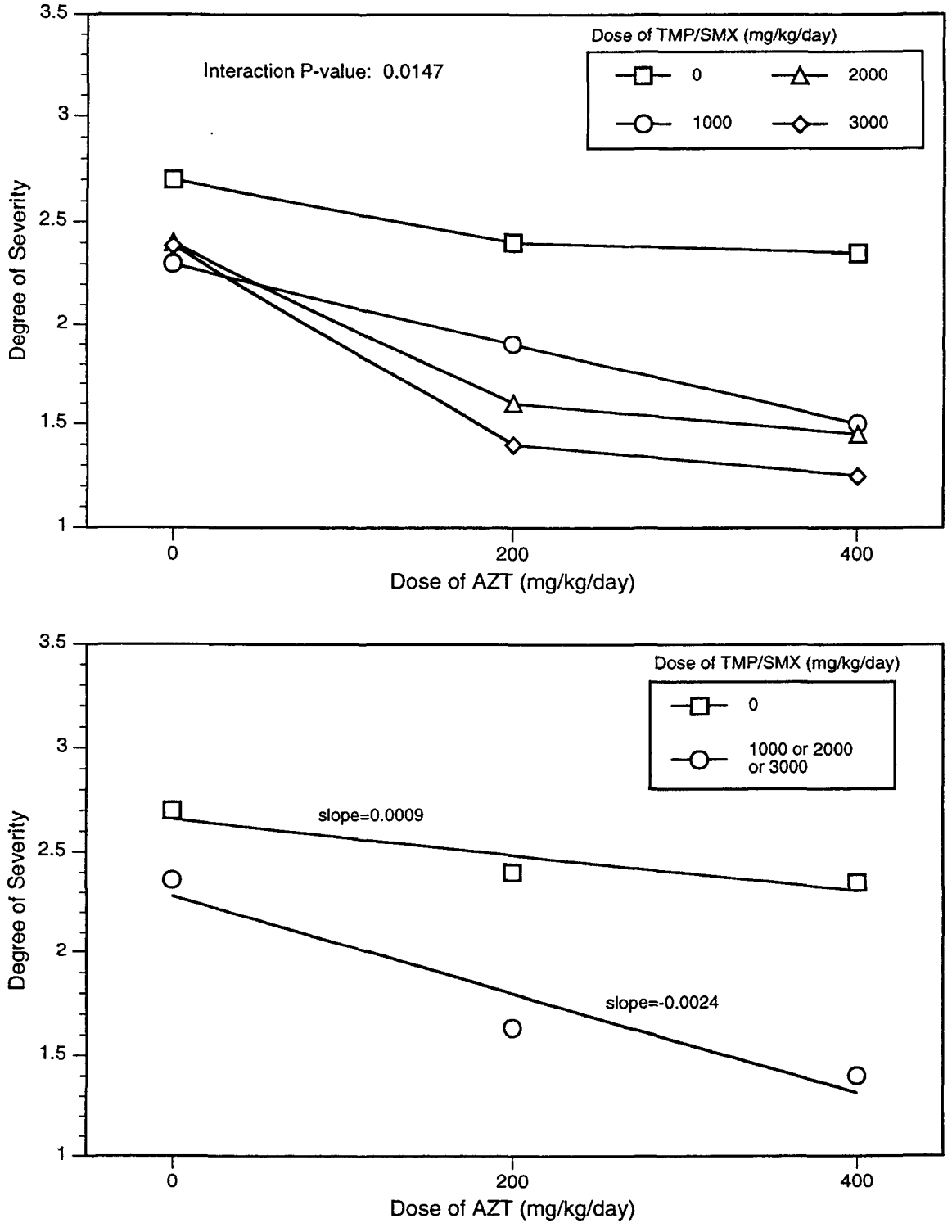


FIGURE 15
Severity of Splenic Hematopoiesis versus Dose for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

Statistically significant declines in hematopoietic activity in the spleen were evident in all female-A groups receiving the test compound combinations, and the dose response, as indicated by the steep negative slope (-0.0024), indicate interaction between AZT and TMP/SMX. Although not statistically significant and in contrast to the male and female-A groups, female-B groups receiving the highest combinations had increased hematopoietic activity that was believed to reflect the short duration of treatment (10 days).

The diminished splenic hematopoiesis in male and female-A mice caused by treatment with AZT + TMP/SMX combinations corresponds with the increased severity of cellular depletion of bone marrow observed in males receiving AZT + TMP/SMX combinations.

Administration of folinic acid had no discernible effect on spleen morphology.

Thymus Lesions

In general, there was a distinct increase in the incidence and severity of thymic atrophy (depletion of cells, mainly lymphocytes) in male and female-A mice receiving AZT and/or TMP/SMX as compared to the control groups.

In the milder forms, thymic atrophy was recognized as a thinning of the thymic cortex mainly due to loss of small lymphocytes. There was accentuation of the normal process of necrobiosis of lymphocytes, with the presence of apparently increased numbers of macrophages, focally distributed, which contained remnants of necrotic lymphocytes including nuclear debris. In the more severe forms of thymic atrophy, there was complete or almost complete loss of identifiable cortex, due principally to loss of lymphocytes. The remaining thymic tissue consisted mainly of some thymic epithelium and stroma, including vascular endothelium, reticular tissue, and fibrous tissue, with some macrophages and a paucity of large and small lymphocytes. Criteria for severity grades of thymic atrophy follow:

Minimal severity - A barely detectable reduction in viable lymphocytes in the cortex. Basic architecture of thymus, including clear distinction between cortex and medulla, is preserved.

Mild severity - A readily apparent reduction in viable lymphocytes in the cortex, but distinction between cortex and medulla is usually preserved.

Moderate severity - Lymphocytes fairly plentiful in thymus, but sufficient lymphocytes depleted from cortex such that concentration of lymphocytes in cortex and medulla is similar, thereby causing blurring of the distinction between cortex and medulla.

Marked severity - Substance of thymic tissue (cortex and medulla) greatly diminished, including lymphocytic, other mesenchymal, and epithelial components. Poor distinction between cortical and medullary zones of thymus.

As compared to the control group, either AZT or TMP/SMX given alone caused dose-related increases in the incidence and severity of thymic atrophy in males (Table 16). Likewise, the AZT + TMP/SMX combinations caused generally dose-related and even greater increases in the incidence and mean severity of thymic atrophy in males.

TABLE 16
Incidence and Mean Severity of Thymic Atrophy in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Group Incidence	Mean Severity[b]
0 + 0 + 0	0/10	—
0 + 0 + 10	0/9	—
200 + 0 + 0	4/10	0.5
400 + 0 + 0	8/10	0.9
400 + 0 + 10	5/10	0.6
0 + 1,000 + 0	1/10	0.1
0 + 2,000 + 0	2/10	0.4
0 + 2,000 + 10	5/10	0.6
0 + 3,000 + 0	3/10	0.5
0 + 3,000 + 10	1/10	0.1
200 + 1,000 + 0	6/10	1.1
400 + 1,000 + 0	9/10	1.0
200 + 2,000 + 0	10/10	2.1
400 + 2,000 + 0	10/10	2.1
400 + 2,000 + 10	9/9	2.6
200 + 3,000 + 0	10/10	2.4
400 + 3,000 + 0	8/8	3.3

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of: 1=minimal, 2=mild, 3=moderate, 4=marked

In female-A mice, AZT alone had no detectable effect at either dose on the incidence or severity of thymic atrophy (Table 17) as compared to the control group. TMP/SMX alone or in combination with AZT caused an increase in the severity of thymic atrophy, which was especially severe at the highest dose combinations of AZT and TMP/SMX. In general, thymic atrophy was more severe in female-A mice than in male mice.

TABLE 17
Incidence and Mean Severity of Thymic Atrophy in Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Group Incidence	Mean Severity[b]
0 + 0 + 0	15/20	1.6
0 + 0 + 10	17/20	1.6
200 + 0 + 0	13/20	1.0
400 + 0 + 0	15/18	1.3
400 + 0 + 10	18/20	1.7
0 + 1,000 + 0	18/18	1.8
0 + 2,000 + 0	18/18	2.6
0 + 2,000 + 10	18/19	1.7
0 + 3,000 + 0	15/17	2.1
0 + 3,000 + 10	14/19	1.8
200 + 1,000 + 0	20/20	2.0
400 + 1,000 + 0	19/19	3.3
200 + 2,000 + 0	18/18	2.8
400 + 2,000 + 0	20/20	3.0
400 + 2,000 + 10	18/18	3.3
200 + 3,000 + 0	18/18	3.6
400 + 3,000 + 0	19/19	3.6

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Mean severity is based on numerical scale of: 1=minimal, 2=mild, 3=moderate, 4=marked

Among female-B mice, no consistent trend in the severity of thymic atrophy due to treatment with AZT or TMP/SMX could be discerned (Table 18), except that in the highest group (400 mg/kg AZT + 3,000 mg/kg TMP/SMX), there was an increase in the incidence (15/16) and mean severity (1.8) as compared to the control group (incidence, 10/13, and mean severity, 0.8). Evaluation of the data in Table 18 reveals, again, a prominent difference between female-B mice dosed for approximately 10 days when compared to male and female-A mice dosed for approximately 20 and 30 days, respectively.

For the male mice, regression analysis of the mean severity grades for thymic atrophy (Figure 16) indicated that a test compound interaction was present that potentiated the development of thymic atrophy. The effects were more than additive.

TABLE 18
Statistical Analysis of Mean Severity of Thymic Atrophy in Swiss (CD-1®) Mice Administered Combinations of AZT and TMP/SMX in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

AZT (mg/kg)	TMP/SMX (mg/kg) 0	1,000	2,000	3,000
Male Mice				
n	10	10	10	10
0	0.00 ± 0.00	0.10 ± 0.10	0.40 ± 0.31	0.50 ± 0.31
200	0.50 ± 0.22	1.10 ± 0.38	2.10 ± 0.23**[b]	2.40 ± 0.27**
400	0.90 ± 0.18	1.00 ± 0.15	2.10 ± 0.28**	3.25 ± 0.25**[c]
Female-A Mice				
n	20	20	18	16
0	1.55 ± 0.23	1.83 ± 0.20[d]	2.61 ± 0.16**	2.06 ± 0.31[e]
200	0.95 ± 0.18	1.95 ± 0.17**	2.83 ± 0.15**	3.56 ± 0.12**
400	1.28 ± 0.19[d]	3.26 ± 0.15**[f]	2.95 ± 0.05**[b]	3.63 ± 0.11**[f]
Female-B Mice				
n	13	13	16	14
0	0.77 ± 0.12	1.00 ± 0.30	0.53 ± 0.22[g]	0.43 ± 0.23
200	0.93 ± 0.16[d]	0.21 ± 0.11**[h]	0.38 ± 0.13**	0.21 ± 0.21**
400	0.63 ± 0.15[l]	0.53 ± 0.17[g]	0.81 ± 0.14	1.75 ± 0.27**[i]

* Significantly different from the control group ($P \leq 0.05$) by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Mean severity is based on numerical scale of: 1=minimal, 2=mild, 3=moderate, 4=marked
[b] n=20 [c] n=8 [d] n=18 [e] n=17 [f] n=19 [g] n=15 [h] n=14 [i] n=16

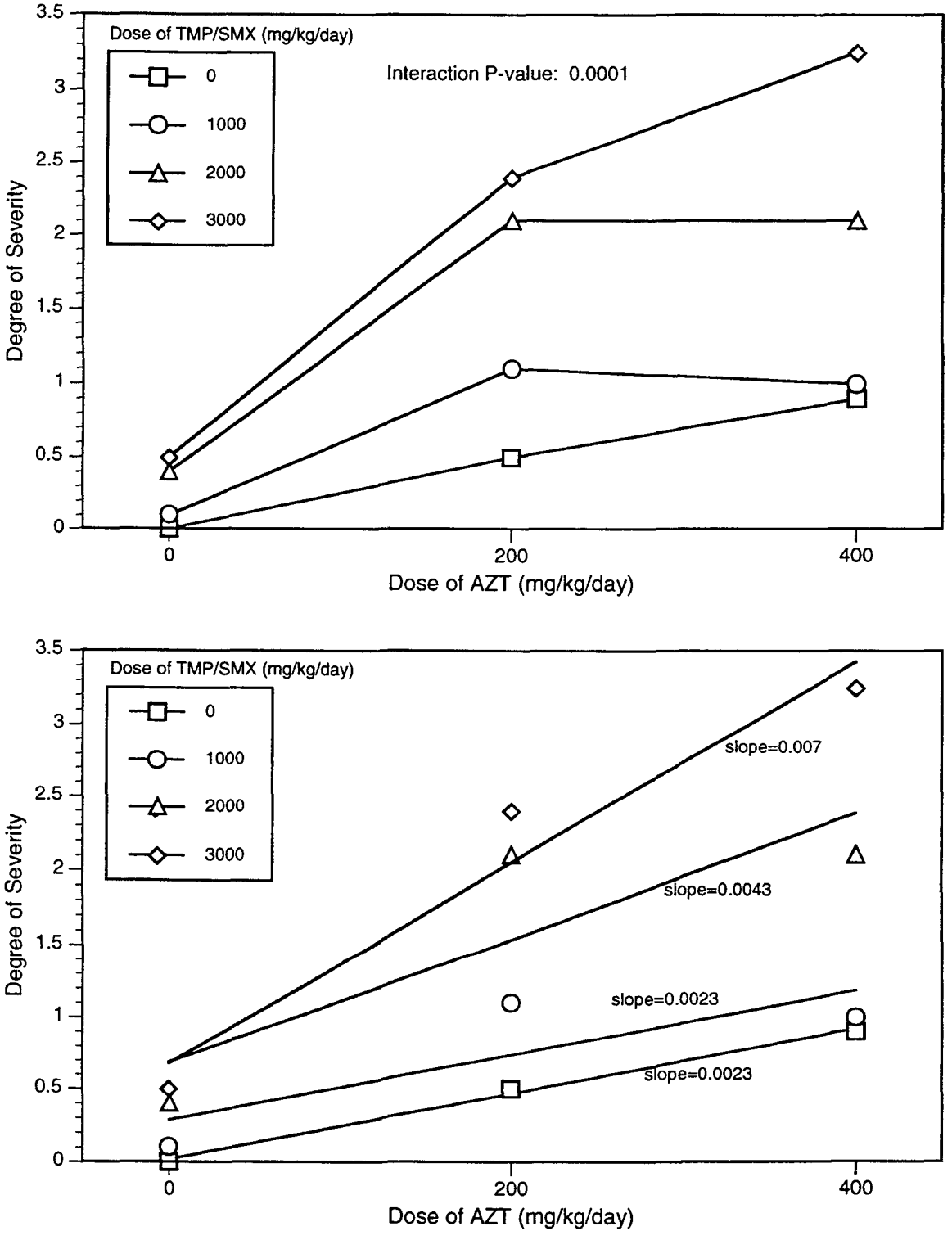


FIGURE 16
Severity of Thymic Atrophy versus Dose for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

For the female-A mice, the stress of pregnancy complicated the statistical analysis of the thymic lesions; however, similar trends indicative of test compound interaction were evident (Figure 17). Administration of AZT alone did not produce thymic atrophy in female-A mice, and the corresponding slope is negative (-0.0007). Administration of combinations of AZT and TMP/SMX resulted in statistically significant elevations ($P \leq 0.01$) in all treatment groups receiving the combinations. Test compound interaction was most evident in the group receiving the highest doses of each compound, and the corresponding dose response had a positive slope (+0.0039).

For the female-B mice dosed for approximately 10 days, thymic atrophy was statistically significant ($P \leq 0.01$) only in the group receiving the highest combinations of AZT and TMP/SMX.

Thymic atrophy was a relatively common occurrence in the control groups and was more severe in the female-A control group than in the female-B control group. Female-A mice were sacrificed near the end of the gestation period. There was a strong association between the number of fetuses in the uterus of each female-A control mouse and the presence and severity of thymic atrophy in the same individual (Table 19).

TABLE 19
Association Between the Occurrence of Thymic Atrophy and the Number of Fetuses in Female-A Control Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Animal Number	Severity	Number of Fetuses in Uterus
86	0	0
87	2	14
88	2	10
89	2	14
90	2	14
91	2	12
92	1	0
93	0	0
94	2	13
95	3	10
511	0	3
512	2	12
513	1	7
514	2	8
515	2	14
516[a]	3	- 8
517	3	13
518	0	0
519	2	10
520	0	0

[a] Animal 516 delivered.

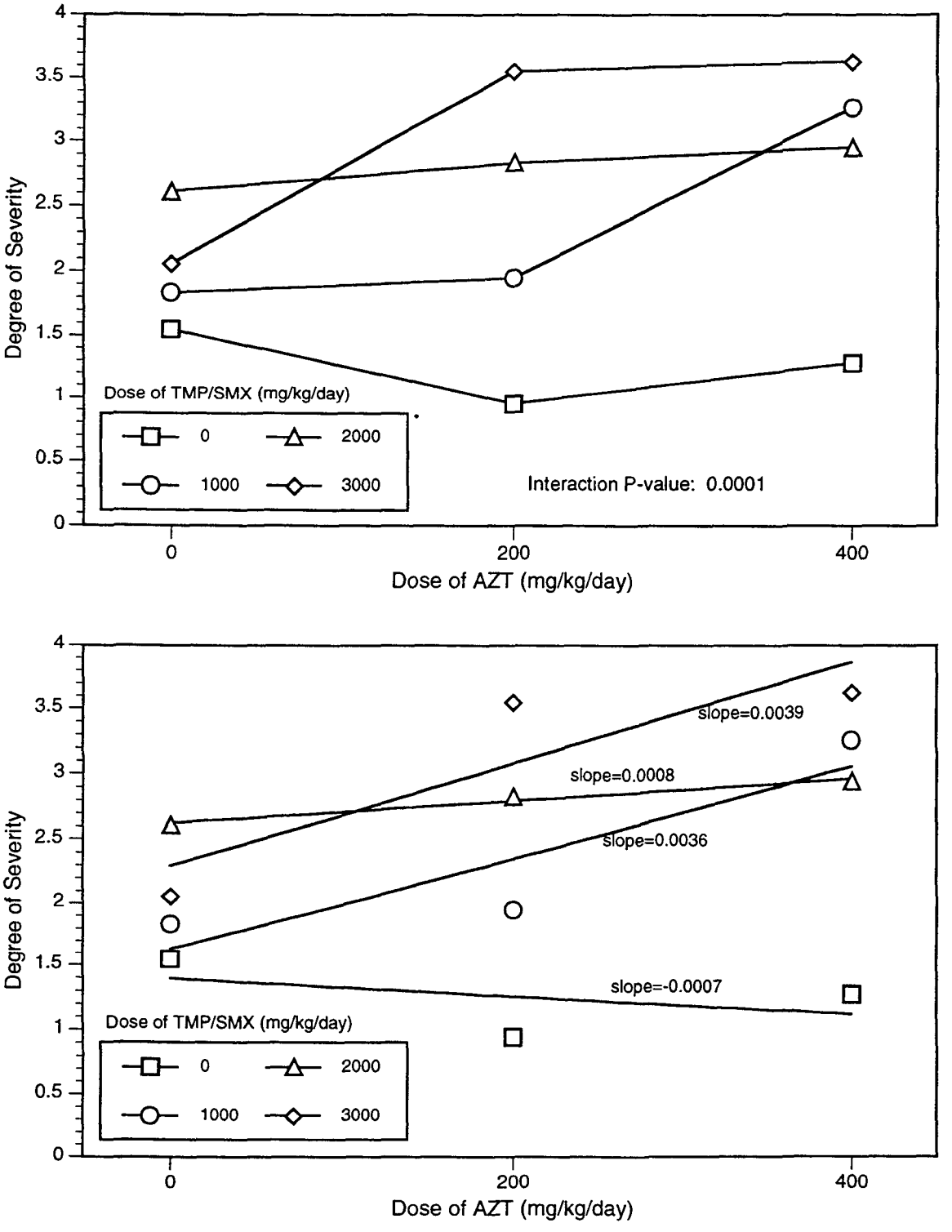


FIGURE 17
Severity of Thymic Atrophy versus Dose for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

A similar but less pronounced correlation was seen in the female-B control group between the number of pups nursing each female and the presence of thymic atrophy in the dam. The occurrence of thymic atrophy in the control group must be considered in interpreting the importance of thymic atrophy in the dosed female groups. No effect of folinic acid on the incidence or severity of thymic atrophy was discernible in males or females.

Thyroid Gland Lesions

Diffuse hyperplasia of the follicular epithelium of the thyroid gland was seen in most of the mice receiving TMP/SMX. Often, the hyperplasia was of sufficient degree to be grossly observable at necropsy as thyroid gland enlargement. In comparison with thyroid glands in the control groups, follicular epithelium of affected thyroid glands was thicker, and individual epithelial cells were enlarged, with larger nuclei. Instead of being low cuboidal or squamous (as in normal thyroid glands of control animals), the follicular epithelial cells in hyperplastic thyroid glands were generally cuboidal or low columnar in shape. The nuclei and cytoplasm of dosed mice with enlarged thyroid glands typically were finely vacuolated. The colloid in each affected follicle in comparison with colloid of thyroid glands of normal control mice was typically less dense and less homogeneous. Criteria for severity grades of thyroid gland hyperplasia follow:

Minimal severity - Majority of follicular epithelial cells are low cuboidal. Colloid has about same color and texture as in normal gland.

Mild severity - Majority of follicular epithelial cells are cuboidal or low cuboidal (about equal distribution of cuboidal and low cuboidal). Colloid shows some rarefaction and vacuolization.

Moderate severity - Majority of follicular epithelial cells are cuboidal but are only rarely thrown up into folds or multilayered papillary projections. Colloid in essentially all follicles is rarefied and vacuolated.

Marked severity - Majority of follicular epithelial cells are cuboidal, and are frequently thrown up in folds or multilayered papillary projections. Follicles contain only sparse remnants of colloid.

AZT given alone at either dose did not cause thyroid gland hyperplasia in any male or female mice. However, most mice in every group (male or female) receiving TMP/SMX, with or without the addition of AZT, developed thyroid gland hyperplasia.

There appeared to be a slight increase in the mean severity of thyroid gland hyperplasia in males with increasing doses of TMP/SMX; however, this was not a consistent trend.

In female-A mice, the severities of thyroid gland hyperplasia (Table 20) appeared to depend mainly on the dosage of TMP/SMX, regardless of whether AZT was also administered. The higher doses of TMP/SMX had

similar effects on the severities of thyroid gland hyperplasia but caused somewhat greater mean severities of thyroid gland hyperplasia than that observed at the lowest dose (1,000 mg/kg) of TMP/SMX.

Incidences and severities of thyroid gland hyperplasia in female-B mice (Table 20) were considerably lower than among female-A mice, which were dosed over a longer time period. The addition of 400 mg/kg AZT to the 3,000 mg/kg TMP/SMX dose caused an apparent increase in the incidence and severity of thyroid gland hyperplasia.

No meaningful effect of folinic acid on the occurrence of thyroid gland hyperplasia was observed in this study.

TABLE 20
Incidence and Mean Severity of Thyroid Gland Hyperplasia in Female Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice[a]

Dose	Female-A Mice		Female-B Mice	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0 + 0	0/20	—	0/14	—
0 + 0 + 10	0/20	—	0/14	—
200 + 0 + 0	0/20	—	0/13	—
400 + 0 + 0	0/20	—	0/16	—
400 + 0 + 10	0/19	—	0/16	—
0 + 1,000 + 0	19/20	2 5	5/15	0 3
0 + 2,000 + 0	20/20	3 0	4/14	0 3
0 + 2,000 + 10	17/17	3 0	8/15	0 7
0 + 3,000 + 0	19/19	3 0	7/14	0 6
0 + 3,000 + 10	18/18	3 0	7/15	0 9
200 + 1,000 + 0	20/20	2 5	5/16	0 4
400 + 1,000 + 0	20/20	2 9	5/15	0 3
200 + 2,000 + 0	19/19	3 0	6/16	0 4
400 + 2,000 + 0	20/20	3 0	8/16	0 6
400 + 2,000 + 10	19/19	3 0	7/14	0 9
200 + 3,000 + 0	14/14	3 0	5/14	0 5
400 + 3,000 + 0	13/14	2 8	13/15	1 5

[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day) Mean severity is based on numerical scale of 1=minimal, 2=mild, 3=moderate, 4=marked

A remarkable similarity was found (Table 21) in the mean severity grades of males and female-A mice dosed for approximately 20 and 30 days, respectively. In contrast, the mean severity grades in female-B groups dosed for approximately 10 days, although statistically significant, were considerably lower. This difference emphasizes the impact of duration of treatment on the development of the lesion.

Chronic inflammation of the thyroid gland, which was focally distributed or involved the wall and adventitia of blood vessels, was seen in 15 mice with hyperplastic thyroid glands. All 15 mice received either 1,000, 2,000, or 3,000 mg/kg TMP/SMX, usually without AZT. All affected mice were in the female-B group; none were in the female-A group. The degree of severity was not affected by increasing doses of TMP/SMX.

No influence of folinic acid on the incidence or severity of inflammation of the thyroid gland was discernible.

TABLE 21
Statistical Analysis of Mean Severity of Hyperplasia of the Thyroid Gland in Swiss (CD-1®) Mice Administered TMP/SMX in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

Group	TMP/SMX[b] (mg/kg)			
	0	1,000	2,000	3,000
Male Mice				
n	29	30	30	28
Mean severity	0.00 ± 0.00	2.50 ± 0 12**	2.93 ± 0.07**	2.93 ± 0 11**
Female-A Mice				
n	60	60	59	47
Mean severity	0.00 ± 0.00	2 58 ± 0 09**	3 00 ± 0 00**	2.94 ± 0.06**
Female-B Mice				
n	43	46	46	43
Mean severity	0.00 ± 0.00	0.37 ± 0 08**	0.44 ± 0.09**	0.88 ± 0.14**

** Significantly different from the control group (P≤0 01) by Williams' or Dunnett's test
[a] Data are presented as mean ± standard error Mean severity is based on numerical scale of 1=minimal, 2=mild, 3=moderate, 4=marked
[b] Since there were no AZT effects, data for all groups with the same TMP/SMX dose were collapsed for statistical evaluation

Other Lesions

Occasional lesions not caused by administered test compounds occurred with random distribution in the various groups of males and females. The only neoplasms observed in the study were a malignant lymphoma of the thymus and an alveolar/bronchiolar adenoma.

Calluses of the rib or vertebra in some mice were attributed to accidental trauma associated with the gavage procedure. Also, inflammation or perforation of the esophagus in a few mice was attributed to the gavage procedure.

Inflammatory changes and/or foreign bodies in the lungs of a few mice, inflammation or foreign bodies in skin or subcutaneous tissue, and lesions of the mucosa or epithelium of the stomach were all attributed to the gavage procedure.

SPERM ENDPOINTS

AZT (200 or 400 mg/kg) and TMP/SMX (2,000 or 3,000 mg/kg) caused decreased sperm motility (Table B1); coadministration of AZT and TMP/SMX further decreased sperm motility. Left testis weight, spermatid heads per testis, and average spermatid counts (Table B1) were significantly decreased by all doses of TMP/SMX alone. Folinic acid did not appear to alleviate or diminish the parameters examined. Left caudal weights (Table B1) showed a significant interaction of all three chemicals, indicating that the dose response relationship of AZT differs across doses of the other two chemicals, TMP/SMX and folinic acid.

NATURAL DELIVERY DATA

The administration of AZT, TMP/SMX, or folinic acid alone or in any combination did not affect the incidence of pregnancy in the female-B mice (Table 22). The lack of pregnancy in the female-B mice dosed with 400 mg/kg AZT + 1,000 mg/kg TMP/SMX was not considered to be test article related because no effects on pregnancy were noted in dose groups receiving high doses of AZT, TMP/SMX, or both. The number of litters delivered was significantly decreased ($P \leq 0.01$) in the groups that received $\geq$ 2,000 mg/kg TMP/SMX in combination with 200 or 400 mg/kg AZT. In addition, the 200 + 1,000 + 0 mg/kg group also had a significantly reduced number of litters. The combination of AZT and TMP/SMX, with or without folinic acid, appeared to potentiate the effect of TMP/SMX; this is supported by the presence of smaller decreases in numbers of litters of groups that received 2,000 or 3,000 mg/kg TMP/SMX alone.

TABLE 22
Occurrence of Pregnancy in Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Number Assigned	Number Pregnant (%)	Number Delivered (%)
0 + 0 + 0	14	14 (100.0%)	14 (100.0%)
0 + 0 + 10	14	14 (100.0%)	13 (92.8%)
200 + 0 + 0	14	13 (92.8%)	13 (100.0%)
400 + 0 + 0	16	15 (93.8%)	14 (93.3%)
400 + 0 + 10	16	14 (87.5%)	11 (78.6%)
0 + 1,000 + 0	15	12 (80.0%)	12 (100.0%)
200 + 1,000 + 0	16	13 (81.2%)	6 (46.2%)**
400 + 1,000 + 0	20	0**	—
0 + 2,000 + 0	15	13 (86.7%)	12 (92.3%)
0 + 2,000 + 10	15	14 (93.3%)	14 (100.0%)
200 + 2,000 + 0	20	15 (75.0%)	4 (26.6%)**
400 + 2,000 + 0	16	16 (100.0%)	0**
400 + 2,000 + 10	15	15 (100.0%)	0**
0 + 3,000 + 0	14	12 (85.7%)	10 (83.3%)
0 + 3,000 + 10	15	15 (100.0%)	12 (80.0%)
200 + 3,000 + 0	14	12 (85.7%)	0**
400 + 3,000 + 0	16	15 (93.8%)	0**

** Significantly different ($P \leq 0.01$) from the control group by the Cochran-Armitage and Fisher exact tests
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)

The duration of gestation was not significantly affected by AZT, TMP/SMX, or folinic acid; a statistically significant increase noted in the 0 + 3,000 + 10 mg/kg group was not considered biologically significant, as similar values were observed in other dosed groups.

The number of implantation sites was not affected by the administration of any test article alone or in combination. The number of dams with stillborn pups was significantly increased in the following groups: 400 + 0 + 10 mg/kg, 200 + 2,000 + 0 mg/kg, 0 + 3,000 + 0 mg/kg, and 0 + 3,000 + 10 mg/kg (Table 23). The number of dams with all liveborn pups dying between days 0 and 4 was significantly increased in the 0 + 2,000 + 0 mg/kg, 0 + 3,000 + 0 mg/kg, and 0 + 3,000 + 10 mg/kg groups.

TABLE 23
Summary of Natural Delivery Litter Data for Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Dams with Stillborn Pups (% of Dams that Delivered)	Dams with All Pups Dying on Days 0-4 (% of Dams with Liveborn Pups)	Mean Live Litter Size	Pups Dying on Days 1-4/Total Alive on Day 1 (%)	Survival/Live Litter Size[b] on Day 4 Postpartum
0 + 0 + 0	1 (7 1)	0 (0 0)	11 3	1/147 (0 7)	11 2/11 3
0 + 0 + 10	1 (7 7)	1 (7 7)	11 1	13/144 (9 0)**	10 1/10 9
200 + 0 + 0	3 (23 1)	1 (8 3)	9 7	6/116 (5 2)	9 2/10 0
400 + 0 + 0	6 (42 8)	2 (14 3)	7 8	8/109 (7 3)*	7 2/8 4
400 + 0 + 10	6 (54 4)*	1 (10 0)	7 9	7/79 (8 9)**	7 2/7 9
0 + 1 000 + 0	3 (25 0)	0 (0 0)	12 2	16/145 (11 0)**	10 8/10 8
200 + 1,000 + 0	3 (50 0)	0 (0 0)	8 7	3/26 (11 5)*	7 7/7 7
400 + 1,000 + 0	—[c]	—	—	—	—
0 + 2,000 + 0	5 (41 7)	5 (45 4)*	11 3	77/116 (66 4)**	3 5*/6 5
0 + 2,000 + 10	4 (28 6)	4 (28 6)	10 7	102/148 (68 9)**	3 3**/4 6*
200 + 2,000 + 0	3 (75 0)*	1 (33 3)	3 7	5/11 (45 4)**	2 0/3 0
400 + 2,000 + 0	—	—	—	—	—
400 + 2,000 + 10	—	—	—	—	—
0 + 3 000 + 0	6 (60 0)*	8 (100 0)**	7 4	68/68 (100 0)**	0**/–
0 + 3,000 + 10	11 (91 7)**	10 (100 0)**	5 8	52/52 (100 0)**	0**/–
200 + 3,000 + 0	—	—	—	—	—
400 + 3 000 + 0	—	—	—	—	—

* Significantly different (P≤0 05) from the control group by the Cochran-Armitage and Fisher exact tests
** P≤0 01
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] Excludes values for litters that had no surviving pups
[c] No litters were delivered

AZT (200 or 400 mg/kg) alone and TMP/SMX (3,000 mg/kg) alone reduced live litter sizes, with or without folinic acid (Table 23) For the remaining combination dose groups that had surviving pups, live litter sizes were also reduced Significant increases in the number of pups dying on days 1 to 4 occurred in groups administered 400 mg/kg AZT alone or with folinic acid, 1,000 mg/kg TMP/SMX alone, 2,000 or 3,000 mg/kg TMP/SMX alone or with folinic acid, or 200 mg/kg AZT in combination with TMP/SMX Although pups from the dose group receiving folinic acid alone had a significantly increased incidence of death, this effect was not

considered to be related to folinic acid because 12 of the 13 deaths that occurred were from a single litter. Significantly decreased values for survival and/or live litter size for groups with surviving litters were noted in the following groups: 0 + 2,000 + 0 mg/kg, 0 + 2,000 + 10 mg/kg, 0 + 3,000 + 0 mg/kg, and 0 + 3,000 + 10 mg/kg. Statistically significant differences in sex ratios (percent males per litter) that occurred were due to the small numbers of surviving litters in a particular group and are not, therefore, considered biologically significant.

Mean pup body weights per litter were decreased ($P \leq 0.05$) in the following groups: 400 + 0 + 0 mg/kg, 400 + 0 + 10 mg/kg, 200 + 1,000 + 0 mg/kg, 0 + 2,000 + 0 mg/kg, 0 + 2,000 + 10 mg/kg, 200 + 2,000 + 0 mg/kg, 0 + 3,000 + 0 mg/kg, and 0 + 3,000 + 10 mg/kg (Figure 18).

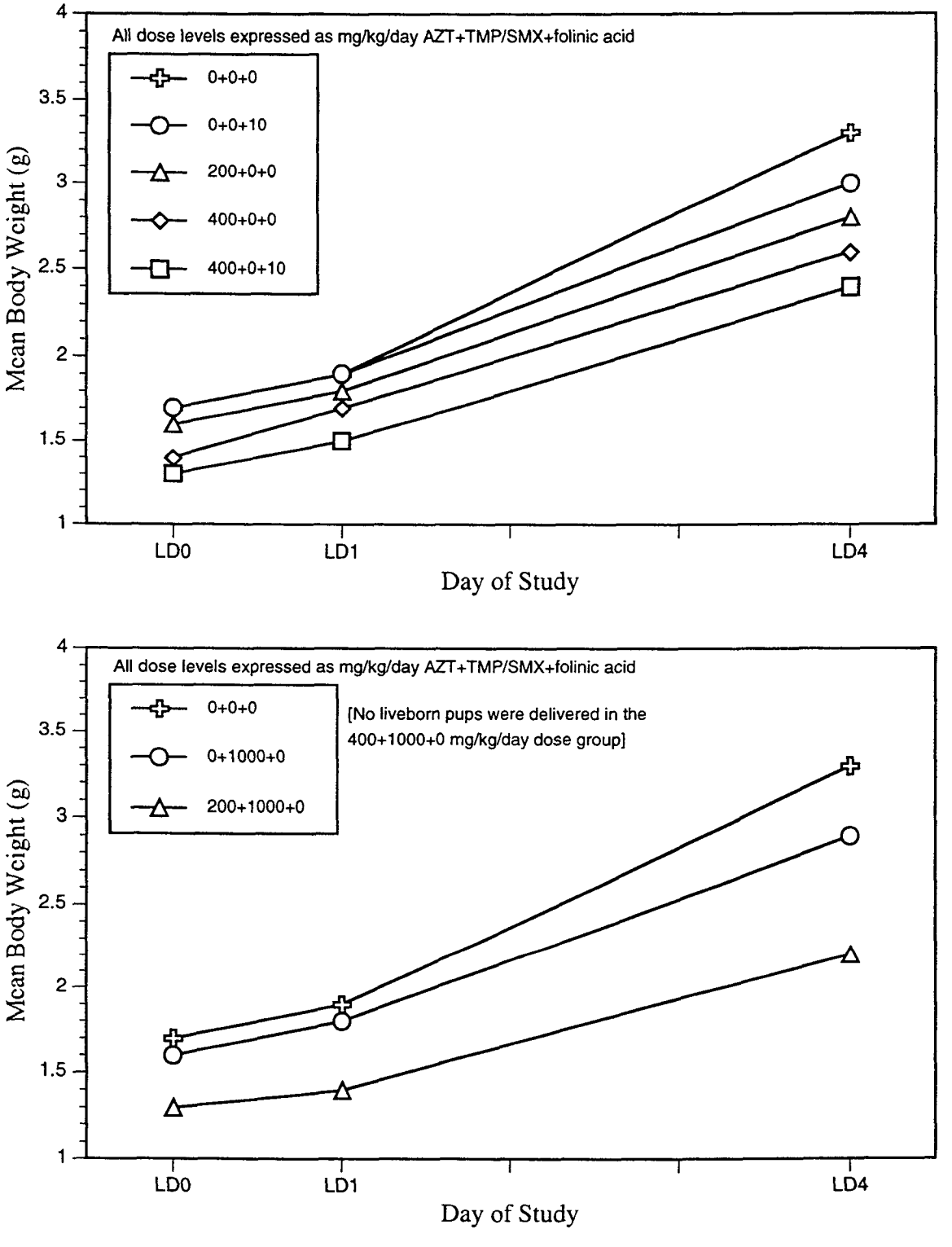


FIGURE 18
Mean Body Weights of Pups of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (LD=lactation day)

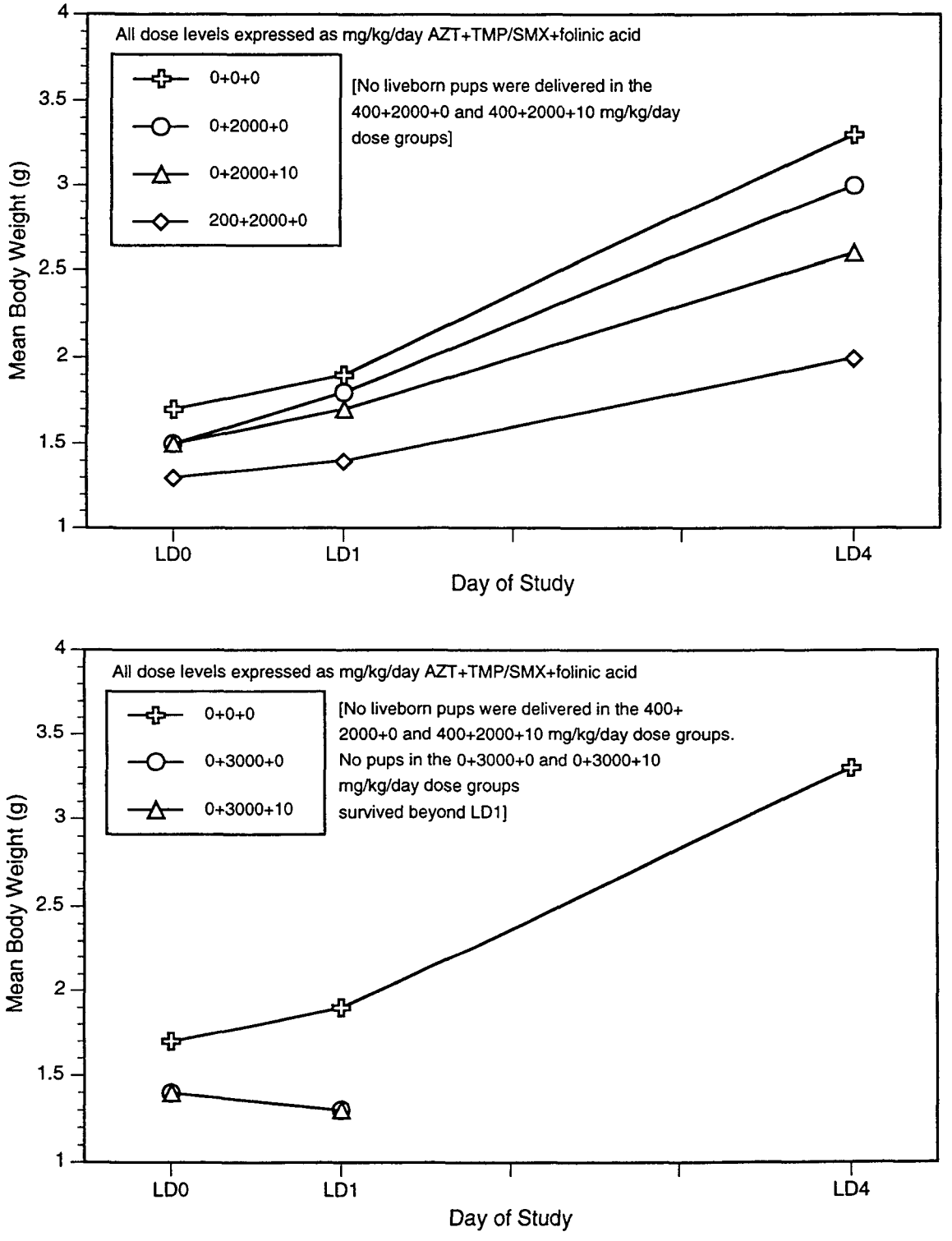


FIGURE 18
Mean Body Weights of Pups of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid (LD=lactation day)

CAESAREAN SECTION DATA

The administration of 200 or 400 mg/kg AZT alone or in combination with any dose of TMP/SMX or folinic acid reduced the number of pregnant mice per group (Table 24). For pregnant dams, the average number of corpora lutea and implantations per litter was not affected by treatment with the test articles. The apparent reduction in corpora lutea and implantation sites per litter in the 200 + 1,000 + 0 mg/kg group was likely related to the absence of live fetuses in this group and the difficulty in counting corpora lutea and implantations of total resorbed litters.

TABLE 24
Occurrence of Pregnancy in Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Number Pregnant (%)[b]
0 + 0 + 0	15 (75.0)
0 + 0 + 10	17 (85.0)
200 + 0 + 0	10 (50.0)
400 + 0 + 0	4 (20.0)**
400 + 0 + 10	8 (40.0)
0 + 1,000 + 0	11 (55.0)
200 + 1,000 + 0	6 (30.0)*
400 + 1,000 + 0	0**
0 + 2,000 + 0	17 (85.0)
0 + 2,000 + 10	10 (50.0)
200 + 2,000 + 0	0**
400 + 2,000 + 0	0**
400 + 2,000 + 10	0**
0 + 3,000 + 0	11 (55.0)
0 + 3,000 + 10	11 (55.0)
200 + 3,000 + 0	0**
400 + 3,000 + 0	0**

* Significantly different ($P \leq 0.05$) from the control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] n = 20

Administration of AZT (200 or 400 mg/kg) alone or in combination with TMP/SMX and/or folinic acid resulted in significant decreases ($P \leq 0.05$) in the number of live fetuses per litter (Table 25). The average number of dead fetuses per litter was increased in groups administered TMP/SMX alone or in combination with folinic acid but was not affected by doses of AZT or folinic acid.

Significant increases in the average number of early and/or late resorptions were noted in the following groups: 200 + 0 + 0 mg/kg, 400 + 0 + 0 mg/kg, and 400 + 0 + 10 mg/kg (Table 25). The 200 + 1,000 + 0 mg/kg group had increases ($P \leq 0.05$) only in early resorptions.

The number of dams with all conceptuses resorbed (Table 25) increased in groups administered 200 or 400 mg/kg AZT.

TABLE 25
Summary of Caesarean-Sectioning Litter Data for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Litter Sizes Mean ± SD[b]	Live Fetuses		Dead Fetuses		Resorptions	
		No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
0 + 0 + 0[c]	11 0 ± 3 2	154	11 0 ± 3 2	0	0 0 ± 0 0	15	1 1 ± 1 4
0 + 0 + 10[d]	10 8 ± 2 8	172	10 8 ± 2 7	1	0 1 ± 0 2	16	1 0 ± 1 2
200 + 0 + 0	4 8 ± 5 5*	48	4 8 ± 5 5*	0	0 0 ± 0 0	83	8 3 ± 4 7**
400 + 0 + 0	0 8 ± 1 5*	3	0 8 ± 1 5*	0	0 0 ± 0 0	36	9 0 ± 1 8*
400 + 0 + 10	0 4 ± 0 7**	3	0 4 ± 0 7**	0	0 0 ± 0 0	79	9 9 ± 2 6**
0 + 1,000 + 0	9 5 ± 3 2	102	9 3 ± 3 1	3	0 3 ± 0 5	15	1 4 ± 1 4
200 + 1,000 + 0	0 0 ± 0 0**	0	0 0 ± 0 0**	0	0 0 ± 0 0	44	7 3 ± 4 6*
400 + 1,000 + 0	—[e]	—	—	—	—	—	—
0 + 2,000 + 0[c,d]	10 4 ± 1 5	141	10 1 ± 1 7	5	0 4 ± 0 6	20	1 4 ± 1 0
0 + 2,000 + 10[c,d,f]	10 8 ± 1 1	70	10 0 ± 1 3	6	0 8 ± 0 9*	9	1 3 ± 1 0
200 + 2,000 + 0	—	—	—	—	—	—	—
400 + 2,000 + 0	—	—	—	—	—	—	—
400 + 2,000 + 10	—	—	—	—	—	—	—
0 + 3,000 + 0[g]	10 0 ± 2 1	92	9 2 ± 1 5	8	0 8 ± 1 1	15	1 5 ± 1 7
0 + 3,000 + 10	8 8 ± 3 3	77	8 6 ± 3 2	2	0 2 ± 0 4	25	2 8 ± 2 4
200 + 3,000 + 0	—	—	—	—	—	—	—
400 + 3,000 + 0	—	—	—	—	—	—	—

* Significantly different (P≤0 05) from the control group by the Cochran-Armitage and Fisher exact tests
** P≤0 01
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] SD=standard deviation
[c] Excludes dams that delivered or began to deliver litters before scheduled sacrifice
[d] Excludes dams that were sacrificed on an estimated day 14 of gestation, due to gestational age of fetuses, genders and viability could not be determined
[e] No pregnancies were detected in this group
[f] Excludes dam that was sacrificed moribund on day 17 of the study
[g] Excludes values for dams that were found dead

Significant reductions ($P \leq 0.05$) in the mean fetal body weight per litter (Table 26) were noted in the groups administered 200 or 400 mg/kg AZT alone or in combination with folinic acid or administered 2,000 or 3,000 mg/kg TMP/SMX alone or in combination with folinic acid.

TABLE 26
Body Weights of Fetuses from Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice

Dose[a]	Mean Fetal Weight (g)
0 + 0 + 0	1.34
0 + 0 + 10	1.33
200 + 0 + 0	0.92**
400 + 0 + 0	0.81*
400 + 0 + 10	0.66**
0 + 1,000 + 0	1.40
200 + 1,000 + 0	—[b]
400 + 1,000 + 0	—
0 + 2,000 + 0	1.06**
0 + 2,000 + 10	1.09*
200 + 2,000 + 0	—
400 + 2,000 + 0	—
400 + 2,000 + 10	—
0 + 3,000 + 0	0.90**
0 + 3,000 + 10	0.94**
200 + 3,000 + 0	—
400 + 3,000 + 0	—

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's or Dunn's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[b] No fetuses were present.

GROSS EXTERNAL ALTERATIONS (FEMALE-B LITTERS)

Administration of 1,000 to 3,000 mg/kg TMP/SMX produced significant increases ($P \leq 0.01$) in the number of fetuses with cleft palate (Table 27 and Plate 14) as well as in the number of litters and the average percentage of fetuses per litter with this alteration. Doses of 2,000 or 3,000 mg/kg TMP/SMX produced cleft palate in

100% of the litters and at least 80% of the fetuses obtained from caesarean section. The frequency of cleft palate was also associated with the presence of distended abdomens (Table 27) in pups of female-B mice. The incidence of cleft palate in pups from examined female-B mice was similar to the incidence observed in the fetuses of female-A mice.

TABLE 27
Association of Cleft Palate and Distended Abdomen in Pups from Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid in Swiss (CD-1®) Mice[a]

Dose[b]	Incidence[c] of Cleft Palate	Incidence[c] of Distended Abdomen
0 + 0 + 0	0/0	0/0
0 + 0 + 10	0/0	0/0
200 + 0 + 0	1/6	0/6
400 + 0 + 0	6/11	1/11
400 + 0 + 10	2/2	0/2
0 + 1,000 + 0	2/2	2/2
200 + 1,000 + 0	0/2	0/2
400 + 1,000 + 0	0/0	0/0
0 + 2,000 + 0	77/81	71/81
0 + 2,000 + 10	57/62	59/62
200 + 2,000 + 0	5/5	0/0
400 + 2,000 + 0	0/0	0/0
400 + 2,000 + 10	0/0	0/0
0 + 3,000 + 0	47/47	27/47
0 + 3,000 + 10	88/91	46/91
200 + 3,000 + 0	0/0	0/0
400 + 3,000 + 0	0/0	0/0

[a] Data obtained from gross necropsy of selected pups
[b] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day)
[c] Number of pups with observation/number of pups in dose group

The fetal incidence of open eyes was also increased in some of these dose groups. Administration of folinic acid did not ameliorate the effects of TMP/SMX. Other alterations not considered to be test article related included hematomas of the head and body, body edema, kyphosis, and club foot. These anomalies are common in this strain of mouse.

DISCUSSION

The most significant measurable evidence of toxicity in adult mice subsequent to the administration of 3′-azido-3′-deoxythymidine (AZT) alone was in the hematopoietic system. A mild dose-related anemia accompanied by macrocytosis occurred in male and female mice. The histopathologic findings of cellular depletion of the bone marrow corresponded well with the clinical anemia. Administration of trimethoprim/ sulfamethoxazole (TMP/SMX) alone to adult mice resulted in only a mild anemia in the 3,000 mg/kg TMP/SMX group of male and female mice.

Combination therapy with AZT and TMP/SMX resulted in hematopoietic toxicity of far greater severity than that detected subsequent to the administration of either compound alone. Male and female-A mice receiving combination therapy developed severe anemia, reticulocytopenia, and thrombocytosis accompanied by cellular depletion of bone marrow and diminished splenic hematopoiesis. The anemia was degenerative, with diminished reticulocyte counts, and surprisingly was microcytic, with elevated MCHC values indicative of hemolysis. In contrast, female-B mice administered combination therapy for only approximately 10 days had only a mild regenerative anemia that was macrocytic and was accompanied by elevated reticulocyte counts. These findings suggest that with the shorter duration of dosing in female-B animals, the macrocytic erythrocytes did not rupture and hemolysis did not occur. The addition of folinic acid had no apparent impact on the hematopoietic toxicity induced by the administration of AZT alone, TMP/SMX alone, or combinations of AZT and TMP/SMX.

Other manifestations of toxicity frequently encountered that are likely secondary to debilitation from the anemia consisted of lower mean body weight and body weight gain, pallor, piloerection, labored breathing, diminished motor activity, and emaciation.

Manifestations of toxicity involving other organ systems were also encountered in adult mice administered TMP/SMX alone or in combination with AZT. Gross enlargement of the thyroid gland, manifested microscopically by thyroid gland hyperplasia, was found in all groups receiving TMP/SMX alone or in combination with AZT. The thyroid gland lesions were believed to be due to SMX, as the goitrogenic properties of sulfonamides in rodents were previously described in the literature (Cohen *et al.*, 1981; Heath and Littlefield, 1984). It has been established that these effects are mediated through the adenohypophysis;

decreased blood levels of thyroid hormone reverse the negative feedback mechanism that inhibits thyrotropic hormone production, resulting in unregulated stimulation of the thyroid gland. Thyroid gland hyperplasia induced by long-term oral administration of SMX to rats progressed to tumor formation with lung metastasis (Swarm *et al.*, 1973). The carcinogenic properties of sulfonamides have been of interest to investigators since the 1930s due to the structural similarity between sulfonamides and aniline dyes (Lewis, 1938). Neoplasms of the thyroid gland were not observed in this study.

TMP/SMX alone and in combination with AZT also resulted in hepatocyte hypertrophy. Minor elevations in alanine aminotransferase activity accompanied the hepatocyte hypertrophy. Thymic atrophy encountered in many control female mice in this study appeared to be associated with pregnancy and the number of fetuses per dam. Administration of AZT in combination with TMP/SMX potentiated the development of thymic atrophy.

Evidence of reproductive toxicity was also observed in this study. The most striking compound-related effect was the incidence of cleft palate noted in the fetuses of female-A mice that received 2,000 or 3,000 mg/kg TMP/SMX alone or in combination with AZT; at least 80% of the fetuses in 100% of the litters had cleft palate. These compounds, TMP and SMX, have distinct inhibitory effects on the function of folic acid, a vitamin required for macromolecular synthesis and cell division. Folic acid antagonists are known to induce developmental anomalies, including cleft palate (Szabo, 1989). The finding of a 100% incidence of cleft palate in litters from females administered two or three times the therapeutic dose of TMP/SMX was unexpected. The lack of any improvement in the dose groups supplemented with folinic acid at 100 times the nutritional requirement was equally surprising; these results suggest that the increased incidence of cleft palate following treatment with TMP/SMX results from a mechanism other than inhibition of folic acid synthesis. These fetuses also exhibited increased incidences of open eyes and distended abdomens. The distended abdomens likely were secondary to the cleft palate and its effect on the ability to swallow.

In female-A and female-B mice, AZT alone produced decreases in uterine weights, live litter sizes, pup body weights, and fetal weight. Administration of AZT also increased pup deaths and resorptions. Administration of 2,000 or 3,000 mg/kg TMP/SMX alone produced mortality in pups and fetuses and slightly reduced the number of litters and the live litter size in the female-A and female-B groups. Administration of 1,000, 2,000, or 3,000 mg/kg TMP/SMX in the female-A and female-B groups decreased litter size, increased the number of pups dying on days 1 through 4, and decreased pup body weights.

Male mice treated with 1,000, 2,000, or 3,000 mg/kg TMP/SMX alone or in combination with 200 or 400 mg/kg AZT had decreased left testicular weights. Diminished sperm motility occurred in groups treated

with 200 or 400 mg/kg AZT alone and in groups treated with 2,000 or 3,000 mg/kg TMP/SMX alone. Diminished sperm motility also occurred in all groups treated with combinations of AZT and TMP/SMX.

In general, the combination of AZT and TMP/SMX at a few times the therapeutic dose appeared to cause synergistic effects, and the basis for this synergism is not known. The liver lesions induced by TMP/SMX are consistent with the hypothesis that TMP/SMX causes liver toxicity and inhibits the metabolism of AZT, resulting in increased plasma levels or half-life. Although the animal model was not folate deficient, administration of folinic acid failed to significantly ameliorate any of the toxic effects of AZT and/or TMP/SMX. Generally, the toxicity observed in this study tended to correspond with the duration of dosing. Overall toxicity was greatest in female-A mice dosed for approximately 30 days. Manifestations of toxicity were less obvious in the males dosed for approximately 20 days and least obvious in female-B mice dosed for approximately 10 days.

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APPENDIX A
CLINICAL PATHOLOGY RESULTS

TABLE A1
Hematology and Clinical Chemistry Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

	Vehicle Control	0 + 0 + 10	200 + 0 + 0	400 + 0 + 0	400 + 0 + 10
n	10	10	10	10	10
Hematology					
Hematocrit (%)	45 5 ± 3 9	46 6 ± 3 9	39 3 ± 5 7	33 6 ± 7 7**	37 8 ± 5 8*
Hemoglobin (g/dL)	15 4 ± 1 4	15 8 ± 1 2	13 3 ± 1 6	11 4 ± 2 5**	12 8 ± 1 5**
Erythrocytes ($10^6/\mu L$)	9 96 ± 1 07	9 90 ± 0 82	7 98 ± 1 02**	6 79 ± 1 42**	7 84 ± 0 84**
Reticulocytes ($10^5/\mu L$)	4 3 ± 1 28[b]	4 2 ± 0 45	3 5 ± 0 96	3 2 ± 1 60	3 4 ± 0 96
Mean cell volume (fL)	45 9 ± 3 5	47 0 ± 2 1	49 1 ± 2 7	49 2 ± 3 6	48 0 ± 3 4
Mean cell hemoglobin (pg)	15 5 ± 1 0	15 9 ± 0 7	16 7 ± 0 8*	16 7 ± 0 8*	16 3 ± 0 9
Mean cell hemoglobin concentration (g/dL)	33 8 ± 1 4	33 9 ± 0 9	34 0 ± 1 3	34 0 ± 1 9	34 1 ± 1 3
Platelets ($10^3/\mu L$)	1,094 ± 250 5	979 ± 206 2	1,161 ± 350 9	1,517 ± 314 0*	1,353 ± 402 7
Leukocytes ($10^3/\mu L$)	7 25 ± 1 93	8 78 ± 3 13	5 99 ± 1 48	5 82 ± 2 51	5 77 ± 2 31
Segmented neutrophils ($10^3/\mu L$)	1 22 ± 0 40	1 28 ± 0 59	0 61 ± 0 25*	0 76 ± 0 53	0 63 ± 0 37*
Lymphocytes ($10^3/\mu L$)	5 65 ± 1 73	7 04 ± 2 53	5 10 ± 1 35	4 76 ± 2 01	4 85 ± 1 92
Monocytes ($10^3/\mu L$)	0 15 ± 0 08	0 18 ± 0 13	0 11 ± 0 07	0 14 ± 0 11	0 09 ± 0 05
Basophils ($10^3/\mu L$)	0 02 ± 0 011	0 03 ± 0 020	0 01 ± 0 008	0 02 ± 0 011	0 01 ± 0 013
Eosinophils ($10^3/\mu L$)	0 18 ± 0 14	0 23 ± 0 14	0 15 ± 0 09	0 14 ± 0 10	0 16 ± 0 09
Large unstained cell ($10^3/\mu L$)	0 03 ± 0 02[b]	0 03 ± 0 03[c]	0 02 ± 0 01[d]	0 02 ± 0 02[c]	0 02 ± 0 02[b]
Clinical Chemistry					
Urea nitrogen (mg/dL)	18 3 ± 3 0	20 8 ± 2 8	17 0 ± 3 3	21 8 ± 3 2	19 9 ± 5 8
Creatinine (mg/dL)	0 26 ± 0 09	0 31 ± 0 12	0 31 ± 0 10	0 28 ± 0 07	0 31 ± 0 11
Alanine aminotransferase (IU/L)	21 ± 14	22 ± 10	19 ± 14	14 ± 4	22 ± 16
Alkaline phosphatase (IU/L)	46 ± 22	39 ± 14	51 ± 41	50 ± 28	35 ± 12
Aspartate aminotransferase (IU/L)	53 ± 17	57 ± 21	57 ± 16	71 ± 29	59 ± 28

TABLE A1
Hematology and Clinical Chemistry Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 1,000 + 0	200 + 1,000 + 0	400 + 1,000 + 0	0 + 2,000 + 0
n	10	9	10	9
Hematology (continued)				
Hematocrit (%)	44 0 ± 3 3	25 9 ± 7 4**	23 3 ± 8 0**	41 5 ± 3 0
Hemoglobin (g/dL)	14 8 ± 1 2	8 8 ± 2 2**	8 2 ± 2 5**	13 9 ± 1 1
Erythrocytes ($10^6/\mu L$)	9 58 ± 1 00	5 39 ± 1 13**	5 09 ± 1 22**	8 88 ± 0 63
Reticulocytes ($10^5/\mu L$)	5 0 ± 1 50	2 8 ± 2 04	2 1 ± 1 60**[b]	5 2 ± 1 26
Mean cell volume (fL)	46 1 ± 2 5	47 4 ± 4 5	45 0 ± 4 1	46 8 ± 2 2
Mean cell hemoglobin (pg)	15 5 ± 0 8	16 3 ± 1 2	16 0 ± 1 0	15 6 ± 0 7
Mean cell hemoglobin concentration (g/dL)	33 7 ± 0 9	34 4 ± 1 8	35 5 ± 1 5	33 4 ± 1 1
Platelets ($10^3/\mu L$)	1,010 ± 191 9	1,708 ± 450 1**	1,595 ± 448 0**	1,082 ± 278 5
Leukocytes ($10^3/\mu L$)	8 28 ± 2 91	5 86 ± 2 88	5 19 ± 1 95	8 00 ± 3 04
Segmented neutrophils ($10^3/\mu L$)	1 47 ± 0 53	0 46 ± 0 25**	0 45 ± 0 46**	1 29 ± 0 48
Lymphocytes ($10^3/\mu L$)	6 41 ± 2 62	5 10 ± 2 52	4 51 ± 1 55	6 26 ± 2 56
Monocytes ($10^3/\mu L$)	0 19 ± 0 05	0 14 ± 0 11	0 10 ± 0 12	0 25 ± 0 10
Basophils ($10^3/\mu L$)	0 03 ± 0 017	0 01 ± 0 012	0 01 ± 0 009	0 03 ± 0 018
Eosinophils ($10^3/\mu L$)	0 15 ± 0 10	0 13 ± 0 13	0 10 ± 0 10	0 13 ± 0 12
Large unstained cell ($10^3/\mu L$)	0 04 ± 0 02[b]	0 03 ± 0 02[d]	0 03 ± 0 02[c]	0 05 ± 0 03*[c]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	21 3 ± 2 7	17 7 ± 3 4[e]	17 0 ± 1 5[b]	20 0 ± 3 8
Creatinine (mg/dL)	0 30 ± 0 07	0 28 ± 0 08[e]	0 28 ± 0 05[b]	0 36 ± 0 17
Alanine aminotransferase (IU/L)	26 ± 13	14 ± 6[e]	23 ± 17[b]	34 ± 19
Alkaline phosphatase (IU/L)	36 ± 11	31 ± 15[e]	31 ± 7[b]	37 ± 14
Aspartate aminotransferase (IU/L)	53 ± 10	53 ± 19[c]	64 ± 30[b]	46 ± 11

TABLE A1
Hematology and Clinical Chemistry Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 2,000 + 10	200 + 2,000 + 0	400 + 2,000 + 0	400 + 2,000 + 10
n	10	10	10	9
Hematology (continued)				
Hematocrit (%)	41 9 ± 2 7	21 2 ± 3 9**	17 5 ± 3 8**	16 2 ± 3 2**
Hemoglobin (g/dL)	14 0 ± 1 1*	7 6 ± 1 3**	6 1 ± 1 2**	5 8 ± 1 1**
Erythrocytes ($10^6/\mu L$)	8 85 ± 1 04*	4 85 ± 0 83**	3 97 ± 0 80**	3 79 ± 0 66**
Reticulocytes ($10^5/\mu L$)	5 2 ± 1 67	1 3 ± 0 53**[b]	1 2 ± 0 61**	0 9 ± 0 30**
Mean cell volume (fL)	47 7 ± 3 8	43 7 ± 2 6	44 0 ± 2 4	42 9 ± 2 2
Mean cell hemoglobin (pg)	15 9 ± 1 0	15 6 ± 0 7	15 5 ± 0 7	15 2 ± 0 7
Mean cell hemoglobin concentration (g/dL)	33 4 ± 0 9	35 8 ± 1 3**	35 3 ± 1 0*	35 5 ± 1 3*
Platelets ($10^3/\mu L$)	1,252 ± 450 4	1,717 ± 353 0**	1,818 ± 434 2**	1,761 ± 423 4**
Leukocytes ($10^3/\mu L$)	8 05 ± 2 80	5 07 ± 1 81	5 53 ± 2 14	5 42 ± 1 97
Segmented neutrophils ($10^3/\mu L$)	1 87 ± 1 14	0 51 ± 0 17*	0 38 ± 0 29**	0 27 ± 0 11**
Lymphocytes ($10^3/\mu L$)	5 66 ± 1 95	4 25 ± 1 78	4 94 ± 2 06	5 01 ± 1 95
Monocytes ($10^3/\mu L$)	0 29 ± 0 17**	0 11 ± 0 05	0 06 ± 0 04	0 05 ± 0 02
Basophils ($10^3/\mu L$)	0 03 ± 0 016	0 02 ± 0 027	0 01 ± 0 008	0 01 ± 0 012
Eosinophils ($10^3/\mu L$)	0 15 ± 0 12	0 13 ± 0 07	0 09 ± 0 14	0 05 ± 0 03*
Large unstained cell ($10^3/\mu L$)	0 05 ± 0 04[b]	0 06 ± 0 07[b]	0 07 ± 0 08[c]	0 03 ± 0 02[c]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	18 7 ± 3 9	19 7 ± 4 4	18 6 ± 3 0	18 6 ± 4 3
Creatinine (mg/dL)	0 32 ± 0 11	0 36 ± 0 10	0 30 ± 0 10	0 39 ± 0 08
Alanine aminotransferase (IU/L)	28 ± 19	31 ± 15	22 ± 12	26 ± 13
Alkaline phosphatase (IU/L)	30 ± 11	38 ± 15	26 ± 10*	29 ± 15
Aspartate aminotransferase (IU/L)	67 ± 30	61 ± 20	54 ± 19	45 ± 13

TABLE A1
Hematology and Clinical Chemistry Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 3,000 + 0	0 + 3,000 + 10	200 + 3,000 + 0	400 + 3,000 + 0
n	8	10	10	7
Hematology (continued)				
Hematocrit (%)	37 4 ± 2 2**	39 3 ± 2 5**	17 4 ± 2 5**	16 7 ± 4 0**
Hemoglobin (g/dL)	12 5 ± 0 9**	13 0 ± 1 0**	6 2 ± 0 9**	5 9 ± 1 3**
Erythrocytes ($10^6/\mu L$)	8 01 ± 0 50**	8 13 ± 0 42**	4 12 ± 0 59**	3 95 ± 0 88**
Reticulocytes ($10^5/\mu L$)	5 1 ± 1 41[d]	5 4 ± 1 40	1 6 ± 1 05**[b]	1 2 ± 0 41**
Mean cell volume (fL)	46 7 ± 2 0	48 5 ± 3 8	42 3 ± 1 9*	42 1 ± 2 1*
Mean cell hemoglobin (pg)	15 6 ± 0 6	16 0 ± 1 1	15 2 ± 0 8	14 9 ± 0 6
Mean cell hemoglobin concentration (g/dL)	33 4 ± 1 1	33 0 ± 1 3	35 8 ± 0 9**	35 4 ± 1 1*
Platelets ($10^3/\mu L$)	1,397 ± 358 9	1,360 ± 198 9	2,077 ± 740 5**	1,866 ± 289 9**
Leukocytes ($10^3/\mu L$)	5 87 ± 1 95	8 02 ± 3 53	3 71 ± 1 49	3 64 ± 1 46
Segmented neutrophils ($10^3/\mu L$)	1 75 ± 0 50	1 79 ± 0 63	0 69 ± 0 38	0 73 ± 0 56
Lymphocytes ($10^3/\mu L$)	3 62 ± 1 57	5 65 ± 3 04	2 81 ± 1 65*	2 79 ± 1 26*
Monocytes ($10^3/\mu L$)	0 29 ± 0 09*	0 34 ± 0 14**	0 10 ± 0 07	0 05 ± 0 06
Basophils ($10^3/\mu L$)	0 03 ± 0 020	0 03 ± 0 017	0 01 ± 0 012	0 01 ± 0 011
Eosinophils ($10^3/\mu L$)	0 13 ± 0 11	0 17 ± 0 08	0 05 ± 0 05*	0 02 ± 0 01**
Large unstained cell ($10^3/\mu L$)	0 06 ± 0 05	0 05 ± 0 02[c]	0 06 ± 0 04[b]	0 05 ± 0 03[f]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	20 2 ± 3 5	21 7 ± 4 0	19 4 ± 5 6	19 2 ± 5 4
Creatinine (mg/dL)	0 41 ± 0 16	0 38 ± 0 20	0 68 ± 0 31**	0 61 ± 0 12**
Alanine aminotransferase (IU/L)	100 ± 59**	53 ± 27*	51 ± 33	42 ± 24
Alkaline phosphatase (IU/L)	15 ± 7**	31 ± 13	30 ± 22	22 ± 8**
Aspartate aminotransferase (IU/L)	66 ± 21	67 ± 33	58 ± 25	55 ± 30

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day) Data are given as mean ± standard error Statistical tests were performed on unrounded data
[b] n = 9
[c] n = 8
[d] n = 7
[e] n = 10
[f] n = 6

TABLE A2
Hematology and Clinical Chemistry Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

	Vehicle Control	0 + 0 + 10	200 + 0 + 0	400 + 0 + 0	400 + 0 + 10
n	20	20	20	20	19
Hematology					
Hematocrit (%)	42 7 ± 2 1	42 3 ± 3 1	35 5 ± 8 9**	29 5 ± 12 0**	29 6 ± 10 5**
Hemoglobin (f/dL)	14 6 ± 0 9	14 4 ± 1 2	11 9 ± 2 7**	9 8 ± 3 7**	10 0 ± 3 3**
Erythrocytes ($10^6/\mu L$)	8 64 ± 0 49	8 65 ± 0 54	6 26 ± 1 42**	5 27 ± 1 77**	5 32 ± 1 55**
Reticulocytes ($10^5/\mu L$)	3 0 ± 0 82	3 5 ± 1 24	3 4 ± 1 75	3 6 ± 2 00	3 0 ± 1 38[b]
Mean cell volume (fL)	49 5 ± 1 4	48 9 ± 2 2	56 3 ± 6 7**	54 0 ± 6 7*	54 3 ± 5 7**
Mean cell hemoglobin (pg)	16 9 ± 0 5	16 7 ± 0 8	18 9 ± 1 6**	18 3 ± 1 4**	18 6 ± 1 4**
Mean cell hemoglobin concentration (g/dL)	34 2 ± 0 9	34 1 ± 1 3	33 8 ± 1 6	34 1 ± 2 2	34 4 ± 1 7
Platelets ($10^3/\mu L$)	1,149 ± 279 3	1,215 ± 202 2	1,325 ± 294 4	1,819 ± 647 1**	1,618 ± 495 1*
Leukocytes ($10^3/\mu L$)	6 69 ± 2 54	6 59 ± 1 63	4 57 ± 1 69**	5 00 ± 1 40*	4 62 ± 1 35**
Segmented neutrophils ($10^3/\mu L$)	1 56 ± 0 79	1 84 ± 1 02	0 72 ± 0 57**	0 50 ± 0 36**	0 51 ± 0 33**
Lymphocytes ($10^3/\mu L$)	4 79 ± 2 18	4 37 ± 1 14	3 59 ± 1 31	4 24 ± 1 23	3 86 ± 1 23
Monocytes ($10^3/\mu L$)	0 17 ± 0 08	0 19 ± 0 09	0 09 ± 0 08**	0 08 ± 0 06**	0 08 ± 0 06**
Basophils ($10^3/\mu L$)	0 02 ± 0 016	0 02 ± 0 009	0 01 ± 0 008**	0 01 ± 0 007**	0 01 ± 0 008**
Eosinophils ($10^3/\mu L$)	0 14 ± 0 09	0 16 ± 0 11	0 14 ± 0 07	0 17 ± 0 15	0 14 ± 0 13
Large unstained cell ($10^3/\mu L$)	0 02 ± 0 01[c]	0 01 ± 0 01	0 01 ± 0 01[d]	0 02 ± 0 01[e]	0 02 ± 0 01[f]
Clinical Chemistry					
Urea nitrogen (mg/dL)	15 9 ± 3 4	14 6 ± 6 0	18 1 ± 4 0[c]	18 3 ± 4 5	17 3 ± 4 1
Creatinine (mg/dL)	0 37 ± 0 05	0 37 ± 0 06[c]	0 33 ± 0 11[c]	0 33 ± 0 08	0 32 ± 0 09
Alanine aminotransferase (IU/L)	22 ± 12	24 ± 12[c]	17 ± 8[c]	13 ± 5*	17 ± 8
Alkaline phosphatase (IU/L)	53 ± 14	53 ± 25	70 ± 24[c]	65 ± 22	71 ± 31*
Aspartate aminotransferase (IU/L)	93 ± 24	77 ± 20	75 ± 17*[c]	67 ± 19**	71 ± 20**

TABLE A2
Hematology and Clinical Chemistry Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 1,000 + 0	200 + 1,000 + 0	400 + 1,000 + 0	0 + 2,000 + 0
n	19	20	20	20
Hematology (continued)				
Hematocrit (%)	42.1 ± 3.9	14.9 ± 8.5**	9.1 ± 1.3**	39.8 ± 4.1
Hemoglobin (g/dL)	14.5 ± 1.4	5.1 ± 2.6**	3.4 ± 0.5**	13.5 ± 1.5
Erythrocytes ($10^6/\mu L$)	8.78 ± 0.92	2.94 ± 1.24**	2.16 ± 0.33**	8.00 ± 0.90
Reticulocytes ($10^5/\mu L$)	2.9 ± 0.85	1.9 ± 2.14[c]	0.6 ± 0.51**	2.8 ± 1.16
Mean cell volume (fL)	48.1 ± 2.4	48.6 ± 7.1	42.4 ± 1.6**	49.9 ± 2.4
Mean cell hemoglobin (pg)	16.6 ± 0.7	17.1 ± 1.6	15.7 ± 0.7**	16.9 ± 0.8
Mean cell hemoglobin concentration (g/dL)	34.5 ± 1.3	35.3 ± 2.0	36.9 ± 0.9**	34.0 ± 1.1
Platelets ($10^3/\mu L$)	1,005 ± 224.3	2,010 ± 559.5**	1,354 ± 763.2	1,303 ± 328.4
Leukocytes ($10^3/\mu L$)	6.51 ± 1.82	4.09 ± 1.88**	3.26 ± 1.19**	6.70 ± 1.55
Segmented neutrophils ($10^3/\mu L$)	1.50 ± 0.81	0.22 ± 0.18**	0.08 ± 0.08**	1.99 ± 0.90
Lymphocytes ($10^3/\mu L$)	4.60 ± 1.58	3.70 ± 1.71	3.13 ± 1.15**	4.29 ± 1.54
Monocytes ($10^3/\mu L$)	0.18 ± 0.09	0.06 ± 0.05**	0.01 ± 0.02**	0.27 ± 0.11**
Basophils ($10^3/\mu L$)	0.02 ± 0.013	0.01 ± 0.008**	0.01 ± 0.006**	0.02 ± 0.009
Eosinophils ($10^3/\mu L$)	0.19 ± 0.13	0.09 ± 0.08	0.02 ± 0.04**	0.11 ± 0.10
Large unstained cell ($10^3/\mu L$)	0.02 ± 0.01[g]	0.02 ± 0.01[c]	0.01 ± 0.01	0.01 ± 0.01[c]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	14.6 ± 3.2[h]	17.4 ± 4.6	18.1 ± 3.6	16.2 ± 3.9
Creatinine (mg/dL)	0.37 ± 0.07	0.32 ± 0.07	0.39 ± 0.09	0.53 ± 0.16**[c]
Alanine aminotransferase (IU/L)	23 ± 9[h]	15 ± 6	19 ± 8	44 ± 18**
Alkaline phosphatase (IU/L)	51 ± 13[h]	39 ± 13	49 ± 17	52 ± 23
Aspartate aminotransferase (IU/L)	70 ± 22**[h]	51 ± 9**	56 ± 15**	81 ± 21

TABLE A2
Hematology and Clinical Chemistry Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 2,000 + 10	200 + 2,000 + 0	400 + 2,000 + 0	400 + 2,000 + 10
n	17	13	12	12
Hematology (continued)				
Hematocrit (%)	40 4 ± 3 3	11 6 ± 7 1**	11 6 ± 9 5**	8 4 ± 1 1**
Hemoglobin (g/dL)	13 9 ± 1 0	4 2 ± 2 3**	4 1 ± 2 8**	3 2 ± 0 4**
Erythrocytes ($10^6/\mu L$)	8 04 ± 0 68	2 49 ± 1 17**	2 40 ± 1 36**	2 00 ± 0 30**
Reticulocytes ($10^5/\mu L$)	3 0 ± 1 32[i]	1 1 ± 1 63**[j]	1 2 ± 1 60**[j]	0 4 ± 0 38**[k]
Mean cell volume (fL)	50 3 ± 2 7	44 7 ± 5 2**	44 9 ± 8 2**	42 1 ± 1 3**
Mean cell hemoglobin (pg)	17 3 ± 1 0	16 6 ± 1 0	16 6 ± 1 6	15 8 ± 0 7**
Mean cell hemoglobin concentration (g/dL)	34 3 ± 1 5	37 4 ± 2 2**	37 5 ± 3 2**	37 5 ± 1 1**
Platelets ($10^3/\mu L$)	1,213 ± 275 6	1,358 ± 482 8	927 ± 711 8	746 ± 655 4
Leukocytes ($10^3/\mu L$)	8 30 ± 2 72	2 62 ± 1 20**	2 49 ± 1 23**	1 56 ± 0 90**
Segmented neutrophils ($10^3/\mu L$)	2 25 ± 1 68	0 09 ± 0 09**	0 10 ± 0 12**	0 04 ± 0 03**
Lymphocytes ($10^3/\mu L$)	5 54 ± 2 45	2 48 ± 1 16**	2 33 ± 1 08**	1 50 ± 0 91**
Monocytes ($10^3/\mu L$)	0 30 ± 0 15**	0 01 ± 0 02**	0 03 ± 0 05**	0 00 ± 0 01**
Basophils ($10^3/\mu L$)	0 03 ± 0 016	0 00 ± 0 005**	0 01 ± 0 007	0 00 ± 0 007**
Eosinophils ($10^3/\mu L$)	0 16 ± 0 15	0 02 ± 0 02**	0 02 ± 0 03**	0 01 ± 0 01**
Large unstained cell ($10^3/\mu L$)	0 03 ± 0 01*	0 01 ± 0 01[l]	0 01 ± 0 01	0 00 ± 0 01*[m]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	15 3 ± 1 7	24 1 ± 14 4[f]	25 4 ± 20 3*	24 0 ± 10 9
Creatinine (mg/dL)	0 44 ± 0 12	0 56 ± 0 24**[f]	0 55 ± 0 19*	0 64 ± 0 12**
Alanine aminotransferase (IU/L)	37 ± 17	36 ± 19[f]	47 ± 44	53 ± 55
Alkaline phosphatase (IU/L)	47 ± 12	34 ± 11**[f]	35 ± 12**	33 ± 12**
Aspartate aminotransferase (IU/L)	87 ± 33	66 ± 26[f]	76 ± 40	90 ± 45

TABLE A2
Hematology and Clinical Chemistry Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 3,000 + 0	0 + 3,000 + 10	200 + 3,000 + 0	400 + 3,000 + 0
n	17	16	4	2
Hematology (continued)				
Hematocrit (%)	38 1 ± 3 3*	38 7 ± 4 5	11 5 ± 2 8**	9 9 ± 0 6**
Hemoglobin (g/dL)	12 9 ± 1 1**	13 2 ± 1 6*	4 3 ± 0 8**	3 8 ± 0 4**
Erythrocytes ($10^6/\mu L$)	7 64 ± 0 66**	7 68 ± 0 95**	2 79 ± 0 49**	2 43 ± 0 13**
Reticulocytes ($10^5/\mu L$)	2 8 ± 0 78	2 8 ± 0 65	1 2 ± 0 64[n]	0 1*[o]
Mean cell volume (fL)	50 0 ± 2 6	50 4 ± 2 7	40 9 ± 3 0**	40 7 ± 0 3*
Mean cell hemoglobin (pg)	16 9 ± 0 9	17 2 ± 0 8	15 3 ± 0 5*	15 6 ± 1 0
Mean cell hemoglobin concentration (g/dL)	33 8 ± 1 1	34 1 ± 1 0	37 5 ± 1 7**	38 4 ± 2 1**
Platelets ($10^3/\mu L$)	1,407 ± 371 4	1,325 ± 189 6	1,382 ± 711 1	972 ± 285 0
Leukocytes ($10^3/\mu L$)	6 37 ± 2 10	6 46 ± 1 77	2 18 ± 0 35**	0 94 ± 0 62**
Segmented neutrophils ($10^3/\mu L$)	1 79 ± 0 95	2 01 ± 0 81	0 18 ± 0 14*	0 02 ± 0 01
Lymphocytes ($10^3/\mu L$)	4 21 ± 1 81	4 08 ± 1 58	1 94 ± 0 49*	0 91 ± 0 59*
Monocytes ($10^3/\mu L$)	0 28 ± 0 12**	0 28 ± 0 12**	0 02 ± 0 03*	0 00 ± 0 00
Basophils ($10^3/\mu L$)	0 02 ± 0 010	0 02 ± 0 012	0 02 ± 0 022	0 00 ± 0 000
Eosinophils ($10^3/\mu L$)	0 05 ± 0 05*	0 05 ± 0 07*	0 01 ± 0 01*	0 00 ± 0 00
Large unstained cell ($10^3/\mu L$)	0 02 ± 0 01	0 02 ± 0 02	0 01 ± 0 01	0 00 ± 0 00
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	15 1 ± 4 6[b]	14 1 ± 1 9	18 5 ± 5 0	11 6 ± 3 5
Creatinine (mg/dL)	0 52 ± 0 14*[b]	0 56 ± 0 21**	0 67 ± 0 25**	0 97 ± 0 00**
Alanine aminotransferase (IU/L)	66 ± 45**[b]	43 ± 24	64 ± 48	59 ± 11
Alkaline phosphatase (IU/L)	45 ± 12[b]	43 ± 11	40 ± 17	24 ± 1*
Aspartate aminotransferase (IU/L)	81 ± 41[b]	72 ± 38	65 ± 24	108 ± 8

* Significantly different (P≤0 05) from the vehicle control group by Dunnett's test
** P≤0 01
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day) Data are given as mean ± standard error Statistical tests were performed on unrounded data
[b] n = 18
[c] n = 19
[d] n = 15
[e] n = 13
[f] n = 14
[g] n = 17
[h] n = 20
[i] n = 16
[j] n = 10
[k] n = 8
[l] n = 12
[m] n = 11
[n] n = 2
[o] n = 1

TABLE A3
Hematology and Clinical Chemistry Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

	Vehicle Control	0 + 0 + 10	200 + 0 + 0	400 + 0 + 0	400 + 0 + 10
n	14	14	14	16	16
Hematology					
Hematocrit (%)	41.9 ± 2.7	43.8 ± 4.3	43.6 ± 2.9	42.1 ± 2.0	43.1 ± 2.9
Hemoglobin (g/dL)	13.8 ± 1.0	14.7 ± 1.7	14.3 ± 1.3	13.7 ± 0.8	14.3 ± 1.1
Erythrocytes ($10^6/\mu L$)	8.31 ± 0.51	8.81 ± 1.09	8.32 ± 0.73	7.77 ± 0.39	8.07 ± 0.78
Reticulocytes ($10^5/\mu L$)	4.7 ± 1.24	4.4 ± 1.44	5.0 ± 1.73	7.9 ± 3.36*	4.9 ± 2.11
Mean cell volume (fL)	50.4 ± 2.0	49.9 ± 2.7	52.6 ± 2.8	54.2 ± 2.1**	53.6 ± 3.4*
Mean cell hemoglobin (pg)	16.6 ± 0.8	16.7 ± 0.7	17.2 ± 0.7	17.7 ± 0.7**	17.8 ± 0.9**
Mean cell hemoglobin concentration (g/dL)	33.0 ± 1.1	33.5 ± 1.2	32.7 ± 1.6	32.7 ± 1.2	33.3 ± 1.4
Platelets ($10^3/\mu L$)	1,403 ± 359.6	1,237 ± 273.4	1,343 ± 228.1	1,421 ± 387.7	1,267 ± 365.0
Leukocytes ($10^3/\mu L$)	4.47 ± 1.54	5.34 ± 2.22	5.04 ± 1.32	5.93 ± 3.68	6.16 ± 2.92
Segmented neutrophils ($10^3/\mu L$)	1.00 ± 0.55	1.07 ± 0.67	0.93 ± 0.33	1.08 ± 0.63	1.10 ± 0.63
Lymphocytes ($10^3/\mu L$)	3.19 ± 1.03	4.00 ± 1.60	3.86 ± 1.05	4.37 ± 2.66	4.70 ± 2.29
Monocytes ($10^3/\mu L$)	0.11 ± 0.05	0.14 ± 0.10	0.10 ± 0.05	0.17 ± 0.13	0.14 ± 0.10
Basophils ($10^3/\mu L$)	0.01 ± 0.008	0.01 ± 0.011	0.01 ± 0.010	0.02 ± 0.031	0.02 ± 0.015
Eosinophils ($10^3/\mu L$)	0.16 ± 0.14	0.11 ± 0.09	0.13 ± 0.08	0.28 ± 0.37	0.19 ± 0.14
Large unstained cell ($10^3/\mu L$)	0.01 ± 0.01[b]	0.01 ± 0.01[c]	0.01 ± 0.01[d]	0.03 ± 0.03[e]	0.02 ± 0.01[f]
Clinical Chemistry					
Urea nitrogen (mg/dL)	29.3 ± 4.2	30.6 ± 2.5	30.4 ± 7.5	27.3 ± 4.8	25.2 ± 5.3
Creatinine (mg/dL)	0.39 ± 0.05[g]	0.42 ± 0.05[g]	0.40 ± 0.10	0.35 ± 0.06[h]	0.37 ± 0.08[h]
Alanine aminotransferase (IU/L)	34 ± 14	33 ± 8	43 ± 25[g]	52 ± 42	32 ± 19
Alkaline phosphatase (IU/L)	37 ± 16	37 ± 8	49 ± 23	37 ± 13	47 ± 20
Aspartate aminotransferase (IU/L)	72 ± 24	72 ± 19	86 ± 27[g]	93 ± 36	76 ± 26

TABLE A3
Hematology and Clinical Chemistry Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 1,000 + 0	200 + 1,000 + 0	400 + 1,000 + 0	0 + 2,000 + 0
n	15	16	15	14
Hematology (continued)				
Hematocrit (%)	42 8 ± 4 0	42 1 ± 3 1	40 1 ± 6 6	44 3 ± 2 3
Hemoglobin (g/dL)	14 2 ± 1 9	13 9 ± 1 3	13 2 ± 2 3	14 8 ± 0 9
Erythrocytes ($10^6/\mu L$)	8 82 ± 1 18	7 98 ± 0 80	7 62 ± 1 25	8 83 ± 0 63
Reticulocytes ($10^5/\mu L$)	5 8 ± 1 76	8 4 ± 4 84**	9 3 ± 5 28**	4 4 ± 1 66[g]
Mean cell volume (fL)	48 8 ± 2 7	53 0 ± 3 6	52 5 ± 2 5	50 4 ± 3 3
Mean cell hemoglobin (pg)	16 2 ± 0 8	17 5 ± 0 8**	17 3 ± 0 6	16 8 ± 0 9
Mean cell hemoglobin concentration (g/dL)	33 3 ± 2 3	33 1 ± 1 5	32 9 ± 1 0	33 3 ± 1 4
Platelets ($10^3/\mu L$)	1,213 ± 330 6	1,435 ± 409 7	1,487 ± 464 9	1,525 ± 325 0
Leukocytes ($10^3/\mu L$)	4 54 ± 1 17	7 05 ± 3 01*	8 14 ± 2 59**	6 06 ± 1 58
Segmented neutrophils ($10^3/\mu L$)	0 75 ± 0 18	1 18 ± 0 45	1 26 ± 0 66	1 01 ± 0 47
Lymphocytes ($10^3/\mu L$)	3 52 ± 1 10	5 29 ± 2 36*	6 40 ± 2 01**	4 74 ± 1 43
Monocytes ($10^3/\mu L$)	0 10 ± 0 05	0 23 ± 0 14**	0 23 ± 0 13**	0 11 ± 0 05
Basophils ($10^3/\mu L$)	0 01 ± 0 011	0 03 ± 0 024	0 04 ± 0 036**	0 02 ± 0 013
Eosinophils ($10^3/\mu L$)	0 15 ± 0 11	0 28 ± 0 20	0 17 ± 0 14	0 16 ± 0 10
Large unstained cell ($10^3/\mu L$)	0 02 ± 0 01[b]	0 04 ± 0 04[g]	0 04 ± 0 04*	0 03 ± 0 01[b]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	29 3 ± 6 6	21 9 ± 3 1**	19 0 ± 5 2**	25 5 ± 4 5
Creatinine (mg/dL)	0 38 ± 0 08[f]	0 29 ± 0 07**	0 28 ± 0 07**	0 36 ± 0 06[b]
Alanine aminotransferase (IU/L)	28 ± 7[f]	63 ± 50*	32 ± 27	28 ± 11
Alkaline phosphatase (IU/L)	46 ± 28	43 ± 13	42 ± 17	50 ± 13
Aspartate aminotransferase (IU/L)	76 ± 27[f]	86 ± 31	71 ± 31	70 ± 24

TABLE A3
Hematology and Clinical Chemistry Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 2,000 + 10	200 + 2,000 + 0	400 + 2,000 + 0	400 + 2,000 + 10
n	15	16	16	15
Hematology (continued)				
Hematocrit (%)	45 8 ± 3 7	40 2 ± 4 3	40 1 ± 4 8	37 5 ± 7 7
Hemoglobin (g/dL)	15 2 ± 1 4	13 2 ± 1 6	12 9 ± 1 7	12 2 ± 2 7
Erythrocytes ($10^6/\mu L$)	9 02 ± 0 78	7 41 ± 0 80	7 37 ± 1 08	7 08 ± 1 60*
Reticulocytes ($10^5/\mu L$)	4 2 ± 1 44	8 8 ± 4 74	11 8 ± 5 12**	12 7 ± 5 79**
Mean cell volume (fL)	50 8 ± 1 8	54 3 ± 2 8**	54 7 ± 3 2**	53 3 ± 3 6
Mean cell hemoglobin (pg)	16 9 ± 0 7	17 8 ± 0 9**	17 5 ± 0 7*	17 2 ± 0 6
Mean cell hemoglobin concentration (g/dL)	33 2 ± 1 0	32 7 ± 1 2	31 9 ± 1 1	32 3 ± 1 5
Platelets ($10^3/\mu L$)	1,289 ± 289 7	1,760 ± 447 5	1,468 ± 380 6	1,541 ± 385 4
Leukocytes ($10^3/\mu L$)	5 10 ± 1 47	8 20 ± 3 10**	8 42 ± 2 54**	8 74 ± 4 26**
Segmented neutrophils ($10^3/\mu L$)	0 70 ± 0 19	1 10 ± 0 44	1 18 ± 0 35	1 15 ± 0 65
Lymphocytes ($10^3/\mu L$)	4 14 ± 1 37	6 54 ± 2 46**	6 70 ± 2 15**	7 09 ± 3 67**
Monocytes ($10^3/\mu L$)	0 10 ± 0 05	0 28 ± 0 23*	0 22 ± 0 13	0 26 ± 0 25*
Basophils ($10^3/\mu L$)	0 02 ± 0 012	0 04 ± 0 038	0 06 ± 0 052*	0 05 ± 0 043
Eosinophils ($10^3/\mu L$)	0 13 ± 0 07	0 21 ± 0 15	0 22 ± 0 20	0 13 ± 0 13
Large unstained cell ($10^3/\mu L$)	0 01 ± 0 01[f]	0 04 ± 0 04[h]	0 05 ± 0 05[h]	0 06 ± 0 08**
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	26 0 ± 7 4	20 6 ± 3 5**	20 9 ± 3 5**	20 1 ± 2 7**
Creatinine (mg/dL)	0 34 ± 0 11[f]	0 30 ± 0 07*	0 29 ± 0 08*	0 29 ± 0 07**
Alanine aminotransferase (IU/L)	28 ± 9	40 ± 34[h]	35 ± 30[h]	48 ± 32
Alkaline phosphatase (IU/L)	46 ± 14	42 ± 14	47 ± 22	44 ± 12
Aspartate aminotransferase (IU/L)	67 ± 17	79 ± 39[h]	78 ± 27[h]	91 ± 38

TABLE A3
Hematology and Clinical Chemistry Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 3,000 + 0	0 + 3,000 + 10	200 + 3,000 + 0	400 + 3,000 + 0
n	13	12	12	13
Hematology (continued)				
Hematocrit (%)	43 8 ± 2 1	44 4 ± 3 8	41 0 ± 6 9	36 4 ± 8 2
Hemoglobin (g/dL)	14 5 ± 0 6	15 2 ± 1 2	13 1 ± 2 4	11 4 ± 2 6**
Erythrocytes ($10^6/\mu L$)	8 80 ± 0 64	8 68 ± 0 79	7 44 ± 1 44	6 48 ± 1 38**
Reticulocytes ($10^5/\mu L$)	4 1 ± 1 13	6 4 ± 8 46[e]	10 0 ± 4 41*	14 2 ± 6 76**
Mean cell volume (fL)	49 9 ± 3 1	51 2 ± 2 5	55 5 ± 3 3**	56 1 ± 5 0**
Mean cell hemoglobin (pg)	16 6 ± 0 8	17 2 ± 0 8	17 7 ± 0 9**	17 4 ± 1 2
Mean cell hemoglobin concentration (g/dL)	33 2 ± 1 7	33 7 ± 0 8	31 9 ± 1 5	31 2 ± 1 0**
Platelets ($10^3/\mu L$)	1,591 ± 428 9	1,870 ± 492 7*	1,680 ± 575 2	1,517 ± 329 0
Leukocytes ($10^3/\mu L$)	6 62 ± 1 83	5 95 ± 2 29	9 32 ± 2 79**	8 31 ± 2 64**
Segmented neutrophils ($10^3/\mu L$)	1 24 ± 0 65	1 10 ± 0 51	1 22 ± 0 33	1 39 ± 1 13
Lymphocytes ($10^3/\mu L$)	4 97 ± 1 58	4 48 ± 1 87	7 61 ± 2 67**	6 44 ± 1 89**
Monocytes ($10^3/\mu L$)	0 17 ± 0 05	0 15 ± 0 07	0 26 ± 0 13	0 27 ± 0 21*
Basophils ($10^3/\mu L$)	0 02 ± 0 013	0 02 ± 0 017	0 05 ± 0 050	0 07 ± 0 071**
Eosinophils ($10^3/\mu L$)	0 20 ± 0 16	0 17 ± 0 19	0 15 ± 0 10	0 09 ± 0 06
Large unstained cell ($10^3/\mu L$)	0 03 ± 0 02[b]	0 03 ± 0 02[e]	0 04 ± 0 03[d]	0 05 ± 0 03[b]
Clinical Chemistry (continued)				
Urea nitrogen (mg/dL)	22 9 ± 4 1**	22 4 ± 2 9**	20 6 ± 3 8**[g]	18 5 ± 3 7**
Creatinine (mg/dL)	0 35 ± 0 10	0 36 ± 0 06[c]	0 30 ± 0 07*[g]	0 27 ± 0 07**
Alanine aminotransferase (IU/L)	50 ± 39	51 ± 32[d]	38 ± 31[g]	24 ± 11
Alkaline phosphatase (IU/L)	49 ± 17	44 ± 9	48 ± 14[g]	39 ± 19
Aspartate aminotransferase (IU/L)	95 ± 28	97 ± 43[e]	76 ± 28[g]	59 ± 22

* Significantly different (P≤0 05) from the vehicle control group by Dunnett's test
** P≤0 01
[a] Daily gavage doses of AZT + TMP/SMX + folinic acid (mg/kg per day) Data are given as mean ± standard error Statistical tests were performed on unrounded data
[b] n = 12
[c] n = 8
[d] n = 10
[e] n = 11
[f] n = 14
[g] n = 13
[h] n = 15

APPENDIX B
REPRODUCTIVE TISSUE EVALUATIONS

TABLE B1
Summary of Reproductive Tissue Evaluations in Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid[a]

	Vehicle Control	0 + 0 + 10[b]	200 + 0 + 0	400 + 0 + 0	400 + 0 + 10[b]
n	10	10	10	10	10
Weights (g)					
Necropsy body wt	34 38 ± 0 99	34 50 ± 1 24	34 44 ± 1 06	34 07 ± 1 09	33 72 ± 0 78
L cauda[c]	0 019 ± 0 001	0 021 ± 0 001	0 021 ± 0 001	0 022 ± 0 001[d]	0 019 ± 0 001
L testis	0 112 ± 0 004	0 121 ± 0 007	0 119 ± 0 004	0 109 ± 0 005[d]	0 119 ± 0 005[d]
Spermatid heads (10^7/testis)	1 57 ± 0 06	1 68 ± 0 07	1 60 ± 0 09	1 56 ± 0 10[d]	1 71 ± 0 05[d]
Epididymal spermatozoal motility (%)	86 52 ± 1 40	85 80 ± 0 95[d]	62 65 ± 8 17*[e]	38 73 ± 11 59*[d,e]	60 05 ± 12 01

	0 + 1,000 + 0	200 + 1,000 + 0	400 + 1,000 + 0	0 + 2,000 + 0
n	10	10	10	9
Weights (g)				
Necropsy body wt	35 03 ± 1 11	34 35 ± 1 18	33 81 ± 0 91	35 21 ± 0 94
L cauda[c]	0 020 ± 0 001	0 020 ± 0 001	0 019 ± 0 001	0 023 ± 0 001
L testis	0 102 ± 0 003*[g]	0 106 ± 0 004*[g]	0 112 ± 0 006*[g]	0 109 ± 0 005*[g]
Spermatid heads (10^7/testis)	1 49 ± 0 08[h]	1 46 ± 0 05[h]	1 54 ± 0 08[d h]	1 35 ± 0 08[f,h]
Epididymal spermatozoal motility (%)	79 50 ± 2 17[d]	28 65 ± 11 12*[e]	51 89 ± 10 00*[d,e]	66 27 ± 9 03*[e f]

TABLE B1
Summary of Reproductive Tissue Evaluations in Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT, TMP/SMX, and Folinic Acid

	0 + 2,000 + 10[b]	200 + 2,000 + 0	400 + 2,000 + 0	400 + 2,000 + 10[b]
n	10	10	10	9
Weights (g)				
Necropsy body wt	34 45 ± 0 88	35.42 ± 0 88	34.64 ± 0.75	34 46 ± 1.28
L. cauda[c]	0.021 ± 0.001	0 023 ± 0.002	0.021 ± 0 001	0.022 ± 0.001
L testis	0.108 ± 0.004*[g]	0 101 ± 0.005*[g]	0.101 ± 0.004*[g]	0.105 ± 0.004*[g]
Spermatid heads (10^7/testis)	1 44 ± 0.05[h]	1 40 ± 0.09[h]	1.45 ± 0.08[h]	1.49 ± 0.07[h]
Epididymal spermatozoal motility (%)	59.85 ± 7 93	44 46 ± 10.16*[e]	19.50 ± 8.00*[e]	17.21 ± 4.05

	0 + 3,000 + 0	0 + 3,000 + 10[b]	200 + 3,000 + 0	400 + 3,000 + 0
n	8	10	10	7
Weights (g)				
Necropsy body wt	33.95 ± 0.82	35.42 ± 0.84	32 05 ± 1.16	32.09 ± 0.98
L cauda[c]	0.022 ± 0.001	0.019 ± 0 001	0 020 ± 0 001	0.018 ± 0.001
L testis	0 108 ± 0.004*[g]	0.102 ± 0.005*[g]	0.108 ± 0.006*[g]	0.094 ± 0.002*[g]
Spermatid heads (10^7/testis)	1 37 ± 0 11[h]	1 47 ± 0.07[h]	1 50 ± 0.05[h]	1.44 ± 0.04[h]
Epididymal spermatozoal motility (%)	66 44 ± 9.89*[e]	68 25 ± 8.44	3.50 ± 1.04*[e]	44.26 ± 14.28*[e]

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test

[a] Daily gavage doses are given as AZT + TMP/SMX + folinic acid (mg/kg per day). Data are given as mean ± standard error

[b] Folinic acid did not influence fertility parameters with either AZT, TMP/SMX, or combinations.

[c] All values showed a significant interaction of all three chemicals ($P=0.01$) by a two-way analysis of variance, indicating the dose response relationship of AZT differs across doses of TMP/SMX and folinic acid.

[d] n=9

[e] Significantly different ($P \leq 0$ 01) from the vehicle control group by Williams' or Dunnett's test for the 200 and 400 mg/kg AZT and the 2,000 and 3,000 mg/kg TMP/SMX groups when data were collapsed over levels of TMP/SMX + folinic acid exposure and AZT + folinic acid exposure, respectively

[f] $n=10$

[g] Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' or Dunnett's test for the 1,000, 2,000, and 3,000 mg/kg TMP/SMX groups when data were collapsed over levels of AZT + folinic acid exposure.

[h] Significantly different from the vehicle control group by Williams' or Dunnett's test for the 1,000 mg/kg TMP/SMX group ($P \leq 0.05$) and the 2,000 and 3,000 mg/kg TMP/SMX groups ($P \leq 0$ 01) when data were collapsed over levels of AZT + folinic acid exposure

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