

**NIEHS AIDS Therapeutics**  
Toxicity Report  
Number 6

**NIEHS Technical Report**  
on the Subchronic Toxicity Study of

**3'-Azido-3'-deoxythymidine (AZT)**  
**and Rifampicin Combinations**

(CAS Nos. 30516-87-1 and 13292-46-1)

**Administered by Gavage**  
**to B6C3F<sub>1</sub> Mice**

**NIH Publication 01-4401**  
**January 2001**

**U.S. Department of Health and Human Services**  
**Public Health Service**  
**National Institutes of Health**

## **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.

**NIEHS AIDS Therapeutics**  
Toxicity Report  
Number 6

**NIEHS Technical Report**  
**on the Subchronic Toxicity Study of**  
**3'-Azido-3'-deoxythymidine (AZT)**  
**and Rifampicin Combinations**

**(CAS Nos. 30516-87-1 and 13292-46-1)**

**Administered by Gavage**  
**to B6C3F<sub>1</sub> Mice**

**NIH Publication 01-4401**  
**January 2001**

**U.S. Department of Health and Human Services**  
**Public Health Service**

## **CONTRIBUTORS**

This report on the subchronic toxicity study of 3'-azido-3'-deoxythymidine (AZT) and rifampicin combinations is based primarily on studies that began in May 1993 and ended in August 1993 at Southern Research Institute, Birmingham, AL.

### **National Institute of Environmental Health Sciences**

*Evaluated experiment, interpreted results, and reported findings*

Ghanta N. Rao, D.V.M., Ph.D., Study Scientist

### **Southern Research Institute**

*Principal contributors*

Daniel R. Farnell, D.V.M., Ph.D.

Herschell D. Giles, D.V.M., Ph.D.

Charles A. Kelley, B.S.

Charles Lindamood III, Ph.D.

Tina S. Rogers, Ph.D.

### **Environmental Health Research and Testing, Inc.**

*Sperm function evaluation*

Linda K. Grimes, D.V.M.

Teresa A. Sexton

M. Thomas

### **Research Triangle Institute**

*Chemical analyses*

D. Brine, B.S.

Robert W. Handy, Ph.D.

Celia D. Keller, M.S.

### **Analytical Sciences, Inc.**

*Statistical analysis of target organ lesions*

Richard Morris, M.S.

## **PEER REVIEW**

The draft report on the subchronic toxicity study of 3'-azido-3'-deoxythymidine (AZT) and rifampicin combinations was evaluated by the following reviewers. These reviewers served 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 subchronic toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were reviewed prior to finalization of this document. Changes were made such that the concerns of the reviewers were addressed to the extent possible.

**Thomas L. Goldsworthy, DVM, Ph.D.**

Integrated Laboratory Systems, Inc.  
Research Triangle Park, NC

**Harold Davis, DVM, Ph.D.**

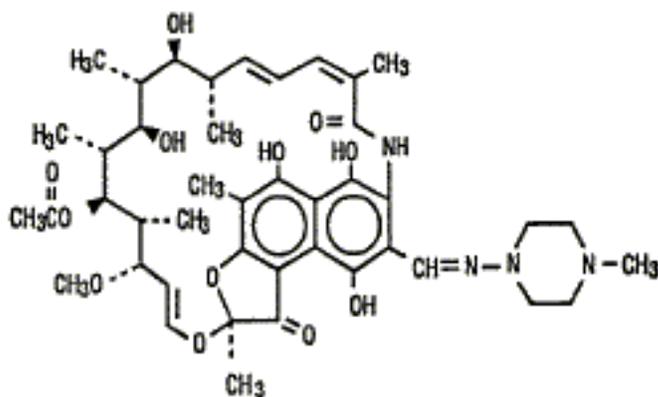
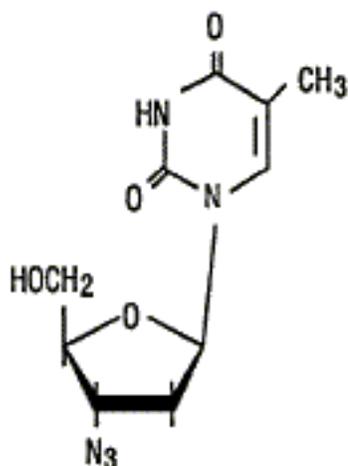
Amgen, Inc.  
Thousand Oaks, CA

# CONTENTS

<b>ABSTRACT</b> .....	5
<b>INTRODUCTION</b> .....	9
Study Rationale .....	13
<b>MATERIALS AND METHODS</b> .....	15
Procurement and Characterization of Chemicals .....	15
Dose Formulations .....	15
Study Design .....	15
Statistical Methods .....	21
<b>RESULTS AND DISCUSSION</b> .....	23
Survival and Clinical Findings .....	23
Body and Organ Weights .....	23
Clinical Pathology .....	33
Hematology .....	33
Clinical Chemistry .....	56
Necropsy Observations .....	63
Histopathologic Observations .....	65
Bone Marrow Lesions .....	65
Spleen Lesions .....	67
Liver Lesions .....	69
Thymus Lesions .....	71
Testis Lesions .....	71
Sperm Morphology and Motility and Vaginal Cytology .....	74
Conclusions .....	76
<b>REFERENCES</b> .....	77
<b>APPENDIX</b>	
Appendix A Clinical Pathology Results .....	A-1

## ABSTRACT

### 3'-Azido-3'-deoxythymidine (AZT) and Rifampicin Combinations



#### AZT

Molecular Formula:  $C_{10}H_{13}N_5O_4$

Molecular Weight: 267.24

CAS No.: 30516-87-1

#### Rifampicin

Molecular Formula:  $C_{43}H_{68}N_4O_{12}$

Molecular Weight: 822.95

CAS No.: 13292-46-1

Male and female B6C3F<sub>1</sub> mice were dosed orally with AZT alone (100, 200, or 400 mg/kg), rifampicin alone (100, 200, or 400 mg/kg), or combinations of AZT and rifampicin for up to 94 days. Mice were evaluated for clinical findings, mean body weight, hematology and clinical chemistry parameters, and sperm function and vaginal cytology. All core study animals and the clinical pathology study animals that died early were necropsied and subjected to histopathological evaluations. A summary of the most significant toxicological parameters is presented in Table 1.

AZT alone or rifampicin alone did not cause significant changes in body weights. Combination therapy with AZT and rifampicin caused marked and treatment-related decreases in body weights.

**TABLE 1**  
**Summary of Significant Treatment-Related Toxicological Parameters in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Parameter	AZT	Rifampicin	AZT + Rifampicin
Mortality	Treatment-related mortality not observed	Treatment-related mortality not observed	Treatment-related mortality corresponding with increasing doses of AZT and rifampicin
Body Weights	Minimal treatment-related decline in body weights	Minimal treatment-related decline in body weights	Marked treatment-related decline in body weights corresponding with increasing doses of AZT and rifampicin
Hematology	Mild anemia	Mild anemia	Marked anemia with severity increasing as doses of AZT and rifampicin increased
Clinical Chemistry	No significant alterations	Treatment-related elevations in liver enzymes	Treatment-related elevations in liver enzymes
Histopathology	Minimal bone marrow atrophy	Mild to marked cytoplasmic vacuolization of hepatocytes	Moderate to marked bone marrow atrophy, cytoplasmic vacuolization of hepatocytes, thymic atrophy, and cellular depletion in the spleen, and mild testicular degeneration

The primary toxicity of AZT was bone marrow suppression manifested by macrocytic anemia, thrombocytosis, and reticulocytopenia followed by reticulocytosis. Bone marrow atrophy was observed microscopically and was considered the major drug-related effect.

Administration of rifampicin alone resulted in both hematological toxicity and hepatotoxicity. Males and females developed a mild microcytic anemia and reticulocytopenia. Hepatotoxicity was manifested by increased liver enzymes in the serum, increased liver weights, and cytoplasmic vacuolization of hepatocytes.

Administration of AZT and rifampicin in combination resulted in hematological toxicity of far greater magnitude than that observed subsequent to the administration of either drug alone. Marked anemia and bone marrow atrophy contributed to significant mortality in groups treated with the higher concentration combinations. Females were more sensitive to the hematological toxicity of combination therapies than the males, and the female mice died earlier than the males.

## **AZT and Rifampicin 7**

Combination therapy with AZT and rifampicin did not exacerbate the hepatotoxicity induced by rifampicin alone. Serum enzyme levels, liver weights, and cytoplasmic vacuolization of hepatocytes were similar to those observed in mice treated with rifampicin alone.

Treatment-related decreases occurred in absolute thymus weights of mice treated with the higher concentration combinations of AZT and rifampicin, and the decreased thymus weights corresponded with thymic atrophy.

Testicular degeneration was observed in groups treated with AZT and rifampicin at 400 mg/kg and 400 mg of AZT + 200 mg of rifampicin. However, testicular degeneration was generally associated with anemia, lower testis weights, and decreased sperm motility.



## INTRODUCTION

Acquired immunodeficiency syndrome (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; Siegle *et al.*, 1981). The etiological agent of AIDS is a retrovirus that is now referred to as the human immunodeficiency virus (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 life span (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<sup>®</sup> mice were dosed daily by gavage with AZT doses of 100, 250, 500, or 1,000 mg/kg 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 1,000 mg/kg. Pathologic findings in the AZT-treated mice were consistent with the hematological results and included lymphoid depletion, reticuloendothelial hyperplasia in spleen and thymus, and bone marrow hypocellularity.

In a 14-week subchronic study (NTP, 1999), B6C3F<sub>1</sub> mice were treated with 0, 25, 50, 100, 400, or 1,000 mg of AZT/kg body weight in 0.5% methylcellulose by gavage. All doses were administered twice daily as one half of the total daily dose. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in 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 doses of 0, 50, 400, or 1,000 mg of AZT/kg twice daily for 14 weeks 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 observation

## 10 AZT and Rifampicin

that affected AZT-treated B6C3F<sub>1</sub> mice was a darkening of the skin on the tail, feet, and/or muzzle (Rao *et al.*, 1998).

Oral bioavailability of AZT was determined in female B6C3F<sub>1</sub> mice by comparison of the area under the curve (AUC) obtained from an oral dose to that of an intravenous dose at the same concentration (Trang *et al.*, 1993). Bioavailability (F) 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 ( $t_{1/2}$ ) 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.

Standard teratology tests 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  $\mu$ g/g or 76 times the antiviral ID<sub>50</sub>. Rabbits were orally dosed 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  $\mu$ g/g or 50 times the antiviral ID<sub>50</sub>.

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 weight gain, food consumption, hematological parameters, and growth or survival of offspring were observed. AZT concentration measurements 30 minutes after the last dose were 62.6  $\mu$ g/mL in maternal plasma and 21.1  $\mu$ g/g in fetal tissue.

Studies in mice concluded that AZT has a direct toxic effect on the developing mouse embryo (Toltzis *et al.*, 1991). Female C3H/He mice were exposed to 0, 0.25, 0.5, or 2.5 mg/mL of AZT in the 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). As a result, the treatment of AIDS is increasingly one of combination therapy of antiretroviral drugs and antimicrobial drugs (Goldschmidt and Dong, 1992). Tuberculosis (TB) is one of the opportunistic diseases leading to mortality in AIDS patients (Nolan, 1992). AIDS patients with TB receive combination therapy with AZT and antituberculosis drugs. Treatment for TB involves combination therapy with multiple antibacterial agents in order to eliminate the strains of organisms inducing TB, including those resistant to isoniazid, the primary drug used in

treating TB. The standard treatment regimen is isoniazid (300 mg), rifampicin (600 mg, or 450 mg for persons weighing less than 50 kg), and pyrazinamide (20 to 30 mg/kg) for the first 2 months of treatment. Isoniazid and rifampicin are continued for another 7 months, for a total therapy duration of 9 months (CDC, 1987; Barnes *et al.*, 1991).

Rifampicin is a well-tolerated drug (Editorial Staff, *Drugs*, 1971). The most serious problem is the development of jaundice (Scheuer *et al.*, 1974). Sixteen deaths associated with this reaction have been recorded in 500,000 treated patients. Hepatitis from rifampicin seldom occurs in patients with normal hepatic function. Similarly, the combination of isoniazid and rifampicin appears to be safe in patients with normal hepatic function (Grosset and Levintis, 1983). Chronic liver disease, alcoholism, and old age appear to increase the incidence of severe hepatic problems when rifampicin is given alone or concurrently with isoniazid (Gronhagen-Riska *et al.*, 1978).

Administration of rifampicin on an intermittent schedule (twice weekly or less) is associated with frequent adverse effects, and rifampicin should not be used in this manner. A flu-like syndrome with fever, chills, and myalgia develops in 20% of patients treated in this way. The syndrome may also include eosinophilia, interstitial nephritis, acute tubular necrosis, thrombocytopenia, hemolytic anemia, and shock (Girling and Hitze, 1979).

Rifampicin [rifampin, 3-4-methylpiperazinyl-1-iminomethylrifamycin SV] is a semisynthetic derivative of rifamycin-B, which is one of a group of antibiotics produced by *Streptomyces mediterranei* (Radner, 1973). Rifampicin is a tasteless, red-orange, crystalline powder (Binda *et al.*, 1971; IARC, 1986). Rifampicin has the molecular formula  $C_{43}H_{58}N_4O_{12}$ , a molecular weight of 823, and decomposes at 183° C to 188° C. It is highly soluble in methanol, ethyl acetate, chloroform, DMSO, methylene chloride, and tetrahydrofuran and is slightly soluble in acetone and  $CCl_4$ . Water solubility (25° C) is 0.28% at pH 7.5 and 9.95% at pH 2.0. Rifampicin is a “zwitterion” with a  $pK_a$  1.7 related to the 4-hydroxy and a  $pK_a$  7.9 related to the 3-piperazine nitrogen group at position 3. It is stable in dimethylsulfoxide and relatively stable in water at different pH levels. The stability curves have been determined at pH 2.3 and 8.0. In acid medium (pH 2.3), slight hydrolysis occurs with release of 1-amino-4-methylpiperazine and formation of 3-formyl rifamycin SV; at pH levels above 7.0, and in the presence of atmospheric oxygen, rifampicin is oxidized in small quantities to rifampicin quinone. This reaction may be prevented by adding ascorbic acid.

Rifampicin at a concentration of 0.5  $\mu$ g/mL inhibits the growth of *Mycobacterium tuberculosis in vitro*. This is an easily achieved serum level because, when given orally, this antibiotic is readily absorbed on an empty stomach. A single 600 mg dose given on an empty stomach produces

## 12 AZT and Rifampicin

blood levels many times that needed to inhibit tubercle bacilli *in vitro*, and the level is maintained for 12 to 24 hours (Radner, 1973). The oral administration of rifampicin produces peak concentrations in plasma in 2 to 4 hours; after ingestion of 600 mg, this value is approximately 71  $\mu$ g/mL, but there can be considerable variance. Para-aminosalicylic acid (PAS) may delay the absorption of rifampicin and adequate plasma concentrations may not be reached. If these agents are concurrently used, they should be separately administered at an interval of 8 to 12 hours (Radner, 1973).

The principal metabolite of rifampicin found in humans is the deacetylated form that is also active against *M. tuberculosis* (Furesz, 1970). Rifampicin diffuses readily and penetrates tissues such as lung, liver, kidney, and bone. High levels have been demonstrated in the lung (including the walls of tuberculous cavities), liver, urine, and spinal fluid in patients receiving one daily oral dose. Animal experiments using small doses of rifampicin have demonstrated two peaks in the blood levels; the second peak is due to reabsorption of the drug from the bile. Higher dosage results in such high blood levels that the second peak is obscured. After 6 hours, almost 100% of the rifampicin in the bile is in the deacetylated form; in urine, deacetylated rifampicin represents 30 to 60% of total rifampicin (Furesz, 1970). Intestinal reabsorption is affected by the presence of food as well as deacetylation; thus enterohepatic circulation does not indefinitely continue (Curci, 1969). Rifampicin is slowly excreted. The duration of blood levels varies in different animal species. Blood levels disappear within 24 hours in rats and guinea pigs, but in dogs, even small doses lead to detectable blood levels for as long as 56 hours. Studies performed on organs of patients show penetration of rifampicin in all examined organs, and higher concentrations are found in the lung than in the serum. Biliary concentrations reach about 10 times the blood levels, whereas urine concentrations are usually more than twice the biliary levels (Radner, 1973).

The LD<sub>50</sub> in the mouse following oral administration in different vehicles was 1,220 and 1,445 mg/kg within 24 hours (Editorial Staff, *Drugs*, 1971). The values obtained within 5 days were 885 mg/kg using oral, 640 mg/kg using intraperitoneal, and 260 mg/kg using intravenous administration. The LD<sub>50</sub> values in the rat were 1,720 mg/kg with oral, 550 mg/kg with intraperitoneal, and 330 mg/kg with intravenous administration. The LD<sub>50</sub> value in the guinea pig was 639 mg/kg with intraperitoneal administration and in the rabbit was 2,120 mg/kg with oral administration (Furesz, 1970).

In rats, administration of 400 mg/kg per day rifampicin by gastric intubation for 8 days produced a transient decrease in body weight, anorexia, apathy, ataxia, and marked dorsal hair loss. This dose of rifampicin induced fatty liver and a significant increase in the total cholesterol in the

livers of male and female rats and in total lipids and triglycerides in the livers of female rats (Piriou *et al.*, 1979).

In a 4-week toxicity study, rabbits that received 400 mg/kg body weight per day by oral administration exhibited significant weight decrease and severe jaundice, together with fatty and hydropic degeneration of the liver and kidneys (Furesz, 1970).

There was no appreciable toxicity evident in rats given 50 or 100 mg/kg body weight daily by oral administration for 26 weeks, but 200 mg/kg caused dose-related histological changes of the liver (slight increase of liver weight, slight cloudy swelling, and hydropic degeneration in liver). There were no adverse effects in rabbits given 100 mg/kg body weight daily by oral administration for 4 weeks, but overt toxicity (mortality 3 of 5), including jaundice and fatty and hydropic degenerations of liver and kidneys, occurred at a dose of 400 mg/kg body weight over the same period (Editorial Staff, *Drugs*, 1971).

Results of animal studies suggest that a combination of rifampicin-isoniazid can produce liver damage, whereas rifampicin alone does not. Hugues *et al.* (1969) found no histological evidence of hepatic lesions in control rats or in rats treated with rifampicin alone at a daily dose of 15 or 30 mg/kg body weight. The addition of isoniazid (5 mg/kg body weight) to these dosages of rifampicin resulted in severe hepatocellular lesions in 7 of 31 animals after 2 weeks of treatment. Weight loss and general debility, although present in all seven rats, were more severe in those given the higher dose of rifampicin. Fatty degeneration and hepatic enlargement occurred in 2 of the 31 rats treated with isoniazid (5 mg/kg body weight) alone.

Rifampicin has been associated with a significant increase in hepatomas in female C3Hf mice (Della Porta *et al.*, 1978). An increased incidence of tumors did not occur in male and female Wistar rats treated with rifampicin in the drinking water for 104 weeks (Della Porta *et al.*, 1978). In a one-year study, malignant tumors did not occur in male and female BALB/c mice given 20 subcutaneous injections of 0.3 mg of rifampicin in saline (Bichel, 1973). Rifampicin has been suspected of teratogenicity and embryotoxicity because of results from rodent studies, but there is no proof of damages to children when moderate therapeutic doses are administered (Warkany, 1979)

## STUDY RATIONALE

Tuberculosis is a common opportunistic infection in AIDS patients. AIDS patients with TB receive combination therapy of antiretroviral drug AZT with antibacterial drugs such as rifampicin. Information on toxicity of AZT alone and rifampicin alone is available. However, toxic potential of AZT with rifampicin combination therapy in animal models is not available. This subchronic toxicity study of the combination of AZT and rifampicin in animal models was conducted by the NIEHS as a part of the program to evaluate the toxicity of drugs, especially combination therapies used in the treatment of AIDS patients with opportunistic infections.

## 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. Rifampicin (lots 7232-150-03 and 122H2518) was procured from SAF Bulk Chemicals (St. Louis, MO) and supplied as a powder.

The relative purity of AZT was determined to be 99.7% by high-performance liquid chromatography. The purity of rifampicin was determined to be 97% to 98.5% by high-performance liquid chromatography. The vehicle used in this study was 0.1M potassium phosphate buffer. The potassium phosphate (lots 31183142 and 32181233) was manufactured by EM Science (Gibbstown, NJ).

### DOSE FORMULATIONS

Dose formulations were prepared by combining both test articles with the vehicle. The formulations were then stirred until in solution or visually homogeneous. A stability study conducted on refrigerated formulations indicated no significant loss of either chemical for at least 30 days. Dose formulations for this study were refrigerated, protected from light, and used within 24 days. Samples at each dose level from the initial, midpoint, and final formulations were analyzed prior to dosing. The found concentrations of AZT ranged from 90% to 105% of the target concentrations; concentrations of rifampicin formulations were 85.6% to 106% of the target concentrations. Residual dosing formulations taken from the animal room after dosing with the midpoint and final mixes were also analyzed. Found concentrations of AZT and rifampicin in the residual formulations were 79% to 109% and 90% to 114% of the target concentrations, respectively.

### STUDY DESIGN

B6C3F<sub>1</sub> mice were obtained from a pathogen-free production colony of NIEHS at Taconic Farms (Germantown, NY), and were approximately 6 weeks old when placed on study. The mice were housed five per cage during quarantine before randomization and were housed individually (males) or five per cage (females) after randomization.

The mice were housed in polycarbonate cages with solid bottoms and sides. Average temperature in the animal room was 71.8\_ F, with a standard deviation of 1.0\_ F; average relative humidity was 58.1%, with a standard deviation of 3.9%.

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

AIDS patients frequently receive rifampicin in combination with AZT to treat tuberculosis, one of the complicating opportunistic infections in these immunologically suppressed individuals. At present, no acceptable alternatives to animal models provide adequate toxicity information regarding this combination therapy. The AZT dose concentrations for this study were based on results of previous toxicity studies conducted in mice, which demonstrate that measurable evidence of toxicity will be obtained at levels of 200 and 400 mg AZT/kg per day. The human therapeutic dose for AZT is 10 mg/kg per day (*PDR*, 1996). The selected doses for this study were 100, 200, and 400 mg/kg body weight. These doses are 10, 20, and 40 times the human dose, but on a body surface area basis (Freirich *et al.*, 1966), they are close to one, two, and four times the therapeutic dose. The human therapeutic dose for rifampicin is 600 mg per day (Editorial Staff, *Drugs*, 1971). The doses selected for this study are 100, 200, and 400 mg/kg body weight. On a body surface area basis (Freirich *et al.*, 1966), these doses are approximately one, two, and four times the therapeutic dose.

A brief summary of the study design is provided in Table 2. The B6C3F<sub>1</sub> mouse was selected as the experimental animal because this animal model is routinely utilized for toxicity evaluations of this type by the NIEHS/NTP. The oral route of administration was selected for this study because it is the route used in humans. Combinations of AZT and rifampicin were administered by gavage as a single formulation in aqueous solution. The total daily dose was divided into two equal doses of 10 mL/kg given approximately 6 hours apart. The control group and the treated groups each consisted of 20 males and 20 females. Core animals (10 per group) were dosed until the day prior to sacrifice (91 to 94 days). Clinical pathology study animals (10 per group) were dosed until day 59. All mice were observed twice daily (morning and afternoon) for signs of mortality/moribundity.

Blood was drawn for clinical pathology evaluations on study days 4, 30, and 60 (clinical pathology study animals) or prior to sacrifice on study days 92 to 95 (core study animals). Vaginal cytology evaluations were conducted on all core study females on days 77 to 88, and sperm count, function, and motility were assessed on core study males on days 92 to 95. All surviving clinical pathology study animals were euthanatized on day 60 using 100% CO<sub>2</sub> and discarded; all surviving core study animals were sacrificed on days 92 to 95 using 100% CO<sub>2</sub>.

### ***Clinical Pathology***

All blood samples were collected from the retroorbital sinus under CO<sub>2</sub>/O<sub>2</sub> (70/30) anesthesia. Blood for hematology analyses was collected into tubes containing EDTA, and blood for clinical chemistry analyses was collected into tubes 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; mean cell hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); mean cell volume (MCV); and leukocyte differentials were determined on 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 leukocyte and platelet parameters if necessary. Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities; bile acid concentrations; and sorbitol dehydrogenase activities were evaluated using a Roche Cobas Fara™ automated analyzer. Depending on the amount of each serum sample, the order of priority for clinical chemistry parameters were as listed above.

### ***Vaginal Cytology and Sperm Function Evaluations***

Samples of vaginal fluid were taken from females using a medicine dropper moistened with neutral buffered saline. Samples from 12 consecutive days were placed on two slides per animal and were evaluated for relative frequency of estrous phases and the estrous cycle length.

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) and weighed. Modified Tyrode's buffer was applied to slides, and a small incision was made at the 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 weighing the left testis, then removing the tunica albuginea and homogenizing the testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

### ***Histopathology***

A complete necropsy was performed on all core study animals and early death clinical pathology study animals. Selected organs (Table 2) from core study animals were weighed. Histopathology was conducted on the tissues listed in Table 2. Tissues were preserved in the appropriate fixative, trimmed to a maximum thickness of 0.3 cm for processing, embedded in paraffin, and sectioned at 4 to 6  $\mu$ m. Testes were stained with PAS and other tissues were stained with hematoxylin and eosin. All tissues were examined by light microscopy.

**TABLE 2**  
**Experimental Design and Materials and Methods in the 13-Week Toxicity Study of AZT and Rifampicin Combinations in B6C3F<sub>1</sub> Mice**

---

**Study Laboratory**

Southern Research Institute, Birmingham, AL

**Strain and Species**

B6C3F<sub>1</sub> mice

**Animal Source**

Taconic Farms, Germantown, NY

**Time Held Before Study**

11 to 19 days

**Age When Placed on Study**

42 to 43 days

**Duration of Dosing**

Core study: 91 to 94 days

Clinical pathology study: 59 days

**Necropsy Dates**

Core study: days 92 to 95

Clinical pathology study: day 60

**Size of Study Groups**

10 males and 10 females

**Method of Animal Distribution**

Animals were assigned to groups using a stratified weight method and then assigned to study groups in random order.

**Animals per Cage**

One animal per cage (males) or five animals per cage (females)

**Method of Animal Identification**

Microchip implant (Biomedic Data Systems, Inc., Maywood, NJ)

**Diet**

NIH-07 pelleted feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*

**Water Distribution**

Tap water (Birmingham, AL), available *ad libitum* via automatic watering system (Edstrom Industries, Inc., Waterford, WI)

**Cages**

Polycarbonate cages with solid bottoms and sides (Lab Products, Inc., Maywood, NJ)

**Bedding**

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

**Cage Filters**

Remay® spun-bonded polyester (Andico, Birmingham, AL)

**Racks**

Stainless steel (Lab Products, Maywood, NJ)

---

**TABLE 2**  
**Experimental Design and Materials and Methods in the 13-Week Toxicity Study of AZT and Rifampicin Combinations in B6C3F<sub>1</sub> Mice**

---

#### **Animal Room Environment**

Temperature: 71.8 Å 1.0\_F  
 Relative humidity: 58.1% Å 3.9%  
 Fluorescent light: 12 hours fluorescent light/day  
 Room air: minimum of 10 changes/hour

#### **Doses**

Doses were administered 7 days a week in two equal doses approximately 6 hours apart. Daily doses in potassium phosphate buffer:

0 mg AZT + 0 mg rifampicin per kg body weight per day  
 0 mg AZT + 100 mg rifampicin per kg body weight per day  
 0 mg AZT + 200 mg rifampicin per kg body weight per day  
 0 mg AZT + 400 mg rifampicin per kg body weight per day  
 100 mg AZT + 0 mg rifampicin per kg body weight per day  
 100 mg AZT + 100 mg rifampicin per kg body weight per day  
 100 mg AZT + 200 mg rifampicin per kg body weight per day  
 100 mg AZT + 400 mg rifampicin per kg body weight per day  
 200 mg AZT + 0 mg rifampicin per kg body weight per day  
 200 mg AZT + 100 mg rifampicin per kg body weight per day  
 200 mg AZT + 200 mg rifampicin per kg body weight per day  
 200 mg AZT + 400 mg rifampicin per kg body weight per day  
 400 mg AZT + 0 mg rifampicin per kg body weight per day  
 400 mg AZT + 100 mg rifampicin per kg body weight per day  
 400 mg AZT + 200 mg rifampicin per kg body weight per day  
 400 mg AZT + 400 mg rifampicin per kg body weight per day

#### **Type and Frequency of Observation**

Mortality/morbidity: twice daily  
 Clinical findings: once weekly (core study animals only)  
 Body weights: once weekly (weights reported for core study animals; clinical pathology study animals were weighed for dosing purposes only)

#### **Method of Sacrifice**

Carbon dioxide asphyxiation

#### **Necropsy**

Complete necropsies were performed on all core study animals and early death clinical pathology study animals. All tissues were fixed in formalin except the testes, which were fixed in Bouin's fluid. The liver, right kidney, heart, lungs, thymus, and right testis were weighed for all core study mice sacrificed at the end of the study.

#### **Histopathology**

The following tissues from all mice in the 0 + 0 control group and the 200 + 200, 200 + 400, 400 + 200, and 400 + 400 groups and all mice that died early in the remaining groups were examined for histopathological changes: adrenal glands, brain, clitoral glands, esophagus, femur, gallbladder, gross lesions, heart and aorta, large intestines, small intestines, kidneys, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, mammary glands, nose, ovaries, pancreas, parathyroid glands, pituitary glands, prostate, salivary glands, seminal vesicle, spleen, stomach, testes with epididymis, thymus, thyroid gland, tissue masses, trachea, urinary bladder, and uterus. Bone marrow, liver, spleen, thymus, testes, and gross lesions were evaluated from remaining mice in all other dose groups.

#### **Clinical Pathology**

Hematology and clinical chemistry evaluations were conducted on all core study animals at terminal sacrifice and on all clinical pathology animals on days 4, 30, and 60.

**Hematology:** erythrocyte, reticulocyte, and platelet counts; hematocrit; hemoglobin; mean cell hemoglobin; mean cell hemoglobin concentration; mean cell volume; leukocyte counts and differentials; erythrocyte and platelet morphology (if necessary)

**Clinical Chemistry:** alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and sorbitol dehydrogenase and bile acid concentrations

#### **Sperm Morphology and Motility and Vaginal Cytology Evaluations**

Sperm samples were collected from all core study males at terminal sacrifice. The parameters evaluated included spermatid heads, spermatid counts, and motility. The left epididymis and cauda epididymis were weighed. Vaginal samples from 12 consecutive days were collected from all core study females. The parameters evaluated included estrous phases and estrous cycle length.

---

## STATISTICAL METHODS

Group means and standard deviations were calculated for final mean body weights, clinical pathology parameters, and organ weights. Organ-weight-to-body weight ratios were also calculated. Final mean body weights, mean organ weights, and organ-weight-to-body weight ratios 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. Final mean body weights were also evaluated using Jonckheere's (1954) test for trend; Williams' (1971, 1972) test was applied if there was an indication of trend, and Dunnett's (1955) test was used in the absence of a trend. Dunnett's test was also used for evaluation of hematology data.

Sperm measurements were first analyzed for interaction using a nonparametric test. If the interaction was not statistically significant, averages were taken from each drug over the levels of the other drug, and control and dosed group means were evaluated using Jonckheere's (1954) test for trend; Williams' (1971, 1972) test was applied if there was an indication of trend, and Dunnett's (1955) test was used in the absence of a trend. The outlier test of Dixon and Massey (1951) was used to detect extreme values. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that were indicated to be inadequate due to measurement problems were eliminated from analysis. Final mean body weights and organ weights associated with sperm measurements were evaluated using a two-way analysis of variance.

Treatment effects on vaginal cytology data were investigated by applying a multivariate analysis of variance (using Wilke's Criterion [Stevens, 1986] as the test statistic) to test for the simultaneous equality of measurements across dose concentrations. Since the data are proportions (the proportion of the observation period that an animal was in a given estrous phase), an arcsine transformation was used to bring the data into closer conformance with the normality assumptions required for the multivariate analysis of variance.

Severity grades for lesions in liver, spleen, thymus, testes, and bone marrow were analyzed for interaction using a two-way analysis of variance. Dunnett's (1955) test was used to detect treatment-related effects.



## RESULTS AND DISCUSSION

### SURVIVAL AND CLINICAL FINDINGS

Administration of AZT alone or rifampicin alone did not result in mortality. A total of 79 core study animals (34 males and 45 females) died from combination treatment with AZT and rifampicin. In general, mortality was increased and occurred earlier in females compared to males.

Seven male mice in the 400 mg AZT + 200 mg rifampicin group died during weeks 8 and 9; eight males that received 100 mg AZT + 400 mg rifampicin died during weeks 8 through 12. Nine and ten male mice, respectively, in the combination groups that received 200 or 400 mg AZT + 400 mg rifampicin died during weeks 7 and 8. Clinical findings were noted primarily in dose groups in which mortality occurred. These signs of toxicity included thinness, tremors, abnormal breathing, ataxia, lethargy, and ruffled fur and were noted in males in the 100 + 400, 200 + 400, 400 + 200, and 400 + 400 (AZT + rifampicin) groups beginning on day 50 of the study.

In general, female mice died earlier than males. One female that received 400 mg AZT + 100 mg rifampicin and three females that received 200 mg AZT + 200 mg rifampicin died during week 8. An additional female in the 200 mg AZT + 200 mg rifampicin group died during week 11. All females in the following dose groups died by week 7: 400 + 200, 100 + 400, 200 + 400, and 400 + 400 (AZT + rifampicin). Clinical findings related to treatment were similar to those noted in males (thinness, tremors, abnormal breathing, ataxia, lethargy, ruffled fur) and were observed beginning on day 45 of the study.

### BODY AND ORGAN WEIGHTS

Administration of 100, 200, or 400 mg of AZT alone did not result in consistent treatment-related decreases in body weights of male or female mice (Table 3 and Figures 1 and 2). Respective final mean body weights of male mice treated with 200 or 400 mg of AZT alone were approximately 8% (34.66 grams;  $P < 0.05$ ) and 4% (36.05 grams) lower than the mean (37.71 grams) of the male control group. Respective final mean body weights of female mice treated with 100, 200, or 400 mg of AZT alone were approximately 7% (26.95 grams), 2% (28.39 grams), and 4% (27.73 grams) lower than the mean (28.89 grams) of the female control group.

TABLE 3  
Final Mean Body Weights, Organ Weights, and Organ-Weight-to-Body Weight Ratios of Mice  
in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose <sup>a</sup>	n <sup>b</sup>	Mean Body Weight (g)	Organ Weight <sup>c</sup> (g)	Organ to Body Ratio x 1,000 <sup>c</sup>	Organ Weight (g)	Organ to Body Ratio x 1,000
<b>Male</b>						
		<b>Heart</b>			<b>Right Kidney</b>	
0 + 0	10	37.71	0.151 ± 0.120	4.0 ± 0.3	0.290 ± 0.025	7.7 ± 0.7
100 + 0	10	38.32	0.150 ± 0.007(-0.7)	4.0 ± 0.4(-1.9)	0.294 ± 0.018(+1.4)	7.8 ± 0.8(+0.1)
200 + 0	10	34.66**	0.145 ± 0.010(-4.0)	4.2 ± 0.3(+4.1)	0.285 ± 0.020(-1.7)	8.3 ± 0.8(+6.7)
400 + 0	10	36.05	0.149 ± 0.012(-1.3)	4.2 ± 0.4(+3.1)	0.280 ± 0.025(-3.4)	7.8 ± 0.7(+0.6)
0 + 100	10	35.00**	0.146 ± 0.010(-3.3)	4.2 ± 0.2(+3.6)	0.294 ± 0.023(+1.4)	8.4 ± 0.5(+8.5)*
100 + 100	10	35.88	0.152 ± 0.009(+0.7)	4.2 ± 0.2(+5.1)	0.289 ± 0.009(-0.3)	8.1 ± 0.4(+4.1)
200 + 100	10	36.32	0.148 ± 0.009(-2.0)	4.1 ± 0.3(+1.3)	0.294 ± 0.025(+1.4)	8.1 ± 0.7(+4.6)
400 + 100	10	35.53	0.150 ± 0.015(-0.7)	4.2 ± 0.4(+5.0)	0.297 ± 0.026(+2.4)	8.4 ± 0.8(+8.4)*
0 + 200	10	35.71	0.140 ± 0.013(-7.3)	3.9 ± 0.2(-2.7)	0.296 ± 0.028(+2.1)	8.3 ± 0.5(+7.0)
100 + 200	9	34.12*	0.144 ± 0.010(-4.3)	4.2 ± 0.2(+5.2)	0.284 ± 0.022(-1.9)	8.3 ± 0.5(+7.7)*
200 + 200	10	32.69**	0.147 ± 0.008(-2.6)	4.5 ± 0.2(+11.6)**	0.271 ± 0.022(-6.6)	8.3 ± 0.3(+6.9)
400 + 200	3	34.87	0.183 ± 0.015(+21.4)**	5.3 ± 0.4(+30.3)**	0.293 ± 0.012(+1.1)	8.4 ± 0.2(+8.6)
0 + 400	10	32.50**	0.132 ± 0.004(-12.6)**	4.1 ± 0.2(+0.9)	0.262 ± 0.017(-9.7)**	8.1 ± 0.6(+4.2)
100 + 400	2	30.25**	0.155 ± 0.007(+2.6)	5.1 ± 0.3(+27.4)**	0.235 ± 0.021(-19.0)**	7.8 ± 0.0(+0.3)
200 + 400	1	22.70**	0.210(+39.1)**	9.3(+129.4)**	0.230(-20.7)*	10.1(+30.8)**
		<b>Liver</b>			<b>Lung</b>	
0 + 0	10	37.71	1.677 ± 0.295	43.3 ± 3.1	0.228 ± 0.050	6.1 ± 1.2
100 + 0	10	38.32	1.676 ± 0.354(-0.1)	43.5 ± 3.9(-1.9)	0.223 ± 0.045(-2.2)	5.9 ± 1.1(-3.8)
200 + 0	10	34.66**	1.505 ± 0.010(-10.3)	43.5 ± 3.0(-1.8)	0.222 ± 0.039(-2.6)	6.4 ± 1.2(+5.9)
400 + 0	10	36.05	1.600 ± 0.134(-4.6)	44.5 ± 2.4(+0.3)	0.216 ± 0.035(-5.3)	6.0 ± 0.9(-1.1)
0 + 100	10	35.00**	2.568 ± 0.197(+53.1)**	73.3 ± 2.4(+65.4)**	0.229 ± 0.056(+0.4)	6.5 ± 1.5(+7.4)
100 + 100	10	35.88	2.661 ± 0.219(+58.7)**	74.1 ± 4.3(+67.2)**	0.212 ± 0.046(-7.0)	5.9 ± 1.3(-2.9)
200 + 100	10	36.32	2.709 ± 0.219(+61.5)**	74.5 ± 3.3(+68.1)**	0.235 ± 0.035(+3.1)	6.5 ± 0.9(+6.3)
400 + 100	10	35.53	2.818 ± 0.357(+68.0)**	79.2 ± 4.5(+78.5)**	0.213 ± 0.032(-6.6)	6.0 ± 1.0(-0.8)
0 + 200	10	35.71	3.519 ± 0.532(+109.8)**	98.3 ± 9.8(+121.6)**	0.233 ± 0.048(+2.3)d	6.5 ± 1.4(+7.0)
100 + 200	9	34.12*	3.151 ± 0.342(+87.9)**	92.3 ± 7.2(+108.3)**	0.208 ± 0.024(-8.9)	6.1 ± 0.6(+0.2)
200 + 200	9	32.69**	2.980 ± 0.126(+77.7)**	90.6 ± 3.0(+104.3)**	0.209 ± 0.045(-8.4)	6.4 ± 1.4(+4.6)
400 + 200	3	34.87	3.183 ± 0.091(+89.8)**	91.4 ± 4.3(+106)**	0.203 ± 0.012(-10.8)	5.8 ± 0.3(-4.1)
0 + 400	10	32.50**	4.184 ± 0.239(+149.5)**	128.9 ± 7.6(+190.6)**	0.196 ± 0.031(-14.0)	6.0 ± 0.9(-0.8)
100 + 400	2	30.25**	3.810 ± 0.509(+127.2)**	125.7 ± 4.8(+183.5)**	0.160 ± 0.000(-99.8)*	5.3 ± 0.5(-12.6)
200 + 400	1	22.70**	2.680(+59.8)**	118.1(+166.3)**	0.130(-43.0)*	5.7(-5.8)
		<b>Right Testicle</b>			<b>Thymus</b>	
0 + 0	10	37.71	0.118 ± 0.011	3.2 ± 0.5	0.036 ± 0.004	1.0 ± 0.1
100 + 0	10	38.32	0.117 ± 0.009(-1.1)	3.1 ± 0.5(-2.3)	0.034 ± 0.005(-6.3)	0.9 ± 0.1(-8.2)
200 + 0	10	34.66**	0.113 ± 0.005(-4.3)	3.3 ± 0.2(+3.3)	0.030 ± 0.004(-17.9)**	0.9 ± 0.1(-10.7)
400 + 0	10	36.05	0.112 ± 0.009(-5.5)	3.1 ± 0.3(-2.0)	0.034 ± 0.004(-6.7)e	1.0 ± 0.2(-1.0)
0 + 100	10	35.00**	0.119 ± 0.010(+0.3)	3.4 ± 0.2(+6.8)	0.035 ± 0.003(-3.0)	1.0 ± 0.1(+3.6)
100 + 100	10	35.88	0.109 ± 0.007(-7.5)*	3.0 ± 0.2(-3.9)	0.034 ± 0.007(-7.4)	0.9 ± 0.2(-3.2)
200 + 100	10	36.32	0.114 ± 0.008(-3.6)	3.1 ± 0.3(-1.0)	0.034 ± 0.004(-6.9)	0.9 ± 0.1(-4.3)
400 + 100	10	35.53	0.103 ± 0.008(-13.2)**	2.9 ± 0.4(-8.2)	0.032 ± 0.005(-10.7)	0.9 ± 0.1(-5.8)
0 + 200	10	35.71	0.115 ± 0.010(-2.4)	3.2 ± 0.4(+2.3)	0.037 ± 0.003(+1.1)	1.0 ± 0.1(+6.5)
100 + 200	9	34.12*	0.111 ± 0.008(-6.4)	3.3 ± 0.3(+2.6)	0.034 ± 0.003(-6.6)	1.0 ± 0.1(+2.4)
200 + 200	10	32.69**	0.107 ± 0.014(-9.7)**	3.3 ± 0.4(+3.1)	0.032 ± 0.004(-13.2)*	1.0 ± 0.1(-0.5)
400 + 200	3	34.87	0.098 ± 0.009(-17.0)**	2.8 ± 0.3(-11.3)	0.028 ± 0.006(-22.9)**	0.8 ± 0.2(-17.1)
0 + 400	10	32.50**	0.108 ± 0.011(-8.9)*	3.3 ± 0.3(+4.3)	0.034 ± 0.007(-5.5)	1.1 ± 0.2(+8.8)
100 + 400	2	30.25**	0.088 ± 0.003(-25.5)**	2.9 ± 0.2(-8.0)	0.022 ± 0.006(-39.4)**	0.7 ± 0.1(-25.7)*
200 + 400	1	22.70**	0.070(-40.0)	3.1(-2.7)	—f	—

TABLE 3

Final Mean Body Weights, Organ Weights, and Organ-Weight-to-Body Weight Ratios of Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	n	Mean Body Weight (g)	Organ Weight (g)	Organ to Body Ratio x 1,000	Organ Weight (g)	Organ to Body Ratio x 1,000
<b>Female</b>						
			<b>Heart</b>		<b>Right Kidney</b>	
0 + 0	10	28.89	0.125 ± 0.010	4.4 ± 0.4	0.189 ± 0.023	6.6 ± 0.5
100 + 0	10	26.95	0.112 ± 0.010(-10.4)*	4.2 ± 0.5(-4.4)	0.172 ± 0.015(-9.0)*	6.4 ± 0.7(-2.5)
200 + 0	10	28.39	0.119 ± 0.009(-4.8)	4.2 ± 0.3(-3.6)	0.184 ± 0.016(-2.6)	6.5 ± 0.7(-0.8)
400 + 0	10	27.73	0.120 ± 0.013(-4.0)	4.4 ± 0.5(-0.2)	0.184 ± 0.017(-2.6)	6.7 ± 0.7(+1.5)
0 + 100	10	29.86	0.123 ± 0.008(-1.6)	4.1 ± 0.3(-5.6)	0.195 ± 0.019(+3.2)	6.5 ± 0.5(-0.6)
100 + 100	9	28.38	0.128 ± 0.012(+2.2)	4.6 ± 0.6(+4.3)	0.188 ± 0.014(-0.6)	6.7 ± 0.6(+1.6)
200 + 100	10	29.03	0.127 ± 0.125(+1.6)	4.4 ± 0.3(+0.3)	0.190 ± 0.018(+0.5)	6.6 ± 0.3(-0.3)
400 + 100	7	26.96	0.141 ± 0.023(+13.1)**	5.4 ± 0.8(+24.7)**	0.201 ± 0.026(+6.6)	7.5 ± 0.5(+14.3)**
0 + 200	10	27.72	0.117 ± 0.007(-6.4)	4.2 ± 0.3(-2.9)*	0.190 ± 0.008(+0.5)	6.9 ± 0.5(+4.8)
100 + 200	10	25.53*	0.143 ± 0.021(+14.7)**d	5.8 ± 1.7(+32.5)**	0.186 ± 0.026(-1.6)	7.4 ± 0.9(+11.9)**
200 + 200	4	24.08**	0.180 ± 0.010(+44.0)**g	7.3 ± 0.9(+68.0)**	0.200 ± 0.014(+5.8)	8.4 ± 1.2(+27.5)**
0 + 400	10	28.13	0.114 ± 0.007(-8.8)*	4.1 ± 0.3(-7.1)	0.186 ± 0.012(-1.6)	6.6 ± 0.5(+0.7)
			<b>Liver</b>		<b>Lung</b>	
0 + 0	10	28.89	1.240 ± 0.168dc	42.4 ± 3.4	0.190 ± 0.021	6.6 ± 0.8
100 + 0	10	26.95	1.093 ± 0.091(-11.9)d	40.3 ± 3.3(-5.0)	0.176 ± 0.025(-7.2)e	6.5 ± 0.9(-1.9)
200 + 0	10	28.39	1.163 ± 0.110(-6.2)	41.0 ± 2.6(-3.2)	0.170 ± 0.023(-10.5)	6.0 ± 0.9(-9.2)
400 + 0	10	27.73	1.214 ± 0.137(-2.1)	43.9 ± 4.4(+3.6)	0.190 ± 0.049(+0.0)	6.9 ± 1.8(+3.9)
0 + 100	10	29.86	1.723 ± 0.118(+38.9)**	57.7 ± 2.2(+36.1)**	0.178 ± 0.018(-6.3)	6.0 ± 0.6(-10.0)
100 + 100	9	28.38	1.772 ± 0.314(+42.9)**	62.4 ± 6.5(+47.1)**	0.181 ± 0.018(-4.7)	6.5 ± 1.0(-2.5)
200 + 100	10	29.03	1.690 ± 0.163(+36.3)**	58.2 ± 1.5(+37.3)**	0.168 ± 0.015(-11.6)	5.8 ± 0.6(-12.3)
400 + 100	7	26.96	1.659 ± 0.228(+33.7)**	61.6 ± 2.1(+45.0)**	0.174 ± 0.029(-8.3)	6.5 ± 1.0(-1.6)
0 + 200	10	27.72	2.051 ± 0.209(+65.4)**	74.1 ± 5.3(+74.7)**	0.186 ± 0.038(-2.3)d	6.6 ± 1.6(+0.1)
100 + 200	10	25.53*	2.068 ± 0.344(+66.7)**	81.1 ± 5.0(+91.2)**	0.152 ± 0.014(-20.0)**	6.0 ± 0.8(-8.8)*
200 + 200	4	24.08**	1.988 ± 0.083(+60.2)**	82.9 ± 6.6(+95.6)**	0.163 ± 0.015(-14.5)	6.8 ± 0.4(+1.9)
0 + 400	10	28.13	3.353 ± 0.098(+170.3)**	119.2 ± 4.1(+181.2)**	0.173 ± 0.017(-8.9)	6.2 ± 0.6(-7.2)
			<b>Thymus</b>			
0 + 0	9	28.89	0.039 ± 0.007	1.4 ± 0.3		
100 + 0	10	26.95	0.041 ± 0.005(+5.0)	1.5 ± 0.2(+10.1)		
200 + 0	10	28.39	0.038 ± 0.004(-2.0)	1.3 ± 0.2(-1.9)		
400 + 0	10	27.73	0.039 ± 0.005(-0.4)	1.4 ± 0.1(+1.5)		
0 + 100	9	29.86	0.043 ± 0.005(+12.1)	1.5 ± 0.2(+6.7)		
100 + 100	9	28.38	0.037 ± 0.005(-5.2)	1.3 ± 0.2(-4.7)		
200 + 100	10	29.03	0.038 ± 0.005(-0.9)*	1.3 ± 0.2(-3.2)		
400 + 100	7	26.96	0.034 ± 0.005(-12.1)	1.2 ± 0.2(-11.7)		
0 + 200	10	27.72	0.039 ± 0.006(+1.4)	1.4 ± 0.3(+4.7)		
100 + 200	10	25.53*	0.030 ± 0.010(-22.4)**	1.1 ± 0.3(-16.2)*		
200 + 200	4	24.08**	0.023 ± 0.008(-39.7)**	0.9 ± 0.3(-32.1)**		
0 + 400	10	28.13	0.038 ± 0.006(-1.5)	1.4 ± 0.2(-1.1)		

\* Significantly different (P 0.05) from vehicle control by Student's *t*-test for organ and body weights and by Jonckheere's and Williams' or Dunnett's tests for body weights.

\*\* P 0.01

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b n=number examined

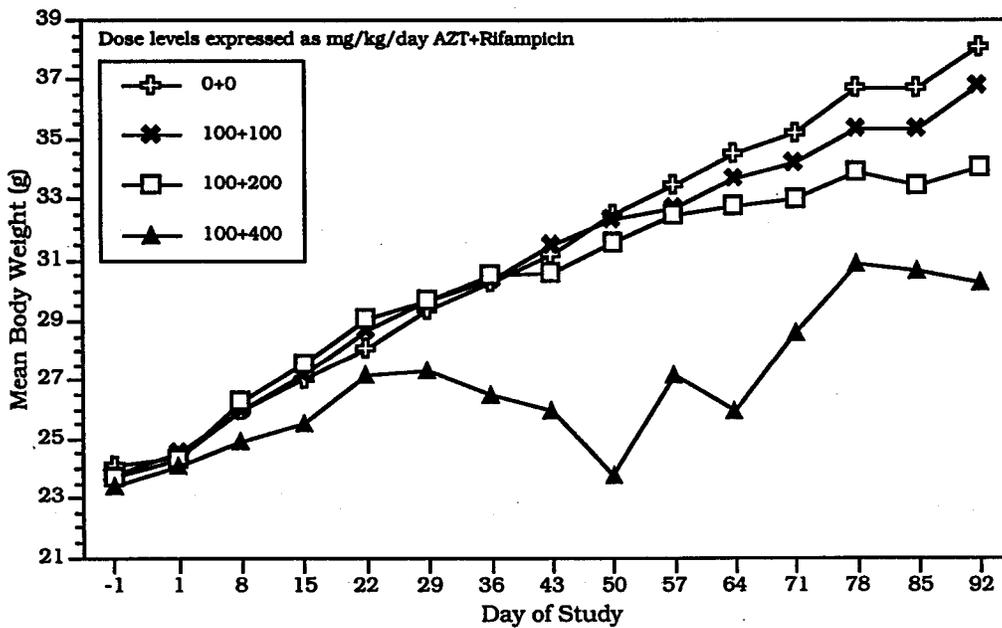
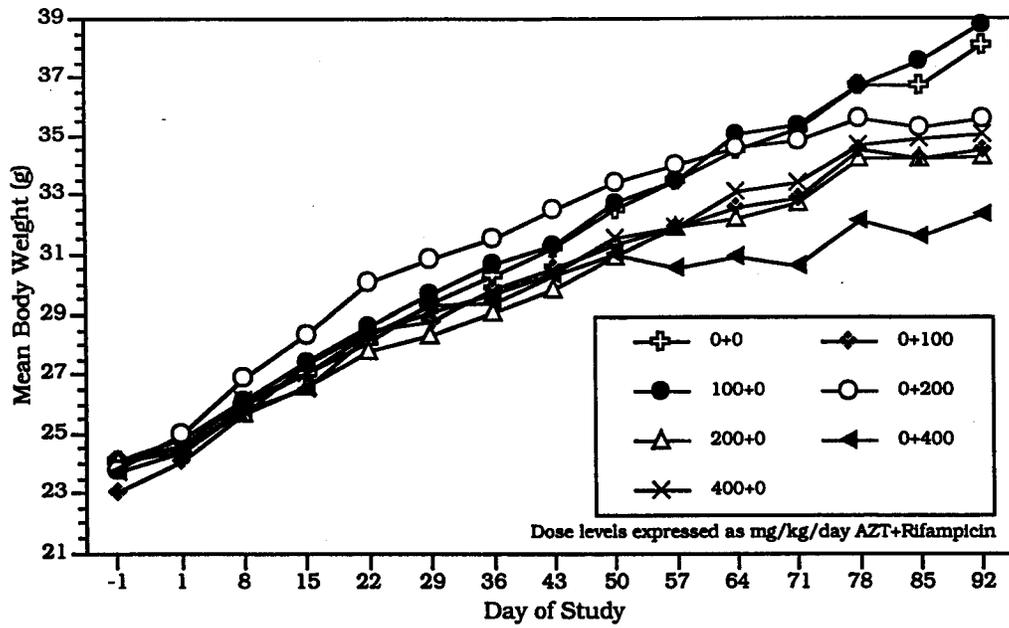
c Data are presented as mean value ± standard deviation (% difference from control)

d n=9

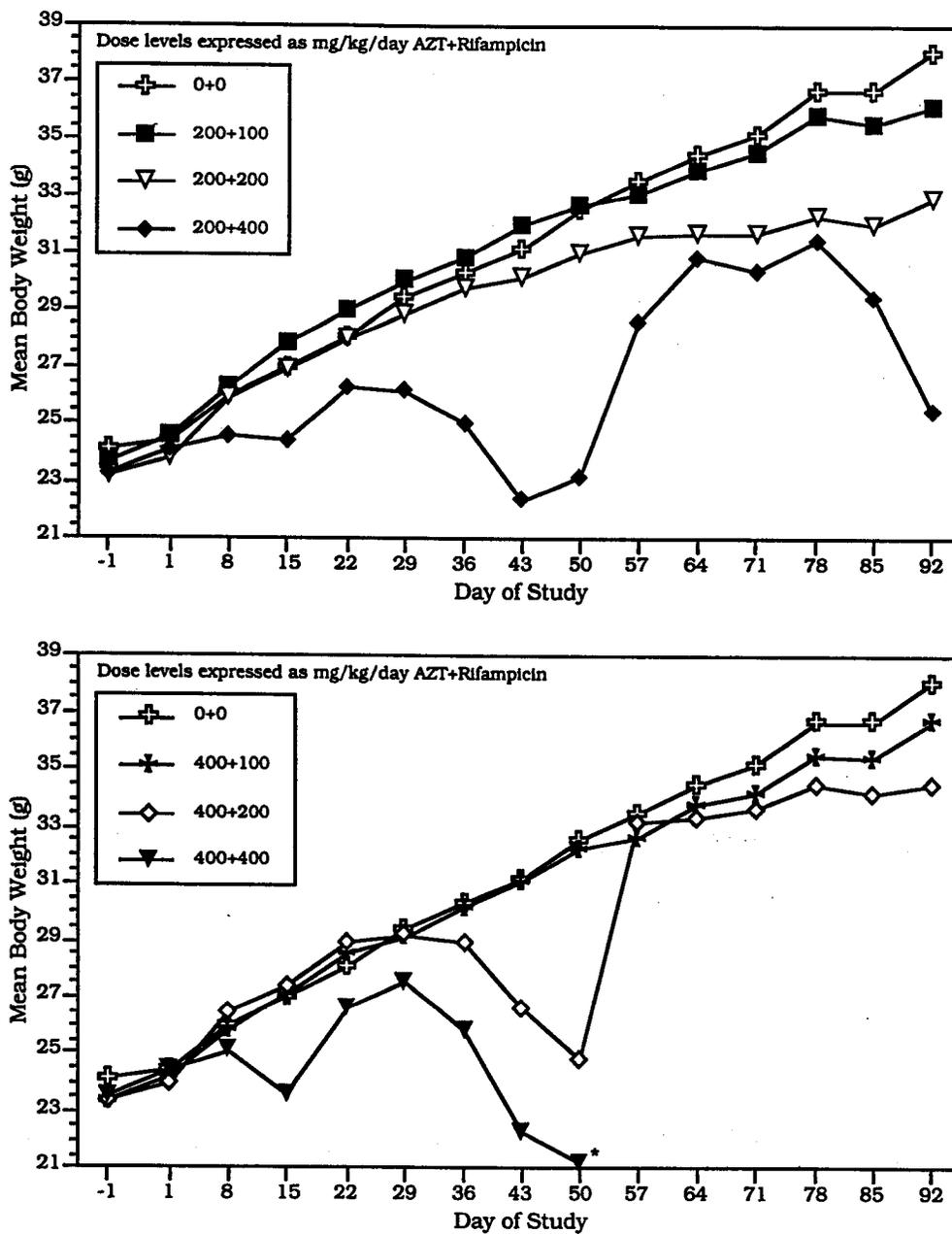
e n=8

f n=0; single available weight excluded as outlier

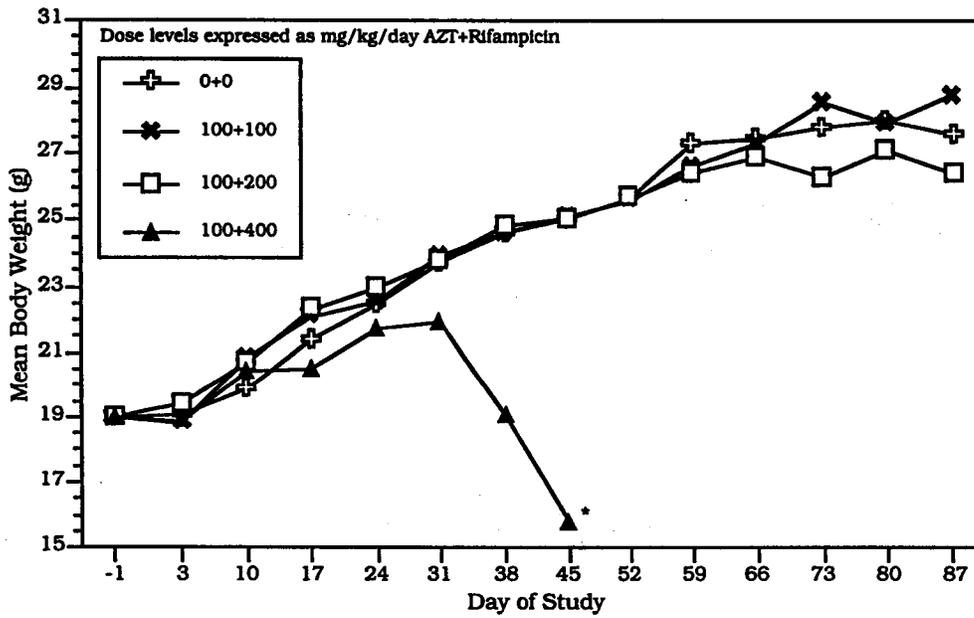
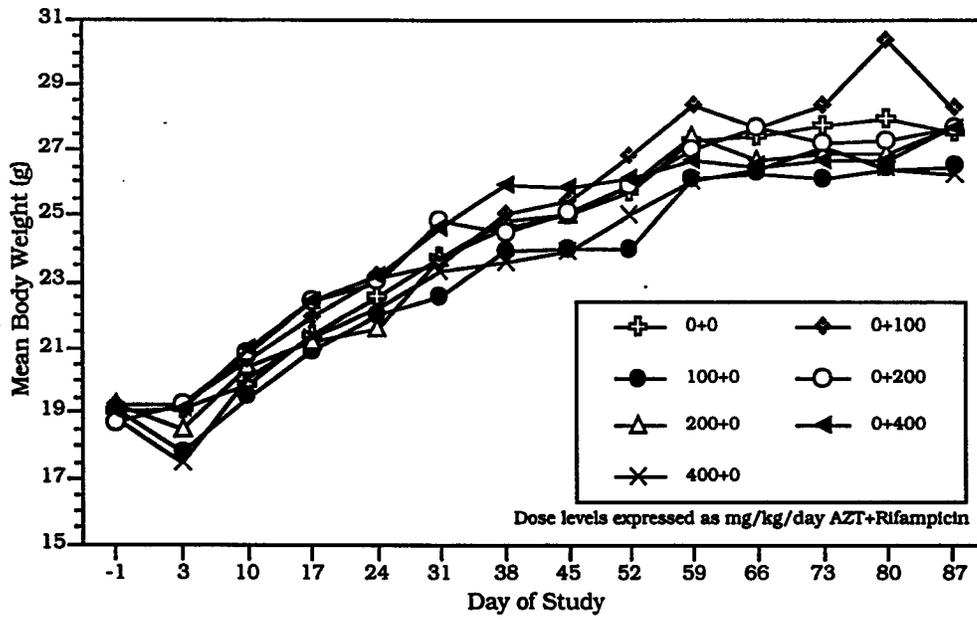
g n=3



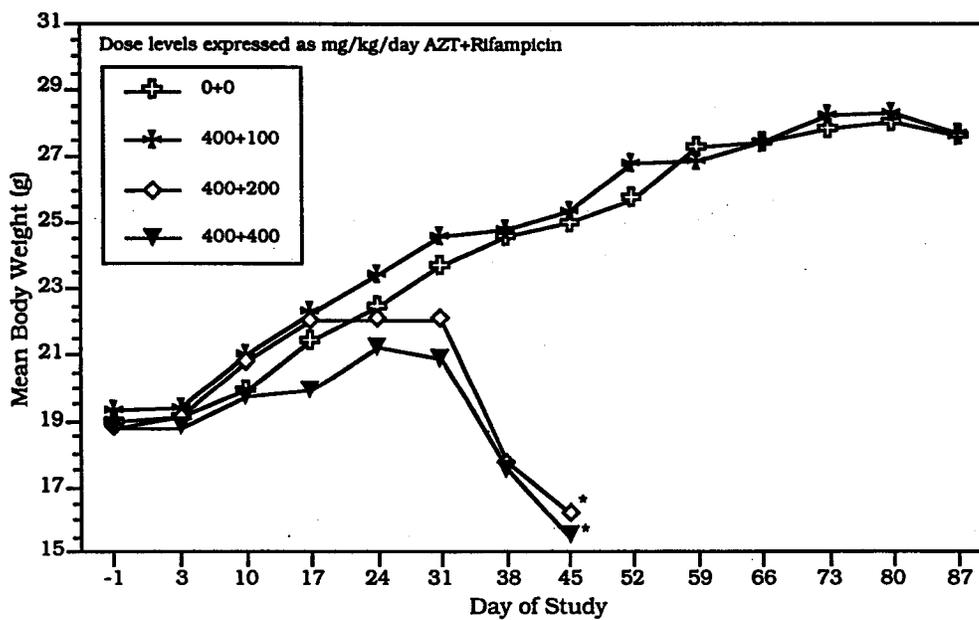
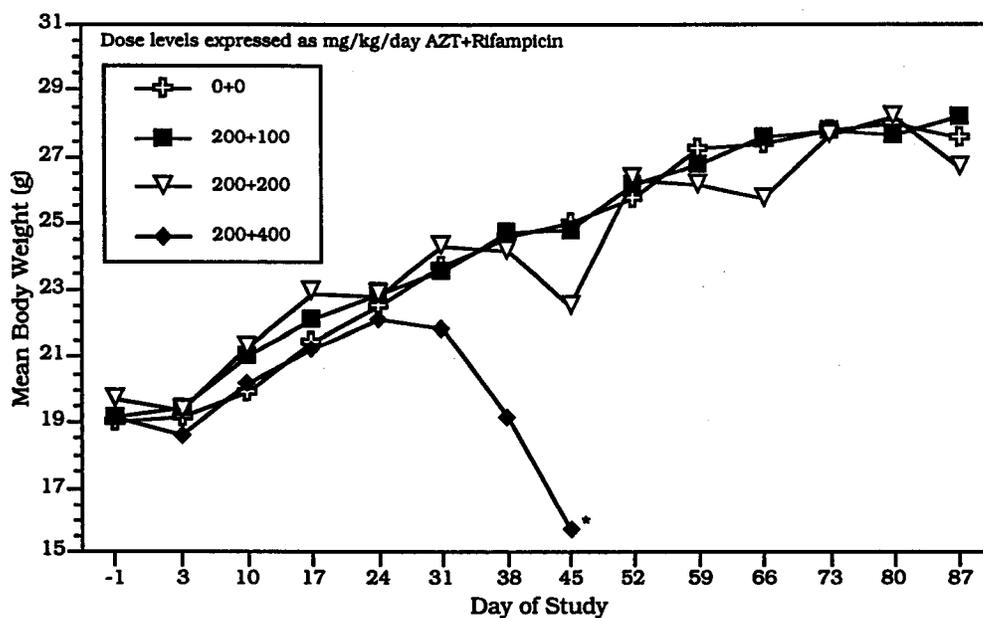
**FIGURE 1**  
**Mean Body Weights of Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**



**FIGURE 1**  
**Mean Body Weights of Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All animals in this group died early)



**FIGURE 2**  
**Mean Body Weights of Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
(\* All animals in this group died early)



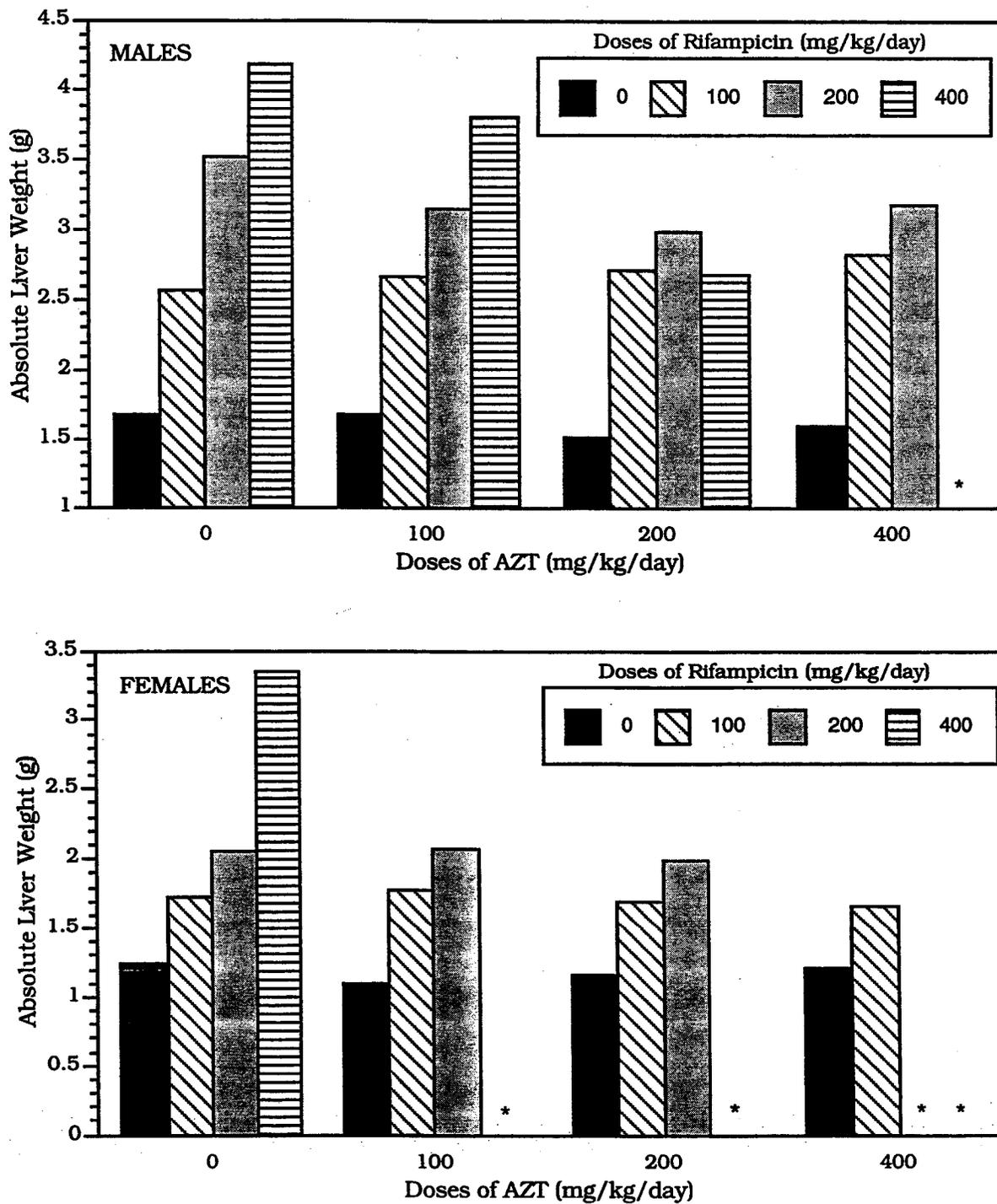
**FIGURE 2**  
**Mean Body Weights of Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All animals in this group died early)

### 30 AZT and Rifampicin

Administration of rifampicin alone caused greater declines in final mean body weights of male mice than of female mice (Table 3 and Figures 1 and 2). Respective final mean body weights of male mice treated with 100, 200, or 400 mg of rifampicin were approximately 7% (35.00 grams;  $P = 0.05$ ), 5% (35.71 grams), and 14% (32.50 grams;  $P = 0.01$ ) lower than the mean (37.71) of the control group. Female mice treated with 100 mg of rifampicin alone did not have lower body weights. Respective final mean body weights of female groups treated with 200 or 400 mg of rifampicin alone were approximately 4% (27.72 grams) and 3% (28.13 grams) lower than the mean (28.89 grams) of the female control group.

Treatment with 100, 200, or 400 mg of AZT in combination with 100 mg of rifampicin had no impact on body weight gain or final mean body weights of male or female mice (Table 3 and Figures 1 and 2). Male and female groups treated with combination therapy at higher dosages had mean body weights at least 10% lower than body weights of the corresponding control groups. Lower body weights were evident in higher dosage combination groups by day 30. Male groups treated with 100 or 200 mg AZT + 400 mg rifampicin and 400 mg AZT + 200 mg rifampicin had marked decreases in body weights by 6 to 7 weeks and marked recovery in body weights by 8 to 11 weeks. Due to anemia, a number of mice in these groups lost weight and died by week 8. The surviving mice had higher body weights than the mice that died, and the surviving mice showed some recovery in growth resulting in the marked increases in body weights from week 8 to week 11 of the study. Respective final mean body weights for male groups treated with 100 or 200 mg of AZT in combination with 200 mg of rifampicin were approximately 10% (34.12 grams;  $P = 0.05$ ) and 13% (32.69 grams;  $P = 0.01$ ) lower than the mean (37.71 grams) of the male control group. The final mean body weight of two surviving male mice treated with 100 mg of AZT + 400 mg of rifampicin was approximately 20% (30.25 grams;  $P = 0.01$ ) lower than that of the control group (37.71 grams). The final body weight of the single surviving mouse treated with 200 mg of AZT + 400 mg of rifampicin was approximately 40% (22.70 grams;  $P = 0.01$ ) lower than the mean of the male control group (37.71 grams). Final body weights were not available for the male group treated with 400 mg of AZT + 400 mg of rifampicin due to mortality. For the female group treated with 100 mg of AZT in combination with 200 mg of rifampicin, the final mean body weight was approximately 12% (25.53 grams;  $P = 0.05$ ) lower than the mean (28.89 grams) of the control group. The final mean body weight of female mice treated with 200 mg of AZT + 200 mg of rifampicin was approximately 17% (24.07 grams;  $P = 0.01$ ) lower than the mean (28.89 grams) of the controls. Female mice treated with higher combinations did not survive.

Marked increases in absolute and relative liver weights occurred in male and females treated with rifampicin alone, and these increases were dose related (Table 3 and Figure 3). Respective absolute mean liver weights for male mice treated with 100, 200, or 400 mg of rifampicin were approximately 1.5, 2.0, and 2.5 times ( $P = 0.01$ ) the mean of the control group. For the female mice receiving the same doses, respective mean liver weights were



**FIGURE 3**  
**Mean Absolute Liver Weights of Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* No data available due to mortality)

approximately 1.4, 1.7, and 2.7 times the mean of the female control group (P 0.01). The dose-related increases in liver weights corresponded well with elevated serum enzymes indicative of hepatotoxicity. Administration of AZT alone to male and female mice had no impact on liver weights. Treatment with combinations of AZT and rifampicin caused increases in liver weights comparable to the increases observed in mice treated with rifampicin alone.

Administration of AZT alone or rifampicin alone did not result in dose-related alterations in thymus weights (Table 3). Treatment-related decreases in absolute thymus weights did occur in mice treated with higher concentration combinations of AZT and rifampicin, and the decreased thymus weights corresponded with thymic atrophy. Respective mean thymus weights of male groups treated with 200 or 400 mg of AZT in combination with 200 mg of rifampicin were approximately 13% (P 0.05) and 23% lower (P 0.01) than the mean of the male control group. Male mice treated with 100 mg of AZT and 400 mg of rifampicin had a mean thymus weight approximately 40% lower (P 0.01) than the mean weight of the control group. Respective mean thymus weights in female mice treated with 100 and 200 mg of AZT in combination with 200 mg of rifampicin were approximately 22% and 40% lower (P 0.01) than the mean of the female control group.

Increases in absolute and/or relative heart weights were observed in male and female mice treated with higher concentration combinations of AZT and rifampicin (Table 3). These elevated heart weights occurred in groups that were anemic and may reflect a compensatory physiological hypertrophy brought on by chronic increased heart rate or force of contraction in an attempt to oxygenate the body. Respective mean relative heart weights in male groups treated with 200 or 400 mg of AZT in combination with 200 mg of rifampicin were approximately 1.1 and 1.3 times (P 0.01) that of the male controls. For the two surviving male mice treated with 100 mg of AZT + 400 mg of rifampicin, the mean relative heart weight was approximately 1.3 times (P 0.01) the mean of the control group. The single surviving male mouse treated with 200 mg of AZT + 400 mg of rifampicin had a relative heart weight approximately 2.3 times (P 0.01) the control group mean. Mean relative heart weights in female mice treated with 400 mg of AZT + 100 mg of rifampicin were approximately 1.2 times (P 0.01) the mean of the female control group. Respective mean relative heart weights in female mice treated with 100 and 200 mg of AZT in combination with 200 mg of rifampicin were approximately 1.3 and 1.6 times (P 0.01) the mean of the female control group. In general, significant decreases (P 0.05 or P 0.01) in absolute heart weights of male mice treated with 200 or 400 mg of rifampicin alone and female groups treated with 100 mg of AZT alone and 400 mg of rifampicin alone paralleled decreases in body weights.

Significantly lower (P 0.05 or P 0.01) testicular weights were observed in male groups treated with 400 mg of rifampicin alone, 100 mg of AZT + 100 or 400 mg of rifampicin, 200 mg of AZT + 200 or 400 mg of rifampicin, and 400 mg of AZT + 100 or 200 mg of rifampicin (Table 3). In general, testis weights decreased with increasing doses of rifampicin.

Significant ( $P < 0.05$ ) changes in absolute or relative kidney and lung weights observed in some male and female groups (Table 3) were not considered to be biologically significant because the changes generally paralleled decreases in final mean body weights and histopathological lesions were not observed.

## CLINICAL PATHOLOGY

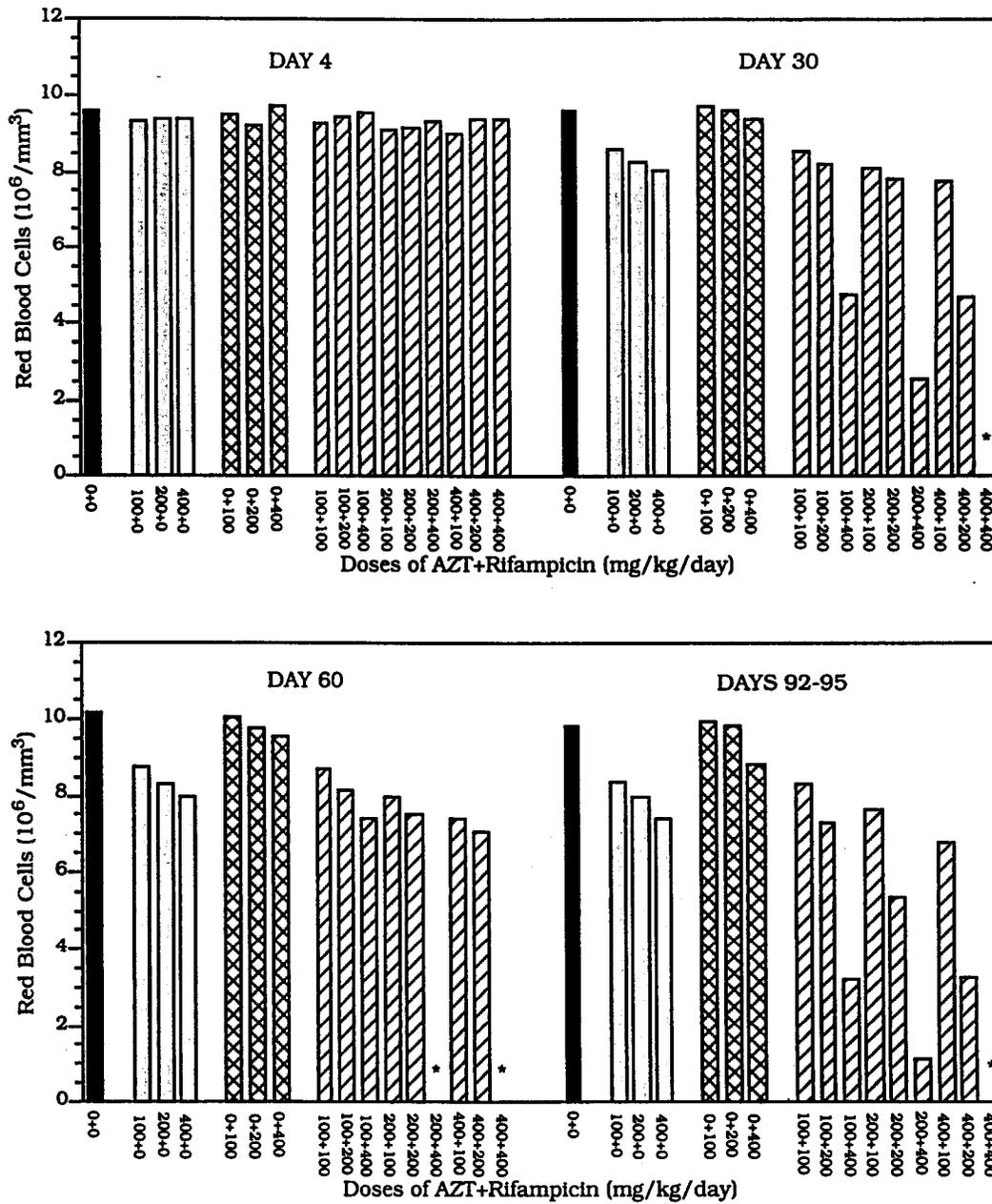
### Hematology

#### AZT Alone

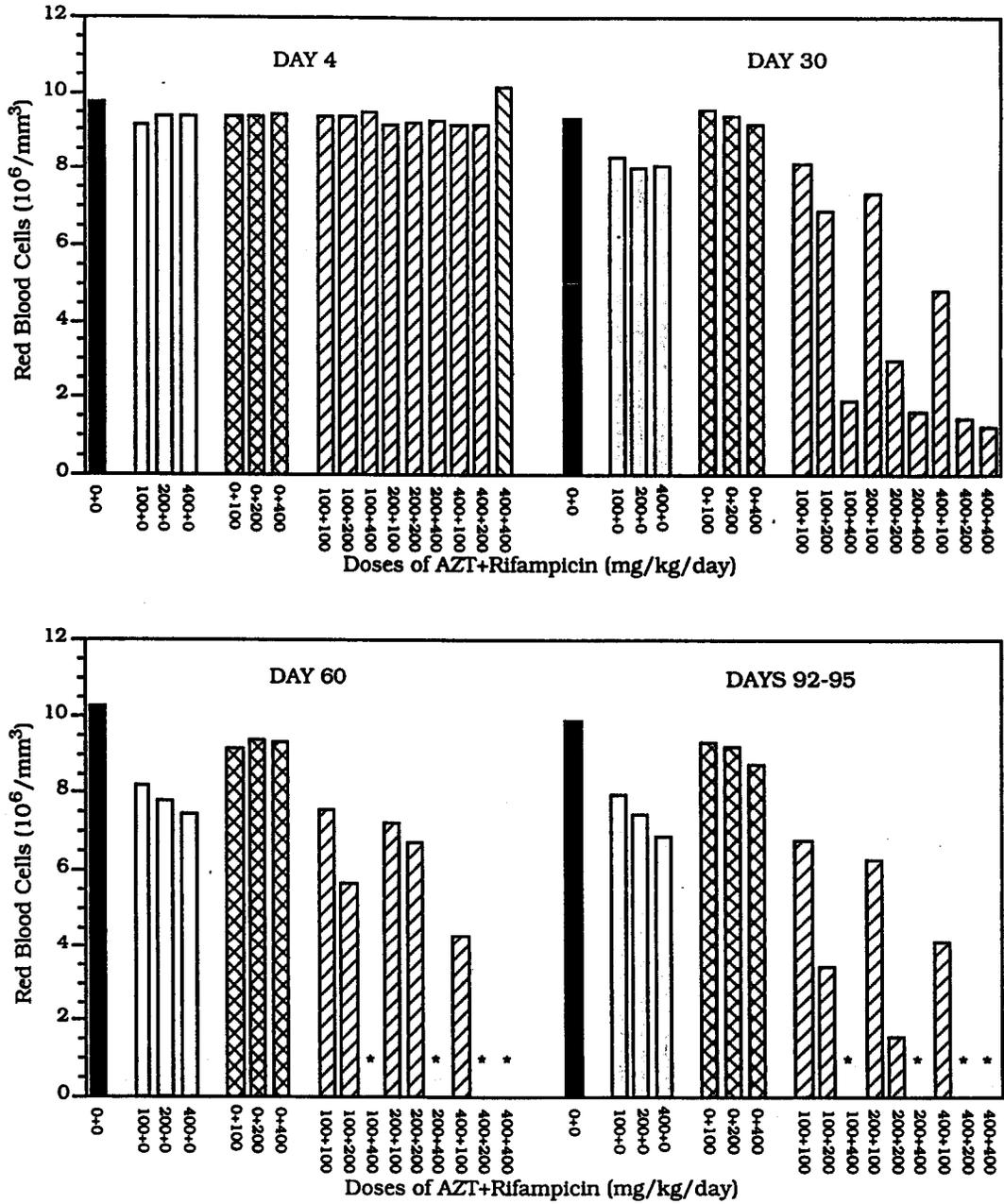
Administration of AZT alone at 100, 200, or 400 mg/kg per day caused anemia, reticulocytopenia followed by reticulocytosis, thrombocytosis, and leukopenia. In general, changes were more prevalent in females than in males. The anemic process was mild, usually dose-related, and accompanied by elevations in mean cell volume (MCV) and mean cell hemoglobin (MCH) values, and as such, could be classified as macrocytic. Hematology values are given in Appendix A.

On day 4, significant alterations in red blood cell (RBC) counts did not occur in male mice (Figure 4) treated with AZT alone. Significant alterations did occur, however, in female mice treated with AZT alone. Respective mean RBC counts on day 4 of female mice (Figure 5) treated with 100, 200, or 400 mg of AZT were approximately 6% ( $9.11 \times 10^6/\text{mm}^3$ ;  $P < 0.01$ ), 4% ( $9.30 \times 10^6/\text{mm}^3$ ;  $P < 0.05$ ), and 4% ( $9.38 \times 10^6/\text{mm}^3$ ) lower than the mean RBC count ( $9.73 \times 10^6/\text{mm}^3$ ) of the female control group. Significant alterations did not occur in MCV, MCH, or mean cell hemoglobin concentration (MCHC) values on day 4, but slight declines in hemoglobin (Hgb) and hematocrit (Hct) accompanied the lower RBC counts.

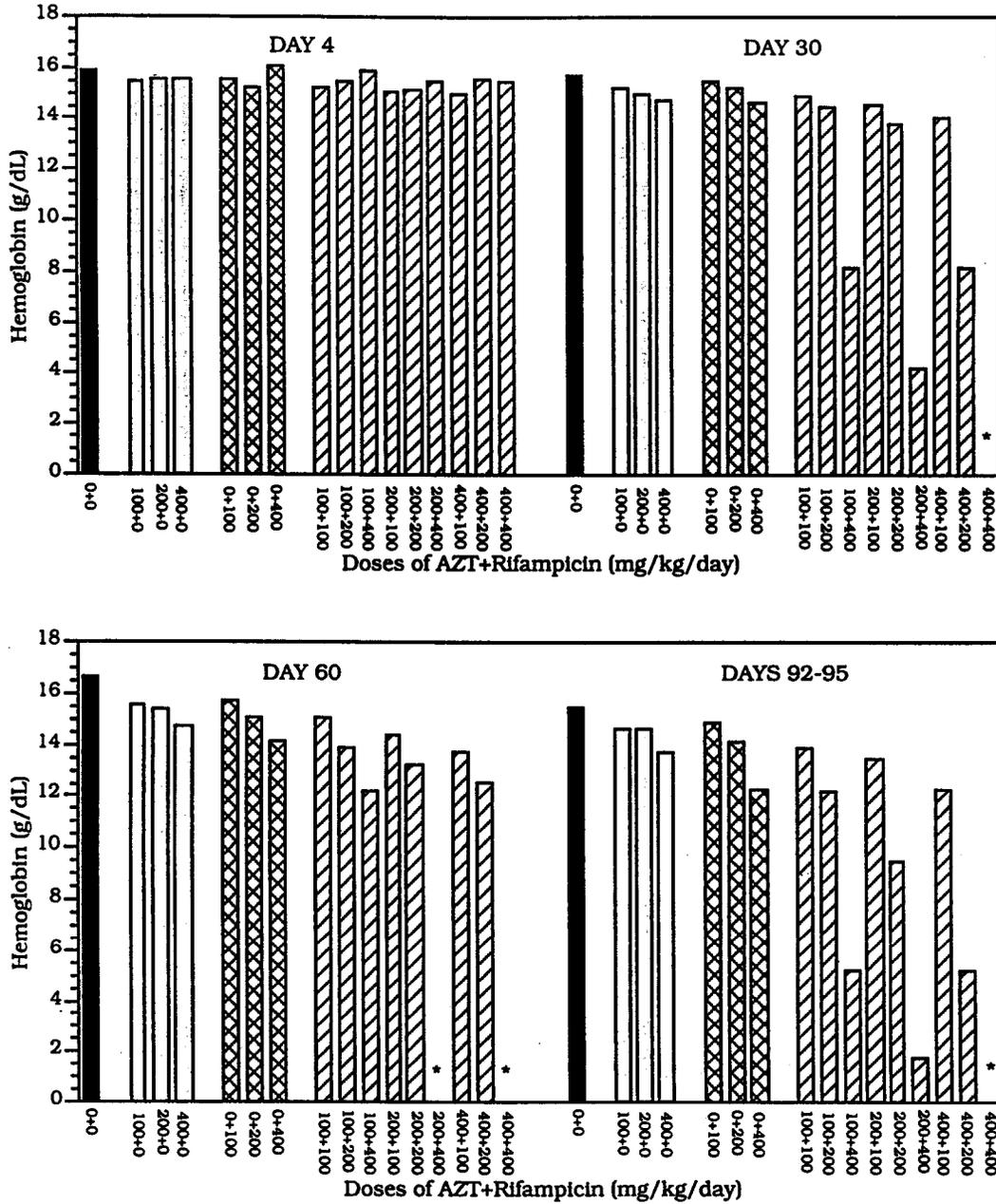
On day 30, a marginal anemia occurred in male and female mice treated with AZT alone. Respective mean RBC counts on day 30 for male mice (Figure 4) treated with 100, 200, or 400 mg of AZT were approximately 10% ( $8.63 \times 10^6/\text{mm}^3$ ), 14% ( $8.32 \times 10^6/\text{mm}^3$ ;  $P < 0.05$ ), and 16% ( $8.06 \times 10^6/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean RBC count ( $9.64 \times 10^6/\text{mm}^3$ ) of the male control group. Declines in Hgb (Figure 6) and Hct values paralleled the decreases in RBC counts. Elevations in MCV and MCH values also accompanied the dose-related anemia. Respective mean MCV values for male mice treated with 100, 200, or 400 mg of AZT were approximately 8% (52.9 fL;  $P < 0.01$ ), 11% (54.1 fL;  $P < 0.01$ ), and 14% (55.7 fL;  $P < 0.01$ ) higher than the mean (48.8 fL) of the male control group. Similar alterations occurred on day 30 in female mice treated with AZT alone. Respective mean



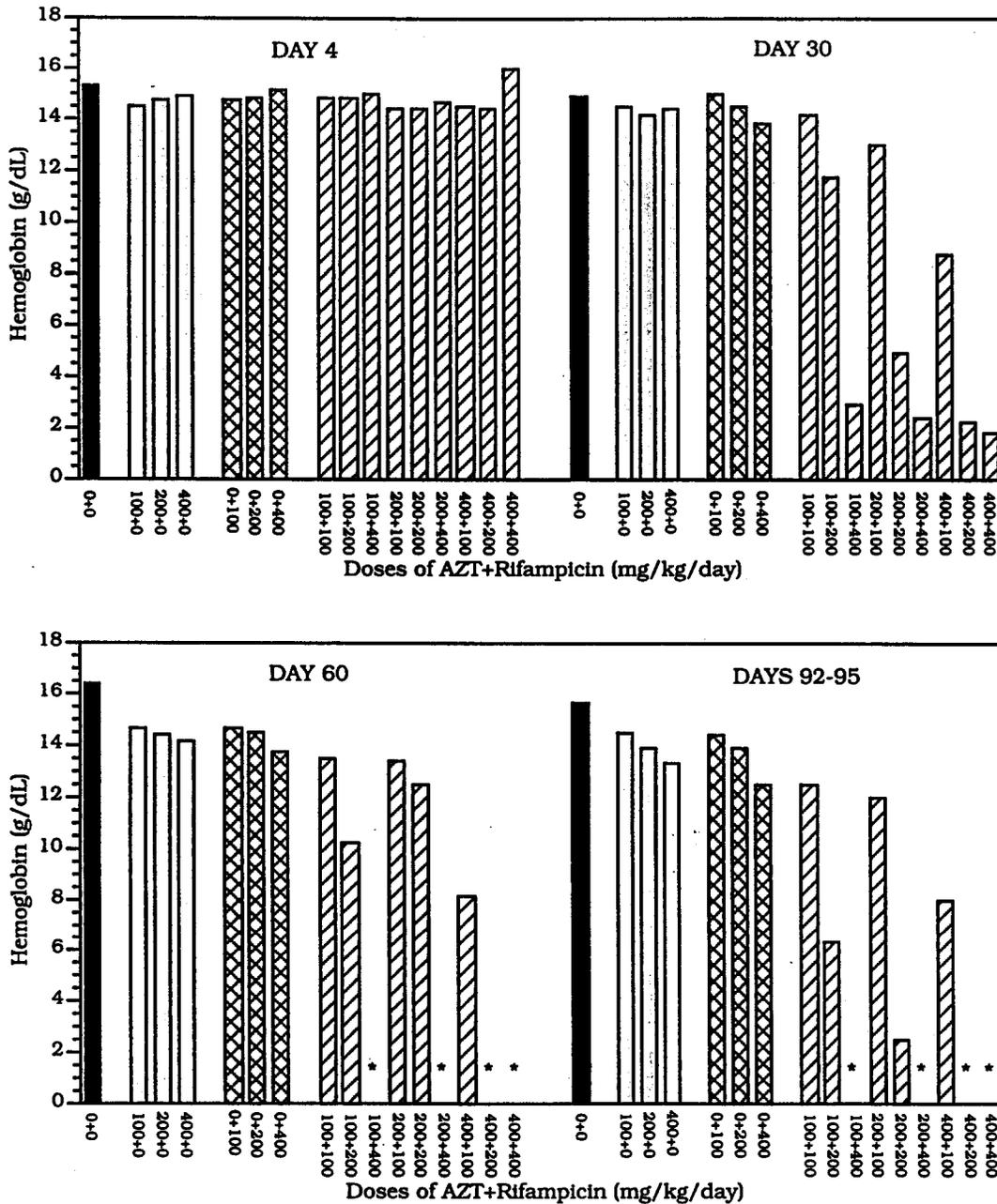
**FIGURE 4**  
**Mean Red Blood Cell Counts for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 5**  
**Mean Red Blood Cell Counts for Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 6**  
**Mean Hemoglobin Values for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 7**  
**Mean Hemoglobin Values for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)

RBC counts on day 30 for female mice (Figure 5) treated with 100, 200, or 400 mg of AZT were approximately 10% ( $8.28 \times 10^6/\text{mm}^3$ ), 14% ( $7.97 \times 10^6/\text{mm}^3$ ; P 0.01), and 13% ( $8.04 \times 10^6/\text{mm}^3$ ; P 0.05) lower than the mean ( $9.25 \times 10^6/\text{mm}^3$ ) of the female control group. Marginal decreases in Hgb (Figure 7) and Hct values accompanied the decreases in RBC counts, and slight dose-related elevations in MCV and MCH values also accompanied the anemia. Respective mean MCV values for the female mice treated with 100, 200, or 400 mg were approximately 10% (53.4 fL; P 0.01), 12% (54.1 fL; P 0.01), and 15% (55.7 fL; P 0.01) higher than the mean (48.5 fL) of the female controls.

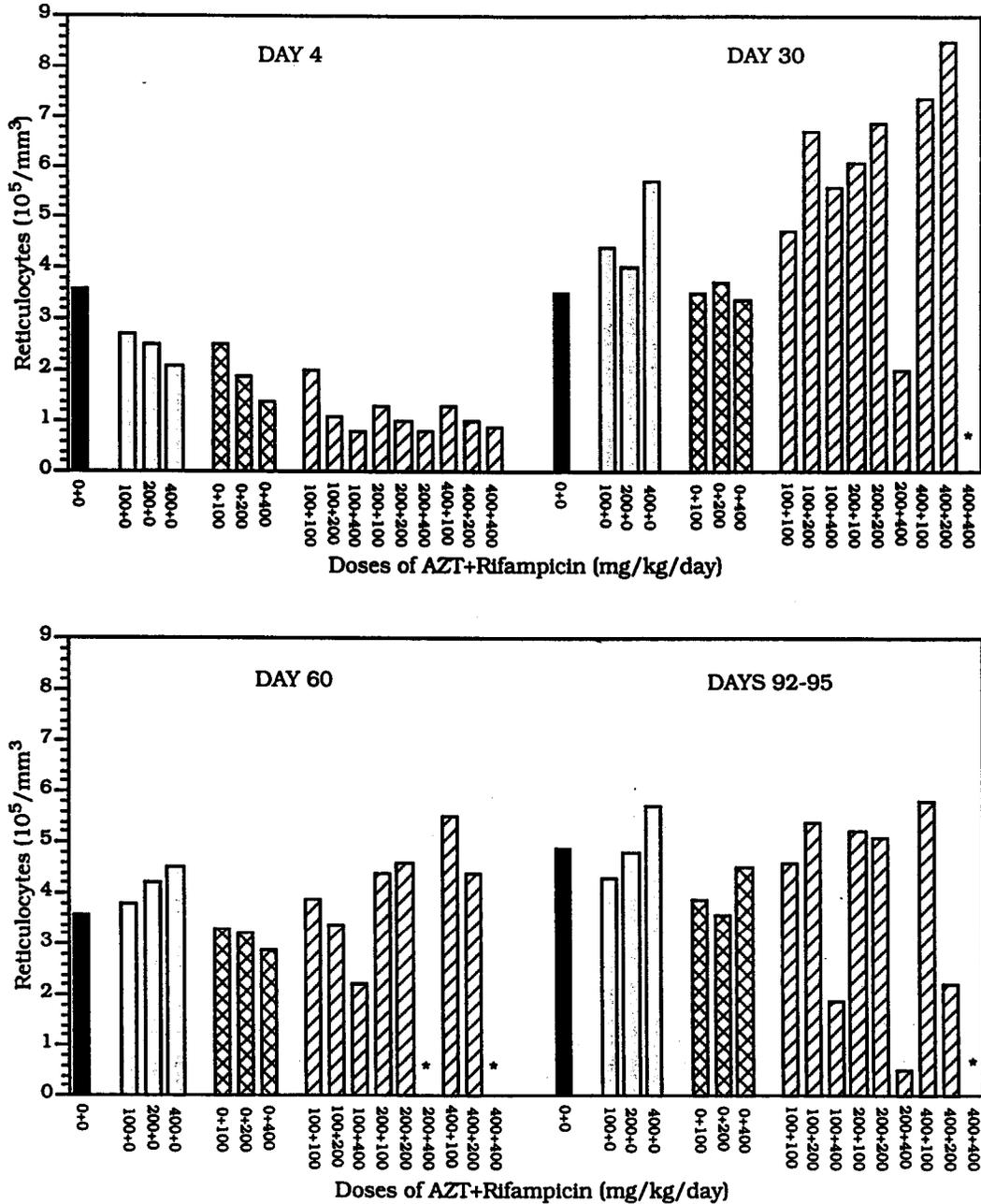
On day 60, the severity of the anemic process was greater than on day 30. Respective mean RBC counts on day 60 of male mice (Figure 4) were approximately 14% ( $8.77 \times 10^6/\text{mm}^3$ ; P 0.01), 18% ( $8.34 \times 10^6/\text{mm}^3$ ; P 0.01), and 21% ( $7.99 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $10.16 \times 10^6/\text{mm}^3$ ) of the male control group. Decreased Hgb (Figure 6) and Hct values accompanied the dose-related declines in RBC counts. Dose-related elevations in MCV and MCH values also accompanied the anemia. Respective mean MCV values for male mice treated with 100, 200, or 400 mg of AZT were approximately 9% (51.2 fL; P 0.01), 14% (53.7 fL; P 0.01), and 17% (54.8 fL; P 0.01) higher than the mean MCV value (47.0 fL) of the male control group. Similar trends occurred on day 60 in female mice treated with AZT alone; however, the severity of the alterations was greater. Respective mean RBC counts on day 60 of female mice (Figure 5) treated with 100, 200, or 400 mg of AZT alone were approximately 20% ( $8.18 \times 10^6/\text{mm}^3$ ; P 0.01), 24% ( $7.76 \times 10^6/\text{mm}^3$ ; P 0.01), and 28% ( $7.42 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean RBC count ( $10.24 \times 10^6/\text{mm}^3$ ) of the female control group. Declines in Hgb (Figure 7) and Hct values paralleled the dose-related decreases in RBC counts. Dose-related elevations in MCV and MCH values were also prominent. Respective mean MCV values for female mice treated with 100, 200, or 400 mg of AZT were approximately 13% (53.4 fL; P 0.01), 18% (55.9 fL; P 0.01), and 22% (57.8 fL; P 0.01) higher than the mean (47.3 fL) of the female control group.

At the time of terminal sacrifice on days 92 to 95, alterations in RBC values were similar to those observed on day 60. Respective mean RBC counts on days 92 to 95 in male mice (Figure 4) treated with 100, 200, or 400 mg of AZT alone were approximately 15% ( $8.40 \times 10^6/\text{mm}^3$ ; P 0.01), 19% ( $8.01 \times 10^6/\text{mm}^3$ ; P 0.01) and 25% ( $7.41 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $9.86 \times 10^6/\text{mm}^3$ ) of the control group. Lower Hgb (Figure 6) and Hct values paralleled the dose-related declines in RBC counts. Treatment-related elevations in MCV and MCH values also occurred. Respective mean MCV values for the 100, 200, and 400 mg groups were approximately 13% (52.7 fL; P 0.01), 19% (55.7 fL; P 0.01), and 22% (57.2 fL; P 0.01) higher than the mean (46.7 fL) of the male control group. The severity of the anemia was slightly greater in female mice than in males. Respective mean RBC counts on days 92 to 95 in female mice (Figure 5) treated with 100, 200, or 400 mg of AZT alone were approximately 19% ( $7.95 \times 10^6/\text{mm}^3$ ; P 0.01), 25% ( $7.40 \times 10^6/\text{mm}^3$ ; P 0.01), and 30% ( $6.88 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $9.85 \times 10^6/\text{mm}^3$ ) of the female control group. Decreases in Hgb (Figure 7) and Hct values paralleled the dose-related declines in RBC counts. Elevations in MCV and MCH values also

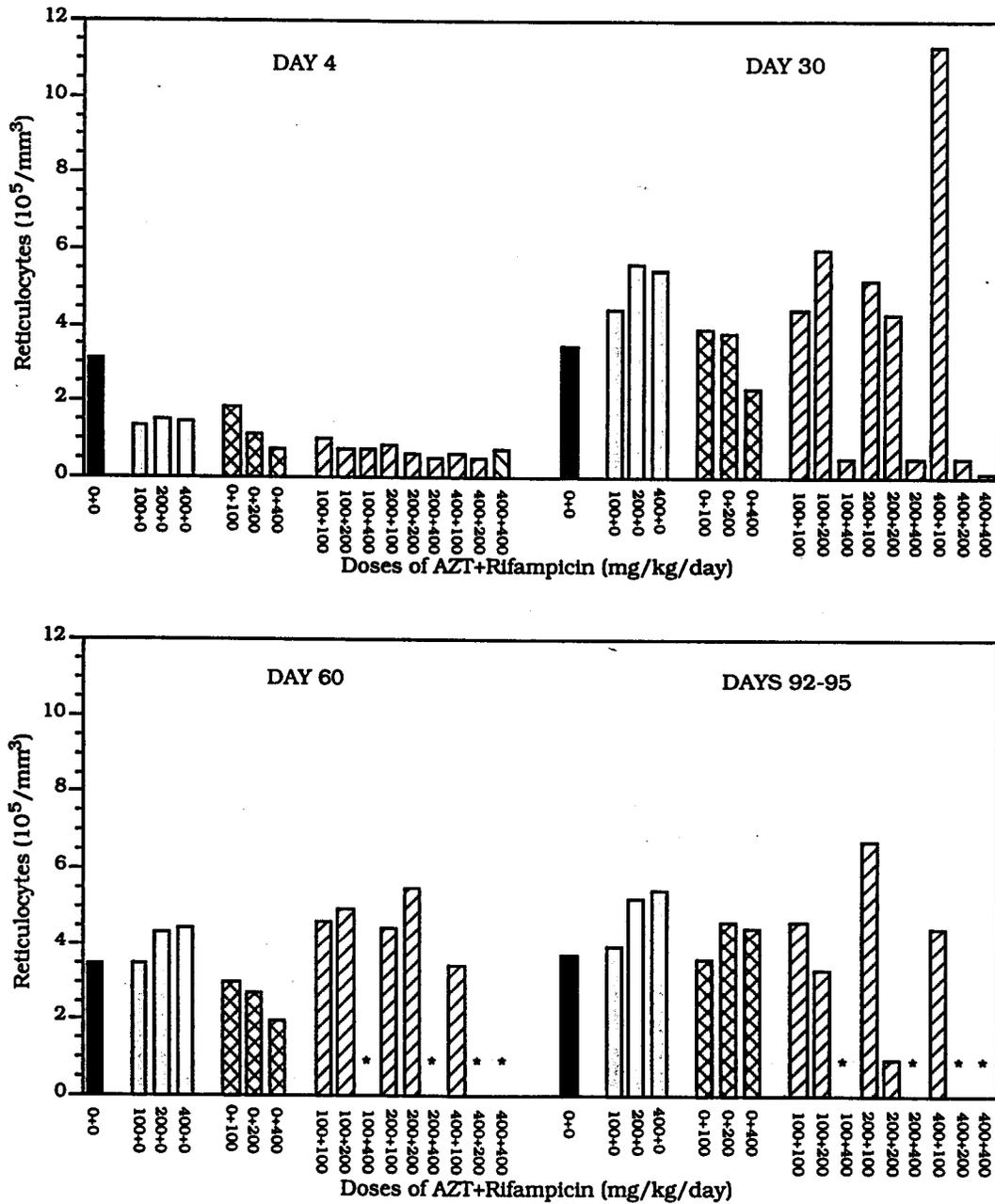
accompanied the anemia. Respective mean MCV values for the 100, 200, and 400 mg female groups were approximately 15% (54.4 fL; P 0.01), 23% (57.9 fL; P 0.01), and 27% (59.6 fL; P 0.01) higher than the mean (47.1 fL) of the female controls.

Administration of AZT alone to male and female mice also produced statistically and biologically significant alterations in reticulocyte counts (Figures 8 and 9). Initially, a reticulocytopenia developed and was followed by a reticulocytosis later in the study. Respective mean reticulocyte counts on day 4 in male mice (Figure 8) treated with 100, 200, or 400 mg of AZT alone were approximately 25% ( $2.7 \times 10^5/\text{mm}^3$ ; P 0.01), 31% ( $2.5 \times 10^5/\text{mm}^3$ ; P 0.01), and 42% ( $2.1 \times 10^5/\text{mm}^3$ ; P 0.01) lower than the mean ( $3.6 \times 10^5/\text{mm}^3$ ) of the male control group. For the female groups (Figure 9) treated identically, respective mean reticulocyte counts were approximately 58% ( $1.3 \times 10^5/\text{mm}^3$ ; P 0.01), 52% ( $1.5 \times 10^5/\text{mm}^3$ ; P 0.01), and 55% ( $1.4 \times 10^5/\text{mm}^3$ ; P 0.01) lower than the mean ( $3.1 \times 10^5/\text{mm}^3$ ) of the control group. Reticulocyte counts rebounded in both males and females, as significant (P 0.05) alterations in reticulocyte counts did not occur on day 30 in male or female mice treated with AZT alone. On day 60, mild elevations in reticulocyte counts occurred. Respective mean reticulocyte counts on day 60 in male mice (Figure 8) treated with 200 or 400 mg of AZT were approximately 1.2 times ( $4.2 \times 10^5/\text{mm}^3$ ; P 0.01) and 1.3 times ( $4.5 \times 10^5/\text{mm}^3$ ; P 0.01) the mean ( $3.6 \times 10^5/\text{mm}^3$ ) of the male control group. Although not statistically significant, respective mean reticulocyte counts for female mice (Figure 9) treated identically were approximately 1.2 times ( $4.3 \times 10^5/\text{mm}^3$ ) and 1.2 times ( $4.4 \times 10^5/\text{mm}^3$ ) the mean ( $3.5 \times 10^5/\text{mm}^3$ ) of the female control group. At the termination of the study on days 92 to 95, reticulocytosis was not evident in male mice treated with AZT alone. For the female mice (Figure 9), however, respective mean reticulocyte counts in the 200 and 400 mg groups were approximately 1.4 times ( $5.2 \times 10^5/\text{mm}^3$ ) and 1.5 times ( $5.4 \times 10^5/\text{mm}^3$ ; P 0.05) the mean ( $3.7 \times 10^5/\text{mm}^3$ ) of the female control group.

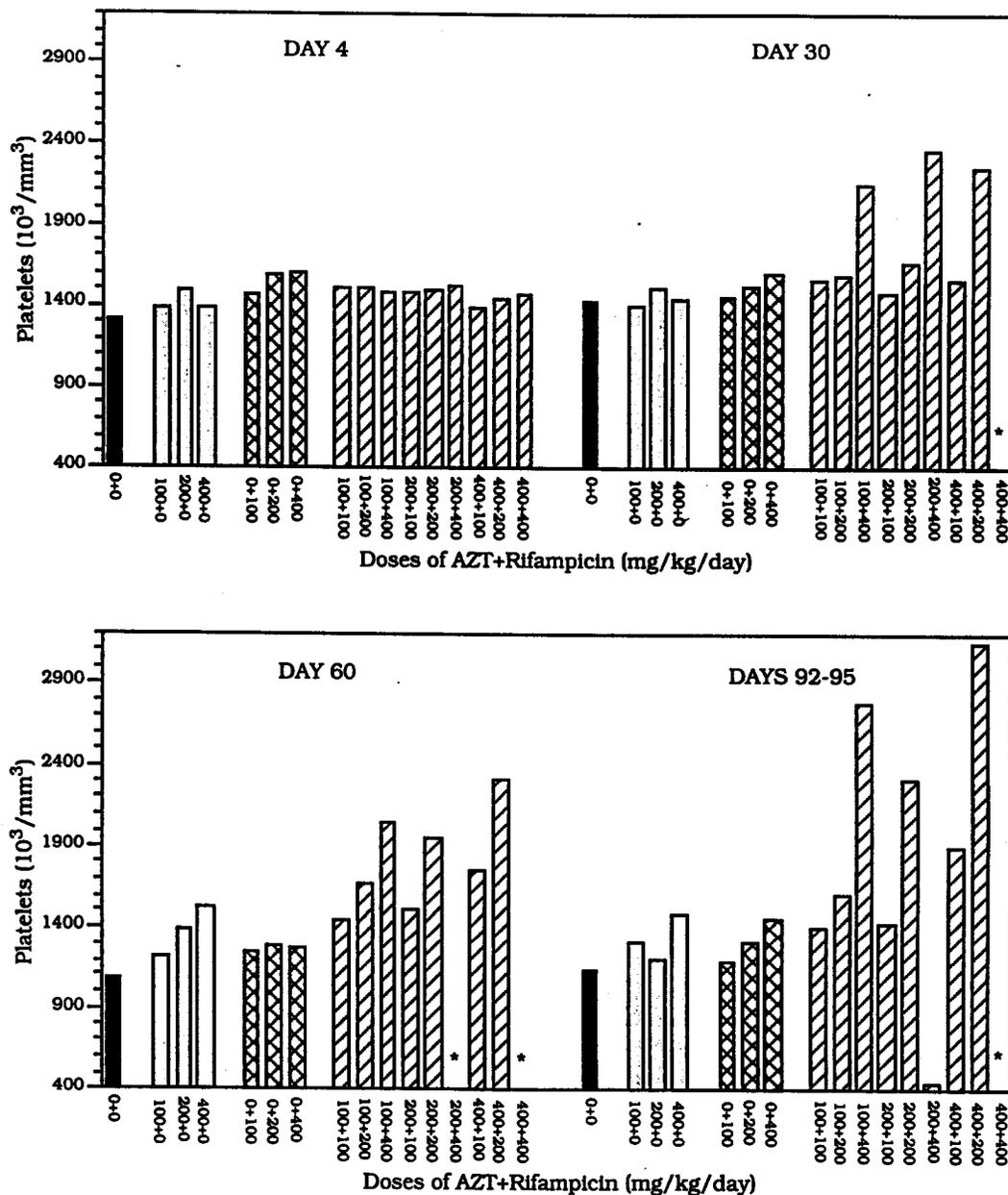
Thrombocytosis also occurred in male and female mice treated with AZT alone (Figures 10 and 11). On day 30, respective mean platelet counts in female mice (Figure 11) treated with 100, 200, or 400 mg of AZT alone were approximately 1.1 times ( $1,282 \times 10^3/\text{mm}^3$ ), 1.3 times ( $1,412 \times 10^3/\text{mm}^3$ ; P 0.05), and 1.2 times ( $1,404 \times 10^3/\text{mm}^3$ ) the mean ( $1,129 \times 10^3/\text{mm}^3$ ) of the female control group. Platelet counts in the corresponding male groups (Figure 10) were within normal limits on day 30. On day 60, respective mean platelet counts in male mice (Figure 10) in the 100, 200, and 400 mg groups were approximately 1.1 times ( $1,216 \times 10^3/\text{mm}^3$ ), 1.3 times ( $1,384 \times 10^3/\text{mm}^3$ ; P 0.01), and 1.4 times ( $1,520 \times 10^3/\text{mm}^3$ ; P 0.01) higher than the mean ( $1,073 \times 10^3/\text{mm}^3$ ) of the male control group. Platelet counts in the female groups (Figure 11) were within a normal range on day 60. At the time of terminal sacrifice, alterations in platelet counts did not occur in male mice (Figure 10) treated with



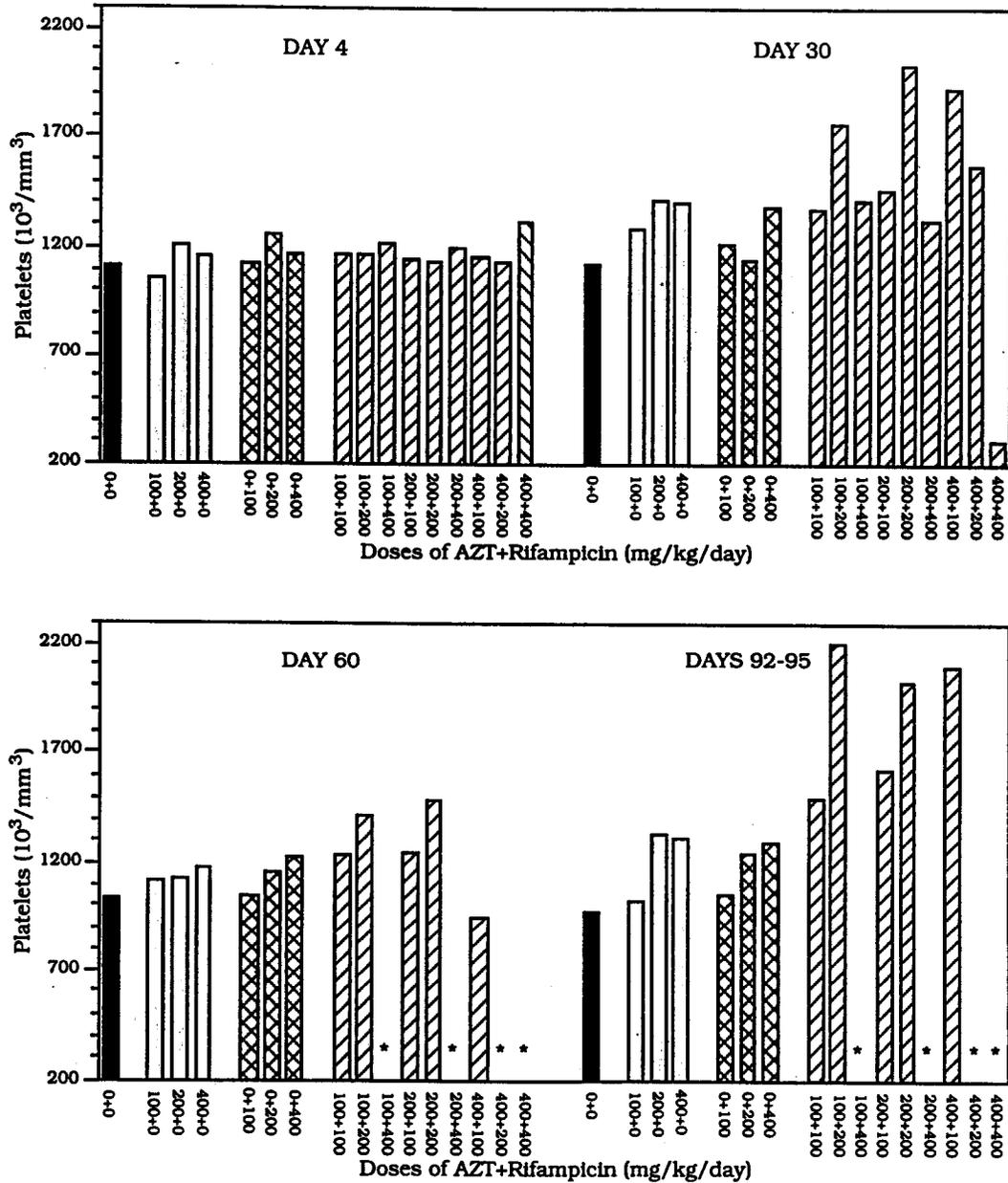
**FIGURE 8**  
**Mean Reticulocyte Counts for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 9**  
**Mean Reticulocyte Counts for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 10**  
**Mean Platelet Counts for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 11**  
**Mean Platelet Counts for Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)

AZT alone. For the female mice (Figure 11) treated with 200 or 400 mg of AZT, respective platelet counts were approximately 1.4 times ( $1,331 \times 10^3/\text{mm}^3$ ; P 0.01) and 1.4 times ( $1,319 \times 10^3/\text{mm}^3$ ; P 0.01) the mean ( $975 \times 10^3/\text{mm}^3$ ) of the female control group at the time of terminal sacrifice.

Macrocytic anemia, reticulocytopenia followed by reticulocytosis, and thrombocytosis that occurred subsequent to the administration of AZT were all similar to those previously described in mice (Thompson *et al.*, 1991).

### Rifampicin Alone

Administration of rifampicin alone at 100, 200, or 400 mg/kg per day caused anemia, reticulocytopenia, and occasional neutrophilia. In general, the anemia was mild and dose related, with reductions in Hgb values of greater magnitude than reductions in RBC counts. The slight anemia was accompanied by dose-related declines in MCV and MCH values and could be classified as microcytic. Hematology values are listed in Appendix A.

On day 4, statistically or biologically significant alterations did not occur in male or female mice treated with rifampicin alone. On day 30, mild declines in Hgb, Hct, MCV, and MCH occurred in male and female mice treated with 400 mg of rifampicin without biologically significant declines in RBC counts. Respective mean Hgb values in male and female mice (Figures 6 and 7) treated with 400 mg of rifampicin were approximately 7% (14.7 g/dL and 13.8 g/dL) lower than the mean Hgb values of the corresponding control groups (15.8 g/dL and 14.9 g/dL). Corresponding mean MCV values for male and female mice treated with 400 mg were approximately 5% (46.3 fL) and 6% (45.8 fL) lower than the mean of the male and female control groups (48.8 fL and 48.5 fL). Significant declines (P 0.01) in corresponding MCH values occurred, with respective mean MCH values approximately 5% (15.6 pg) and 6% (15.1 pg) lower than the means of the male and female control groups (16.4 pg and 16.1 pg). The decreases in Hgb, Hct, MCV, and MCH indicate a hematological microcytic response even though RBC counts were not significantly decreased.

On day 60, a distinct dose-related anemia was evident in male and female mice treated with rifampicin alone, and the severity of the anemia was most evident in the Hgb parameter. Respective mean Hgb values on day 60 in male mice (Figure 6) treated with 100, 200, or 400 mg of rifampicin were approximately 5% (15.8 g/dL; P 0.01), 10% (15.1 g/dL; P 0.01), and 15% (14.2 g/dL; P 0.01) lower than the mean (16.7 g/dL) of the male control group. Corresponding RBC counts (Figure 4) were approximately 1% ( $10.06 \times 10^6/\text{mm}^3$ ), 4% ( $9.79 \times 10^6/\text{mm}^3$ ), and 7% ( $9.55 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean RBC count ( $10.16 \times 10^6/\text{mm}^3$ ) of the male control group. Declines in Hct values accompanied the lower Hgb and RBC values. Dose-related declines in MCV and MCH values depicting the microcytic nature of the anemia also occurred. Respective mean MCV values on day 60 in male mice treated with 100, 200, or 400 mg of rifampicin were approximately 3% (45.6 fL; P 0.01), 4% (45.0 fL; P 0.01), and

8% (43.4 fL; P 0.01) lower than the mean (47.0 fL) of the male control group. A dose-related microcytic anemia of similar severity also occurred on day 60 in female mice treated with rifampicin alone. Respective Hgb values in female mice (Figure 7) treated with 100, 200, or 400 mg of rifampicin were approximately 11% (14.6 g/dL), 12% (14.5 g/dL), and 16% (13.7 g/dL; P 0.05) lower than the mean (16.4 g/dL) of the control group. Corresponding RBC counts (Figure 5) for the same treatment groups were approximately 11% ( $9.15 \times 10^6/\text{mm}^3$ ), 8% ( $9.39 \times 10^6/\text{mm}^3$ ), and 9% ( $9.34 \times 10^6/\text{mm}^3$ ) lower than the mean ( $10.24 \times 10^6/\text{mm}^3$ ) of the female control group. Lower Hct values accompanied the declines in Hgb and RBC values. Although not statistically significant (P 0.05), declines in MCV values occurred in the 200 and 400 mg groups. Respective MCV values were approximately 3% (45.9 fL) and 6% (44.4 fL) lower than the mean of the female control group. Corresponding MCH values for the 200 and 400 mg female groups were approximately 4% (15.4 pg; P 0.01) and 9% (14.6 pg; P 0.01) lower than the mean (16.0 pg) of the control.

At the end of the study, on days 92 to 95, the severity of the microcytic anemia was slightly greater than at day 60. Respective mean Hgb values for male mice treated with 100, 200, or 400 mg of rifampicin (Figure 6) were approximately 4% (14.9 g/dL), 8% (14.2 g/dL; P 0.05), and 21% (12.3 g/dL) lower than the mean (15.5 g/dL) of the male control group. Declines in RBC counts (Figure 4) were not observed in the male groups treated with 100 or 200 mg of rifampicin. However, the mean RBC count in the 400 mg male group was approximately 10% ( $8.84 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $9.86 \times 10^6/\text{mm}^3$ ) of the control group. Declines in Hct values paralleled the decreases in Hgb values. Respective mean MCV values for male mice treated with 100, 200, or 400 mg of rifampicin were approximately 4% (45.0 fL), 7% (43.4 fL; P 0.01), and 7% (43.3 fL; P 0.01) lower than the mean (46.7 fL) of the male control group. Similar alterations occurred in female mice at the end of the study. Respective mean Hgb values (Figure 7) in female mice treated with identical doses of rifampicin were approximately 8% (14.4 g/dL), 11% (13.9 g/dL), and 20% (12.5 g/dL) lower than the mean (15.6 g/dL) of the female control group. Corresponding RBC counts (Figure 5) were approximately 5% ( $9.31 \times 10^6/\text{mm}^3$ ), 6% ( $9.21 \times 10^6/\text{mm}^3$ ), and 11% ( $8.77 \times 10^6/\text{mm}^3$ ; P 0.05) lower than the mean ( $9.85 \times 10^6/\text{mm}^3$ ) of the female control group. Declines in Hct values accompanied the decreases in Hgb and RBC values. Declines in MCV and MCH values typical of a microcytic anemia also occurred in the 400 mg female treatment group. The mean MCV value was approximately 7% (43.9 fL; P 0.05) lower than the mean (47.1 fL) of the control group, and the mean MCH value was approximately 10% (14.3 pg; P 0.01) lower than the mean (15.9 pg) of the control.

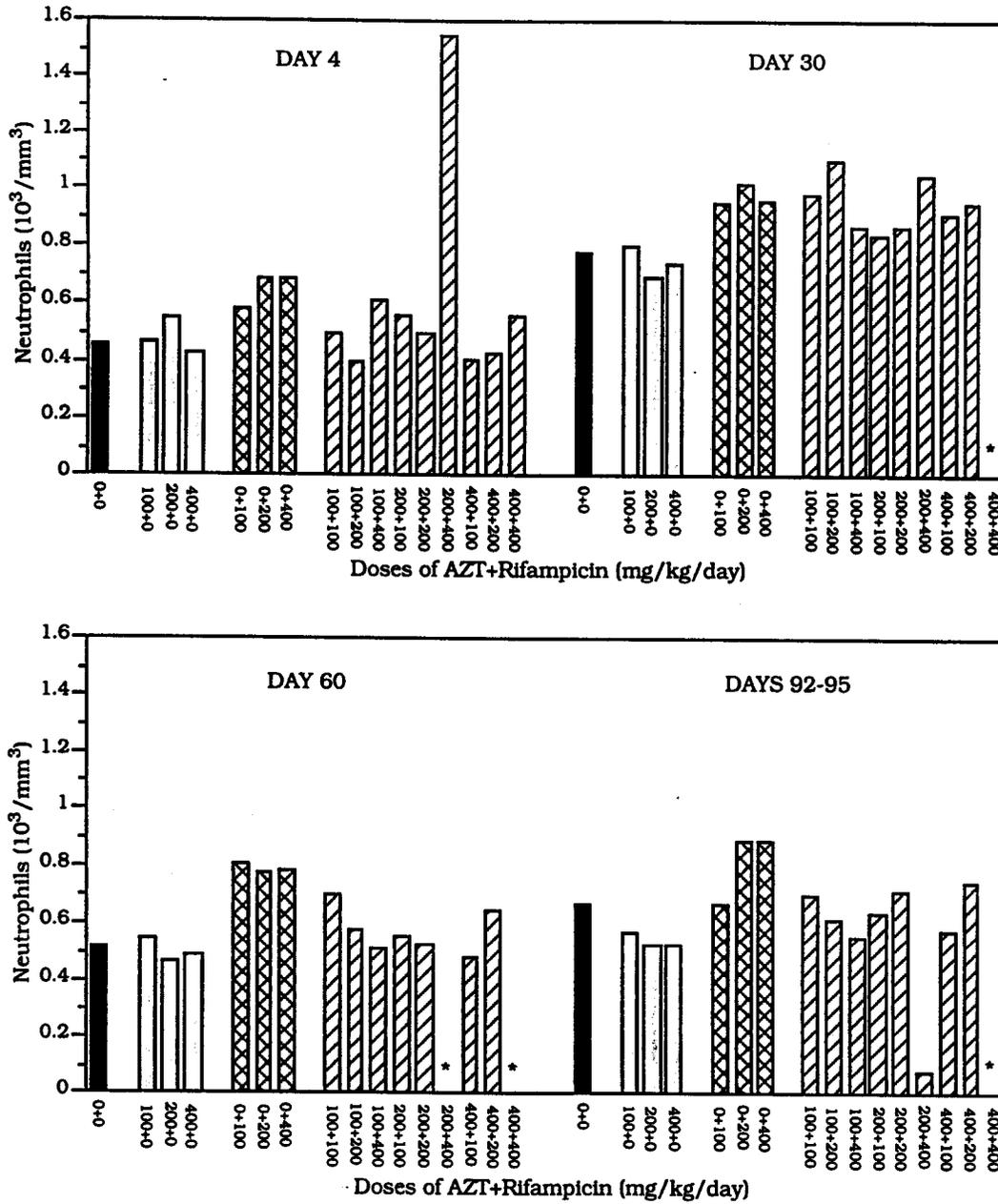
A distinct dose-related reticulocytopenia was observed in male and female mice treated with rifampicin alone. On day 4, respective mean reticulocyte counts in male mice (Figure 8) treated with 100, 200, or 400 mg of rifampicin were approximately 31% ( $2.5 \times 10^5/\text{mm}^3$ ; P 0.01), 47% ( $1.9 \times 10^5/\text{mm}^3$ ; P 0.01), and 61% ( $1.4 \times 10^5/\text{mm}^3$ ; P 0.01) lower than the mean reticulocyte count ( $3.6 \times 10^5/\text{mm}^3$ ) of the male control group. Respective mean reticulocyte counts on day 4 in female mice (Figure 9) treated with same

doses were approximately 42% ( $1.8 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ), 65% ( $1.1 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ), and 77% ( $0.7 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $3.1 \times 10^5/\text{mm}^3$ ) of the female control group. Reticulocyte counts rebounded in all treatment groups of both males and females as statistically significant ( $P < 0.05$ ) alterations did not occur on day 30. On day 60, significant declines ( $P < 0.05$ ) in mean reticulocyte counts were observed in male (Figure 8) and female (Figure 9) mice treated with 400 mg of rifampicin alone. Respective mean reticulocyte counts for male and female mice in the 400 mg/kg group were approximately 19% ( $2.9 \times 10^5/\text{mm}^3$ ;  $P < 0.05$ ) and 46% ( $1.9 \times 10^5/\text{mm}^3$ ;  $P < 0.05$ ) lower than the respective means ( $3.6 \times 10^5/\text{mm}^3$  and  $3.5 \times 10^5/\text{mm}^3$ ) of the male and female control groups. At the end of the study, significant ( $P < 0.05$ ) declines in reticulocyte counts did not occur in male or female groups treated with rifampicin alone.

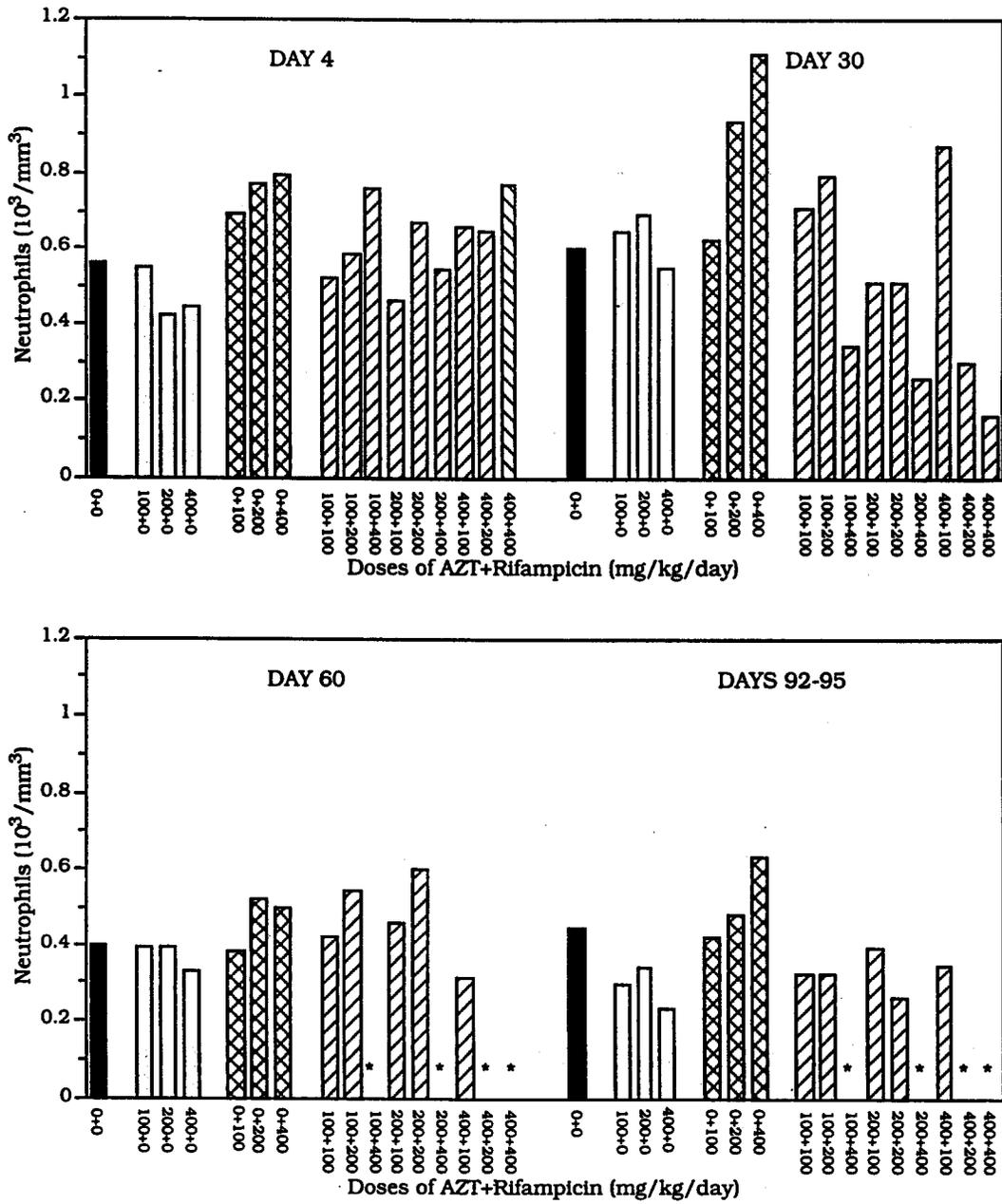
Male and female mice treated with rifampicin alone tended to have minor treatment-related elevations in segmented neutrophil counts. This compound-related neutrophilia was statistically significant on day 30 in female mice and on day 60 in male mice. On day 60, respective mean neutrophil counts in male mice (Figure 12) treated with 100, 200, or 400 mg of rifampicin alone were approximately 1.6 times ( $0.81 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ), 1.5 times ( $0.78 \times 10^3/\text{mm}^3$ ;  $P < 0.05$ ), and 1.5 times ( $0.79 \times 10^3/\text{mm}^3$ ;  $P < 0.05$ ) the mean ( $0.52 \times 10^3/\text{mm}^3$ ) of the male control group. On day 30, respective mean neutrophil counts of female mice (Figure 13) treated with 200 or 400 mg of rifampicin alone were approximately 1.6 times ( $0.93 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) and 1.9 times ( $1.11 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean neutrophil count ( $0.60 \times 10^3/\text{mm}^3$ ) of the female control group. Although not statistically significant, total WBC counts were slightly elevated (Figures 14 and 15), reflecting the impact of the neutrophilia.

A slightly significant ( $P < 0.05$ ) elevation in platelet counts on day 4 in the 200 mg rifampicin female group (Figure 11) was not considered to be biologically significant, as a dose-related pattern was not observed. No other statistically significant ( $P < 0.05$ ) alterations in platelet counts occurred in male or female mice treated with rifampicin alone.

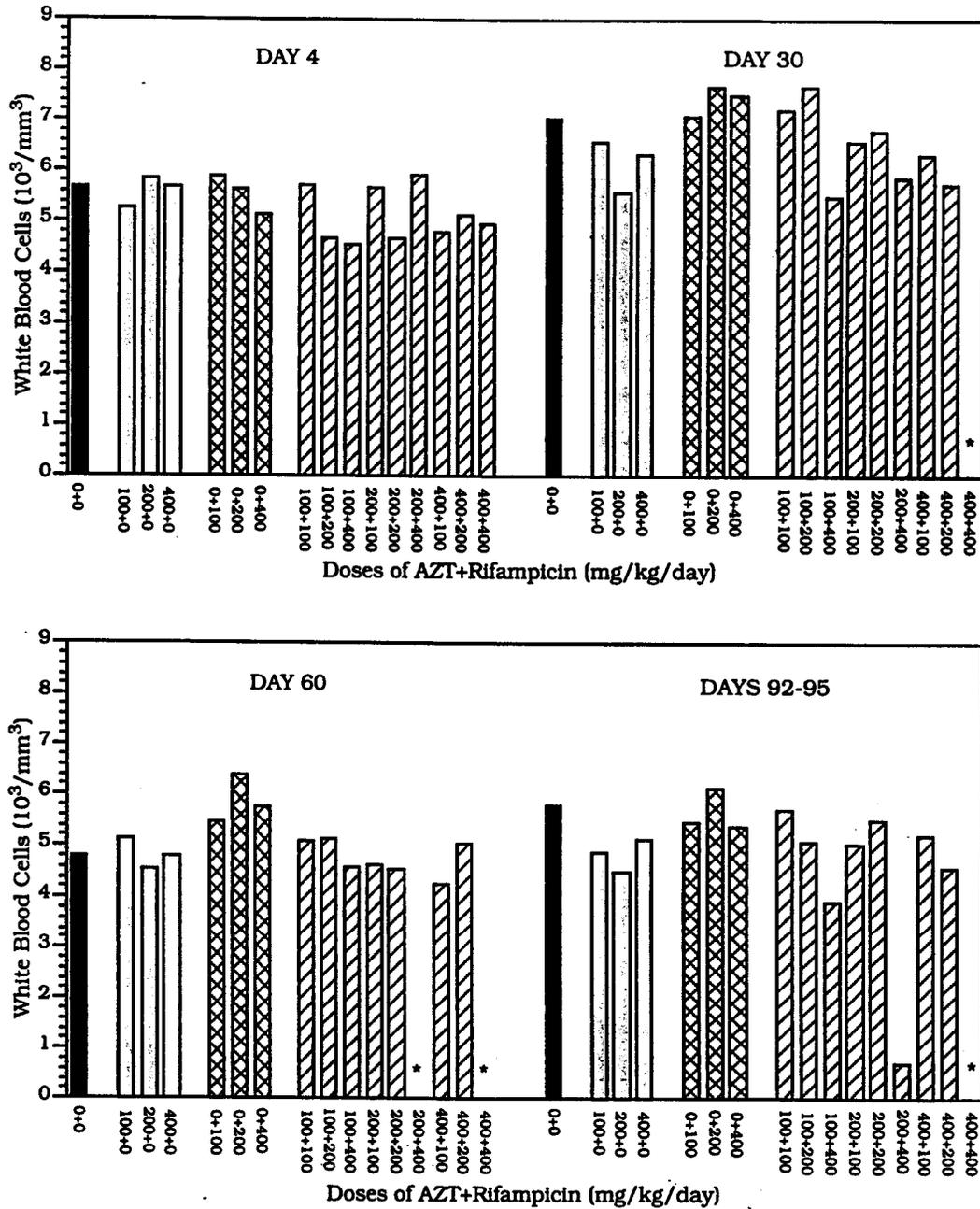
Treatment with rifampicin alone resulted in a dose-related microcytic anemia in contrast to the macrocytic anemia caused by AZT. The microcytic anemia was accompanied by an early reticulocytopenia that tended to rebound by day 30. However, rifampicin-associated microcytic anemia did not cause reticulocytosis.



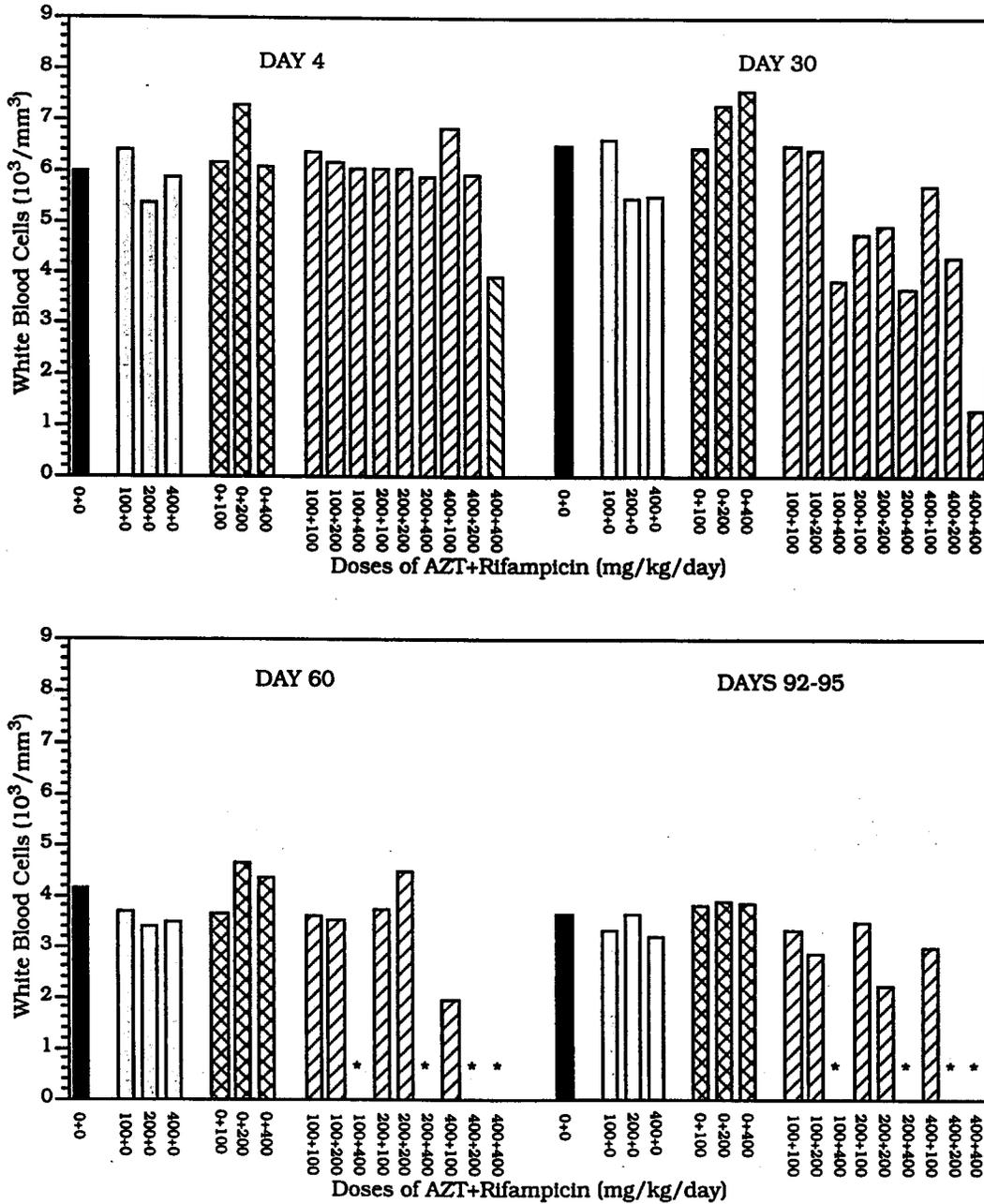
**FIGURE 12**  
**Mean Neutrophil Counts for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 13**  
**Mean Neutrophil Counts for Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 14**  
**Mean White Blood Cell Counts for Male Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 15**  
**Mean White Blood Cell Counts for Female Mice in the 13-Week Toxicity Study**  
**of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)

## AZT and Rifampicin Combinations

In general, administration of combinations of AZT and rifampicin to male and female mice resulted in hematological alterations of far greater severity than those caused by either drug alone. In general, effects in female mice were more severe than in males. Hematology values are given in Appendix A.

On day 4, statistically significant ( $P < 0.05$ ) alterations in erythrocyte parameters were not observed in male mice treated with AZT and rifampicin combinations. Although overt anemia did not occur, significant ( $P < 0.01$ ) declines in RBC counts occurred in several female groups (Figure 5) treated with AZT and rifampicin. The lowest RBC count observed in female mice treated with 400 mg of AZT + 200 mg of rifampicin, and it was approximately 7% ( $9.08 \times 10^6/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $9.73 \times 10^3/\text{mm}^3$ ) of the control group. Approximately 6% decreases occurred in female groups treated with 200 or 400 mg of AZT + 100 mg of rifampicin, 200 mg of AZT + 200 mg of rifampicin, and 200 mg of AZT + 400 mg of rifampicin. Slight decreases in Hgb (Figure 7) and Hct values paralleled the declines in RBC counts.

On day 30, severe dose-related anemia occurred in male and female mice treated with combinations of AZT and rifampicin, and the severity of the anemia contributed to mortality in the 400 mg AZT + 400 mg rifampicin combination groups. The mean RBC count of male mice (Figure 4) treated with 200 mg of AZT + 400 mg of rifampicin was approximately 73% ( $2.56 \times 10^6/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $9.64 \times 10^6/\text{mm}^3$ ) of the male control group. All male mice treated with 400 mg of AZT + 400 mg of rifampicin had died prior to day 30. The mean RBC count of female mice (Figure 5) treated with 400 mg of AZT + 400 mg of rifampicin was approximately 87% ( $1.21 \times 10^6/\text{mm}^3$ ) lower than the mean ( $9.25 \times 10^6/\text{mm}^3$ ) of the vehicle control group. Declines in Hgb (Figures 6 and 7) and Hct values accompanied the lower RBC counts in male and female mice. In general, the anemia was macrocytic in the low-dosage combination groups, reflecting the influence of AZT, and microcytic in the high-dosage combination groups, reflecting the effect of rifampicin. The peak MCV value of males occurred in the group treated with 400 mg of AZT + 100 mg of rifampicin and was approximately 13% (54.9 fL;  $P < 0.01$ ) higher than the mean (48.8 fL) of the control group. For the females, the peak MCV value occurred in the group treated with 200 mg of AZT + 100 mg of rifampicin and was approximately 15% (55.8 fL;  $P < 0.01$ ) higher than the mean (48.5 fL) of the female control. The lowest MCV value on day 30 was observed in the female group treated with 400 mg of AZT + 400 mg of rifampicin and was approximately 18% (39.7 fL;  $P < 0.01$ ) lower than the mean (48.5 fL) of the female control.

On day 60, a dose-related macrocytic anemia was present in male and female mice treated with combinations of AZT and rifampicin, and female mice were more anemic than males. The lowest RBC count in male mice (Figure 4) occurred in the group treated with 400 mg of AZT + 200 mg of rifampicin and was approximately 30% ( $7.09 \times 10^6/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $10.16 \times 10^6/\text{mm}^3$ ) of the male control group. The lowest RBC count in female mice (Figure 5) occurred in the group treated with

400 mg of AZT + 100 mg of rifampicin and was approximately 59% ( $4.24 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $10.24 \times 10^6/\text{mm}^3$ ) of the female control group. The anemia was macrocytic in males and females. The peak MCV value in males occurred in the group treated with 400 mg of AZT + 100 mg of rifampicin and was approximately 18% (55.6 fL; P 0.01) higher than the mean (47.0 fL) of the male control group. For the females, the peak MCV value was observed in the group treated with 200 mg of AZT + 100 mg of rifampicin and was approximately 22% (57.7 fL; P 0.01) higher than the mean of the control group. All male mice treated with 200 or 400 mg of AZT + 400 mg of rifampicin died prior to day 60. All female mice treated with 100, 200, or 400 mg of AZT + 400 mg of rifampicin as well as those treated with 400 mg of AZT + 200 mg of rifampicin died prior to day 60. In general, male and female mice that had microcytic anemia on day 30 died prior to day 60.

The compound-related anemia in male and female mice treated with AZT and rifampicin combinations was more severe at the time of terminal sacrifice (days 92 to 95) than at day 60. The RBC count in the single surviving male mouse (Figure 4) treated with 200 mg of AZT and 400 mg of rifampicin was approximately 89% ( $1.10 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $9.86 \times 10^6/\text{mm}^3$ ) of the male control group. The mean RBC count in the female group (Figure 5) treated with 200 mg of AZT + 200 mg of rifampicin was approximately 84% ( $1.56 \times 10^6/\text{mm}^3$ ; P 0.01) lower than the mean ( $9.85 \times 10^6/\text{mm}^3$ ) of the female control group. Declines in Hgb (Figures 6 and 7) and Hct values paralleled the decreases in RBC counts. Trends in MCV values similar to those described on day 60 occurred at the time of terminal sacrifice. All female mice treated with 400 mg of AZT + 200 mg of rifampicin and 100, 200, or 400 mg of AZT + 400 mg of rifampicin died prior to terminal sacrifice.

Male and female mice treated with combinations of AZT and rifampicin developed a marked reticulocytopenia early (day 4) in the study, and the severity of this alteration was far greater than that subsequent to the administration of either drug alone. Significant declines (P 0.01) in reticulocyte counts occurred in all male groups receiving combination therapy. The lowest values (Figure 8) occurred in male groups treated with 100 or 200 mg of AZT + 400 mg of rifampicin and were approximately 78% ( $0.8 \times 10^5/\text{mm}^3$ ; P 0.01) lower than the mean ( $3.6 \times 10^5/\text{mm}^3$ ) of the male control group. Mean reticulocyte counts in the female groups (Figure 9) treated with 400 mg of AZT + 200 mg of rifampicin and 200 mg of AZT + 400 mg of rifampicin were approximately 84% ( $0.5 \times 10^5/\text{mm}^3$ ; P 0.01) lower than the mean ( $3.1 \times 10^5/\text{mm}^3$ ) of the control group. Reticulocyte counts rebounded in male and female groups treated with lower dose combinations. On day 30, the mean reticulocyte count in the male group (Figure 8) treated with 400 mg of AZT + 200 mg of rifampicin was approximately 2.4 times ( $8.5 \times 10^5/\text{mm}^3$ ; P 0.01) the mean ( $3.5 \times 10^5/\text{mm}^3$ ) of the male control group. Although not statistically significant (P 0.05), the reticulocyte count in the male group treated with 200 mg of AZT + 400 mg of rifampicin was approximately 43% ( $2.0 \times 10^5/\text{mm}^3$ ) lower than the mean ( $3.5 \times 10^5/\text{mm}^3$ ) of the control group. All male mice treated with 400 mg of AZT + 400 mg of rifampicin died by day 30. For the female mice (Figure 9) on day 30, significant (P 0.01) reticulocytosis occurred in only one treatment group. The

mean reticulocyte count in the female group treated with 400 mg of AZT + 100 mg of rifampicin (Figure 9) was approximately 3.3 times ( $11.3 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $3.4 \times 10^5/\text{mm}^3$ ) of the female control group. Although not statistically significant ( $P > 0.05$ ), marked declines in reticulocyte counts occurred in female groups treated with 400 mg of AZT + 200 mg of rifampicin as well as the groups treated with 100, 200, or 400 mg of AZT + 400 mg of rifampicin. On day 60, the reticulocyte count of the male group (Figure 8) treated with 400 mg of AZT + 100 mg of rifampicin was approximately 1.5 times ( $5.5 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $3.6 \times 10^5/\text{mm}^3$ ) of the male control group. In contrast, the mean reticulocyte count in the male group (Figure 8) treated with 100 mg of AZT + 400 mg of rifampicin was approximately 39% ( $2.2 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $3.6 \times 10^5/\text{mm}^3$ ) of the control group. Male mice treated with 200 or 400 mg of AZT + 400 mg of rifampicin died prior to day 60. For the female mice on day 60, statistically significant ( $P < 0.05$ ) alterations in reticulocyte counts did not occur in any of the surviving groups. All female mice treated with 400 mg of AZT + 200 mg of rifampicin as well as those treated with 100, 200 or 400 mg of AZT + 400 mg of rifampicin died prior to day 60.

At the time of terminal sacrifice (days 92 to 95), significant reticulocytopenia ( $P < 0.01$ ) was observed in male groups treated with 400 mg of AZT + 200 mg of rifampicin as well as the groups treated with 100 or 200 mg of AZT + 400 mg of rifampicin. The lowest value occurred in the single surviving male mouse (Figure 8) treated with 200 mg of AZT + 400 mg of rifampicin and was approximately 90% ( $0.5 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $4.9 \times 10^5/\text{mm}^3$ ) of the male control group. All male mice treated with 400 mg of AZT + 400 mg of rifampicin died prior to day 92. For the female mice at terminal sacrifice (days 92 to 95), elevated reticulocyte counts occurred in the low dose combination groups. The mean reticulocyte count of the female group (Figure 9) treated with 200 mg of AZT + 100 mg of rifampicin was approximately 1.8 times ( $6.7 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $3.7 \times 10^5/\text{mm}^3$ ) of the female control group. In contrast, the mean reticulocyte count of the female group (Figure 9) treated with 200 mg of AZT + 200 mg of rifampicin was approximately 86% ( $0.9 \times 10^5/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $3.7 \times 10^5/\text{mm}^3$ ) of the female control group. Female mice treated with 400 mg of AZT + 200 mg of rifampicin as well as 100, 200, or 400 mg of AZT + 400 mg of rifampicin died prior to day 92.

In general, treatment with combinations of AZT and rifampicin caused thrombocytosis in male and female mice except in severely anemic animals, probably due to bone marrow depression. On day 4, only slight elevations in platelet counts occurred. Although not consistently significant ( $P > 0.05$ ), platelet counts of all male and female treatment groups receiving combination therapy were higher than the mean platelet counts of the respective control groups (Figures 10 and 11). The highest mean platelet count in males occurred in the group treated with 200 mg of AZT + 400 mg of rifampicin (Figure 10) and was approximately 1.2 times ( $1,519 \times 10^3/\text{mm}^3$ ) the mean ( $1,310 \times 10^3/\text{mm}^3$ ) of the male control group. For the females, the highest mean platelet count occurred in the group treated with 400 mg of AZT + 400 mg of rifampicin (Figure 11) and was approximately 1.2 times ( $1,318 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $1,124 \times 10^3/\text{mm}^3$ ) of the female control group.

On day 30, mean platelet counts of all male groups (Figure 10) treated with combinations of AZT and rifampicin were higher than the mean of the controls and the elevations were statistically significant in the higher dose combination groups with survivors. The peak mean platelet count occurred in the male group treated with 200 mg of AZT + 400 mg of rifampicin (Figure 10) and was approximately 1.7 times ( $2,359 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $1,427 \times 10^3/\text{mm}^3$ ) of the male control group. Male mice treated with 400 mg of AZT + 400 mg of rifampicin did not survive. For the female mice, the peak platelet count occurred in the group treated with 200 mg of AZT + 200 mg of rifampicin (Figure 11) and was approximately 1.8 times ( $2,036 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $1,129 \times 10^3/\text{mm}^3$ ) of the female control group. A distinct thrombocytopenia occurred in the female group treated with 400 mg of AZT + 400 mg of rifampicin (Figure 11). The mean platelet count of this group was approximately 73% ( $307 \times 10^3/\text{mm}^3$ ;  $P < 0.05$ ) lower than the mean ( $1,129 \times 10^3/\text{mm}^3$ ) of the control. The marked thrombocytopenia corresponds with the severe anemia and generalized bone marrow depression observed in this group.

On day 60, thrombocytosis occurred in all surviving male groups (Figure 10) treated with combinations of AZT and rifampicin. The peak mean platelet value was observed in the male group treated with 400 mg of AZT + 200 mg of rifampicin (Figure 10) and was approximately 2.2 times ( $2,313 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $1,073 \times 10^3/\text{mm}^3$ ) of the male control group. Male mice treated with 200 or 400 mg of AZT + 400 mg of rifampicin did not survive to day 60. For the surviving female groups (Figure 11) on day 60, statistically significant ( $P < 0.05$ ) elevations in platelet counts did not occur. Female mice treated with 400 mg of AZT + 200 mg of rifampicin as well as those treated with 100, 200, or 400 mg of AZT + 400 mg of rifampicin did not survive to day 60.

At the time of terminal sacrifice (days 92 to 95), prominent thrombocytosis occurred in male and female mice treated with combinations of AZT and rifampicin. The peak mean platelet count of the males occurred in the group treated with 400 mg of AZT + 200 mg of rifampicin (Figure 10) and was approximately 2.7 times ( $3,144 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $1,146 \times 10^3/\text{mm}^3$ ) of the control group. For the single surviving male mouse in the group treated with 200 mg of AZT + 400 mg of rifampicin (Figure 10), thrombocytopenia indicative of bone marrow toxicity occurred, and the platelet count was approximately 61% ( $445 \times 10^3/\text{mm}^3$ ) lower than the mean ( $1,146 \times 10^3/\text{mm}^3$ ) of the male control group. Male mice treated with 400 mg of AZT + 400 mg of rifampicin did not survive to day 92. For the female mice, the peak platelet value occurred in the group treated with 100 mg of AZT + 200 mg of rifampicin (Figure 11) and was approximately 2.3 times ( $2,209 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) the mean ( $975 \times 10^3/\text{mm}^3$ ) of the female control group. Female mice treated with 400 mg of AZT + 200 mg of rifampicin as well as those treated with 100, 200, or 400 mg of AZT + 400 mg of rifampicin did not survive to day 92.

Leukopenia, considered to be compatible with bone marrow toxicity, occurred on day 30 in female groups treated with combinations of AZT and rifampicin. The lowest WBC count occurred in the female group

treated with 400 mg of AZT + 400 mg of rifampicin (Figure 15) and was approximately 80% ( $1.29 \times 10^3/\text{mm}^3$ ;  $P < 0.01$ ) lower than the mean ( $6.47 \times 10^3/\text{mm}^3$ ) of the female control group. Evaluation of the corresponding differential data revealed statistically significant ( $P < 0.01$  to  $0.05$ ) declines in both neutrophils and lymphocytes. Significant alterations in leukocyte counts ( $P < 0.05$ ) did not occur in surviving male or female mice on day 60 or at terminal sacrifice. At the time of terminal sacrifice, although not statistically significant, lower WBC and differential counts were observed in the single surviving male mouse (Figure 14) treated with 200 mg of AZT + 400 mg of rifampicin as well as in the four surviving female mice (Figure 15) treated with 200 mg of AZT + 200 mg of rifampicin. These lower WBC counts are compatible with bone marrow depression.

In summary, both AZT and rifampicin produced a mild dose-related anemia when administered alone to male and female mice. The severity of the hematological alterations tended to be greater in females than in males. The anemia produced by AZT was accompanied by macrocytosis, whereas the anemia produced by rifampicin was accompanied by microcytosis. Male and female mice treated with combinations of AZT and rifampicin developed severe anemia, and the severity of the anemia was considered to be a contributing factor to the mortality that occurred in mice treated with the high dose combinations. The severe anemia and mortality were accompanied by bone marrow depression and hematopoietic cell proliferation in the spleen. Significant treatment-related alterations also occurred in reticulocyte counts. A prominent reticulocytopenia developed early (day 4) subsequent to the administration of AZT alone and also rifampicin alone. Combination therapy enhanced the severity of the reticulocytopenia. Reticulocyte counts in general tended to rebound by day 30; however, for those groups that died later in the study from severe anemia, reticulocyte counts decreased. Thrombocytosis tended to occur subsequent to treatment with AZT alone, whereas administration of rifampicin alone had a negligible effect on platelet counts. With the exception of mice treated with the high-dosage combinations, for which bone marrow toxicity was quite prominent, combinations of AZT and rifampicin tended to enhance the magnitude of the thrombocytosis.

## Clinical Chemistry

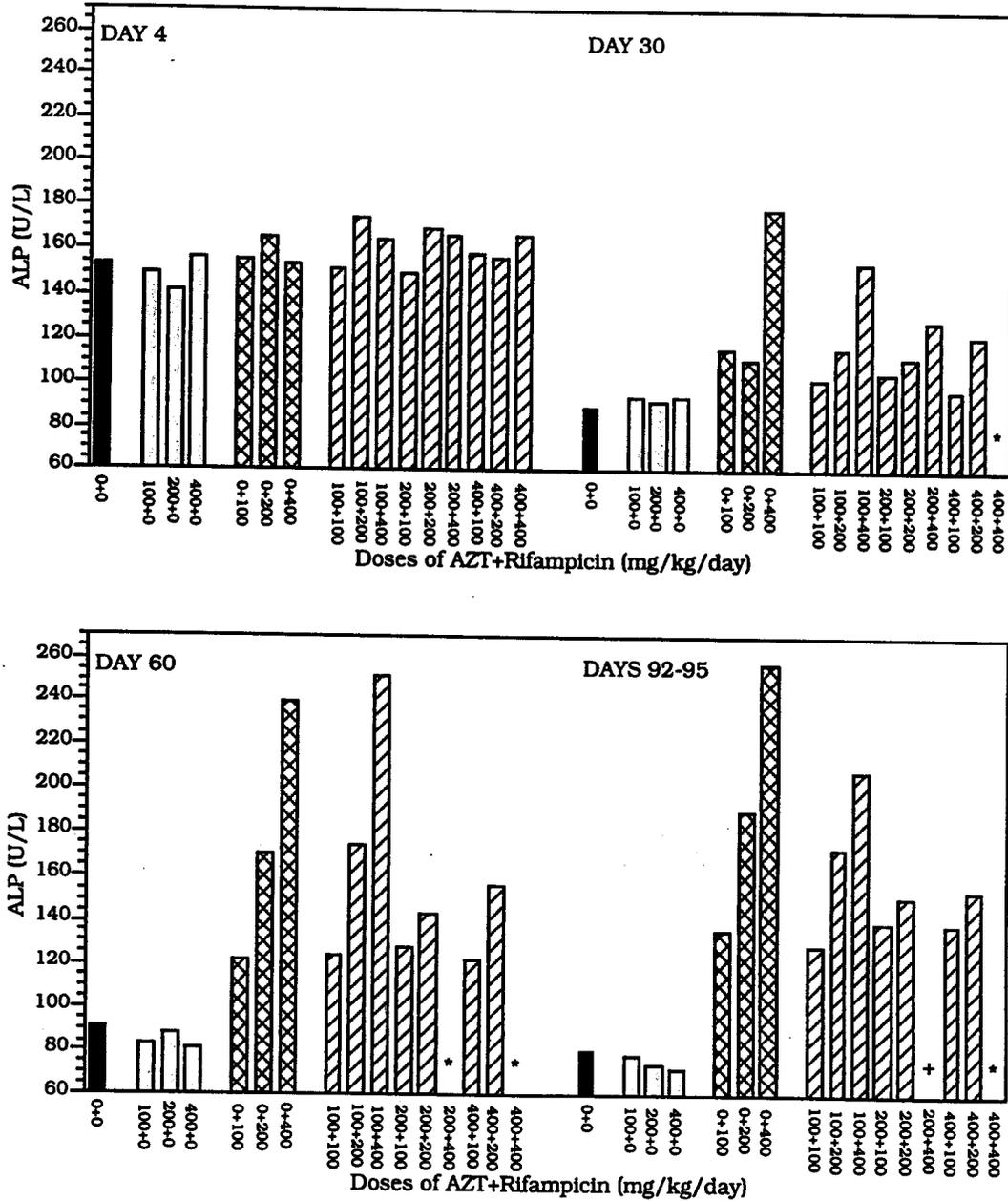
### AZT Alone

Statistically or biologically significant alterations did not occur in any of the clinical chemistry parameters evaluated in male or female mice treated with 100, 200, or 400 mg of AZT alone. Clinical chemistry values are listed in Appendix A.

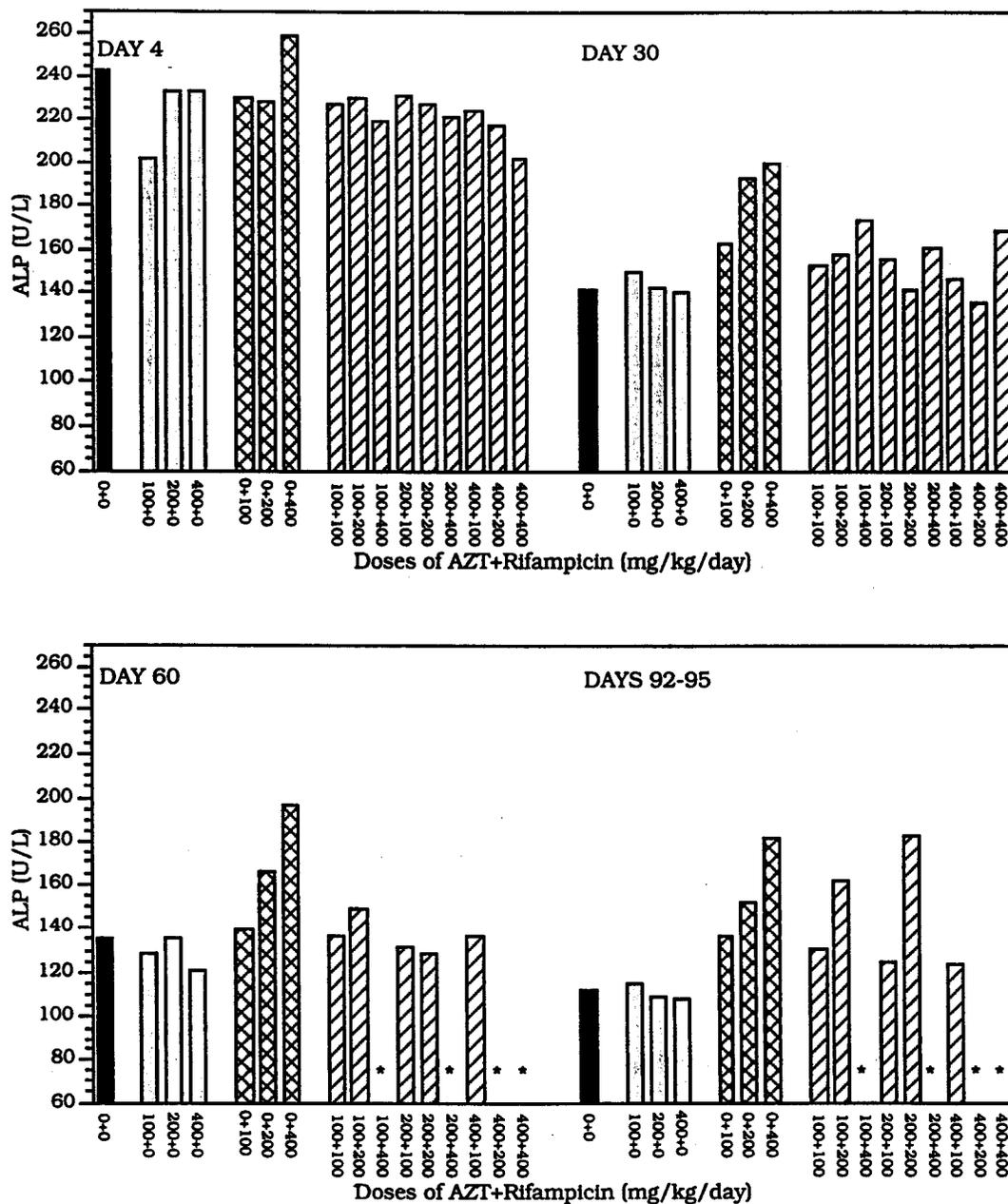
### Rifampicin Alone

Administration of 100, 200, or 400 mg of rifampicin alone resulted in treatment-related elevations in alkaline phosphatase (ALP) activity in male and female mice (Appendix A). On day 30, respective mean ALP values in male mice (Figure 16) treated with 100, 200, or 400 mg of rifampicin were approximately 1.3 times (116 U/L; P 0.01), 1.2 times (111 U/L; P 0.05), and 2.0 times (178 U/L; P 0.01) the mean ALP value (89 U/L) of the male control group. Respective mean ALP values on day 30 in female mice (Figure 17) treated with identical doses of rifampicin alone were approximately 1.1 times (163 U/L), 1.4 times (193 U/L; P 0.01), and 1.4 times (200 U/L; P 0.01) the mean (142 U/L) of the female control group. Similar treatment-related increases in ALP activity occurred on day 60. Respective mean ALP values on day 60 of male mice (Figure 16) treated with the above doses were approximately 1.3 times (122 U/L; P 0.01), 1.9 times (170 U/L; P 0.01), and 2.6 times (239 U/L; P 0.01) the mean (91 U/L) of the male control group. Respective mean ALP values of female mice (Figure 17) treated with same doses were approximately 1.0 times (139 U/L), 1.2 times (166 U/L; P 0.01), and 1.5 times (197 U/L; P 0.01) the mean (135 U/L) of the control group. At the time of terminal sacrifice (days 92 to 95), similar increases in ALP activity occurred. Respective mean ALP values in male mice (Figure 16) treated with 100, 200, or 400 mg of rifampicin alone were approximately 1.7 times (135 U/L; P 0.01), 2.4 times (190 U/L; P 0.01), and 3.2 times (258 U/L; P 0.01) the mean (80 U/L) of the male control group. Respective mean ALP values in female mice (Figure 17) treated with the above doses were approximately 1.2 times (136 U/L), 1.4 times (152 U/L; P 0.01), and 1.6 times (182 U/L; P 0.01) higher than the mean (112 U/L) of the female control group.

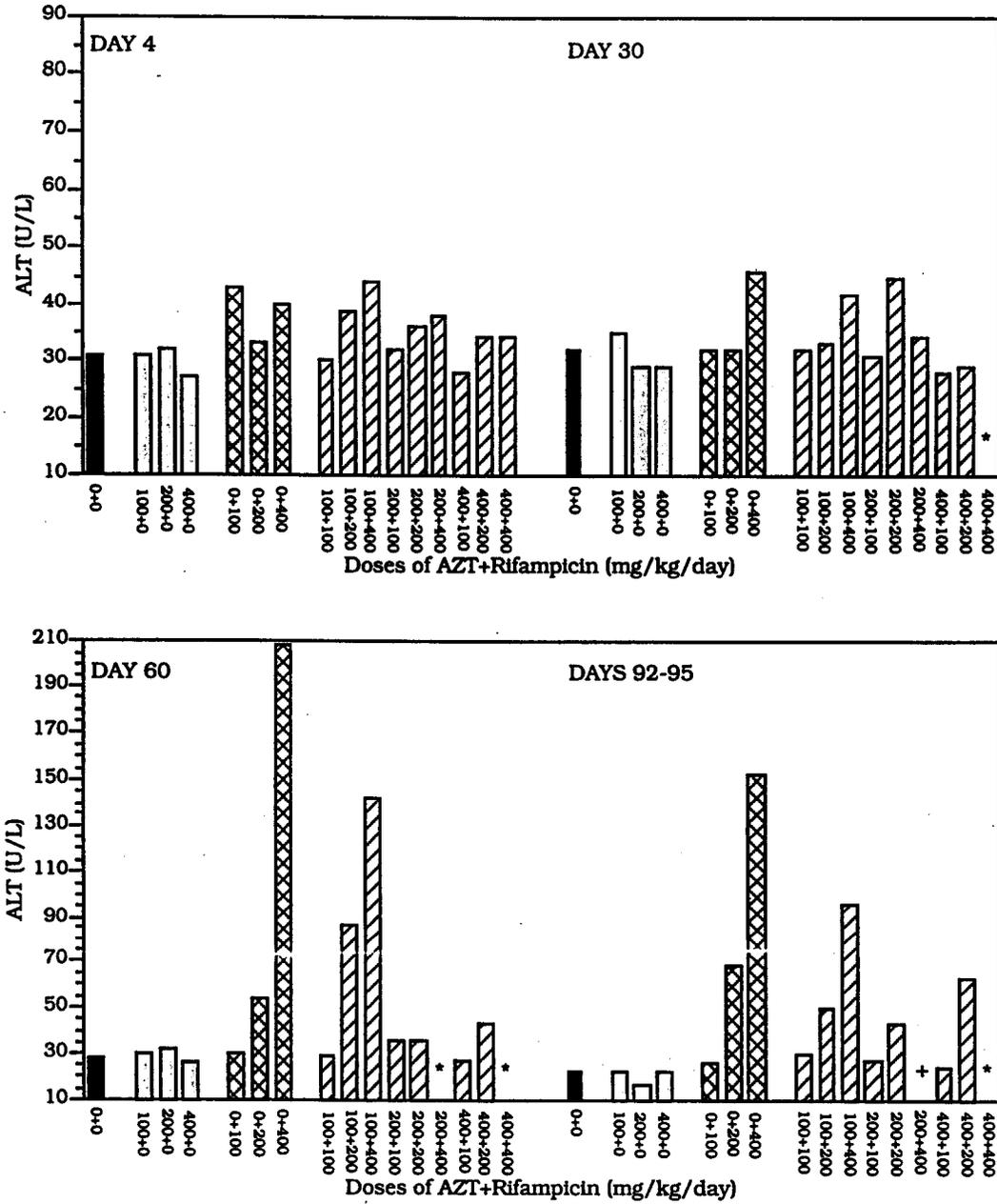
Treatment-related increases in alanine aminotransferase (ALT) activity occurred in male and female mice treated with 200 or 400 mg of rifampicin alone (Figures 18 and 19). On day 30, the mean ALT value of the female group treated with 400 mg of rifampicin alone (Figure 19) was approximately 3.2 times (82 U/L; P 0.01) the mean (26 U/L) of the female control group. Significant (P 0.05) elevations in ALT values did not occur in the male groups on day 30. On day 60, the mean ALT value in the male group (Figure 18) treated with 400 mg of rifampicin alone was approximately 7.4 times (208 U/L; P 0.01) the mean (28 U/L) of the male control group. Significant (P 0.05) increases in ALT values did not occur in the female mice on day 60. At the time of terminal



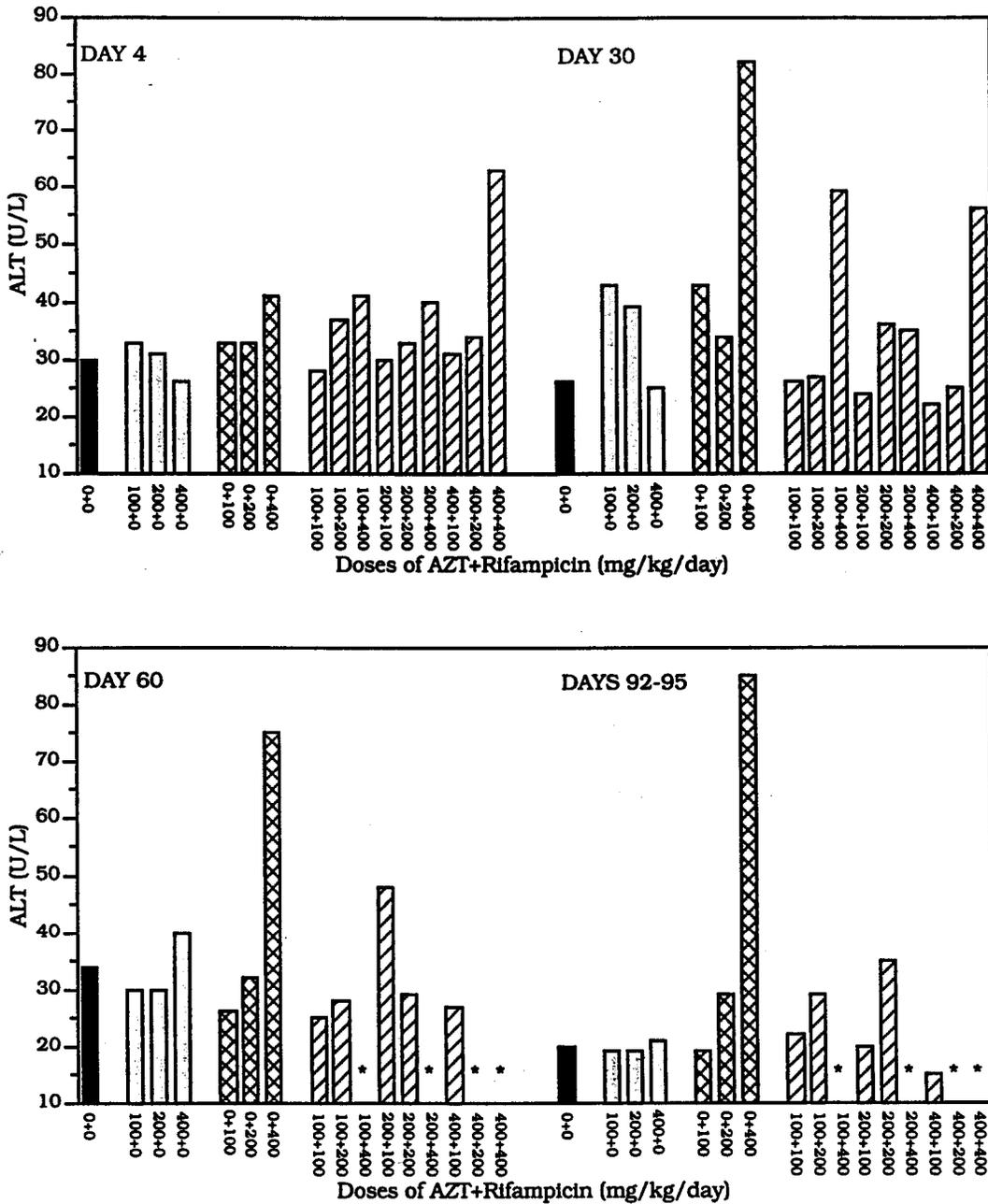
**FIGURE 16**  
**Mean Alkaline Phosphatase Values for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection; + = last animal in this group died prior to collection of clinical chemistry sample.)



**FIGURE 17**  
**Mean Alkaline Phosphatase Values for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)



**FIGURE 18**  
**Mean Alanine Aminotransferase Values for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection; + = last animal in this group died prior to collection of clinical chemistry sample.)



**FIGURE 19**  
**Mean Alanine Aminotransferase Values for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**  
 (\* All mice in this group died prior to blood collection)

sacrifice (days 92 to 95), respective mean ALT values in male mice treated with 200 or 400 mg of rifampicin alone were approximately 3.1 times (69 U/L; P 0.01) and 7.0 times (153 U/L) the mean of the male control (22 U/L) group. The mean ALT value in female mice (Figure 19) treated with 400 mg of rifampicin was approximately 4.3 times (85 U/L; P 0.01) the mean (20 U/L) of the female control group.

Mild compound-related increases in aspartate aminotransferase (AST) activity occurred in male and female mice treated with 400 mg of rifampicin alone. Statistically significant (P 0.05) elevations in AST activity did not occur in male or female mice on day 4 or in male mice on day 30. On day 30 for the female group treated with 400 mg of rifampicin alone, the mean AST value was approximately 1.5 times (103 U/L; P 0.05) the mean (71 U/L) of the female control group. On day 60, the mean AST value in the male group treated with 400 mg of rifampicin alone was approximately 2.5 times (157 U/L; P 0.01) the mean (63 U/L) of the male control. Elevations in AST values did not occur in the female groups on day 60. At the time of terminal sacrifice (days 92 to 95), the mean AST value in male mice treated with 400 mg of rifampicin alone was approximately 2.2 times (148 U/L; P 0.01) the mean of the male control group. Although not statistically significant (P 0.05), female mice treated with 400 mg had a mean AST value approximately 1.4 times (112 U/L) the mean (80 U/L) of the female control group.

With the exception of female mice in the 400 mg group, insufficient serum samples prevented adequate statistical comparisons for sorbitol dehydrogenase (SDH) activity and bile acid levels. The mean SDH value on day 60 in female mice treated with 400 mg of rifampicin alone was approximately 1.5 times (44 U/L; P 0.05) the mean (29 U/L) in the female control group. Although not statistically significant (P 0.05), the mean bile acid level in the same treatment group was approximately 2.1 times (73  $\mu\text{mol/L}$ ) the mean bile acid level (34  $\mu\text{mol}$ ) of two samples in the female control group (Appendix A).

Dose and duration of treatment-related increases in ALP, ALT, and AST activities indicate that rifampicin caused hepatotoxicity.

### **AZT and Rifampicin Combinations**

Increased ALP activity was observed in male and female mice treated with combinations of AZT and rifampicin in a manner similar to mice treated with rifampicin alone (Appendix A). On day 4, ALP values in male and female mice (Figures 16 and 17) treated with AZT and rifampicin were within a normal range. On day 30, ALP values in all male groups (Figure 16) treated with AZT and rifampicin were higher than the mean ALP value of the control group. The peak ALP value occurred in the group treated with 100 mg of AZT + 400 mg of rifampicin and was approximately 1.7 times (155 U/L; P 0.01) the mean (89 U/L) of the male control group. Only slight increases were observed in female groups on day 30. The highest value in female mice (Figure 17) was observed in the group treated with 100 mg of AZT + 400 mg of rifampicin and was approximately 1.2 times (173 U/L; P 0.05) the mean (142 U/L) of the female control group. On day 60, significant elevations (P 0.01) in ALP levels occurred in all

surviving male groups (Figure 16) treated with combination therapy. The peak ALP value occurred in the male group treated with 100 mg of AZT + 400 mg of rifampicin and was approximately 2.8 times (252 U/L; P 0.01) the mean (91 U/L) of the male control group. Male mice treated with 200 or 400 mg of AZT + 400 mg of rifampicin did not survive. Statistically significant (P 0.05) elevations in ALP activity did not occur on day 60 in female mice treated with combinations of AZT and rifampicin. Female groups treated with 400 mg of AZT + 200 mg of rifampicin and 100, 200, or 400 mg of AZT + 400 mg of rifampicin did not survive. At the time of terminal sacrifice (days 92 to 95), significant elevations (P 0.01) in ALP activity occurred in all surviving male groups (Figure 16). The peak value occurred in the male group treated with 100 mg of AZT + 400 mg of rifampicin and was approximately 2.6 times (208 U/L; P 0.01) the mean in the male control group (80 U/L). Male groups treated with 200 or 400 mg of AZT + 400 mg of rifampicin did not survive until terminal sacrifice. For the female mice at terminal sacrifice (days 92 to 95), the peak ALP value occurred in the group treated with 200 mg of AZT + 200 mg of rifampicin (Figure 17) and was approximately 1.6 times (183 U/L; P 0.01) the mean (112 U/L) in the female control group. Female groups treated with 400 mg of AZT + 200 mg of rifampicin and 100, 200, or 400 mg of AZT + 400 mg of rifampicin did not survive to day 92.

Increased ALT values indicative of liver toxicity also occurred in mice treated with AZT and rifampicin combinations, and the most prominent increases were observed in groups that received 400 mg of rifampicin in combination with AZT (Appendix A). On day 4, elevated ALT values occurred in female mice treated with AZT and rifampicin combinations. The peak ALT value occurred in the female group treated with 400 mg of AZT + 400 mg of rifampicin (Figure 19) and was approximately 2.1 times (63 U/L; P 0.01) the mean (30 U/L) in the female control group. Significant elevations (P 0.05) in ALT values did not occur on day 4 in male mice. On day 30, the ALT value in the female group treated with 100 mg of AZT + 400 mg of rifampicin (Figure 19) was approximately 2.3 times (59 U/L; P 0.05) the mean (26 U/L) of the female control group. Significant increases (P 0.05) in ALT values did not occur on day 30 in male mice. On day 60, elevated ALT values occurred in male mice that received 200 or 400 mg of rifampicin in combination with AZT. The peak ALT value occurred in the male group treated with 100 mg of AZT + 400 mg of rifampicin (Figure 18) and was approximately 5.1 times (143 U/L; P 0.01) the mean (28 U/L) of the male control group. Significant increases (P 0.05) did not occur on day 60 in female mice receiving combination therapy. At the time of terminal sacrifice (days 92 to 95), elevated ALT activity was observed only in the male group treated with 100 mg of AZT + 400 mg of rifampicin (Figure 18) and it was approximately 4.4 times (96 U/L; P 0.01) the mean (22 U/L) of the male control group. Significant elevations (P 0.05) in ALT values did not occur in female groups receiving combination therapy.

Statistically significant (P 0.05) increases in AST values did not occur in male or female mice treated with combinations of AZT and rifampicin (Appendix A). Although not statistically significant, elevated AST values indicative of liver toxicity was observed in one female group treated with 200 mg of AZT +

200 mg of rifampicin, with a mean AST value of approximately 1.8 times (143 U/L) the mean (80 U/L) of the female control group.

---

Insufficient serum samples prevented adequate statistical comparisons for SDH activity and bile acid levels in mice treated with combination therapy. Bile acid elevations appeared to occur in at least two of the combination dose groups on day 30. The single serum sample obtained on day 30 from the male group treated with 100 mg of AZT + 400 mg of rifampicin had a bile acid value of 144  $\mu\text{mol/L}$ . Three female mice treated with 400 mg of AZT + 200 mg of rifampicin had a mean bile acid value of 137  $\mu\text{mol/L}$  (Appendix A).

In summary, treatment with AZT alone did not result in any alterations in clinical chemistry parameters. Treatment with rifampicin alone resulted in dose-related increases in serum enzyme (ALP, ALT, and AST) activity, indicative of hepatotoxicity. The elevated serum enzymes corresponded well with increased liver weights and histopathological evidence of hepatotoxicity in the form of increased cytoplasmic vacuolization of hepatocytes. Treatment with combinations of AZT and rifampicin did not appear to exacerbate hepatotoxicity as serum enzyme levels in general were similar to those in mice treated with rifampicin alone.

## **NECROPSY OBSERVATIONS**

Significant treatment-related lesions found at necropsy consisted of enlarged and/or pale livers and pale carcasses. The pale and enlarged livers occurred primarily in male and female groups treated with rifampicin alone or combinations of AZT and rifampicin. The incidence of enlarged pale livers corresponded in general with increased liver weights (Table 3), elevated serum enzymes (Tables A3 and A4), and cytoplasmic vacuolization of hepatocytes (Tables 4 and 5) suggestive of hepatotoxicity. In general, the incidence of pale carcass corresponded with the increased severity of anemia evident in the hematology parameters of groups treated with higher combination doses of AZT and rifampicin.

TABLE 4

**Incidence and Mean Severity of Cytoplasmic Vacuolization of Hepatocytes of the Liver in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Male		Female	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0	10/10	1.6	8/10	1.0
100 + 0	10/10	1.7	10/10	1.0
200 + 0	10/10	1.8	10/10	1.3
400 + 0	10/10	1.5	9/10	1.1
0 + 100	10/10	3.0	10/10	1.3
100 + 100	10/10	3.1	9/9	1.7
200 + 100	10/10	3.1	10/10	1.5
400 + 100	10/10	3.5	10/10	1.6
0 + 200	10/10	4.0	10/10	1.5
100 + 200	9/9	3.7	10/10	2.4
200 + 200	10/10	3.0	9/10	2.8
400 + 200	10/10	2.9	10/10	2.4
0 + 400	10/10	3.6	10/10	2.9
100 + 400	10/10	3.2	10/10	3.1
200 + 400	10/10	3.0	10/10	2.9
400 + 400	10/10	3.1	10/10	2.7

a Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for mice with lesions and is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 5

**Statistical Analysis of Mean Severity of Cytoplasmic Vacuolization of Hepatocytes of the Liver in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>	
	Male <sup>c</sup>	Female <sup>c</sup>
0 + 0	1.60 ± 0.22	0.90 ± 0.11 <sup>d</sup>
100 + 0	1.70 ± 0.21	1.00 ± 0.00
200 + 0	1.80 ± 0.13	1.30 ± 0.15**
400 + 0	1.50 ± 0.17	1.00 ± 0.15
0 + 100	3.00 ± 0.00**	1.30 ± 0.15**
100 + 100	3.10 ± 0.10**	1.70 ± 0.17**
200 + 100	3.10 ± 0.10**	1.50 ± 0.17**
400 + 100	3.50 ± 0.17**	1.60 ± 0.27*
0 + 200	4.00 ± 0.00**	1.50 ± 0.17**
100 + 200	3.70 ± 0.17** <sup>d</sup>	2.40 ± 0.22**
200 + 200	3.00 ± 0.00**	2.50 ± 0.34**
400 + 200	2.90 ± 0.10**	2.40 ± 0.16**
0 + 400	3.60 ± 0.16**	2.90 ± 0.10**
100 + 400	3.20 ± 0.13**	3.10 ± 0.10**
200 + 400	3.00 ± 0.15**	2.90 ± 0.10**
400 + 400	3.10 ± 0.18**	2.70 ± 0.15**

\* Significantly different from the control group (P < 0.05) by Dunnett's test

\*\* P < 0.01

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

c n=10                      d n=9

## HISTOPATHOLOGIC OBSERVATIONS

Treatment-related lesions were observed in bone marrow, liver, spleen, thymus, and testis. No treatment-related lesions were observed in the other organs examined. Photomicrographs of treatment-related lesions are illustrated in Plates 1 through 10 and summarized in Table 6.

**TABLE 6**  
**List of Photomicrographs (Plates) of Compound-Related Lesions in Male B6C3F<sub>1</sub> Mice**  
**in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

<b>Dose<sup>a</sup></b>	<b>Tissue</b>	<b>Magnification</b>	<b>Plate Number</b>	<b>Treatment-Related Lesions</b>
0 + 0	Bone Marrow	450x	1	None
200 + 400	Bone Marrow	450x	2	Cellular depletion (marked)
0 + 0	Liver	450x	3	None
0 + 400	Liver	450x	4	Cytoplasmic vacuolization (marked)
0 + 0	Spleen	400x	5	None
200 + 400	Spleen	400x	6	Cellular depletion (moderate)
0 + 0	Thymus	400x	7	None
200 + 400	Thymus	400x	8	Atrophy (marked)
0 + 0	Testis	450x	9	None
200 + 400	Testis	450x	10	Degeneration of germinal epithelium (mild)

a AZT + rifampicin (mg/kg per day)

### ***Bone Marrow Lesions***

Cellular depletion of the bone marrow occurred in all groups treated with AZT alone (Table 7) and involved primarily cells of the erythrocytic series. Alterations in the bone marrow seldom occurred in mice treated with rifampicin alone. Criteria for severity grades of cellular depletion of the bone marrow were as follow:

*Minimal* - approximately 5% or less of the hematopoietic cells of the bone marrow

*Mild* - approximately 6% to 20% of the hematopoietic cells of the bone marrow

*Moderate* - approximately 21% to 50% of the hematopoietic cells of the bone marrow

*Marked* - more then 50% of the hematopoietic cells of the bone marrow

**TABLE 7**  
**Incidence and Mean Severity of Cellular Depletion of the Bone Marrow in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Male		Female	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0	0/10	—	0/10	—
100 + 0	2/10	1.0	5/10	1.0
200 + 0	6/10	1.0	8/10	1.0
400 + 0	6/10	1.0	7/10	1.3
0 + 100	0/10	—	0/10	—
100 + 100	0/10	—	6/9	1.0
200 + 100	7/10	1.0	10/10	1.1
400 + 100	4/10	1.0	7/10	2.0
0 + 200	0/10	—	0/10	—
100 + 200	9/9	1.4	10/10	1.9
200 + 200	6/10	1.2	10/10	2.9
400 + 200	10/10	3.0	10/10	4.0
0 + 400	1/10	1.0	0/10	—
100 + 400	10/10	3.0	10/10	3.6
200 + 400	10/10	3.7	10/10	3.7
400 + 400	10/10	3.7	10/10	3.9

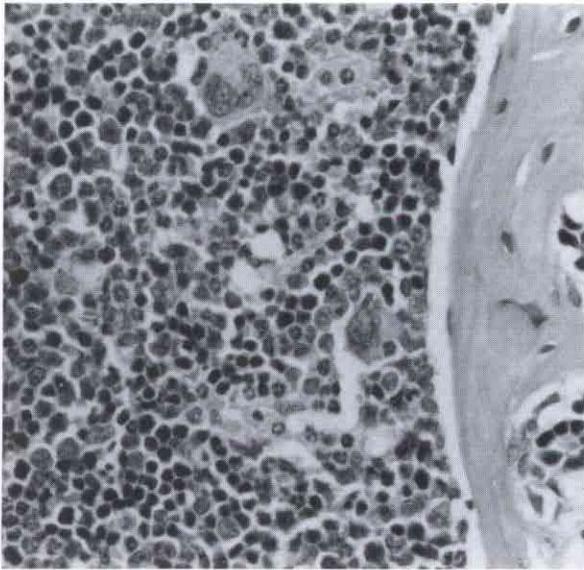
a Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for mice with lesions and is based on the numeric scale of 1=mimimal, 2=mild, 3=moderate, 4=marked

The incidence of cellular depletion of bone marrow increased in male and female mice treated with combinations of AZT and rifampicin. Significant increases (P 0.05 to P 0.01) in the severity of this lesion occurred in the groups treated with combination therapy, and the degree of severity increased (Table 8) as dose levels of AZT and rifampicin increased. The increased severity of bone marrow depletion corresponded in general with decreased erythrocyte counts in blood.

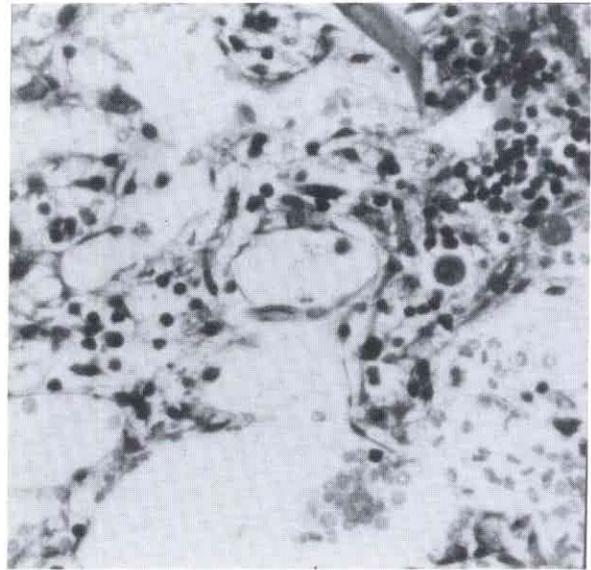
Hematopoietic cell proliferation in the red pulp of the spleen was a treatment-related lesion, and it was observed in male and female mice. Criteria for severity grades of hematopoietic cell proliferation were as follows:

*Minimal* - the number of hematopoietic cells in the red pulp is estimated to be increased, but these cells do not occupy more than 15% of the red pulp

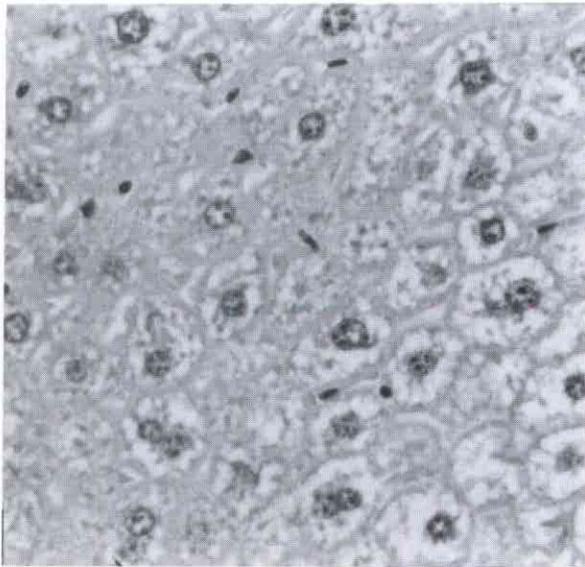
*Mild* - hematopoietic cells are estimated to occupy approximately 16% to 50% of the splenic red pulp



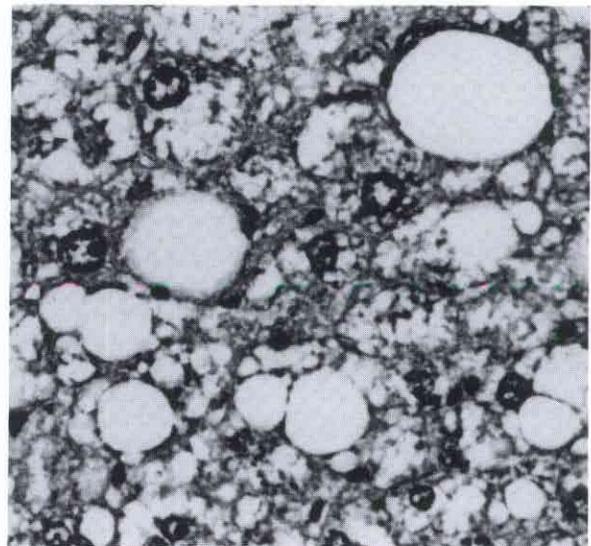
**PLATE 1**  
Bone marrow of a vehicle control male B6C3F<sub>1</sub> mouse showing no lesions. H&E; 450×



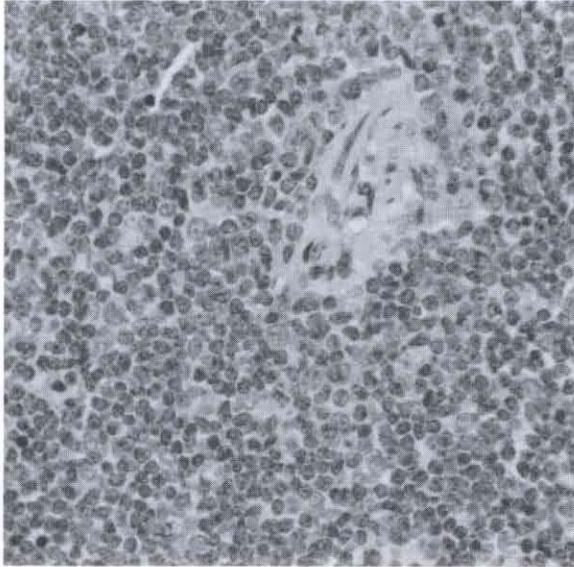
**PLATE 2**  
Bone marrow of a male B6C3F<sub>1</sub> mouse given 200 mg AZT + 400 mg rifampicin per kg body weight per day by gavage for 13 weeks showing marked cellular depletion. H&E; 450×



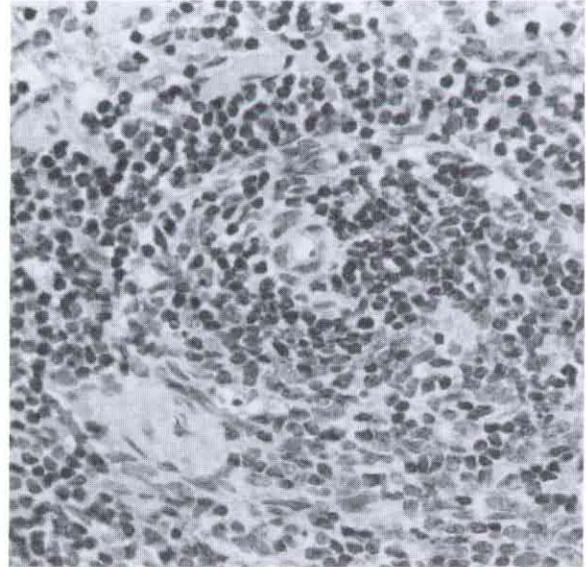
**PLATE 3**  
Liver of a vehicle control male B6C3F<sub>1</sub> mouse showing no lesions. H&E; 450×



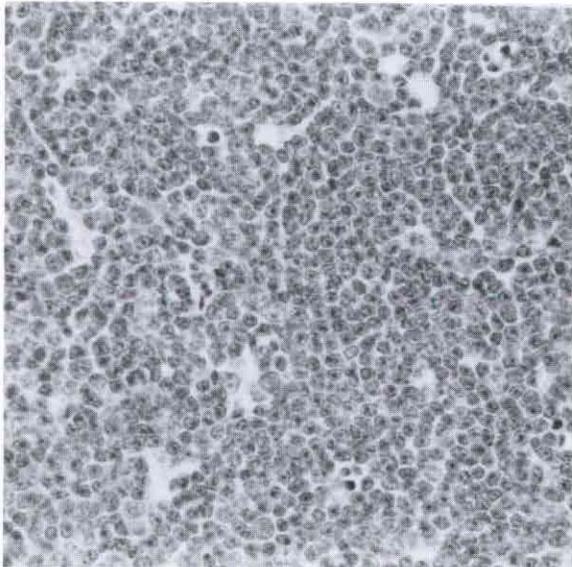
**PLATE 4**  
Liver of a male B6C3F<sub>1</sub> mouse given 400 mg rifampicin per kg body weight per day by gavage for 13 weeks showing marked cytoplasmic vacuolization. H&E; 450×



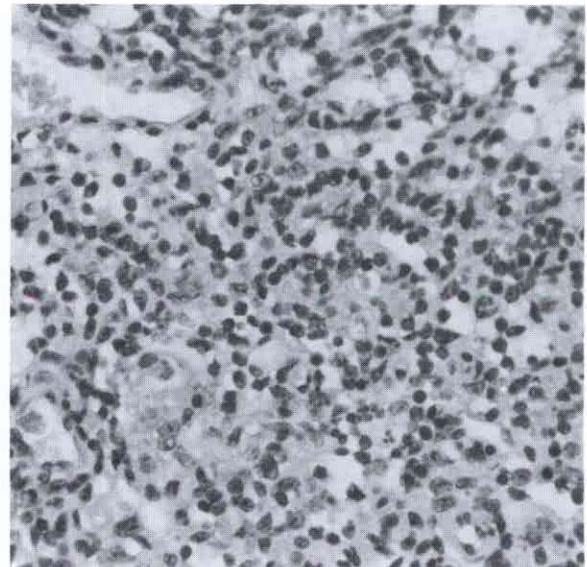
**PLATE 5**  
Spleen of a vehicle control male B6C3F<sub>1</sub> mouse showing no lesions. H&E; 400×



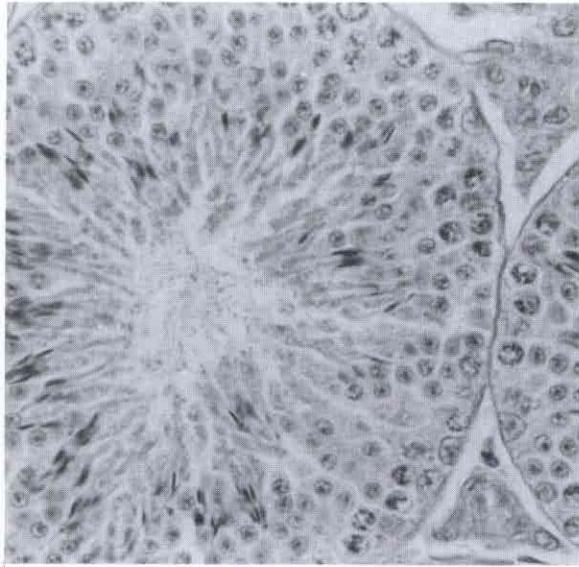
**PLATE 6**  
Spleen of a male B6C3F<sub>1</sub> mouse given 200 mg AZT + 400 mg rifampicin per kg body weight per day for 13 weeks showing moderate cellular depletion. H&E; 400×



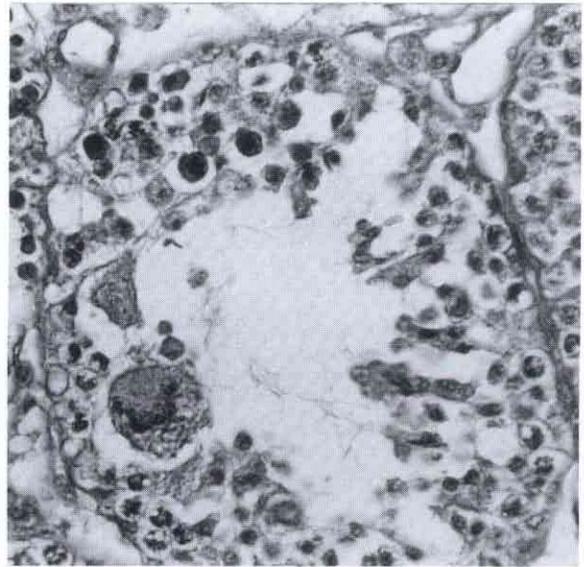
**PLATE 7**  
Thymus of a vehicle control male B6C3F<sub>1</sub> mouse showing no lesions. H&E; 400×



**PLATE 8**  
Thymus of a male B6C3F<sub>1</sub> mouse given 200 mg AZT + 400 mg rifampicin per kg body weight per day for 13 weeks showing marked atrophy. H&E; 400×



**PLATE 9**  
Testis of a vehicle control male B6C3F<sub>1</sub> mouse showing no lesions. H&E; 450×



**PLATE 10**  
Testis of a male B6C3F<sub>1</sub> mouse given 200 mg AZT + 400 mg rifampicin per kg body weight per day for 13 weeks showing mild degeneration of germinal epithelium. H&E; 450×

**TABLE 8**  
**Statistical Analysis of Mean Severity of Cellular Depletion of the Bone Marrow in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>	
	Male <sup>c</sup>	Female <sup>c</sup>
0 + 0	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>
100 + 0	0.20 ± 0.13	0.50 ± 0.17*
200 + 0	0.60 ± 0.16**	0.80 ± 0.13**
400 + 0	0.50 ± 0.17**	0.90 ± 0.23**
0 + 100	0.00 ± 0.00	0.00 ± 0.00
100 + 100	0.00 ± 0.00	0.67 ± 0.17**
200 + 100	0.70 ± 0.15**	1.10 ± 0.10**
400 + 100	0.40 ± 0.16*	1.40 ± 0.48*
0 + 200	0.00 ± 0.00	0.00 ± 0.00
100 + 200	1.44 ± 0.18** <sup>d</sup>	1.90 ± 0.31**
200 + 200	0.70 ± 0.21**	2.90 ± 0.31**
400 + 200	3.00 ± 0.45**	4.00 ± 0.00**
0 + 400	0.10 ± 0.10	0.00 ± 0.00
100 + 400	3.00 ± 0.39**	3.60 ± 0.16**
200 + 400	3.70 ± 0.15**	3.70 ± 0.15**
400 + 400	3.70 ± 0.15**	3.90 ± 0.10**

\* Significantly different from the control group (P 0.05) by Dunnett's test

\*\* P 0.01

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

c n=10

d n=9

### *Spleen Lesions*

Both AZT and rifampicin given alone resulted in a dose-related increase in the incidence (Table 9) and severity (Table 10) of splenic hematopoiesis in male and female mice. Significant increases in severity (P 0.05 to P 0.01) occurred in male mice treated with 100, 200, or 400 mg of AZT in combination with 100 mg of rifampicin. Male mice treated with higher combinations of AZT and rifampicin did not have increased hematopoiesis in the red pulp, as many of these mice died early from severe anemia. For the female mice, significant increases (P 0.01) in severity occurred in groups treated with 100 or 200 mg of AZT in combination with 100 mg of rifampicin or 100 mg of AZT + 200 mg of rifampicin. Female groups treated with higher combinations of AZT and rifampicin developed severe anemia accompanied by increased mortality rates. Lower incidence and severity of hematopoiesis in the red pulp occurred in these groups with severe anemia and increased mortality.

**TABLE 9**  
**Incidence and Mean Severity of Hematopoietic Cell Proliferation of the Spleen in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Male		Female	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0	2/10	1.5	5/10	1.0
100 + 0	5/10	1.0	8/10	1.0
200 + 0	7/10	1.3	9/10	1.7
400 + 0	7/10	1.4	9/10	1.7
0 + 100	9/10	1.0	10/10	2.0
100 + 100	8/10	1.4	9/9	2.0
200 + 100	10/10	1.6	10/10	2.0
400 + 100	10/10	1.8	6/10	2.0
0 + 200	6/10	1.2	10/10	1.9
100 + 200	9/9	1.8	8/10	2.0
200 + 200	9/10	1.8	3/10	1.0
400 + 200	2/10	1.0	0/10	—
0 + 400	10/10	1.6	10/10	2.0
100 + 400	2/10	1.0	0/10	—
200 + 400	0/10	—	0/10	—
400 + 400	0/10	—	0/10	—

<sup>a</sup> Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for mice with lesions and is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

**TABLE 10**  
**Statistical Analysis of Mean Severity of Hematopoietic Cell Proliferation of the Spleen in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>	
	Male <sup>c</sup>	Female <sup>c</sup>
0 + 0	0.30 ± 0.21	0.56 ± 0.18d
100 + 0	0.50 ± 0.17	0.80 ± 0.13
200 + 0	0.90 ± 0.23	1.50 ± 0.22**
400 + 0	1.00 ± 0.26	1.50 ± 0.22**
0 + 100	0.90 ± 0.10*	2.00 ± 0.00**
100 + 100	1.10 ± 0.23*	2.00 ± 0.00**d
200 + 100	1.60 ± 0.16**	2.00 ± 0.00**
400 + 100	1.80 ± 0.13**	1.20 ± 0.33
0 + 200	0.70 ± 0.21	1.90 ± 0.10**
100 + 200	1.78 ± 0.15**d	1.60 ± 0.27**
200 + 200	1.60 ± 0.22**	0.30 ± 0.15
400 + 200	0.20 ± 0.13	0.00 ± 0.00**
0 + 400	1.60 ± 0.16**	2.00 ± 0.00**
100 + 400	0.20 ± 0.13	0.00 ± 0.00**
200 + 400	0.00 ± 0.00	0.00 ± 0.00**
400 + 400	0.00 ± 0.00	0.00 ± 0.00**

\* Significantly different from the control group (P < 0.05) by Dunnett's test

\*\* P < 0.01

<sup>a</sup> Daily gavage doses of AZT + rifampicin (mg/kg per day)

<sup>b</sup> Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>c</sup> n=10

<sup>d</sup> n=9

Cellular depletion of the spleen occurred only in male and female mice treated with the higher combinations of AZT and rifampicin (Table 11). The depletion of cells occurred in both the white and red pulp, and the criteria for severity grades were as follows:

*Minimal* - estimated depletion of 1% to 20% of the cells of the spleen

*Mild* - estimated depletion of 21% to 40% of the cells of the spleen

*Moderate* - estimated depletion of 41% to 60% of the cells of the spleen

*Marked* - estimated depletion of more than 60% of the cells of the spleen

In male mice, significant increases ( $P < 0.01$ ) in the severity of cellular depletion (Table 12) occurred in the group treated with 400 mg of AZT + 200 mg of rifampicin and in the groups treated with 100, 200, or 400 mg of AZT in combination with 400 mg of rifampicin. For the female groups, significant increases ( $P < 0.01$ ) in severity of cellular depletion of the spleen occurred in the groups treated with 200 or 400 mg of AZT + 200 mg rifampicin as well as in the groups treated with 100, 200, or 400 mg of AZT + 400 mg of rifampicin. In general, cellular depletion of the spleen was observed in male and female mice with severe anemia and high mortality due to severe hematological toxicity.

### ***Liver Lesions***

An increase in the severity ( $P < 0.05$  to  $P < 0.01$ ) of cytoplasmic vacuolization of hepatocytes occurred in male and female groups treated with rifampicin alone or in combination with AZT (Tables 4 and 5). Small cytoplasmic vacuolizations, believed to be the result of glycogen deposition, are a normal finding in well-nourished mice. With increasing doses of rifampicin, there was a corresponding increase in round cytoplasmic vacuoles typical of fat vacuoles, and the vacuolization was most prominent in the centrilobular areas of the liver. Criteria for the severity grades of cytoplasmic vacuolization were as follows:

*Minimal* - approximately 1% to 15% of hepatocytes containing round cytoplasmic vacuoles

*Mild* - approximately 16% to 35% of hepatocytes containing round cytoplasmic vacuoles

*Moderate* - approximately 36% to 60% of hepatocytes containing round cytoplasmic vacuoles

*Marked* - more than 60% of hepatocytes containing round cytoplasmic vacuoles

In general, cytoplasmic vacuolization of hepatocytes was more prominent in male mice than in females. Hepatocyte cytoplasmic vacuolization in the liver corresponded with increased liver-to-body-weight ratios and increases in serum enzymes typical of hepatotoxicity.

TABLE 11

**Incidence and Mean Severity of Cellular Depletion of the Spleen in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Male		Female	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0	0/10	—	0/10	—
100 + 0	0/10	—	0/10	—
200 + 0	0/10	—	0/10	—
400 + 0	0/10	—	0/10	—
0 + 100	0/10	—	0/10	—
100 + 100	0/10	—	0/9	—
200 + 100	0/10	—	0/10	—
400 + 100	0/10	—	2/10	1.5
0 + 200	0/10	—	0/10	—
100 + 200	0/9	—	1/10	2.0
200 + 200	0/10	—	8/10	2.5
400 + 200	7/10	3.0	10/10	3.1
0 + 400	0/10	—	0/10	—
100 + 400	8/10	2.6	10/10	2.9
200 + 400	10/10	2.8	9/10	2.8
400 + 400	9/10	2.6	10/10	2.7

a Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for mice with lesions and is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 12

**Statistical Analysis of Mean Severity of Cellular Depletion of the Spleen in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>	
	Male <sup>c</sup>	Female <sup>c</sup>
0 + 0	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>
100 + 0	0.00 ± 0.00	0.00 ± 0.00
200 + 0	0.00 ± 0.00	0.00 ± 0.00
400 + 0	0.00 ± 0.00	0.00 ± 0.00
0 + 100	0.00 ± 0.00	0.00 ± 0.00
100 + 100	0.00 ± 0.00	0.00 ± 0.00
200 + 100	0.00 ± 0.00	0.00 ± 0.00
400 + 100	0.00 ± 0.00	0.30 ± 0.21
0 + 200	0.00 ± 0.00	0.00 ± 0.00
100 + 200	0.00 ± 0.00 <sup>d</sup>	0.20 ± 0.20
200 + 200	0.00 ± 0.00	2.00 ± 0.42**
400 + 200	2.10 ± 0.46**	3.10 ± 0.10**
0 + 400	0.00 ± 0.00	0.00 ± 0.00
100 + 400	2.10 ± 0.41**	2.90 ± 0.28**
200 + 400	2.80 ± 0.13**	2.50 ± 0.34**
400 + 400	2.30 ± 0.30**	2.70 ± 0.21**

\*\*Significantly different from the control group (P < 0.01) by Dunnett's test

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

c n=10

d n=9

***Thymus Lesions***

An increase in the incidence and the degree of severity (P 0.05 to P 0.01) of atrophy of the thymus was observed in male and female groups treated with the higher combinations of AZT and rifampicin (Tables 13 and 14). Significant alterations in the thymus did not occur in groups treated with AZT or rifampicin alone. Thymic atrophy was characterized by a reduction in the size of the thymus alone with a reduction in the number of lymphocytes. Criteria for severity grades of thymic atrophy were as follows:

*Minimal* - reduction of 10% or less lymphocytes in the cortex

*Mild* - reduction of approximately 11% to 25% of lymphocytes in the cortex

*Moderate* - reduction of approximately 26% to 50% of lymphocytes in the cortex

*Marked* - reduction of approximately 51% or greater lymphocytes in the cortex

Atrophy of the thymus was more prominent in female groups treated with drug combinations than in males and, in general, corresponded with treatment-related decreases in thymus weights. Decreases in thymus weights and severity of thymic atrophy appear to be due to dose-related loss or decrease in body weights and severity of anemia. Stress of anemia appears to have contributed to thymic atrophy.

***Testis Lesions***

Testicular degeneration was observed in the group treated with 400 mg of AZT in combination with 200 mg of rifampicin and in the groups treated with 100, 200, or 400 mg of AZT in combination with 400 mg of rifampicin (Tables 15 and 16). Microscopically, testicular degeneration was characterized by disruption of the orderly arrangement of germinal cells in seminiferous tubules with decreased numbers and necrosis of germinal cells, and the formation of multinucleated giant cells. Criteria for severity grades were as follows:

*Minimal* - degeneration in approximately 5% or less of seminiferous tubules

*Mild* - degeneration in approximately 6% to 40% of seminiferous tubules

*Moderate* - degeneration in approximately 41% to 80% of seminiferous tubules

The degree of severity (P 0.01) of testicular degeneration was similar in all of the affected groups. In general, the testicular degeneration was accompanied by lower testis weights and sperm motility.

TABLE 13

**Incidence and Mean Severity of Thymic Atrophy in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Male		Female	
	Group Incidence	Mean Severity	Group Incidence	Mean Severity
0 + 0	0/10	—	0/10	—
100 + 0	0/10	—	0/10	—
200 + 0	0/10	—	0/10	—
400 + 0	1/9	1.0	1/10	1.0
0 + 100	0/10	—	0/10	—
100 + 100	0/10	—	0/9	—
200 + 100	0/10	—	0/10	—
400 + 100	0/10	—	4/10	3.3
0 + 200	0/10	—	0/10	—
100 + 200	0/9	—	2/9	2.0
200 + 200	0/10	—	9/9	3.1
400 + 200	7/10	3.4	10/10	3.6
0 + 400	0/10	—	0/10	—
100 + 400	7/8	3.3	8/8	3.5
200 + 400	9/9	3.3	8/8	3.3
400 + 400	5/5	3.6	8/8	3.5

a Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for mice with lesions and is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

TABLE 14

**Statistical Analysis of Mean Severity of Thymic Atrophy in Male and Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>	
	Male <sup>c</sup>	Female <sup>c</sup>
0 + 0	0.00 ± 0.00	0.00 ± 0.00 <sup>d</sup>
100 + 0	0.00 ± 0.00	0.00 ± 0.00
200 + 0	0.00 ± 0.00	0.00 ± 0.00
400 + 0	0.10 ± 0.10	0.10 ± 0.10
0 + 100	0.00 ± 0.00	0.00 ± 0.00
100 + 100	0.00 ± 0.00	0.00 ± 0.00
200 + 100	0.00 ± 0.00	0.00 ± 0.00
400 + 100	0.00 ± 0.00	1.30 ± 0.54*
0 + 200	0.00 ± 0.00	0.00 ± 0.00
100 + 200	0.00 ± 0.00 <sup>d</sup>	0.40 ± 0.31
200 + 200	0.00 ± 0.00	2.80 ± 0.36**
400 + 200	2.40 ± 0.54**	3.60 ± 0.16**
0 + 400	0.00 ± 0.0	0.00 ± 0.00
100 + 400	2.30 ± 0.52**	2.80 ± 0.49**
200 + 400	3.00 ± 0.37**	2.60 ± 0.45**
400 + 400	1.80 ± 0.31**	2.80 ± 0.49**

\* Significantly different from the control group (P < 0.05) by Dunnett's test

\*\* P < 0.01

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked

c n=10                      d n=9

**TABLE 15**  
**Incidence and Mean Severity of Testicular Degeneration in Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>**

Dose	Group Incidence	Mean Severity
0 + 0	0/10	—
100 + 0	0/10	—
200 + 0	0/10	—
400 + 0	0/10	—
0 + 100	0/10	—
100 + 100	0/10	—
200 + 100	0/10	—
400 + 100	0/10	—
0 + 200	0/10	—
100 + 200	0/9	—
200 + 200	0/10	—
400 + 200	7/10	1.9
0 + 400	0/8	—
100 + 400	7/9	2.0
200 + 400	8/10	1.8
400 + 400	8/10	1.9

a Daily gavage doses of AZT + rifampicin (mg/kg per day). Mean severity is for male mice with lesions and is based on the numeric scale of 1=minimal, 2=mild

**Table 16**  
**Statistical Analysis of Mean Severity of Testicular Degeneration in Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Mean Severity <sup>b</sup>
0 + 0	0.00 ± 0.00
100 + 0	0.00 ± 0.00
200 + 0	0.00 ± 0.00
400 + 0	0.10 ± 0.10
0 + 100	0.00 ± 0.00
100 + 100	0.00 ± 0.00
200 + 100	0.00 ± 0.00
400 + 100	0.00 ± 0.00
0 + 200	0.00 ± 0.00
100 + 200	0.00 ± 0.00 <sup>c</sup>
200 + 200	0.00 ± 0.00
400 + 200	1.30 ± 0.33**
0 + 400	0.00 ± 0.00
100 + 400	1.40 ± 0.31**
200 + 400	1.40 ± 0.31**
400 + 400	1.50 ± 0.31**

\*\* Significantly different from the control group (P < 0.01) by Dunnett's test

a Daily gavage doses of AZT + rifampicin (mg/kg per day); n=10

b Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild

c n=9

## SPERM MORPHOLOGY AND MOTILITY AND VAGINAL CYTOLOGY

For left epididymal weights, testicular weights, and epididymal sperm motility in surviving animals (70% to 100% mortality in groups treated with 400 mg of rifampicin + all doses of AZT or with 400 mg of AZT + 200 mg of rifampicin), the dose response relationship of AZT differed across doses of rifampicin (Table 17). The values, in general, decreased with increasing doses of rifampicin. For left caudal weights, epididymal sperm count per gram of caudal tissue, total spermatid heads per testis, and total spermatid heads per gram of testis, there were no significant AZT or rifampicin effects and no interaction effect.

**TABLE 17**  
**Summary of Significant Reproductive Tissue Parameters in Male Mice Treated with AZT and Rifampicin Combinations**

Dose <sup>a</sup>	Left Epididymal Weight <sup>b</sup>	Left Testicular Weight <sup>b</sup>	Epididymal Sperm Motility <sup>b</sup>
0 + 0	0.043 ± 0.001(10)	0.118 ± 0.002(10)	86.25 ± 0.82(10)
100 + 0	0.043 ± 0.002(10)	0.119 ± 0.004(10)	84.69 ± 0.73(10)
200 + 0	0.044 ± 0.001(10)	0.113 ± 0.002(10)	85.01 ± 0.79(10)
400 + 0	0.043 ± 0.001(10)	0.109 ± 0.003(10)	81.45 ± 1.68(10)
0 + 100	0.043 ± 0.001(10)	0.112 ± 0.002(10)	83.64 ± 1.17(9)
100 + 100	0.043 ± 0.001(10)	0.109 ± 0.001(10)	84.22 ± 1.05(10)
200 + 100	0.044 ± 0.001(10)	0.109 ± 0.003(10)	77.95 ± 4.18(10)
400 + 100	0.043 ± 0.001(10)	0.101 ± 0.003(10)	80.89 ± 1.64(9)
0 + 200	0.043 ± 0.001(10)	0.113 ± 0.003(10)	85.06 ± 0.70(9)
100 + 200	0.043 ± 0.001(9)	0.111 ± 0.002(9)	80.65 ± 2.01(8)
200 + 200	0.041 ± 0.001(9)	0.101 ± 0.003(9)	83.38 ± 1.41(8)
400 + 200	0.042 ± 0.001(3)	0.095 ± 0.004(3)	55.10 ± 22.72(3)
0 + 400	0.041 ± 0.003(10)	0.107 ± 0.004(10)	78.90 ± 3.69(10)
100 + 400	0.036 ± 0.002(2)	0.092 ± 0.005(2)	39.05 ± 22.45(2)
200 + 400	0.022 ± (1)	0.072 ± (1)	0.00 ± (1)
400 + 400 <sup>c</sup>	—	—	—

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Results of a two-way analysis of variance indicate that the dose response relationship of AZT differs across doses of rifampicin; data are presented as mean ± standard error(number of animals providing data); left epididymal weight and left testicular weight expressed in grams; epididymal sperm motility expressed as percent (%)

c Data were not available due to 100% mortality in this group.

The average estrous cycle length was not altered by AZT alone, rifampicin alone, or combinations of AZT and rifampicin. Results of a two-way analysis of variance indicated a significant interaction effect (P 0.05) in the duration of estrus, metestrus, and diestrus (Table 18). This analysis indicated that the dose response relationship of AZT differed across doses of rifampicin. There was no significant change in the duration of proestrus. However, the reason for changes in the other phases of the estrous cycle in groups treated with the combinations of AZT and rifampicin is not known.

**TABLE 18**  
**Estrous Cycle Phases in Female Mice Treated with AZT and Rifampicin Combinations**

<b>Dose<sup>a</sup></b>	<b>Proestrus</b>	<b>Estrus<sup>b</sup></b>	<b>Metestrus<sup>b</sup></b>	<b>Diestrus<sup>b</sup></b>
0 + 0	15.8	22.5	20.0	41.7
100 + 0	15.0	32.5	21.7	30.8
200 + 0	16.7	29.2	22.5	31.7
400 + 0	18.3	30.0	20.0	31.7
0 + 100	16.7	21.7	15.8	45.8
100 + 100	17.6	28.7	23.1	30.6
200 + 100	21.7	25.0	24.2	29.2
400 + 100	19.0	28.6	25.0	27.4
0 + 200	16.7	29.2	22.5	30.8
100 + 200	18.3	21.7	15.8	42.5
200 + 200	22.9	16.7	14.6	45.8
400 + 200 <sup>c</sup>	—	—	—	—
0 + 400	19.2	22.5	18.3	40.0
100 + 400 <sup>c</sup>	—	—	—	—
200 + 400 <sup>c</sup>	—	—	—	—
400 + 400 <sup>c</sup>	—	—	—	—

a Daily gavage doses of AZT + rifampicin (mg/kg per day)

b Results of a two-way analysis of variance show a significant interaction effect in the duration of estrus, metestrus, and diestrus, indicating that the dose response relationship of AZT differs across doses of rifampicin; data are presented as percent (%)

c Data were not available due to 100% mortality in this group.

## CONCLUSIONS

AZT alone or rifampicin alone did not cause mortality. There was a dose-related increase in mortality with increasing doses of combinations of AZT and rifampicin. In general, female mice died earlier than males. Rifampicin alone caused significant and dose-related increases in liver weights, and AZT did not affect the increases in liver weights caused by rifampicin. Testis weights decreased with increasing dose of rifampicin, especially when given in combination with AZT.

AZT alone caused dose- and duration of treatment-related anemia, reticulocytopenia followed by reticulocytosis, thrombocytosis and leukopenia. Rifampicin alone caused mild but dose-related anemia and reticulocytopenia. Hematological toxicity in males and females increased with increasing dose and duration of treatment with AZT alone or in combination with rifampicin. Combinations of AZT and rifampicin resulted in hematological changes of far greater severity than those caused by either drug alone. Most or all mice treated with 400 mg rifampicin + 100, 200, or 400 mg AZT or 200 mg rifampicin + 400 mg AZT either had severe anemia or died due to anemia, indicating that rifampicin synergized the hematological toxicity of AZT. Females were more sensitive to the hematological toxicity of combination therapies than the males. AZT alone did not cause significant changes in serum ALP and ALT activities. Rifampicin alone caused dose-related increases in ALP and ALT activities, indicating hepatotoxicity. AZT did not markedly influence the increases in serum ALP and ALT activities caused by rifampicin.

AZT alone caused dose-related bone marrow cellular depletion, and rifampicin alone did not cause consistent changes in bone marrow cellularity. Combination of AZT and rifampicin caused dose-related increases in severity of bone marrow cellular depletion, and bone marrow cellular depletion corresponded with decreases in blood erythrocyte counts. An increase in severity of cytoplasmic vacuolization of hepatocytes typical of fat vacuoles was observed in groups treated with rifampicin alone or in combination with AZT. In general, cytoplasmic vacuolization was more severe in male mice than in females. Hepatocyte cytoplasmic vacuolization corresponded with increases in liver weights and serum enzymes consistent with hepatotoxicity. Testicular degeneration was observed in groups treated with 100, 200, or 400 mg AZT + 400 mg rifampicin and 400 mg AZT + 200 mg rifampicin. Testicular degeneration was generally associated with anemia and lower testis weights and sperm motility.

## REFERENCES

- Amin, N.M. (1989). Zidovudine for treating AIDS: What physicians need to know. *Postgrad. Med.* **86**, 195-208.
- Ayers, K.M. (1988). Preclinical toxicology of Zidovudine: An overview. *Am. J. Med.* **85**, 186-188.
- Barnes, P.F., Bloch, A.B., Davidson, P.T., and Snider, D.E., Jr. (1991). Tuberculosis in patients with human immunodeficiency virus infection. *N. Engl. J. Med.* **324**, 1644-1650.
- Bichel, J. (1973). Rifampicin in a carcinogenic experiment. *Lancet* **2**, 1209.
- Binda, G., Domenichini, E., Gottardi, A., Orlandi, B., Orтели, E., Pacini, B., and Fowst, G. (1971). Rifampicin, a general review. *Arzneimittelforschung* **21**, 1907-1977.
- Centers for Disease Control (CDC) (1987). Diagnosis and management of mycobacterial infection and disease in persons with human immunodeficiency virus infection. *Ann. Intern. Med.* **106**, 254-256.
- Coffin, J.M. (1986). Genetic variation in AIDS viruses. *Cell* **46**, 1-4.
- Curci, C. (1969). Some aspects of the pharmacokinetics of rifampicin. *Acta Tuberc. Pneumol. Belg.* **60**, 276-287.
- Della Porta, G., Cabral, J.R., and Rossi, L. (1978). Carcinogenicity study of rifampicin in mice and rats. *Toxicol. Appl. Pharmacol.* **43**, 293-302.
- Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.
- Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.
- Editorial Staff (1971). Rifampicin: A review. *Drugs* **1**, 354-398.
- Freirich, E.J., Gehon, E.A., Rall, D.P., Schmidt, L.H., and Skipper, H.E. (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother. Rep.* **50**, 219-244.
- Furesz, S. (1970). Chemical and biological properties of rifampicin. *Antibiot. Chemother.* **16**, 316-351.
- Girling, D.J., and Hitze, K.L. (1979). Adverse reactions to rifampicin. *Bull. World Health Organ.* **57(1)**, 45-49.
- Goldschmidt, R.H., and Dong, B.J. (1992). Current report-HIV treatment of AIDS and HIV-related conditions: 1992. *J. Am. Board Fam. Pract.* **5**, 335-350.

- Gottlieb, M.S., Schroff, R., Schanker, H.M., Weisman, J.D., Fan, P.T., Wolf, R.A., and Saxon, A. (1981). *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **305**, 1425-1431.
- Greene, J.A., Ayers, K.M., deMiranda, P., and Tucker, W.E. (1990). Postnatal survival in Wistar rats following oral dosage with zidovudine on gestation day 10. *Fundam. Appl. Toxicol.* **15**, 201-206.
- Gronhagen-Riska, C., Hellstrom, P.E., and Forseth, B. (1978). Predisposing factors in hepatitis induced by isoniazid-rifampin treatment of tuberculosis. *Am. Rev. Respir. Dis.* **188**, 461-466.
- Grosset, J., and Levintis, S. (1983). Adverse effects of rifampin. *Rev. Infect. Dis.* **5** (Suppl. 3), S440-S446.
- Hardy, W.D. (1991). Prophylaxis of AIDS-related opportunistic infections (OIs). *AIDS Clin. Rev.* 145-180.
- Harkins, T., and Herriot, K.B. (1992). Medical management of acquired immune deficiency syndrome patients: A review. *J. Am. Optom. Assoc.* **63**, 35-42.
- Hugues, F.-C., Marche, C., and Marche, J. (1969). Effets hépato-biliaires de l'association rifampicine-isoniazide. *Thérapie* **24**, 899.
- International Agency for Research on Cancer (IARC) (1986). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 24. IARC, Lyon, France.
- Jeffries, D.J. (1989). Targets for antiviral therapy of human immunodeficiency virus infection. *J. Infect.* **18** (Suppl. 1), 5-13.
- Jonckheere, A.R. (1954). A distribution-free k-sample test against ordered alternatives. *Biometrika.* **41**, 133-145.
- Mansuri, M.M., Hitchcock, M.J., Buroker, R.A., Bregman, C.L., Ghazzouli, I., Desiderio, J.V., Starrett, J.E., Sterzycki, R.Z., and Martin, J.C. (1990). Comparison of *in vitro* biological properties and mouse toxicities of three thymidine analogs active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **34**, 637-641.
- Masur, H., Michelis, M.A., Greene, J.B., Onorato, I., Vande Stouwe, R.A., Holzman, R.S., Wormser, G., Brettman, L., Lange, M., Murray, H.W., and Cunningham-Rundles, S. (1981). An outbreak of community-acquired *Pneumocystis carinii* pneumonia: Initial manifestations of cellular immune dysfunction. *N. Engl. J. Med.* **305**, 1431-1438.
- National Toxicology Program (NTP) (1999). Toxicology and Carcinogenesis Studies of AZT (CAS No. 30516-87-1) and AZT/Interferon A/D in B6C3F<sub>1</sub> Mice (Gavage Studies). Technical Report Series No. 469. NIH Publication No. 99-3959. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health. Research Triangle Park, N.C.
- Nolan, C.M. (1992). Human immunodeficiency syndrome-associated tuberculosis: A review with an emphasis on infection control issues. *Am. J. Infect. Control* **20**, 30-34.
- Physicians' Desk Reference* (PDR) (1996). 50th ed., pp. 1158-1163. Medical Economics Data, Medical Economics Company, Inc., Montvale, NJ.
- Piriou, A., Warnet, J.M., Jacqueson, A., Clause, J.R., and Truhaut, R. (1979). Fatty liver induced by high doses of rifampicin in the rat: possible relation with an inhibition of RNA polymerases in eukariotic cells. *Arch. Toxicol Suppl.* **2**, 333-337.

Radner, D.B. (1973). Toxicologic and pharmacologic aspects of rifampin. *Chest* **64**, 213-216.

Rao, G. N., Lindamood, C., III., Heath, J. E., Farnell, D. R., and Giles, H. D. (1998). Subchronic toxicity of human immunodeficiency virus and tuberculosis combination therapies in B6C3F<sub>1</sub> mice. *Toxicol. Sci.* **45**, 113-127.

- Richman, D.D. (1988). The treatment of HIV infection. Azidothymidine (AZT) and other new antiviral drugs. *Infect. Dis. Clin. North Am.* **2**, 397-497.
- Scheuer, P.J., Summerfield, J.A., Lai, S., and Sherlock, S. (1974). Rifampin hepatitis. *Lancet* **1**, 421-425.
- Siegle, F.P., Lopez, C., Hammer, C.S., Brown, A.E., Kornfeld, S.J., Gold, J., Hassett, J., Hirschman, S.Z., Cunningham-Rundles, C., Adelsberg, B.R., Parham, D.M., Siegal, M., Cunningham-Rundles, S., and Armstrong, D. (1981). Severe acquired immunodeficiency in male homosexuals manifested by chronic perianal ulcerative herpes simplex lesions. *N. Engl. J. Med.* **305**, 1439-1444.
- Stevens, J. (1986). *Applied Multivariate Statistics for the Social Sciences*. Chapter 3. LEA Publishers.
- Thompson, M.B., Dunnick, J.K., Sutphin, M.E., Giles, H.D., Irwin, R.D., and Prejean, J.D. (1991). Hematologic toxicity of AZT and ddC administered as single agents and in combination to rats and mice. *Fundam. Appl. Toxicol.* **17**, 159-176.
- Toltzis, P., Marx, C.M., Kleinman, N., Levine, E.M., and Schmidt, E.V. (1991). Zidovudine-associated embryonic toxicity in mice. *J. Infect. Dis.* **163**, 1212-1218.
- Trang, J.M., Prejean, J.D., James, R.H., Irwin, R.D., Goehl, T.J., and Page, J.G. (1993). Zidovudine bioavailability and linear pharmacokinetics in female B6C3F1 mice. *Drug Metab. Dispos.* **21**, 189-193.
- Vince, R., Hua, M., Brownell, J., Daluge, S., Lee, F.C., Shannon, W.M., Lavelle, G.C., Qualls, J., Weislow, O.S., Kiser, R., Canonico, P.G., Schultz, R.H., Narayanan, V.L., Mayo, J.G., Shoemaker, R.H., and Boyd, M.R. (1988). Potent and selective activity of a new carbocyclic nucleoside analog (Carbovir: NSC 614846) against human immunodeficiency virus *in vitro*. *Biochem. Biophys. Res. Commun.* **156**, 1046-1053.
- Warkany, J. (1979). Antituberculosis drugs. *Teratology* **20**, 133-138.
- Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.
- Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

## APPENDIX A

### CLINICAL PATHOLOGY RESULTS

TABLE A1 Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations.....	A-2
TABLE A2 Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations.....	A-10
TABLE A3 Clinical Chemistry Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations.....	A-18
TABLE A4 Clinical Chemistry Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations.....	A-22

TABLE A1

Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>

Dose	WBC <sup>b</sup> (10 <sup>3</sup> /mm <sup>3</sup> )	RBC 10 <sup>6</sup> /mm <sup>3</sup>	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10 <sup>3</sup> /mm <sup>3</sup> )
Day 4								
0 + 0 <sup>c</sup>	5.67	9.61	15.9	47.6	49.5	16.5	33.3	1,310
	0.803	0.566	0.85	2.92	1.15	0.20	0.77	227.9
100 + 0	5.26	9.34	15.5	46.2	49.5	16.6	33.6	1,384
	0.995	0.455	0.63	2.60	1.46	0.24	1.34	186.4
200 + 0 <sup>c</sup>	5.85	9.38	15.6	46.5	49.6	16.6	33.5	1,490
	0.842	0.347	0.55	2.48	1.49	0.13	1.08	159.9
400 + 0	5.68	9.38	15.6	46.2	49.2	16.6	33.8	1,387
	0.932	0.313	0.54	2.22	1.45	0.17	1.04	155.4
0 + 100	5.88	9.53	15.6	46.6	48.9	16.3	33.5	1,462
	0.786	0.429	0.68	1.79	1.28	0.15	1.01	194.8
100 + 100	5.71	9.28	15.3	45.9	49.5	16.5	33.3	1,499
	0.749	0.299	0.43	1.84	1.43	0.20	1.12	112.3
200 + 100	5.67	9.13	15.1	44.5	48.7	16.5	33.9	1,484
	0.950	0.445	0.69	2.34	0.91	0.20	0.88	167.6
400 + 100	4.81	9.04	15.0	44.3	49.0	16.5	33.8	1,390
	1.159	0.368	0.58	1.61	1.02	0.19	0.57	162.1
0 + 200	5.63	9.26	15.3	45.5	49.3	16.5	33.6	1,579
	0.580	0.519	0.78	1.81	1.36	0.25	1.04	172.3
100 + 200	4.67	9.44	15.5	46.0	48.8	16.5	33.8	1,509
	0.781	0.364	0.71	2.07	1.33	0.14	1.05	209.0
200 + 200 <sup>c</sup>	4.69	9.19	15.2	44.9	48.8	16.6	34.0	1,487
	1.155	0.254	0.45	1.47	1.57	0.17	1.30	114.4
400 + 200	5.12	9.43	15.6	46.3	49.1	16.6	33.8	1,445
	0.596	0.266	0.47	1.57	0.82	0.22	0.78	180.4
0 + 400 <sup>c</sup>	5.13	9.75	16.1	47.7	49.0	16.5	33.7	1,598
	0.756	0.432	0.70	2.14	1.12	0.32	1.00	241.7
100 + 400	4.57	9.56	15.9	47.0	49.2	16.7	33.9	1,475
	0.917	0.257	0.32	1.94	1.86	0.27	1.17	162.2
200 + 400	5.92	9.34	15.5	45.7	48.9	16.6	33.9	1,519
	3.069	0.652	1.16	3.54	1.71	0.27	1.59	197.4
400 + 400	4.95	9.41	15.5	46.1	49.0	16.5	33.6	1,470
	0.846	0.313	0.52	1.44	1.04	0.20	0.98	184.5

TABLE A1

## Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC <sup>d</sup> (10 <sup>3</sup> /mm <sup>3</sup> )
Day 4							
0 + 0 <sup>c</sup>	3.6	0.46	4.95	0.14	0.09	0.02	0.02
	0.92	0.124	0.719	0.048	0.035	0.005	0.011
100 + 0	2.7**	0.47	4.57	0.13	0.06	0.01	0.02 <sup>c</sup>
	0.83	0.208	0.811	0.039	0.023	0.009	0.007
200 + 0 <sup>c</sup>	2.5**	0.55	5.06	0.16	0.08	0.01	0.02 <sup>e</sup>
	0.60	0.249	0.680	0.084	0.042	0.007	0.008
400 + 0	2.1**	0.43	4.97	0.17	0.08	0.02	0.02
	0.63	0.173	0.695	0.067	0.030	0.009	0.011
0 + 100	2.5**	0.58	5.02	0.17	0.08	0.01	0.02
	0.83	0.166	0.697	0.053	0.030	0.005	0.008
100 + 100	2.0**	0.50	4.94	0.17	0.08	0.01	0.02
	0.52	0.135	0.591	0.050	0.036	0.008	0.006
200 + 100	1.3**	0.56	4.85	0.14	0.09	0.02	0.02 <sup>c</sup>
	0.25	0.365	0.746	0.038	0.044	0.016	0.010
400 + 100	1.3**	0.41	4.19	0.12	0.07	0.01	0.02
	0.40	0.155	1.015	0.030	0.028	0.006	0.008
0 + 200	1.9**	0.68	4.74	0.12	0.07	0.01	0.02 <sup>c</sup>
	0.47	0.213	0.682	0.048	0.025	0.007	0.010
100 + 200	1.1**	0.40	4.03	0.12	0.08	0.01	0.01
	0.38	0.092	0.738	0.028	0.024	0.003	0.005
200 + 200 <sup>c</sup>	1.0** <sup>e</sup>	0.50	3.96*	0.11	0.09	0.01	0.01
	0.35	0.113	1.125	0.020	0.039	0.010	0.009
400 + 200	1.0**	0.43	4.49	0.09	0.09	0.02	0.01 <sup>c</sup>
	0.29	0.131	0.489	0.027	0.028	0.010	0.009
0 + 400 <sup>c</sup>	1.4** <sup>e</sup>	0.68	4.21	0.12	0.09	0.01	0.01
	0.43	0.254	0.629	0.042	0.052	0.005	0.007
100 + 400	0.8**	0.61	3.72**	0.13	0.09	0.01	0.01
	0.39	0.227	0.760	0.065	0.048	0.005	0.004
200 + 400	0.8** <sup>c</sup>	1.55	4.12	0.15	0.07	0.02	0.02
	0.32	3.150	0.356	0.061	0.028	0.008	0.008
400 + 400	0.9**	0.56	4.20	0.11	0.06	0.01	0.02 <sup>e</sup>
	0.29	0.194	0.800	0.068	0.039	0.007	0.005



TABLE A1

## Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Day 30							
0 + 0c	3.5	0.78	5.84	0.20	0.18	0.03	0.02
	0.22	0.272	1.410	0.062	0.066	0.012	0.007
100 + 0 <sup>c</sup>	4.4	0.80	5.43	0.18	0.15	0.02	0.02
	0.58	0.137	1.203	0.039	0.045	0.007	0.011
200 + 0 <sup>c</sup>	4.0	0.69	4.60	0.12*	0.16	0.02	0.02e
	1.07	0.347	1.394	0.076	0.091	0.011	0.007
400 + 0	5.7	0.74	5.22	0.17	0.17	0.02	0.02
	0.97	0.132	1.011	0.036	0.038	0.007	0.005
0 + 100 <sup>c</sup>	3.5	0.95	5.78	0.18	0.17	0.02	0.02
	0.55	0.365	1.173	0.051	0.064	0.007	0.007
100 + 100	4.7	0.98	5.81	0.20	0.16	0.02	0.02
	0.58	0.313	0.692	0.042	0.042	0.007	0.009
200 + 100	6.1	0.84	5.39	0.17	0.17	0.02	0.02
	0.98	0.203	1.052	0.028	0.048	0.011	0.007
400 + 100 <sup>c</sup>	7.4 <sup>ac</sup>	0.91	5.02	0.18	0.20	0.02	0.01
	0.82	0.357	1.057	0.068	0.088	0.008	0.007
0 + 200 <sup>c</sup>	3.7	1.02	6.23	0.22	0.16	0.02	0.02
	0.28	0.230	1.578	0.046	0.037	0.012	0.007
100 + 200e	6.7	1.10	6.12	0.20	0.20	0.03	0.02
	1.02	0.346	1.164	0.057	0.059	0.012	0.007
200 + 200f	6.9	0.87	5.53	0.18	0.18	0.02	0.01
	0.95	0.092	0.805	0.039	0.045	0.010	0.005
400 + 200 <sup>c</sup>	8.5 <sup>**</sup>	0.95	4.53	0.14	0.12	0.01	0.02
	8.44	0.240	0.694	0.027	0.093	0.005	0.012
0 + 400e	3.4	0.96	6.17	0.17	0.19	0.02	0.02
	1.06	0.215	1.110	0.033	0.051	0.005	0.009
100 + 400e	5.6	0.87	4.43	0.12*	0.06 <sup>**</sup>	0.01	0.02
	4.64	0.382	1.464	0.034	0.026	0.005	0.012
200 + 400f	2.0 <sup>h</sup>	1.05	4.64	0.13	0.04 <sup>**</sup>	0.01	0.04 <sup>h</sup>
	0.65	0.541	1.758	0.079	0.018	0.010	0.016
400 + 400g	—	—	—	—	—	—	—



TABLE A1

## Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Day 60							
0 + 0 <sup>c</sup>	3.6	0.52	4.06	0.11	0.10	0.01	0.01
	0.33	0.228	0.872	0.022	0.045	0.010	0.006
100 + 0 <sup>c</sup>	3.8e	0.55	4.40	0.09	0.09	0.01	0.01
	0.38	0.097	0.677	0.016	0.038	0.005	0.005
200 + 0 <sup>c</sup>	4.2	0.47	3.88	0.07	0.10	0.01	0.01
	0.35	0.108	0.662	0.019	0.065	0.007	0.005
400 + 0	4.5**	0.49	4.15	0.08	0.07	0.01	0.01
	0.32	0.161	0.727	0.027	0.032	0.006	0.005
0 + 100 <sup>c</sup>	3.3	0.81**	4.44	0.11	0.09	0.01	0.01
	0.36	0.219	0.633	0.034	0.027	0.004	0.010
100 + 100	3.9	0.70	4.17	0.09	0.10	0.01	0.01
	0.41	0.189	0.589	0.029	0.037	0.003	0.005
200 + 100	4.4**	0.56	3.89	0.07	0.09	0.01	0.01
	0.53	0.236	0.647	0.025	0.033	0.006	0.013
400 + 100 <sup>c</sup>	5.5**e	0.48	3.62	0.06**	0.09	0.01	0.01e
	0.55	0.137	0.765	0.017	0.042	0.006	0.005
0 + 200 <sup>c</sup>	3.2	0.78*	5.38	0.12	0.10	0.02	0.01
	0.27	0.220	1.466	0.026	0.055	0.007	0.005
100 + 200 <sup>c</sup>	3.4	0.58	4.40	0.07	0.07	0.01	0.01
	0.27	0.124	0.631	0.015	0.017	0.004	0.009
200 + 200 <sup>f</sup>	4.6**	0.53	3.88	0.07*	0.06	0.01	0.01
	0.68	0.129	0.650	0.010	0.010	0.006	0.005
400 + 200 <sup>i</sup>	4.4k	0.65	4.26	0.07	0.07	0.01	0.01
	1.33	0.178	0.449	0.029	0.021	0.000	0.005
0 + 400 <sup>f</sup>	2.9*h	0.79*	4.76	0.09	0.10	0.01	0.01
	0.58	0.251	1.322	0.048	0.097	0.008	0.000
100 + 400 <sup>j</sup>	2.2**	0.51	3.97	0.05*	0.04	0.02	0.02
	0.21	0.064	0.177	0.007	0.021	0.007	0.007
200 + 400 <sup>g</sup>	—	—	—	—	—	—	—
400 + 400 <sup>g</sup>	—	—	—	—	—	—	—



TABLE A1

## Hematology Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Days 92 to 95							
0 + 0 <sup>c</sup>	4.9 <sup>e</sup> 2.48	0.67 0.261	4.88 0.697	0.12 0.040	0.11 0.045	0.02 0.010	0.01 0.011
100 + 0 <sup>c</sup>	4.3 <sup>f</sup> 0.31	0.57 0.161	4.11 0.974	0.08 0.023	0.10 0.054	0.01 0.005	0.01 0.011
200 + 0 <sup>c</sup>	4.8 0.32	0.53 0.271	3.72 0.897	0.08 0.049	0.18 0.211	0.01 0.007	0.01 0.008
400 + 0	5.7 0.50	0.53 0.161	4.39 0.994	0.08 0.036	0.12 0.043	0.01 0.007	0.01 0.008
0 + 100 <sup>c</sup>	3.9 0.36	0.67 0.184	4.57 1.370	0.10 0.020	0.11 0.037	0.01 0.009	0.01 0.007
100 + 100	4.6 0.77	0.70 0.372	4.78 1.334	0.07 0.031	0.16 0.065	0.02 0.007	0.01 0.017
200 + 100	5.2 0.46	0.64 0.219	4.25 1.149	0.06 0.017	0.11 0.031	0.01 0.007	0.01 0.010
400 + 100	5.8 <sup>c</sup> 0.34	0.58 0.159	4.45 0.695	0.07 0.024	0.11 0.038	0.01 0.011	0.01 0.006
0 + 200	3.6 0.31	0.89 0.242	5.01 1.435	0.09 0.037	0.12 0.053	0.02 0.013	0.01 0.008
100 + 200 <sup>c</sup>	5.4 0.96	0.62 0.222	4.32 0.978	0.08 0.021	0.08 0.025	0.01 0.006	0.01 0.008
200 + 200	5.1 1.42	0.71 0.279	4.58 1.309	0.08 0.045	0.10 0.031	0.01 0.008	0.01 0.006
400 + 200 <sup>k</sup>	2.2 <sup>**</sup> 0.47	0.75 0.180	3.67 1.272	0.07 0.032	0.08 0.040	0.01 0.006	0.00 0.000
0 + 400	4.5 1.26	0.89 0.216	4.27 1.082	0.11 0.032	0.09 0.076	0.01 0.007	0.01 0.007
100 + 400 <sup>j</sup>	1.9 <sup>**</sup> 0.00	0.56 0.057	3.28 0.940	0.05 0.021	0.02 0.000	0.01 0.007	0.01 0.007
200 + 400 <sup>l</sup>	0.5 <sup>**</sup>	0.08	0.64	0.00	0.02	0.00	0.00
400 + 400 <sup>g</sup>	—	—	—	—	—	—	—

\* Significantly different (P<0.05) from the vehicle control group using analysis of variance followed by Dunnett's test

\*\* P<0.01

a Daily gavage doses of AZT + rifampicin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=10, unless otherwise noted.

b WBC counts corrected for reticulocyte (nucleated erythrocyte) counts greater than 10 per 100 WBCs

<sup>c</sup> n=9      <sup>d</sup> Large unstained cells      <sup>e</sup> n=8      <sup>f</sup> n=7

<sup>g</sup> No data were available due to 100% mortality in this group.

<sup>h</sup> n=6      <sup>i</sup> n=4      <sup>j</sup> n=2      <sup>k</sup> n=3

<sup>l</sup> n=1; standard deviation not calculated due to high mortality

TABLE A2

Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>

Dose	WBC <sup>b</sup> (10 <sup>3</sup> /mm <sup>3</sup> )	RBC 10 <sup>6</sup> /mm <sup>3</sup>	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10 <sup>3</sup> /mm <sup>3</sup> )
Day 4								
0 + 0	5.99	9.73	15.3	47.3	48.6	15.8	32.4	1,124
	1.488	0.440	0.42	1.56	0.98	0.44	0.55	74.4
100 + 0	6.39	9.11**	14.5*	44.5**	48.8	15.9	32.6	1,048
	0.991	0.211	0.61	1.76	1.13	0.45	0.43	153.0
200 + 0	5.34	9.30*	14.7	45.4	48.8	15.8	32.4	1,206
	1.123	0.236	0.47	1.48	1.19	0.38	0.44	63.9
400 + 0	5.88	9.38	14.9	45.6	48.6	15.9	32.8	1,159
	1.210	0.274	0.40	1.36	1.11	0.37	0.78	69.1
0 + 100	6.17	9.34	14.7	45.4	48.6	15.8	32.5	1,128
	1.303	0.103	0.39	1.34	1.32	0.43	0.28	102.0
100 + 100	6.37	9.32	14.8	45.7	49.0	15.9	32.5	1,164
	1.429	0.306	0.46	1.46	1.00	0.35	0.48	92.9
200 + 100	6.01	9.11**	14.4**	44.4**	48.7	15.8	32.4	1,150
	1.527	0.217	0.50	1.58	1.16	0.38	0.50	60.1
400 + 100	6.83	9.10**	14.5*	44.1**	48.4	15.9	32.8	1,163
	1.750	0.307	0.49	2.00	1.30	0.37	0.77	63.0
0 + 200	7.26	9.37	14.8	45.6	48.6	15.8	32.6	1,258*
	1.526	0.426	0.77	2.21	1.58	0.43	0.44	107.8
100 + 200	6.17	9.33	14.8	45.5	48.7	15.9	32.5	1,172
	1.275	0.240	0.44	1.13	1.02	0.37	0.46	90.9
200 + 200	6.03	9.12**	14.4*	44.2**	48.4	15.8	32.7	1,140
	1.411	0.257	0.62	1.99	1.25	0.44	0.55	59.4
400 + 200	5.89	9.08**	14.4*	44.0**	48.5	15.9	32.8	1,137
	0.923	0.273	0.44	1.19	1.13	0.38	0.42	81.6
0 + 400	6.08	9.45	15.1	46.1	48.7	16.0	32.7	1,170
	1.603	0.360	0.58	1.90	1.33	0.36	0.40	194.2
100 + 400 <sup>c</sup>	6.03	9.50	15.0	45.9	48.3	15.8	32.7	1,218
	1.390	0.164	0.41	1.12	1.44	0.47	0.58	88.1
200 + 400	5.88	9.18**	14.6	44.6*	48.6	16.0	32.9	1,202
	1.181	0.252	0.42	1.26	1.08	0.37	0.44	79.0
400 + 400	3.90	10.12	16.0	47.8	47.2	15.8	33.4	1,318**
	1.743	0.759	1.36	3.88	1.32	0.43	0.56	96.1

TABLE A2

Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC <sub>d</sub> (10 <sup>3</sup> /mm <sup>3</sup> )
Day 4							
0 + 0	3.1 <sup>c</sup>	0.56	5.07	0.12	0.19	0.02	0.02
	0.49	0.150	1.329	0.030	0.107	0.009	0.007
100 + 0	1.3**	0.55	5.49	0.17	0.14	0.02	0.02
	0.32	0.178	0.854	0.065	0.049	0.004	0.007
200 + 0	1.5** <sup>c</sup>	0.42	4.64	0.10	0.15	0.02	0.02
	0.21	0.115	1.011	0.033	0.044	0.007	0.008
400 + 0	1.4**	0.44	5.11	0.13	0.16	0.02	0.02
	0.50	0.089	1.072	0.042	0.064	0.010	0.009
0 + 100	1.8**	0.69	5.10	0.17	0.18	0.02	0.02
	0.78	0.209	1.163	0.050	0.062	0.009	0.006
100 + 100	1.0**	0.52	5.51	0.13	0.18	0.02	0.02
	0.29	0.160	1.234	0.059	0.061	0.005	0.005
200 + 100	0.8**	0.46	5.23	0.12	0.17	0.02	0.02
	0.29	0.187	1.356	0.034	0.065	0.005	0.007
400 + 100	0.6** <sup>c</sup>	0.66	5.82	0.12	0.17	0.02	0.03
	0.10	0.190	1.571	0.034	0.062	0.007	0.012
0 + 200	1.1**	0.77	6.05	0.16	0.23	0.02	0.02
	0.45	0.122	1.416	0.022	0.101	0.014	0.012
100 + 200	0.7**	0.58	5.28	0.12	0.17	0.02	0.02
	0.16	0.198	1.041	0.044	0.057	0.007	0.005
200 + 200	0.6**	0.67	5.03	0.14	0.15	0.02	0.02
	0.15	0.244	1.234	0.040	0.067	0.009	0.005
400 + 200	0.5**	0.64	4.96	0.11	0.14	0.01	0.02
	0.23	0.248	0.725	0.034	0.058	0.005	0.008
0 + 400	0.7**	0.79	4.98	0.12	0.14	0.02	0.02
	0.17	0.241	1.474	0.039	0.059	0.008	0.010
100 + 400 <sup>c</sup>	0.7**	0.76	4.92	0.12	0.19	0.02	0.02
	0.24	0.287	1.109	0.055	0.118	0.009	0.008
200 + 400	0.5**	0.54	5.03	0.11	0.17	0.02	0.02
	0.10	0.125	1.018	0.031	0.060	0.011	0.009
400 + 400	0.7**	0.77	2.93**	0.10	0.08	0.01	0.01 <sup>c</sup>
	0.17	0.370	1.731	0.053	0.068	0.010	0.008

TABLE A2

Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	WBC (10 <sup>3</sup> /mm <sup>3</sup> )	RBC 10 <sup>6</sup> /mm <sup>3</sup>	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10 <sup>3</sup> /mm <sup>3</sup> )
Day 30								
0 + 0 <sup>c</sup>	6.47	9.25	14.9	44.9	48.5	16.1	33.2	1,129
	1.090	0.125	0.23	0.85	0.82	0.31	0.59	95.9
100 + 0	6.60	8.28	14.5	44.2	53.4**	17.5**	32.8	1,282
	1.045	0.367	0.47	1.72	0.69	0.43	0.55	108.8
200 + 0	5.43	7.97**	14.1	43.1	54.1**	17.6**	32.6	1,412*
	1.632	0.223	0.45	1.25	0.77	0.24	0.32	45.5
400 + 0	5.47	8.04*	14.4	44.8	55.7**	17.9**	32.1	1,404
	1.217	0.171	0.28	0.99	0.97	0.31	0.44	121.8
0 + 100	6.45	9.54	15.0	46.0	48.3	15.8	32.7	1,221
	0.864	0.421	0.46	1.74	0.79	0.41	0.53	127.8
100 + 100 <sup>e</sup>	6.48	8.11*	14.1	43.3	53.5**	17.4**	32.6	1,376
	1.044	0.336	0.64	1.65	0.53	0.16	0.55	113.6
200 + 100 <sup>c</sup>	4.73*	7.33**	13.0	40.9	55.8**	17.8**	31.8	1,469**
	0.696	0.245	0.45	1.09	0.88	0.35	0.53	131.3
400 + 100	5.68	4.79**	8.7**	27.6**	54.4**	17.7**	33.0	1,929**
	1.581	2.330	4.59	15.60	8.74	1.55	2.83	326.4
0 + 200	7.27	9.32	14.5	44.2	47.4	15.6	32.9	1,153
	1.694	0.390	0.56	1.82	0.66	0.29	0.57	177.5
100 + 200	6.40	6.89**	11.7**	36.6**	53.1**	17.0*	31.9	1,762**
	1.210	0.388	0.72	2.42	1.06	0.30	0.57	139.9
200 + 200	4.92*	2.91**	4.9**	14.9**	48.6	16.5	34.2	2,036**
	1.253	1.578	2.95	10.09	6.32	0.84	2.76	292.9
400 + 200	4.26**	1.42**	2.2**	6.1**	42.8**	15.5	36.3**	1,577**
	0.814	0.095	0.16	0.41	1.05	0.44	1.01	161.8
0 + 400	7.56	9.11	13.8	41.7	45.8	15.1**	33.1	1,381
	0.743	0.185	0.36	1.02	0.73	0.30	0.83	172.4
100 + 400	3.81**	1.85**	2.9**	7.8**	42.3**	15.5	36.7**	1,415*
	0.593	0.350	0.48	1.53	0.73	0.51	1.24	168.8
200 + 400 <sup>c</sup>	3.67**	1.58**	2.4**	6.7**	42.4**	15.6	36.8**	1,327
	1.442	0.378	0.57	1.66	1.02	0.21	1.26	523.7
400 + 400 <sup>f</sup>	1.29**	1.21**	1.8**	4.8**	39.7**	15.3	38.7**	307**
	0.826	0.271	0.40	1.29	2.14	0.46	3.29	238.0

TABLE A2

Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes (10 <sup>5</sup> /mm <sup>3</sup> )	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Day 30							
0 + 0c	3.4g	0.60	5.53	0.15	0.17	0.02	0.02
	0.42	0.148	0.979	0.046	0.089	0.009	0.007
100 + 0	4.4	0.65	5.59	0.14	0.19	0.02	0.02
	0.53	0.203	0.832	0.043	0.082	0.007	0.005
200 + 0	5.6	0.69	4.48	0.13	0.10	0.01	0.01
	0.65	0.298	1.372	0.054	0.050	0.007	0.005
400 + 0	5.4	0.55	4.64	0.11	0.14	0.01	0.02
	0.68	0.168	1.002	0.022	0.081	0.005	0.007
0 + 100	3.9	0.62	5.50	0.15	0.15	0.02	0.02
	0.51	0.159	0.741	0.031	0.047	0.005	0.011
100 + 100e	4.4h	0.71	5.46	0.14	0.13	0.02	0.02
	0.64	0.247	0.894	0.036	0.034	0.006	0.011
200 + 100 <sup>c</sup>	5.2e	0.51	3.99*	0.10	0.10	0.01	0.01
	0.80	0.117	0.613	0.032	0.031	0.005	0.007
400 + 100	11.3**c	0.87*	4.57	0.08**	0.12	0.01	0.02
	7.49	0.340	1.218	0.051	0.109	0.010	0.009
0 + 200	3.8e	0.93**	5.97	0.20*	0.13	0.02	0.02
	0.97	0.220	1.478	0.050	0.052	0.008	0.008
100 + 200	6.0 <sup>c</sup>	0.79	5.31	0.14	0.12	0.02	0.02
	1.24	0.249	0.976	0.045	0.063	0.007	0.006
200 + 200	4.3 <sup>c</sup>	0.51	4.24	0.07**	0.06**	0.01	0.02
	4.13	0.223	1.209	0.052	0.030	0.007	0.020
400 + 200	0.5	0.30*	3.90**	0.02**	0.02**	0.01	0.02
	0.09	0.100	0.725	0.009	0.008	0.005	0.012
0 + 400	2.3 <sup>c</sup>	1.11**	6.11	0.17	0.14	0.02	0.02
	0.73	0.258	0.880	0.050	0.080	0.012	0.006
100 + 400	0.5	0.34	3.41**	0.03**	0.03**	0.01	0.01
	0.20	0.143	0.484	0.013	0.018	0.003	0.007
200 + 400 <sup>c</sup>	0.5e	0.26**	3.34**	0.02**	0.02**	0.01*	0.01
	0.16	0.130	1.341	0.009	0.013	0.005	0.007
400 + 400f	0.1	0.16*	1.08**	0.02**	0.03**	0.01	0.00
	0.06	0.076	0.789	0.006	0.006	0.006	0.006



TABLE A2

## Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Day 60							
0 + 0 <sup>c</sup>	3.5 <sup>e</sup>	0.40	3.58	0.09	0.08	0.01	0.01
	0.49	0.212	1.147	0.047	0.044	0.006	0.009
100 + 0	3.5	0.39	3.11	0.08	0.10	0.01	0.01
	0.51	0.164	1.096	0.037	0.021	0.007	0.006
200 + 0	4.3	0.39	2.85	0.07	0.09	0.01	0.01 <sup>e</sup>
	0.86	0.146	0.964	0.024	0.056	0.005	0.009
400 + 0 <sup>c</sup>	4.4	0.33	2.96	0.07	0.12	0.01	0.01
	0.62	0.126	0.738	0.026	0.068	0.006	0.005
0 + 100	3.0	0.38	3.11	0.09	0.08	0.01	0.01
	0.57	0.134	0.633	0.028	0.047	0.006	0.005
100 + 100	4.6	0.42	3.02	0.08	0.09	0.01	0.01
	1.18	0.109	0.505	0.037	0.042	0.003	0.000
200 + 100	4.4	0.46	3.10	0.07	0.08	0.01	0.01
	0.77	0.175	0.700	0.026	0.028	0.004	0.004
400 + 100 <sup>i</sup>	3.4	0.31	1.56	0.05	0.04	0.00	0.011
	2.80	0.302	1.001	0.060	0.040	0.000	0.007
0 + 200	2.7	0.52	3.97	0.09	0.08	0.01	0.01
	0.53	0.207	1.092	0.030	0.035	0.007	0.005
100 + 200	4.9	0.54	2.85	0.07	0.08	0.01	0.01
	2.16	0.185	0.940	0.028	0.086	0.008	0.004
200 + 200 <sup>j</sup>	5.5	0.60	3.76	0.06	0.04	0.01	0.01
400 + 200 <sup>k</sup>	—	—	—	—	—	—	—
0 + 400	1.9 <sup>*</sup>	0.50	3.71	0.08	0.07	0.01	0.01
	0.42	0.148	0.909	0.027	0.044	0.007	0.007
100 + 400 <sup>k</sup>	—	—	—	—	—	—	—
200 + 400 <sup>k</sup>	—	—	—	—	—	—	—
400 + 400 <sup>k</sup>	—	—	—	—	—	—	—



TABLE A2

## Hematology Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	Reticulocytes 10 <sup>5</sup> /mm <sup>3</sup>	Neutrophils (10 <sup>3</sup> /mm <sup>3</sup> )	Lymphocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Monocytes (10 <sup>3</sup> /mm <sup>3</sup> )	Eosinophils (10 <sup>3</sup> /mm <sup>3</sup> )	Basophils (10 <sup>3</sup> /mm <sup>3</sup> )	LUC (10 <sup>3</sup> /mm <sup>3</sup> )
Days 92 to 95							
0 + 0	3.7 0.65	0.45 0.186	3.06 0.762	0.07 0.031	0.06 0.015	0.01 0.006	0.01 0.005
100 + 0	3.9 0.81	0.29 0.097	2.91 0.675	0.04 0.008	0.07 0.022	0.01 0.006	0.00 0.003
200 + 0	5.2 0.68	0.34 0.117	3.14 0.831	0.06 0.028	0.09 0.045	0.01 0.006	0.01 0.007
400 + 0 <sup>c</sup>	5.4* 0.67	0.23* 0.100	2.85 0.784	0.05 0.025	0.06 0.025	0.01 0.004	0.00 0.005
0 + 100	3.6 0.80	0.42 0.166	3.27 0.678	0.06 0.019	0.07 0.028	0.01 0.000	0.00 0.005
100 + 100 <sup>c</sup>	4.6 0.70	0.32 0.169	2.87 0.793	0.05 0.025	0.09 0.052	0.01 0.008	0.00 0.005
200 + 100	6.7** 0.89	0.39 0.165	2.95 0.885	0.05 0.035	0.08 0.042	0.01 0.003	0.01 0.005
400 + 100 <sup>g</sup>	4.4 1.77	0.35 0.156	2.56 0.854	0.05 0.035	0.04 0.020	0.00 0.005	0.00 0.005
0 + 200	4.6 <sup>c</sup> 2.02	0.48 0.217	3.30 0.661	0.05 0.020	0.06 0.037	0.01 0.007	0.01 <sup>c</sup> 0.005
100 + 200	3.3 1.64	0.32 0.168	2.39 0.840	0.06 0.058	0.05 0.034	0.00 0.005	0.00 0.004
200 + 200 <sup>m</sup>	0.9** 0.28	0.26 0.172	1.96 0.923	0.01 0.010	0.02 0.010	0.00 0.000	0.00 0.005
400 + 200 <sup>k</sup>	—	—	—	—	—	—	—
0 + 400	4.4 1.82	0.63 0.168	3.12 1.092	0.06 0.029	0.05 0.031	0.01 0.009	0.01 <sup>c</sup> 0.005
100 + 400 <sup>k</sup>	—	—	—	—	—	—	—
200 + 400 <sup>k</sup>	—	—	—	—	—	—	—
400 + 400 <sup>k</sup>	—	—	—	—	—	—	—

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group using analysis of variance followed by Dunnett's test

\*\*  $P \leq 0.01$

a Daily gavage doses of AZT + rifampicin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=10, unless otherwise noted.

b WBC counts corrected for reticulocyte (nucleated erythrocyte) counts greater than 10 per 100 WBCs

<sup>c</sup> n=9

<sup>d</sup> Large unstained cells

<sup>e</sup> n=8

<sup>f</sup> n=3

<sup>g</sup> n=7

<sup>h</sup> n=6

<sup>i</sup> n=5

<sup>j</sup> n=1; standard deviation not calculated due to high mortality

<sup>k</sup> No data were available due to 100% mortality in this group.

<sup>l</sup> n=2

<sup>m</sup> n=4

TABLE A3

Clinical Chemistry Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
<b>Day 4</b>					
0 + 0	154 20.2	31b 5.6	65 <sup>c</sup>	— <sup>d</sup>	—
100 + 0	150 24.5	31b 6.1	103e 49.4	42 <sup>c</sup>	38 <sup>c</sup>
200 + 0	142f 23.3	32e 8.1	96 <sup>c</sup>	27 <sup>c</sup>	—
400 + 0	157f 13.5	27g 6.0	61 <sup>c</sup>	—	—
0 + 100	156f 14.7	43 23.4	80 <sup>c</sup>	19 <sup>c</sup>	—
100 + 100	152 20.4	30h 2.4	64 <sup>c</sup>	—	—
200 + 100	150 9.7	32 4.6	72i 5.7	43 <sup>c</sup>	—
400 + 100	158j 10.8	28b 3.3	71 <sup>c</sup>	—	—
0 + 200	165f 6.4	33g 1.0	70i 1.4	—	—
100 + 200	174j 14.8	39 5.8	77i 2.1	30 <sup>c</sup>	29 <sup>c</sup>
200 + 200	169f 22.2	36f 3.6	85h 19.4	33 <sup>c</sup>	34i 1.4
400 + 200	157 18.2	34k 4.8	82 <sup>c</sup>	—	—
0 + 400	154f 22.6	40k 2.8	87i 12.0	—	—
100 + 400	164f 21.3	44h 6.6	80 <sup>c</sup>	—	—
200 + 400	166b 17.7	38h 8.0	81i 28.3	—	—
400 + 400	166b 15.2	34e 3.6	57 <sup>c</sup>	—	—

TABLE A3

Clinical Chemistry Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
Day 30					
0 + 0	89 11.7	32 6.4	77g 17.8	—	10 <sup>c</sup>
100 + 0	94 7.5	35 12.6	67b 13.1	34i 8.5	12i 1.4
200 + 0	92b 9.9	29b 4.0	73h 13.4	—	—
400 + 0	94j 14.5	29j 11.0	64f 12.5	20 <sup>e</sup>	5i 3.5
0 + 100	116** 8.0	32 5.2	76h 16.2	36i 6.4	10 <sup>c</sup>
100 + 100	102 7.5	32 15.1	74b 20.4	46 <sup>c</sup>	20 <sup>c</sup>
200 + 100	105 12.1	31 8.5	53g 13.6	—	15 <sup>c</sup>
400 + 100	97 12.6	28 5.3	68b 29.3	37i 4.9	8 <sup>c</sup>
0 + 200	111*b 8.9	32b 4.8	71g 10.1	26e 7.5	26i 6.4
100 + 200	116*f 15.9	33f 4.7	71g 10.0	36i 7.1	51 <sup>c</sup>
200 + 200	112*b 7.5	45b 33.7	73k 11.5	—	12i 4.9
400 + 200	122*f 18.2	29f 6.8	67f 12.1	32e 19.5	63e 40.8
0 + 400	178*b 29.4	46b 9.1	82b 17.9	55 <sup>c</sup>	72i 21.2
100 + 400	155*b 9.8	42b 5.4	83b 24.7	35k 5.4	144 <sup>c</sup>
200 + 400	128**b 16.7	34b 8.7	68b 14.1	38g 6.6	116e 33.2
400 + 400i	—	—	—	—	—

TABLE A3

Clinical Chemistry Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
<b>Day 60</b>					
0 + 0	91 10.8	28 9.0	63f 20.8	23 <sup>c</sup>	22k 1.0
100 + 0	83 6.4	30 7.3	63 13.1	36k 1.9	41h 30.5
200 + 0	88 9.1	32 9.1	69f 13.5	38i 0.7	40h 28.8
400 + 0	81j 5.6	26j 5.5	57j 14.8	32h 7.0	23h 7.5
0 + 100	122** 9.8	30 2.9	65 14.1	32k 7.4	24k 4.2
100 + 100	123**j 11.8	29j 2.9	63 25.0	36 <sup>c</sup>	36h 23.3
200 + 100	127**j 8.1	36j 7.4	73j 19.8	36e 6.1	27h 3.3
400 + 100	122** 10.2	27 5.3	58 7.5	36h 6.1	27h 5.4
0 + 200	170** 11.8	54 13.7	74f 16.1	38h 1.8	30g 3.5
100 + 200	174**f 8.9	86*f 35.8	97f 23.1	—	38k 10.7
200 + 200	144**b 13.0	36b 6.9	73b 13.8	42e 7.9	45b 7.9
400 + 200	157**k 6.4	44k 7.9	83k 31.9	35i 9.2	37i 4.9
0 + 400	239**f 15.7	208**f 147.9	157**f 109.3	42 <sup>c</sup>	35i 4.9
100 + 400	252**i 4.9	143**i 3.5	100i 15.6	—	85 <sup>c</sup>
200 + 400i	—	—	—	—	—
400 + 400i	—	—	—	—	—

TABLE A3

Clinical Chemistry Data for Male Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu$ mol/L)
Days 92 to 95					
0 + 0	80 17.4	22 5.6	67b 15.8	27 <sup>c</sup>	30 <sup>c</sup>
100 + 0	78j 17.1	22j 7.2	78j 21.6	39e 10.5	23i 4.9
200 + 0	74f 10.1	17f 7.2	68g 16.5	22 <sup>c</sup>	20 <sup>c</sup>
400 + 0	72j 15.0	22j 7.2	75f 22.1	39k 11.6	31g 18.6
0 + 100	135***f 15.1	26f 4.3	70b 17.0	28i 2.8	56i 49.5
100 + 100	128** 8.9	30 6.8	75f 13.0	36e 5.5	27k 7.2
200 + 100	140** 11.3	27 4.8	69b 16.4	30 <sup>c</sup>	32h 12.5
400 + 100	139** 10.4	24 4.6	98b 26.2	37e 11.1	29h 2.9
0 + 200	190***j 13.1	69***j 17.0	86g 13.8	43i 1.4	44i 14.8
100 + 200	173** 11.6	50* 14.6	90f 22.3	40i 7.8	30k 14.2
200 + 200	152** 14.4	44 15.4	95 20.5	38i 14.1	32e 10.1
400 + 200	155***e 14.2	63*e 8.0	98e 26.9	50 <sup>c</sup>	28e 22.1
0 + 400	258** 20.0	153** 62.2	148***b 46.4	—	84e 49.4
100 + 400	208***c	96***c	110 <sup>c</sup>	—	—
200 + 400i	—	—	—	—	—
400 + 400i	—	—	—	—	—

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group using analysis of variance followed by Dunnett's test\*\*  $P \leq 0.01$ 

a Daily gavage doses of AZT + rifampicin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=9, unless otherwise noted.

b n=7

c n=1; standard deviation not calculated due to high mortality

d Data were not available due to insufficient number of samples or insufficient sample.

e n=3

f n=8

g n=6

h n=5

i n=2

j n=10

k n=4

l No data were available due to 100% mortality in this group.

TABLE A4

Clinical Chemistry Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations<sup>a</sup>

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
Day 4					
0 + 0	243b	30 <sup>c</sup>	81 <sup>d</sup>	23 <sup>d</sup>	— <sup>e</sup>
	25.2	2.7	19.8	7.1	
100 + 0	202f	33f	84g	—	—
	29.4	10.0			
200 + 0	234b	31f	75h	15 <sup>d</sup>	—
	19.5	6.1	20.1	15.6	
400 + 0	234f	26 <sup>c</sup>	73g	19g	—
	16.6	2.0			
0 + 100	231f	33b	97h	27g	—
	29.9	6.2	28.4		
100 + 100	227	28f	91g	25g	—
	26.9	1.3			
200 + 100	232	30b	82i	14 <sup>d</sup>	—
	50.5	4.1	6.6	13.4	
400 + 100	224b	31	67h	—	—
	24.4	3.5	7.0		
0 + 200	229f	33f	93g	—	—
	28.5	6.3			
100 + 200	231f	37 <sup>c</sup>	—	—	—
	13.1	4.5			
200 + 200	228f	33	88 <sup>d</sup>	20g	—
	26.2	5.0	6.4		
400 + 200	217	34 <sup>c</sup>	85g	21g	—
	32.6	3.5			
0 + 400	259j	41h	—	—	—
	25.4	1.2			
100 + 400	219b	41* <sup>b</sup>	91i	31 <sup>d</sup>	—
	24.0	4.4	21.5	0.0	
200 + 400	221f	40* <sup>k</sup>	87 <sup>d</sup>	28 <sup>d</sup>	—
	22.7	12.3	12.7	2.1	
400 + 400	202h	63* <sup>*i</sup>	—	—	—
	9.0	18.9			

TABLE A4

## Clinical Chemistry Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu$ mol/L)
<b>Day 30</b>					
0 + 0	142 17.0	26 5.2	71j 11.9	33g	79g
100 + 0	150j 17.6	43k 37.2	100g	—	58g
200 + 0	143f 24.3	39f 29.9	66i 22.6	5g	—
400 + 0	140b 17.5	25b 5.2	82k 19.2	—	19 <sup>d</sup> 2.8
0 + 100	163 <sup>c</sup> 17.8	43 <sup>c</sup> 28.9	56h 13.1	—	130g
100 + 100	153 15.7	26 5.0	62i 9.3	21g	—
200 + 100	156b 13.1	24b 2.4	73j 6.0	21g	39 <sup>d</sup> 17.7
400 + 100	147 31.5	22 3.0	62j 13.7	24g	74 <sup>d</sup> 47.4
0 + 200	193 <sup>**</sup> 26.5	34 8.7	79k 6.6	23g	40 <sup>d</sup> 1.4
100 + 200	158 14.3	27 3.7	77 <sup>c</sup> 23.2	43 <sup>d</sup> 9.9	109 <sup>d</sup> 41.0
200 + 200	141b 30.3	36b 21.1	61f 14.9	18j 7.8	108h 43.2
400 + 200	135f 15.7	25f 4.9	57k 6.9	25h 3.1	137h 52.5
0 + 400	200 <sup>**c</sup> 19.9	82 <sup>**c</sup> 76.7	103 <sup>**i</sup> 15.8	—	—
100 + 400	173* 17.1	59* 19.4	73f 20.0	38 <sup>d</sup> 12.0	—
200 + 400	161k 12.2	35k 6.4	55k 4.4	27 <sup>d</sup> 0.0	—
400 + 400	169 <sup>d</sup> 38.2	56 <sup>d</sup> 19.8	77g	—	—

TABLE A4

## Clinical Chemistry Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
<b>Day 60</b>					
0 + 0	135	34	97f	29 <sup>d</sup>	34 <sup>d</sup>
	9.5	17.7	20.4	0.7	10.6
100 + 0	129	30	78b	29j	24i
	15.8	10.8	20.7	3.1	3.1
200 + 0	135b	30b	76b	27i	28i
	21.9	9.5	17.5	5.6	7.7
400 + 0	121	40	73f	26j	33j
	14.4	45.2	7.4	3.4	17.0
0 + 100	139	26	75	20k	33 <sup>c</sup>
	13.9	3.5	19.2	6.2	8.1
100 + 100	136	25	67	22j	43i
	15.9	3.7	21.2	7.3	20.1
200 + 100	132	48	79	32j	44j
	12.4	59.7	19.8	4.7	10.1
400 + 100	136i	27i	72i	26h	32h
	8.5	1.4	15.6	7.6	13.9
0 + 200	166**	32	74	27i	45j
	12.7	5.8	17.9	9.7	5.3
100 + 200	149b	28b	69b	26k	68 <sup>c</sup>
	12.5	6.7	15.5	4.5	40.9
200 + 200	129g	29g	108g	39g	37g
400 + 200i	—	—	—	—	—
0 + 400	197**	75	98	44* <sup>c</sup>	73b
	27.5	26.4	20.8	7.8	34.4
100 + 400i	—	—	—	—	—
200 + 400i	—	—	—	—	—
400 + 400i	—	—	—	—	—

TABLE A4

## Clinical Chemistry Data for Female Mice in the 13-Week Toxicity Study of AZT and Rifampicin Combinations

Dose	ALP (U/L)	ALT (U/L)	AST (U/L)	SDH (U/L)	Bile Acids ( $\mu\text{mol/L}$ )
Days 92 to 95					
0 + 0	112 10.9	20 4.3	80j 15.7	—	30h 8.5
100 + 0	115f 8.5	19f 3.7	77 <sup>c</sup> 11.9	—	18g
200 + 0	109 <sup>c</sup> 14.5	19 <sup>c</sup> 5.5	74k 24.9	21g	32 <sup>d</sup> 9.2
400 + 0	108b 19.0	21b 10.5	90f 23.3	37g	40h 31.2
0 + 100	136 <sup>c</sup> 11.7	19 <sup>c</sup> 2.6	67j 12.9	—	35 <sup>d</sup> 2.8
100 + 100	131f 16.2	22f 10.2	77 <sup>c</sup> 17.1	27 <sup>d</sup> 6.4	39i 18.5
200 + 100	125f 9.1	20f 10.3	79 <sup>c</sup> 23.7	32h 12.5	33h 4.7
400 + 100	124 <sup>c</sup> 22.9	15 <sup>c</sup> 4.0	79 <sup>c</sup> 32.9	31i 5.7	46j 27.2
0 + 200	152 <sup>**b</sup> 16.6	29b 6.3	86 <sup>c</sup> 20.2	38 <sup>d</sup> 3.5	34h 5.0
100 + 200	162 <sup>**</sup> 25.9	29 16.1	82 18.8	34 <sup>c</sup> 7.1	75b 43.0
200 + 200	183 <sup>**i</sup> 25.9	35i 19.2	143i 70.9	51 <sup>d</sup> 3.5	80i 26.4
400 + 200i	—	—	—	—	—
0 + 400	182 <sup>**b</sup> 32.7	85 <sup>**b</sup> 57.6	112f 63.8	—	—
100 + 400i	—	—	—	—	—
200 + 400i	—	—	—	—	—
400 + 400i	—	—	—	—	—

\* Significantly different ( $P \leq 0.05$ ) from the vehicle control group using analysis of variance followed by Dunnett's test

\*\*  $P \leq 0.01$

a Daily gavage doses of AZT + rifampicin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=10, unless otherwise noted

b n=9                      c n=7                      d n=2

e Data were not available due to insufficient number of samples or insufficient sample.

f n=8

g n=1; standard deviation not calculated due to high mortality

h n=3                      i n=4                      j n=5                      k n=6

l No data were available due to 100% mortality in this group.

## **Other NIEHS Reports and Publications on Toxicology of AIDS Therapeutics:**

National Institute of Environmental Health Sciences (NIEHS) (1997). Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide Administered by Gavage to Swiss (CD-1<sup>®</sup>) Mice. NIEHS AIDS Therapeutics Toxicity Report No. 1. NIH Publication No. 97-3938. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Institute of Environmental Health Sciences (NIEHS) (1998). Reproductive, Developmental, and General Toxicity Studies of 3'-Azido-3'-Deoxythymidine (AZT), Trimethoprim (TMP)/Sulfamethoxazole (SMX), and Folinic Acid Combinations Administered by Gavage to Swiss (CD-1<sup>®</sup>) Mice. NIEHS AIDS Therapeutics Toxicity Report No. 2. NIH Publication No. 99-3940. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Institute of Environmental Health Sciences (NIEHS) (1999). Reproductive, Developmental, and General Toxicity Studies of 3'-Azido-3'-Deoxythymidine (AZT)/Isoniazid Combinations Administered by Gavage to Swiss (CD-1<sup>®</sup>) Mice. NIEHS AIDS Therapeutics Toxicity Report No. 3. NIH Publication No. 99-3941. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Institute of Environmental Health Sciences (NIEHS) (2000). Reproductive, Developmental, and General Toxicity Studies of 3'-Azido-3'-Deoxythymidine(AZT)/Rifabutin Combinations Administered by Gavage to Swiss (CD-1<sup>®</sup>) Mice. NIEHS AIDS Therapeutics Toxicity Report No. 4. NIH Publication No. 00-3948. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. (In press.)

National Institute of Environmental Health Sciences (NIEHS) (2000). Subchronic Toxicity Studies of 3'-Azido-3'- Deoxythymidine (AZT)/Pyrazinamide Combinations Administered by Gavage to B6C3F<sub>1</sub> Mice. NIEHS AIDS Therapeutics Toxicity Report No. 5. NIH Publication No. 00-3949. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Rao, G.N., Collins, B.J., Giles, H.D., Heath, J.E., Foley, J.F., May, R.D., and Buckley, L.A. (1996). Carcinogenicity of 2',3'-dideoxycytidine in mice. *Cancer Res.* **56**, 4666-4672.

Rao, G.N., Lindamood, C., Heath, J.E., Farnell, D.R., and Giles, H.D. (1998). Subchronic toxicity of human immunodeficiency virus and tuberculosis combination therapies in B6C3F<sub>1</sub> mice. *Toxicol. Sci.* **45**, 113 -127.

Sanders, V.M., Elwell, M.R., Heath, J.E., Collins, B.J., Dunnick, J.K., Rao, G.N., Prejean, D., Lindamood, C., and Irwin, R.D. (1995). Induction of thymic lymphoma in mice administered the dideoxynucleoside ddC. *Fundam. Appl. Toxicol.* **27**, 263-269.

Zhuang, S.-M., Eklund, L.K., Cochran, C., Rao, G.N., Wiseman, R.W., and Soderkvist, P. (1996). Allelotype analysis of 2',3'-dideoxycytidine- and 1,3-butadiene-induced lymphomas in B6C3F<sub>1</sub> Mice. *Cancer Res.* **56**, 3338-3343.

These reports may be accessed at the NIEHS AIDS World Wide Web site:

[http://ntp-server.niehs.nih.gov/Main\\_Pages/AIDS/AIDSpa.html](http://ntp-server.niehs.nih.gov/Main_Pages/AIDS/AIDSpa.html)