NIEHS AIDS Therapeutics
Toxicity Report
Number 3

NIEHS Technical Report
on the Reproductive, Developmental,
and General Toxicity Study of

3′-Azido-3′-Deoxythymidine (AZT) and Isoniazid Combinations

(CAS Nos. 30516-87-1 and 54-85-3)

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

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This report on the reproductive, developmental, and general toxicity studies of 3′-azido-3′-deoxythymidine (AZT) and isoniazid combinations is based primarily on studies that began in March 1993 and ended in April 1993 at Southern Research Institute, Birmingham, Alabama.

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PEER REVIEW

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

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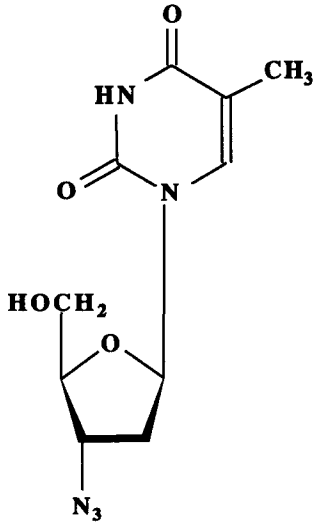
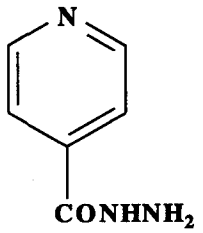
3′-Azido-3′-Deoxythymidine (AZT) and Isoniazid Combinations



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



Isoniazid
Molecular Formula: $C_6H_7N_3C$
Molecular Weight: 137.14
CAS No.: 54-85-3

The toxicity of AZT and isoniazid combination therapy was assessed in Swiss (CD-1®) mice. Gavage doses of AZT (100, 200, or 400 mg/kg) were administered alone or in combination with isoniazid (50, 100, or 150 mg/kg). Male mice (10 per group) were dosed from day 5 until the day prior to sacrifice on day 25 or 26. Prior to dosing (days 0 to 4), the male mice were cohabited with a group of female mice (20 per group), hereafter referred to as female-B mice. The sperm-positive female-B mice were dosed on days 6 through 15 of gestation and sacrificed on scheduled day 4 of lactation. A second group of female mice (20 per group), hereafter referred to as female-A mice, were dosed from day 0 throughout mating on days 9 to 13 until sacrifice on day 18 of gestation. Adult mice were evaluated for clinical findings, mean body weights, and hematologic parameters. Offspring were evaluated for viability, external anomalies, and mean fetal weight.

Administration of isoniazid alone did not produce toxicity in male mice. The primary toxicity observed in male mice administered AZT alone or in combination with isoniazid was anemia. Coadministration of 400 mg/kg AZT + 150 mg/kg isoniazid exacerbated the anemia induced by AZT administered alone. AZT caused a minor decrease in epididymal sperm motility at 400 mg/kg. Isoniazid alone did not have a significant effect on epididymal sperm motility. A significant decrease in sperm motility was caused by 400 mg/kg AZT + 100 mg/kg isoniazid.

In female mice, hematologic, reproductive, and developmental toxicities were observed. In female-A mice, AZT administered alone induced anemia and leukopenia. Coadministration of 150 mg/kg isoniazid with any dose of AZT exacerbated the hematologic toxicity of AZT, with thrombocytosis being induced in addition to anemia and leukopenia. Other than the hematologic toxicity, 100 or 200 mg/kg AZT alone produced minimal, if any, maternal toxicity. The toxicity that was produced (decreased mean body weights and body weight gains) was probably related to the reduced litter sizes, increased resorptions, and reduced pup weights that occurred in these groups, as the mean body weights corrected for gravid uterine weights were not reduced in these two groups. Mice administered 400 mg/kg AZT had both maternal and developmental toxicity.

Isoniazid administered alone at doses as high as 150 mg/kg produced no maternal toxicity. Administration of 50, 100, or 150 mg/kg isoniazid alone produced some developmental toxicity: slight increases in the incidence of dams with any resorptions and percentage of dead or resorbed fetuses per litter.

Both isoniazid and AZT, when administered alone, appeared more toxic to the developing fetus and pup than to adult mice. Doses of 100, 200, or 400 mg/kg of AZT alone and 50, 100, or 150 mg/kg of isoniazid alone produced developmental toxicity. Administered in combination, AZT and isoniazid increased both maternal and developmental toxicity.

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

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Hematology			
AZT alone	Mild anemia	Minimal anemia Mild leukopenia	No significant alterations
Isoniazid alone	No significant alterations	No significant alterations	No significant alterations
AZT + isoniazid	Moderate anemia	Moderate anemia Moderate leukopenia Thrombocytosis	No significant alterations
Histopathology, Spleen			
AZT alone	No significant alterations	No significant alterations	No significant alterations
Isoniazid alone	No significant alterations	No significant alterations	No significant alterations
AZT + isoniazid	No significant alterations	Splenic enlargement	No significant alterations
Reproductive/Developmental			
AZT alone		Minimal maternal and developmental toxicity	Minimal developmental toxicity
Isoniazid alone		Minimal developmental toxicity	Minimal developmental toxicity
AZT + isoniazid		Mild maternal and developmental toxicity	Minimal developmental toxicity
Epididymal Sperm Motility			
AZT alone	Slight decrease		
Isoniazid alone	No significant alterations		
AZT + isoniazid	No significant alterations		

[a] Daily gavage doses of AZT + isoniazid (mg/kg per day)

INTRODUCTION

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

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

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

In a 14-week subchronic study (NTP, 1999), B6C3F$_1$ mice were treated with 0, 25, 50, 100, 400, or 1,000 mg AZT/kg body weight in 0.5% methylcellulose by gavage twice daily. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in 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 after treatment ended, additional groups were administered 0, 50, 400, or 1,000 mg/kg AZT twice daily for 92 days and then held without additional treatment for 29 days. Improvement of hematology parameters indicated recovery of the bone marrow after treatment stopped. An apparently nontoxic, treatment-related clinical finding that affected all AZT-treated B6C3F$_1$ mice was a darkening of the skin on the tail, feet, and/or muzzle.

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

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

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

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

Because AIDS is a disease of immune suppression, the majority of AIDS patients actually die from characteristic opportunistic infections (Hardy, 1991; Harkins and Herriot, 1992). Thus, AIDS is increasingly treated with a combination of antiretroviral and antimicrobial drugs (Goldschmidt and Dong, 1992). One of the opportunistic infections leading to mortality in AIDS patients is tuberculosis (Nolan, 1992). AIDS patients with tuberculosis receive combination therapy with AZT and antituberculosis drugs, the primary one of which is isoniazid. Therapy for tuberculosis involves combinations of multiple antibacterial agents in order to eliminate the strains of organisms inducing the disease, including those resistant to isoniazid. The standard daily regimen is isoniazid (300 mg), rifampin (600 mg, or 450 mg for persons weighing less than 50 kg), and pyrazinamide (20 to 30 mg) for the first 2 months of treatment. Isoniazid and rifampin are continued for another 7 months, for a total therapy duration of 9 months (CDC, 1987; Barnes *et al.*, 1991).

Isoniazid is used alone for chemoprophylaxis of tuberculosis at a daily dose of 5 to 10 mg/kg for 12 months. This therapeutic regimen is relevant to AIDS in that up to 31% of new cases of tuberculosis are subsequently found to be HIV seropositive (Jacobson, 1988). Therefore, it is now recommended that all HIV-seropositive patients, with or without clinical evidence of AIDS, be evaluated for the presence of latent tuberculosis infection and the need for chemoprophylaxis.

Isoniazid is water soluble and is readily absorbed into all body fluids and cells following administration by any route. Peak plasma concentrations of 3 to 5 μg/mL are obtained in humans 1 to 2 hours after an oral dose of 5 mg/kg (*Goodman and Gilman's*, 1990). Thirty minutes after an oral dose of 144 mg/kg in albino mice, mean plasma concentrations of 44 μg/mL isoniazid were achieved (Rubin *et al.*, 1952). The absorption, distribution, and excretion of isoniazid appear to be similar between rodents, dogs, and humans (Rubin and Burke, 1953; Peretti *et al.*, 1987). The drug is excreted in the urine within 24 hours, primarily as acetylisoniazid and isonicotinic acid, products of acetylation and hydrolysis, respectively. Smaller quantities of isonicotinyl conjugates (e.g., glycine), isonicotinyl hydrazones, and N-methylisoniazid are also detected in urine (*Goodman and Gilman's*, 1990). Isoniazid does not appear to accumulate following daily administration for up to 8 months (Rubin and Burke, 1953).

Acetylation by the enzyme N-acetyltransferase represents a major route of metabolism for isoniazid in rodents, dogs, and humans (Timbrell, 1981). The metabolism, pharmacokinetics, and toxicity of isoniazid are determined, in part, by the inheritance of a "rapid" or "slow" acetylator phenotype. The plasma concentrations of acetylisoniazid and isonicotinic acid are increased in rapid acetylators, while concentrations of unchanged isoniazid and hydrazones are increased in slow acetylators. The half-life of isoniazid in humans is approximately 3 hours in slow acetylators and 70 minutes in the rapid phenotype (*Goodman and Gilman's*, 1990). Slow acetylators are more likely to develop isoniazid neurotoxicity, which results from the competitive inhibition of pyridoxal phosphokinase (reviewed by Blakemore, 1986). This enzyme phosphorylates vitamin B6 to form an active coenzyme that participates in a variety of enzymatic reactions. Timbrell *et al.* (1980) reviewed studies showing that the association of acetylator phenotype and hepatotoxicity is less clear. The metabolite acetylhydrazine is transformed by cytochrome P_{450} to an intermediate that covalently bonds to liver proteins. Acetylhydrazine can be further acetylated to diacetylhydrazine, a nontoxic moiety, and acetylation and P_{450}-dependent biotransformation are inhibited by isoniazid. Therefore, hepatotoxicity appears to be dependent upon acetylator phenotype, which will determine the relative concentrations of isoniazid, acetylhydrazine, and diacetylhydrazine and on the relative activities of other metabolic pathways. In mice (or rodents), the acetylator phenotype is strain specific (Tannen and Weber, 1979). Swiss (CD-1®) mice are reported to be slow acetylators compared to Wistar rats (Mate *et al.*, 1981). The authors reported that slow

acetylation resulted in prolonged (18 hours) circulating levels of mono- and di-acetylhydrazine in mice; these metabolites were not detected in rats after 3 hours. The fact that mice, and not rats, develop lung tumors after long-term exposure to isoniazid led the investigators to correlate the slow acetylator phenotype with tumorigenicity.

Isoniazid has been used for the treatment of tuberculosis since 1951; clinical toxicities are fairly well known and include rash (2% incidence), fever (1.2%), jaundice (0.6%), and peripheral neuritis (0.2%) (*Goodman and Gilman's*, 1990). Hypersensitivity, hematological effects (agranulocytosis, eosinophilia, thrombocytopenia, and anemia), vasculitis, arthritic symptoms, and neurologic effects occur at lesser incidences. Hepatotoxicity resulting in death can occur in some individuals. The peripheral neuritis and hepatotoxicity observed clinically with isoniazid use are reproducible in rodents (Timbrell, 1979; Blakemore, 1986).

The following oral lethal doses causing 50% mortality (LD_{50}) for isoniazid in mice were determined by Benson *et al.* (1952): oral, 133 mg/kg; subcutaneous, 177 mg/kg; intravenous, 153 mg/kg; intraperitoneal, 130 mg/kg; and intramuscular, 137 mg/kg. In rats, the oral LD_{50} was 1,435 mg/kg, and in rabbits, the oral LD_{50} was 200 mg/kg and the intravenous LD_{50} 94 mg/kg. Male and female Sprague-Dawley rats were administered 0%, 0.025%, 0.05%, 0.25%, or 0.5% isoniazid in feed for 52 weeks (Harper and Worden, 1966). The authors reported that the 0.5% concentration was equivalent to a daily dose of 500 mg/kg; the majority of animals that received this dose died within a few weeks, and hepatic necrosis and ovarian or testicular hypertrophy were observed. Ovarian or testicular hypertrophy and reduced mean body weight gains were observed at 0.25%. No adverse effects were observed at 0.025% or 0.05%.

The carcinogenic potential of isoniazid in Swiss (CD-1®) mice has been studied by Menon and Bhide (1983). Beginning at 10 weeks of age, male and female mice were treated with 0 or 1.1 mg isoniazid by gavage in distilled water 5 days per week until death. A significant increase in the incidences of adenocarcinoma of the lung were observed in treated males and females. In a second experiment, 1.1 mg/day was administered in distilled water by gavage to pregnant mice throughout gestation and lactation. Eight weeks after weaning, F_1 males and F_1 females were administered 0 (distilled water), 0.55, 1.1, or 2.2 mg isoniazid per day. Four weeks after initiation of treatment, F_1 males and F_1 females were mated. The dose of 2.2 mg/day was highly toxic to the F_1 mice and resulted in mortality; therefore, no mating occurred at this dose. Pregnant F_1 females received isoniazid treatment during gestation and lactation and were then divided into a group that was dosed postweaning and a group that was not. At study termination, the incidences of lung neoplasms were significantly increased in the F_1 mice at all three doses and in both postweaning groups. The F_2 generations from two groups were raised: a) from F_1 mice given 1.1 mg/day with no treatment postweaning, and b) from

F_1 mice given 1.1 mg/day and 0.55 mg/day postweaning. The incidence of lung neoplasms was 11% in F_2 mice from the first group, 70% in F_2 mice from the second group, and 5% in the control group.

Reproductive and developmental toxicity studies of isoniazid have been conducted in Swiss (CD-1®) mice (Menon and Bhide, 1980). Groups of pregnant female mice were administered 0 (distilled water), 1.1, or 2.2 mg/day by gavage during gestation days 1 to 4, gestation days 10 to 13, or gestation days 1 to 19. The results indicated that isoniazid induced early resorptions and embryolethality, but no teratogenicity. Hemorrhages were observed in some of the progeny from the isoniazid-treated dams. In a second experiment (Menon and Bhide, 1983), pregnant females were administered 0 (distilled water), 0.55, 1.1, or 2.2 mg isoniazid per day by gavage during gestation days 1 to 19. There were no adverse effects in the offspring of the dams administered 0.55 mg/day, while embryolethality and reduced mean body weights were observed in the offspring of the dams administered 1.1 or 2.2 mg/day. The F_1 offspring were mated and then dosed during gestation days 1 to 19. Successful mating of the F_1 offspring occurred only in the F_1 group from dams that received 0.55 mg/day. Embryolethality was observed in the F_2 generation of the group administered 0.55 mg/day.

STUDY RATIONALE

This study of the combination of AZT and isoniazid was conducted by the NIEHS as part of its program to evaluate the toxicity of drugs used in the treatment of AIDS or the opportunistic infections accompanying AIDS in pregnant women and in the developing conceptus. Tuberculosis is a frequent complication of AIDS and is commonly treated with isoniazid. Additionally, effects on the motility and density of sperm were also evaluated. The National Toxicology Program has developed a protocol, published elsewhere (Morrissey *et al.*, 1989; Harris *et al.*, 1992), to facilitate the screening of chemicals for reproductive and developmental toxicity. Because the liver may be a target of isoniazid toxicity, based on the effects of isoniazid in humans, liver enzyme determinations were included, depending on sample availability.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3′-Azido-3′-deoxythymidine (AZT; lot 1401-R-7) was manufactured by Raylo Chemicals (Edmonton, Alberta) and supplied as a fine, off-white powder. Isoniazid (lot 7232-74-02) was manufactured by Fluka Chemical Corporation (Ronkonkoma, NY) and supplied as a white crystalline substance. Identification of both compounds was confirmed by nuclear magnetic resonance and infrared spectroscopy. The purity of both AZT and isoniazid was determined to be approximately 99% by high-performance liquid chromatography.

DOSE FORMULATIONS

The required amounts of AZT and isoniazid were combined with the required amount of the vehicle, 0.5% methylcellulose in deionized water. Each dose formulation was stirred until a solution or homogeneous suspension was observed.

Stability studies conducted on 0.5% methylcellulose solutions containing 5.0 mg/mL AZT and 1.5 mg/mL isoniazid indicated no significant loss of either test chemical after storage for 30 days at ambient temperature (23° to 28° C) or refrigerated (2° to 5° C) or for 3 hours at room temperature, open to air and light. Dose formulations of AZT and isoniazid used in this study were stored refrigerated in the dark for up to 20 days.

Samples of formulations from each dose concentration from the first mix indicated all formulations analyzed prior to dosing were within the acceptable range (within 10% of theoretical values) except for one isoniazid dose formulation, which was 87% of the target dose. Animal room samples contained 94% to 105% of the target AZT concentrations and 97% to 118% of the target isoniazid concentrations (3 of 15 samples were outside the acceptable range). The animal room samples that were out of specification were not regarded as having an adverse effect on the results of the study.

STUDY DESIGN

Male and female Swiss (CD-1®) mice were obtained from Charles River Laboratories (Raleigh, NC) and were about 13 weeks of age when the study began. At terminal sacrifice, blood samples were collected from five

male and five female sentinel animals as part of the animal disease screening program. Results indicated all animals were free of viral antibodies.

The mice were housed five males or five females per cage during quarantine before randomization and were individually housed after randomization, except during cohabitation. Solid-bottom polycarbonate cages were used to house the animals. Two animal rooms were used in this study; the average temperature and relative humidity in one room were 22.0° C and 51% and in the other were 22.4° C and 47%.

The design of this study is a modification of a design published elsewhere (Harris *et al.*, 1992). A brief summary of the study design is provided in Table 2. The oral route of administration was selected because it is the route used in humans. The Swiss (CD-1®) mouse was chosen for this study because it is one of the mouse models routinely used for reproductive and developmental toxicity studies by the NIEHS. AZT was administered at concentrations of 100, 200, or 400 mg/kg and isoniazid at concentrations of 50, 100, or 150 mg/kg by gavage in 0.5% methylcellulose. Each drug was given alone and in combination with each concentration of the other drug. Total daily dose volumes of 20 mL/kg were divided into two equal dose volumes of 10 mL/kg that were administered to the animals approximately 6 hours apart. Mice were divided into three groups as follows:

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

Female-A Mice: Twenty females were assigned to each dose group. Female-A mice were dosed from study day 0 to the day prior to sacrifice. Males were cohabited with female-A mice on study days 9 to 13 to identify any effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. During the cohabitation period, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually, and that day was designated as day 0 of gestation. At the end of the cohabitation period, all animals were housed individually. Prior to parturition on day 18 of presumed gestation (study days 28 to 32), all female-A mice were weighed, and blood samples were taken from the retroorbital sinus for hematology and clinical chemistry evaluations.

The female-A mice were then euthanized with CO_2, and necropsy and caesarean section evaluations were conducted. Live fetuses were removed, weighed, anesthetized on ice, and preserved in Bouin's fixative. The uteri of all sperm-negative females were examined for evidence of unsuccessful pregnancy and then press-plated between two heavy plates of glass to visualize implantation sites. Additional endpoints for all female-A mice included gravid uterine weight and number of implantation sites, resorptions, corpora lutea, and dead and live fetuses.

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

Clinical Pathology

All blood samples were collected from the retroorbital sinus under carbon dioxide:oxygen anesthesia. Blood for hematology analyses was collected into a tube containing EDTA, and blood for clinical chemistry analyses was collected in a tube without anticoagulant. Animals were selected in random order for blood collection, and samples were analyzed in the order collected.

Erythrocyte and platelet counts, hematocrit, hemoglobin concentration, mean cell hemoglobin, mean cell volume, mean cell hemoglobin concentration, leukocyte count and differentials, and erythrocyte and platelet morphologies were determined on whole blood using a Technicon H·1™ automated hematology analyzer (Technicon Corporation, Tarrytown, NY). Reticulocytes were counted by a Coulter Model Elite® Flow Cytometer (Coulter Electronics, Inc., Hialeah, FL). Blood smears were prepared to manually verify leukocyte differentials and erythrocyte and platelet morphologies, if necessary.

Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and sorbitol dehydrogenase activities and bile acid concentrations were determined using the Roche Cobas Fara® automated analyzer (Roche Diagnostic Systems, Inc., Montclair, NJ). Priority for clinical chemistry tests was assigned in the order listed above.

Sperm Function Evaluations

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 distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in a 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, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Histopathology

A necropsy was performed on all animals except sperm-negative female-B mice. If gross lesions were present, the tissue was fixed in formalin, trimmed to a maximum thickness of 0.3 cm for processing, embedded in paraffin, sectioned to a thickness of 4 to 6 μm, stained with hematoxylin and eosin, and examined by light microscopy.

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

Study Laboratory
Southern Research Institute, Birmingham, AL

Strain and Species
Swiss (CD-1®) mice

Animal Source
Charles River Laboratories, Raleigh, NC (Area R03)

Time Held Before Studies
17 to 18 days

Average Age When Studies Began
88 to 89 days

Date of First Dose
Male mice: 17 or 24 March 1993
Female-A mice: 12 or 19 March 1993
Female-B mice: 18-22 or 25-29 March 1993

Duration of Dosing
Male mice: day 5 to day prior to sacrifice (day 24 or 25)
Female-A mice: day 0 to day prior to sacrifice (days 27 to 31)
Female-B mice: gestation days 6 through 15

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

Date of Last Dose
Male mice: 5-6 or 12-13 April 1993
Female-A mice: 8-12 or 15-19 April 1993
Female-B mice: 27-31 March or 3-7 April 1993

Necropsy Dates
Male mice: day 25 or 26
Female-A mice: days 28 to 32
Female-B mice: postnatal day 4 (sperm-positive)

Average Age at Necropsy
Male mice: 113 to 115 days
Female-A mice: 116 to 121 days
Female-B mice: 112 to 117 days

Size of Study Groups
Male mice: 10
Female-A mice: 20
Female-B mice: 20

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

Animals per Cage
One animal per cage, except during cohabitation

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

Method of Animal Identification
Tail tattoo

Diet
NIH-07 Open Formula pelleted diet (Zeigler Brothers Inc., Gardners, PA), available *ad libitum*, changed weekly or as necessary

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

Cages
Polycarbonate cages with solid bottom and sides

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

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

Racks
Stainless steel (Lab Products, Maywood, NJ)

Animal Room Environment
Temperature: 20.9° C to 23.6° C
Relative humidity: 37% to 66%
Fluorescent light: 12 hours fluorescent light/day
Room air: minimum of 10 changes/hour

Doses
Daily doses in 0.5% methylcellulose gavage:
0 mg AZT + 0 mg isoniazid per kg body weight per day
0 mg AZT + 50 mg isoniazid per kg body weight per day
0 mg AZT + 100 mg isoniazid per kg body weight per day
0 mg AZT + 150 mg isoniazid per kg body weight per day
100 mg AZT + 0 mg isoniazid per kg body weight per day
100 mg AZT + 50 mg isoniazid per kg body weight per day
100 mg AZT + 100 mg isoniazid per kg body weight per day
100 mg AZT + 150 mg isoniazid per kg body weight per day
200 mg AZT + 0 mg isoniazid per kg body weight per day
200 mg AZT + 50 mg isoniazid per kg body weight per day
200 mg AZT + 100 mg isoniazid per kg body weight per day
200 mg AZT + 150 mg isoniazid per kg body weight per day
400 mg AZT + 0 mg isoniazid per kg body weight per day
400 mg AZT + 50 mg isoniazid per kg body weight per day
400 mg AZT + 100 mg isoniazid per kg body weight per day
400 mg AZT + 150 mg isoniazid per kg body weight per day

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

Method of Sacrifice
Carbon dioxide asphyxiation

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

Necropsy
Complete necropsies were performed on all breeder animals except sperm-negative female-B mice. The epididymis was weighed for all male mice.

Histopathology
Gross lesions of F_0 mice were collected, fixed in 10% formalin, and microscopically examined. Pups were examined externally only.

Clinical Pathology
Hematology and clinical chemistry evaluations were conducted on all animals at terminal sacrifice.
Hematology: erythrocyte and platelet counts, hematocrit, hemoglobin concentration, mean cell hemoglobin, mean cell volume, mean cell hemoglobin concentration, leukocyte count and differentials, and erythrocyte and platelet morphologies
Clinical Chemistry: alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and sorbitol dehydrogenase activities and bile acid concentrations

Sperm Function Evaluations
Sperm samples were collected on all males at terminal sacrifice. The parameters evaluated included spermatid heads, spermatid counts, and motility. The left epididymis and cauda epididymis were weighed.

STATISTICAL METHODS

Paternal and maternal body weights were analyzed using Bartlett's test of homogeneity of variances (Sokal and Rohlf, 1969a) and the analysis of variance (Snedecor and Cochran, 1967a). If Bartlett's test was not significant ($P>0.05$) and the analysis of variance was significant ($P\leq0.05$), then Scheffe's test (Scheffe, 1953) was used to identify the statistical significance of individual groups. If Bartlett's test was significant ($P\leq0.05$), the Kruskal-Wallis test (Sokal and Rohlf, 1969b) was used; in cases in which the Kruskal-Wallis test was significant ($P\leq0.05$), Dunn's (1964) method of multiple comparisons was used to identify the statistical significance of individual groups. These methods were also used to analyze fetal body weight and pup body weight per litter as well as all other evaluations involving continuous data. Observations of delivered and dead fetuses of the female-A dams and the fetuses from female-A dams caesarean-sectioned on an estimated day 14 of gestation were excluded from fetal body weight summaries and statistical analyses.

Group means and standard deviations were calculated for hematology and clinical chemistry parameters and for final mean body and epididymis weights. Epididymis-to-body-weight ratios were also calculated. Final mean body weights of males and females and mean epididymis weights and epididymis-to-body-weight ratios of males for each dosed group were compared to those of the control group by a two-tailed Student's *t*-test. The standard deviations used in the *t*-tests were obtained by pooling the individual values for the control and dosed groups. Hematology and clinical chemistry data were evaluated using Dunnett's test (Dunnett, 1955).

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

Testis and body weight data obtained during sperm function evaluations were analyzed using a two-way analysis of variance. Sperm measurements were analyzed using the nonparametric test for interaction; if the interaction was not statistically significant, averages were taken for each compound over the concentrations of the second compound. Control and dosed group means were compared using either Shirley's (1977) nonparametric multiple comparison procedure if a trend was present or Dunn's (1964) nonparametric multiple comparison procedure if no trend was observed. The application of Shirley's or Dunn's procedure was determined by application of Jonckheere's test (Jonckheere, 1954) for evidence of a dose-related trend. The outlier test of Dixon and Massey (1951) was employed to detect extreme values. Sperm motility was evaluated using the nonparametric comparison of Wilcoxon's rank sum test (Wilcoxon, 1945).

RESULTS AND DISCUSSION

SURVIVAL AND CLINICAL FINDINGS

Male Mice

All males survived to the end of the study with the exception of one death due to a dosing accident in the 100 + 100 mg/kg group. No clinical findings of toxicity were observed in male mice.

Female-A Mice

Five female-A mice died during the study; three of the deaths resulted from dosing accidents. Of the two remaining deaths, one death occurred on day 18 in the 200 + 150 mg/kg group and the other death occurred on day 30 in the group administered 400 + 150 mg/kg; the cause of these two deaths was undetermined. No clinical findings of toxicity were observed in any of the female-A mice.

Female-B Mice

All female-B mice survived to the end of the study. No clinical findings of toxicity were observed in any of the female-B mice.

BODY AND ORGAN WEIGHTS

Male Mice

The mean body weights of male mice were unaffected by the administration of AZT alone or in combination with isoniazid (Figure 1). Absolute right epididymal weights were increased in all dose groups relative to the vehicle controls, and the increases were significant ($P \leq 0.05$) in all groups except the 100 + 0 and 0 + 50 mg/kg groups. The effects of the combined administration of AZT and isoniazid did not appear to be biologically significant, as they were not additive or synergistic (Table 3).

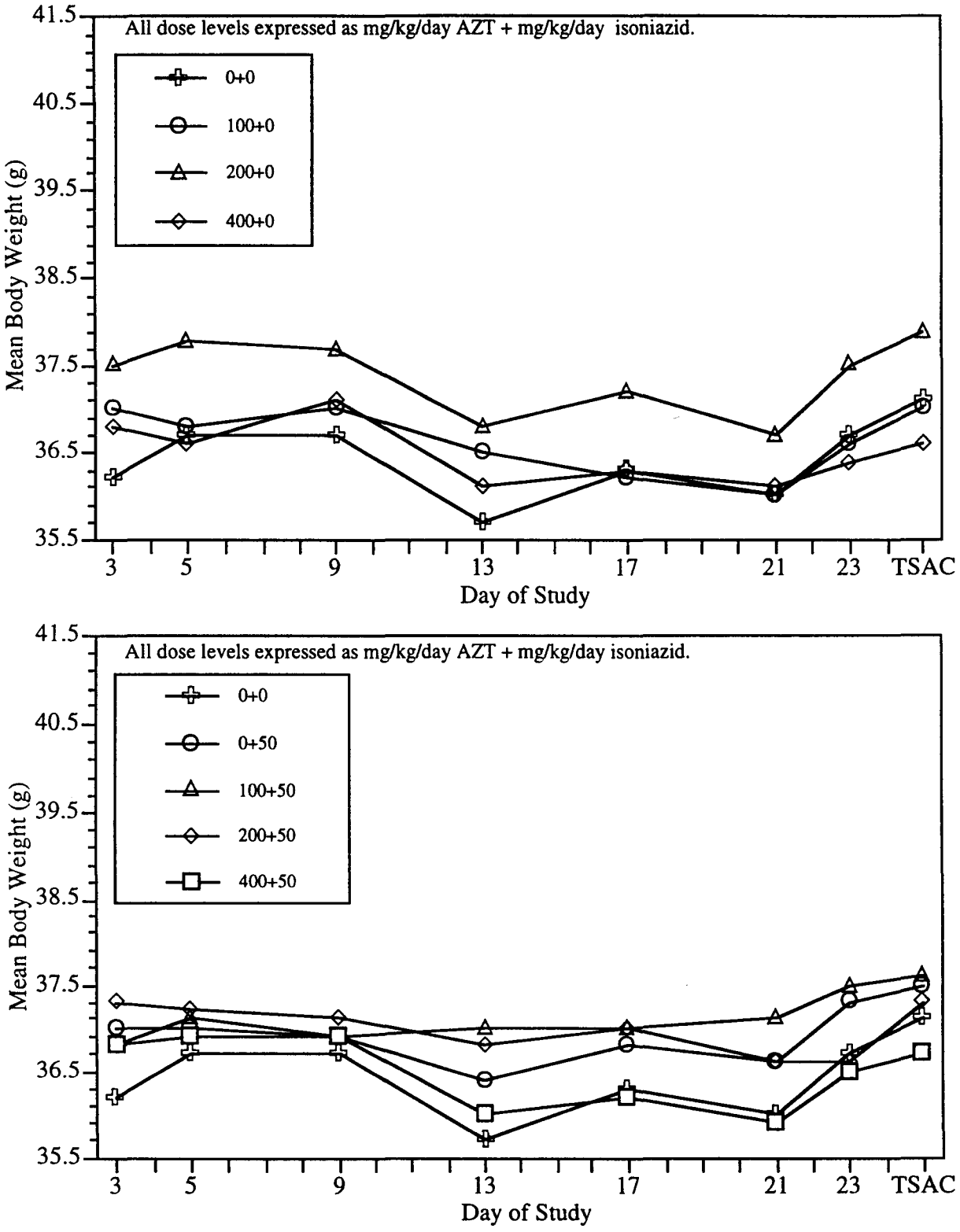


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (TSAC=terminal sacrifice)

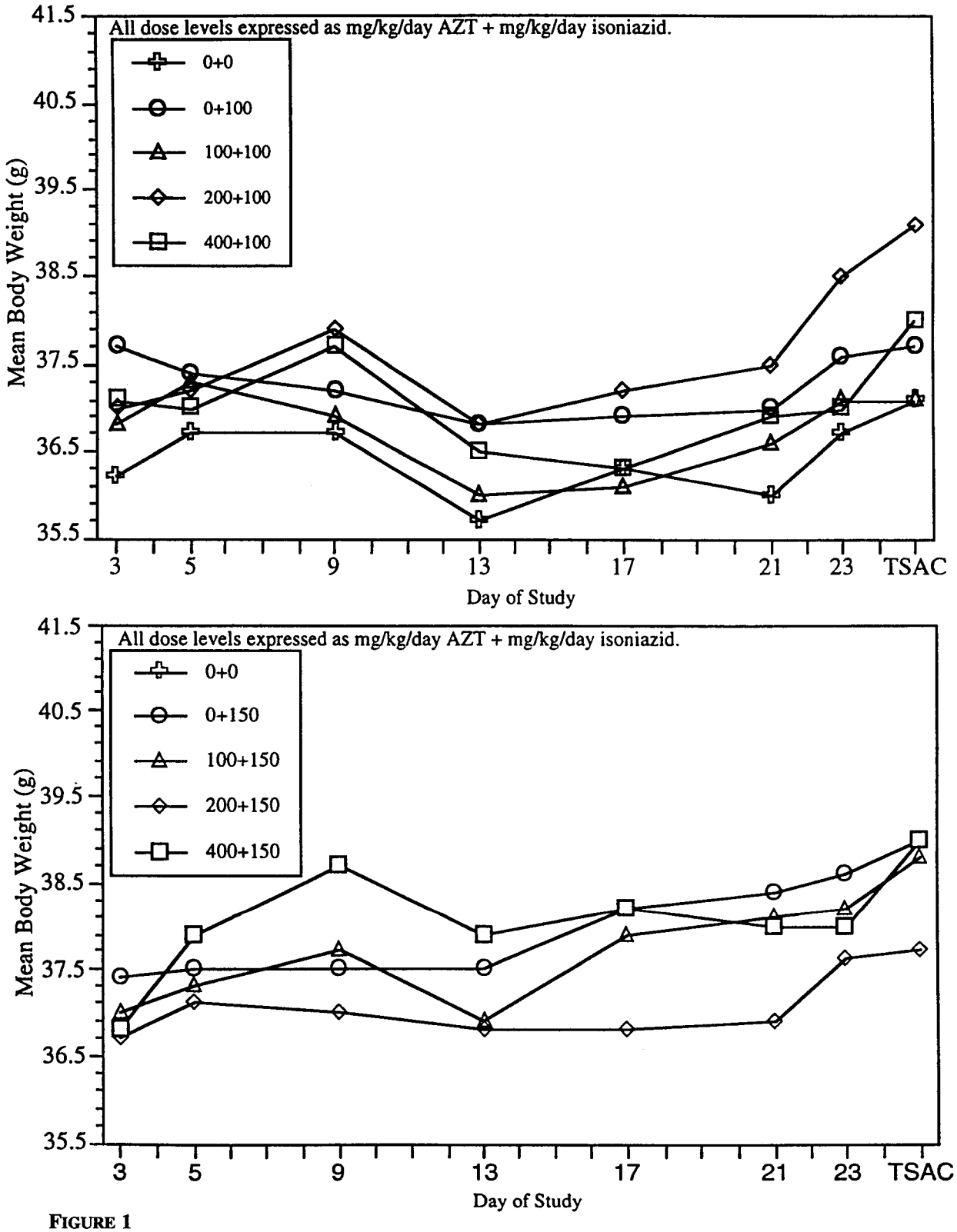


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (TSAC = terminal sacrifice)

TABLE 3
Right Epididymal Weights of Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

Dose[b]	Mean Body Weight	Right Epididymis		
		Absolute	% Difference	Relative
0 + 0	37.12	0.03906 ± 0.00518		1.1 ± 0.1
100 + 0	36.97	0.04122 ± 0.00266	(+5.5)	1.1 ± 0.1
200 + 0	37.88	0.04446 ± 0.00413	(+13.8)*	1.2 ± 0.1*
400 + 0	36.59	0.04659 ± 0.00683	(+19.3)*	1.3 ± 0.2*
0 + 50	37.52	0.04222 ± 0.00278	(+8.1)	1.1 ± 0.1
100 + 50	37.58	0.04362 ± 0.00206	(+11.7)*[c]	1.2 ± 0.0*[c]
200 + 50	37.25	0.04413 ± 0.00528	(+13.0)*	1.2 ± 0.1*
400 + 50	36.69	0.04338 ± 0.00401	(+11.1)*	1.2 ± 0.1*
0 + 100	37.66	0.04335 ± 0.00280	(+11.0)*	1.2 ± 0.1
100 + 100	37.11	0.04574 ± 0.00439	(+17.1)*	1.2 ± 0.1*
200 + 100	39.11*	0.04370 ± 0.00543	(+11.9)*	1.1 ± 0.1
400 + 100	37.97	0.04592 ± 0.00368	(+17.6)*	1.2 ± 0.1*
0 + 150	38.96	0.04229 ± 0.00475	(+8.3)*	1.1 ± 0.1
100 + 150	38.77	0.04652 ± 0.00365	(+19.1)*	1.2 ± 0.1*
200 + 150	37.70	0.04465 ± 0.00278	(+14.3)*	1.2 ± 0.1*
400 + 150	39.00	0.04478 ± 0.00600	(+14.6)*	1.2 ± 0.1*

* Significantly different from the vehicle control group ($P \leq 0.05$) by two-tailed Student's *t*-test

[a] Organ weights (absolute weights) and body weights are given in grams; organ-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

[b] Daily gavage doses of AZT + isoniazid (mg/kg per day)

[c] n=9

Female-A Mice

A decrease in mean body weights was apparent on approximately day 23 of the study in all groups of female-A mice receiving 100, 200, or 400 mg/kg AZT alone or in combination with 50, 100, or 150 mg/kg isoniazid (Figure 2). By day 26, these mean body weight decreases were significant ($P \leq 0.05$) in groups receiving 200 or 400 mg/kg AZT (alone or with isoniazid) compared to the vehicle control group or groups administered isoniazid alone. These decreases reflected reduced gravid uterine weights ($P \leq 0.05$), especially in the female-A mice receiving 200 or 400 mg/kg AZT. Mean gravid uterine weights of female-A mice receiving isoniazid alone were similar to those of the vehicle control group. For all groups of female-A mice administered AZT alone or with isoniazid, final mean body weights were corrected by subtracting the gravid uterine weights. The corrected final mean body weights of dosed and vehicle control females were similar. Thus, the principal AZT effect in female-A mice was upon gravid uterine weights.

Administration of isoniazid at doses up to 150 mg/kg did not produce statistically significant differences in mean body weights, mean body weight gains, terminal body weights, or gravid uterine weights.

Female-B Mice

On gestation day 15, the mean body weight of the 400 + 150 mg/kg group was significantly reduced ($P \leq 0.05$) compared to groups administered isoniazid alone (Figure 3). The 400 + 100 mg/kg group also had a reduced mean body weight compared to the vehicle control group, 0 + 100 mg/kg group, and groups administered 100, 200, or 400 mg/kg AZT + 100 mg/kg isoniazid, but the difference was not statistically significant. Otherwise, mean body weights of female-B mice administered AZT alone, AZT with isoniazid, or isoniazid alone were similar to that of the vehicle control group.

During the lactation period, maternal mean body weights and body weight gains were generally reduced for the 400 mg/kg AZT + 100 or 150 mg/kg isoniazid dams with surviving litters compared to the vehicle control group and the groups administered isoniazid alone.

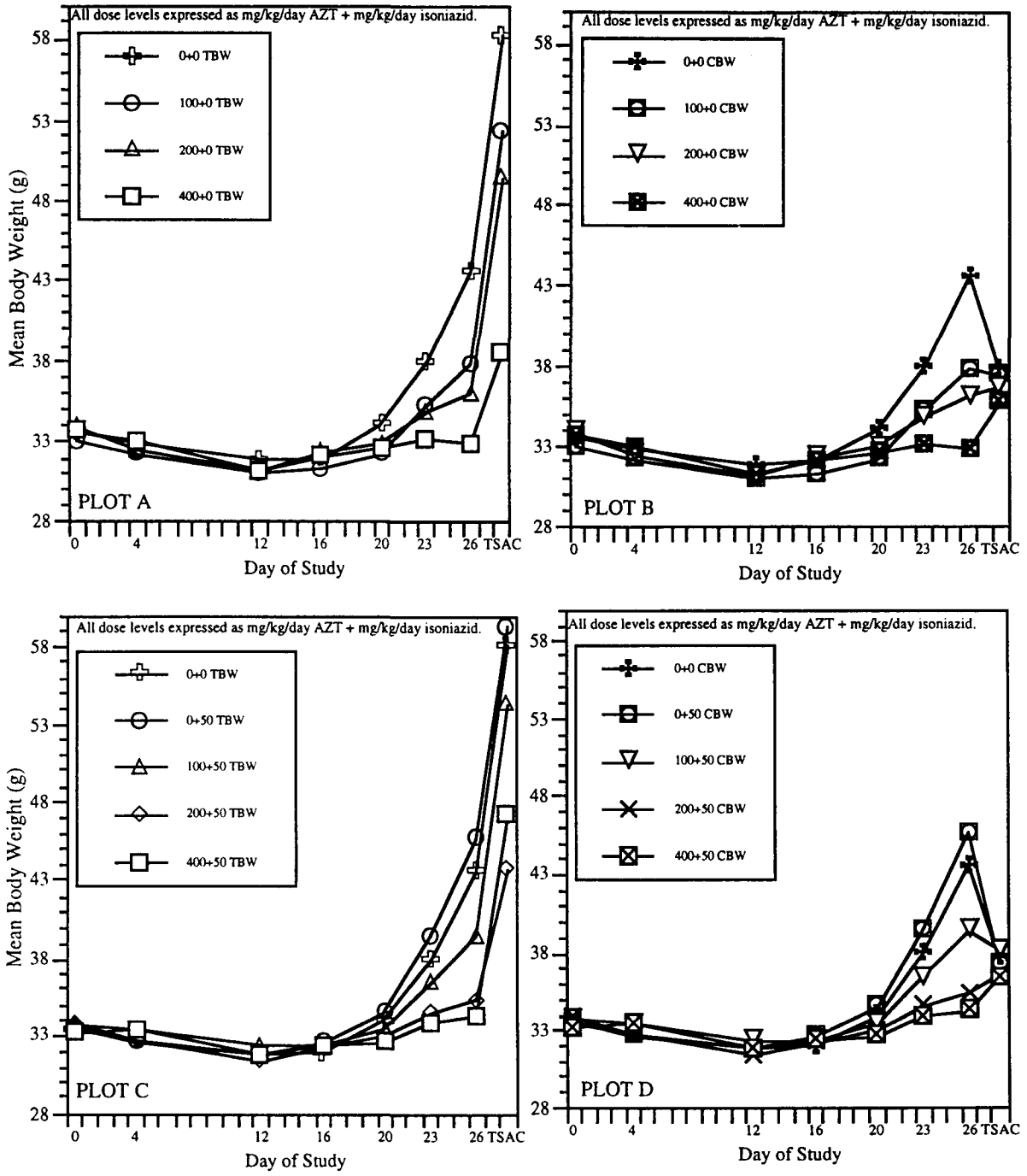


FIGURE 2

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (Plots A and C show terminal body weights [TBW] from day 0 through terminal sacrifice [TSAC]. Plots B and D show corrected body weights [CBW; without gravid uterus] at TSAC. TBW and CBW include only values for dams that were actually pregnant and that were sacrificed and caesarean-sectioned on presumed day 19 of gestation.)

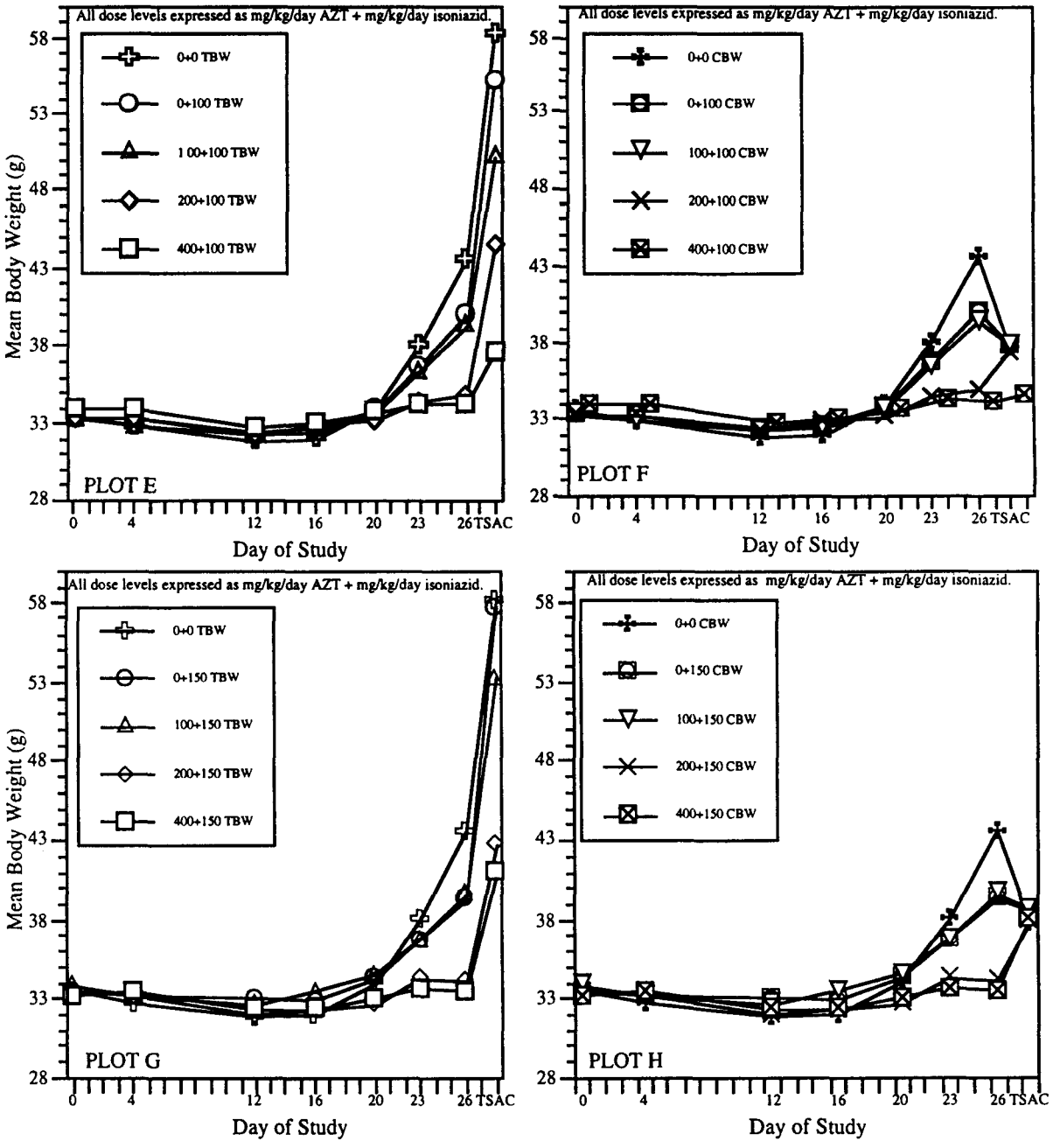


FIGURE 2

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (Plots E and G show terminal body weights [TBW] from day 0 through terminal sacrifice [TSAC]. Plots F and H show corrected body weights [CBW; without gravid uterus] at TSAC. TBW and CBW include only values for dams that were actually pregnant and that were sacrificed and caesarean-sectioned on presumed day 19 of presumed gestation.)

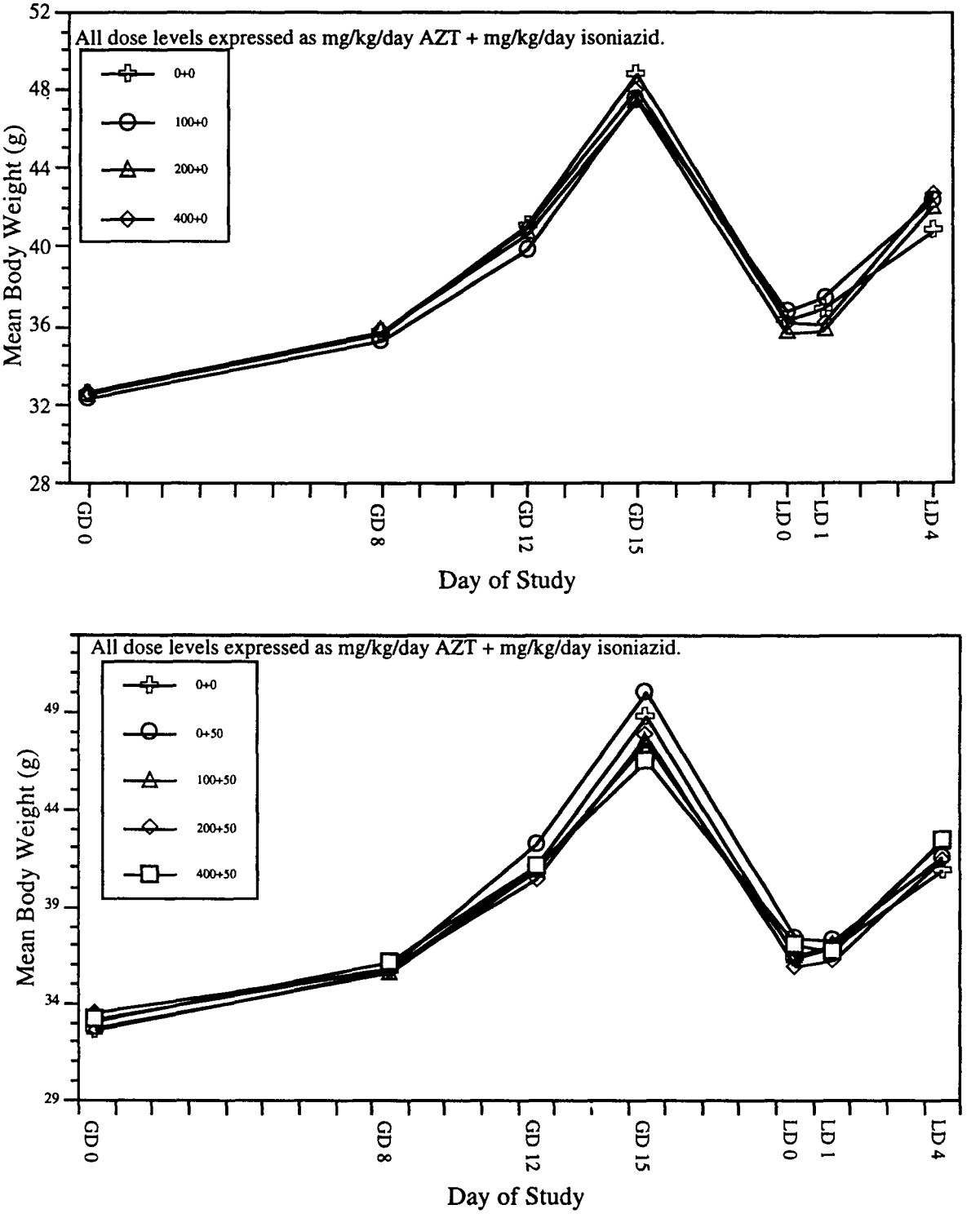
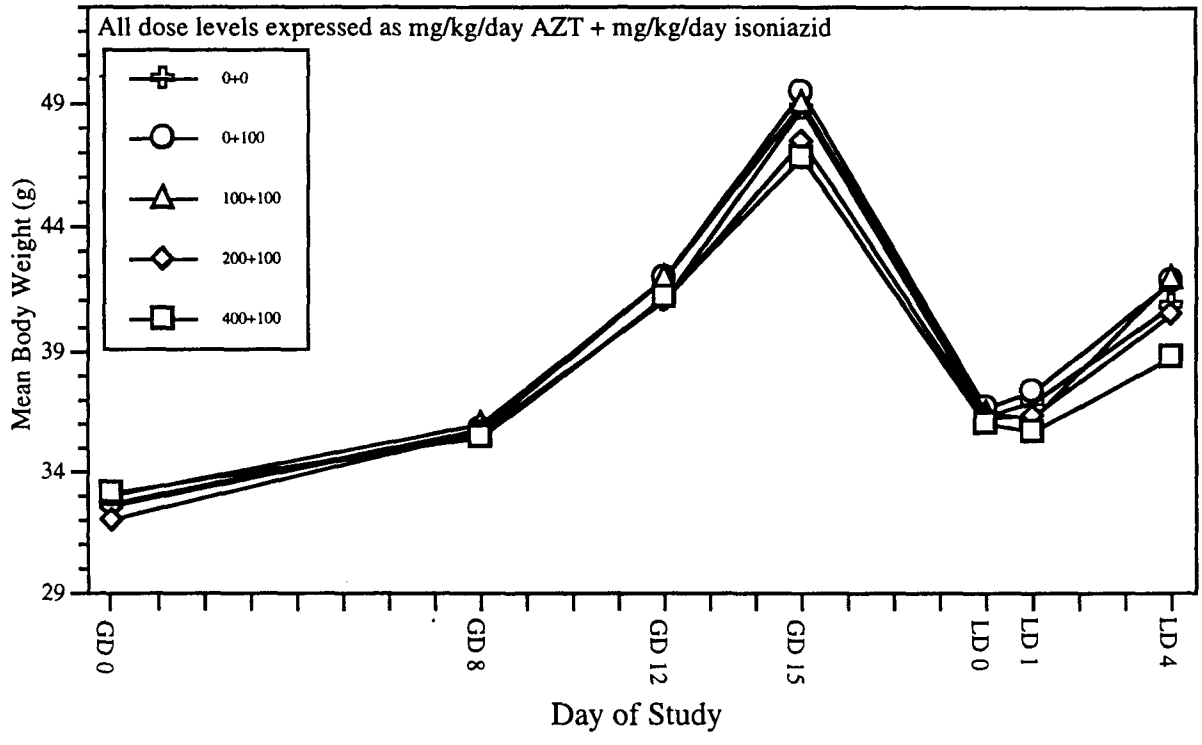


FIGURE 3
Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (GD=gestation day; LD=lactation day.
Note: Mean body weights include only values for those dams that were actually pregnant, that survived to LD 4, and that delivered pups that survived to LD 4.)



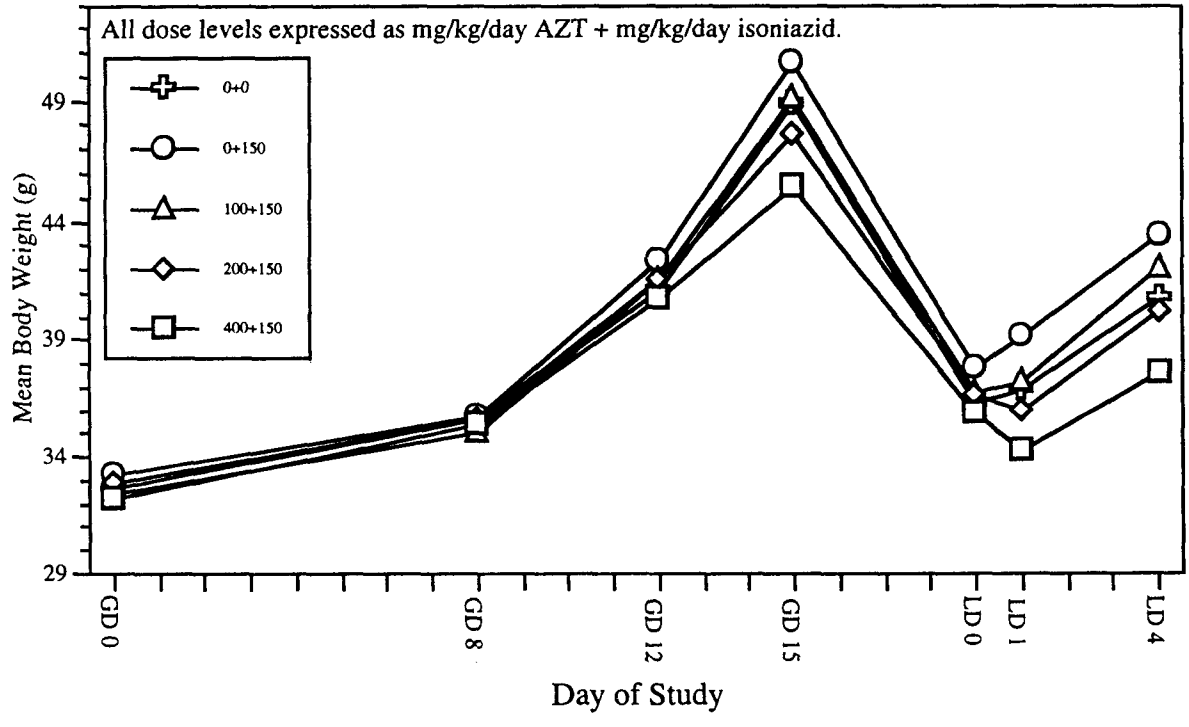


FIGURE 3

Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations in Swiss (CD-1®) Mice (GD=gestation day; LD=lactation day. Note: Mean body weights include only values for those dams that were actually pregnant, that survived to LD 4, and that delivered pups that survived to LD 4.)

CLINICAL PATHOLOGY

Hematology

Male Mice

Administration of AZT alone to male mice produced significant decreases in erythrocyte (RBC) counts ($P \leq 0.01$) and hemoglobin (Hgb) concentrations ($P \leq 0.05$), slight decreases in hematocrit (Hct) values, and slight increases in platelet counts (Figures 4 through 7 and Table A1). Respective RBC counts for the 100, 200, and 400 mg/kg AZT groups were approximately 15%, 14%, and 18% less than the RBC count of the vehicle control group.

Isoniazid administered alone did not result in biologically significant alterations in any of the parameters evaluated. The statistically significant increase in the reticulocyte count ($P \leq 0.01$) of males administered 150 mg/kg isoniazid alone was not considered biologically significant because there was no evidence of anemia in this group (Table A1).

The reduction in RBC counts observed in males administered AZT alone was more pronounced in males administered AZT in combination with 150 mg/kg isoniazid. The administration of 100, 200, or 400 mg/kg AZT + 150 mg/kg isoniazid resulted in RBC counts that were 21%, 25%, or 36% less ($P \leq 0.01$) than that of the vehicle control group, respectively. In general, Hgb concentrations and Hct values paralleled the decreases in RBC counts and were accompanied by marginal elevations in mean cell volume (MCV) and mean cell hemoglobin (MCH) values for combined doses of AZT and isoniazid. Variations in cell size and shape consistent with anemia were evident. Respective platelet counts (Figure 7 and Table A1) of the 100, 200, and 400 mg/kg AZT + 150 mg/kg isoniazid groups were 19%, 29%, and 56% greater ($P \leq 0.01$) than the platelet count of the vehicle control group. Sporadic elevations in reticulocyte counts were observed; however, the inconsistency of this finding precluded any interpretations. No biologically significant alterations were detected in any of the other hematologic parameters evaluated in male mice in this study (Table A1).

Female-A Mice

Female-A mice administered AZT alone developed marginally reduced RBC counts (Figure 4 and Table A2) accompanied by minor decreases in Hgb concentrations and Hct values and slightly elevated ($P \leq 0.01$) MCV and MCH values. A 24% reduction occurred in the leukocyte (WBC) count of the 400 + 0 mg/kg group compared to that of the vehicle control group (Figure 8 and Table A2). Administration of isoniazid alone did not result in any biologically significant alterations (Table A2).

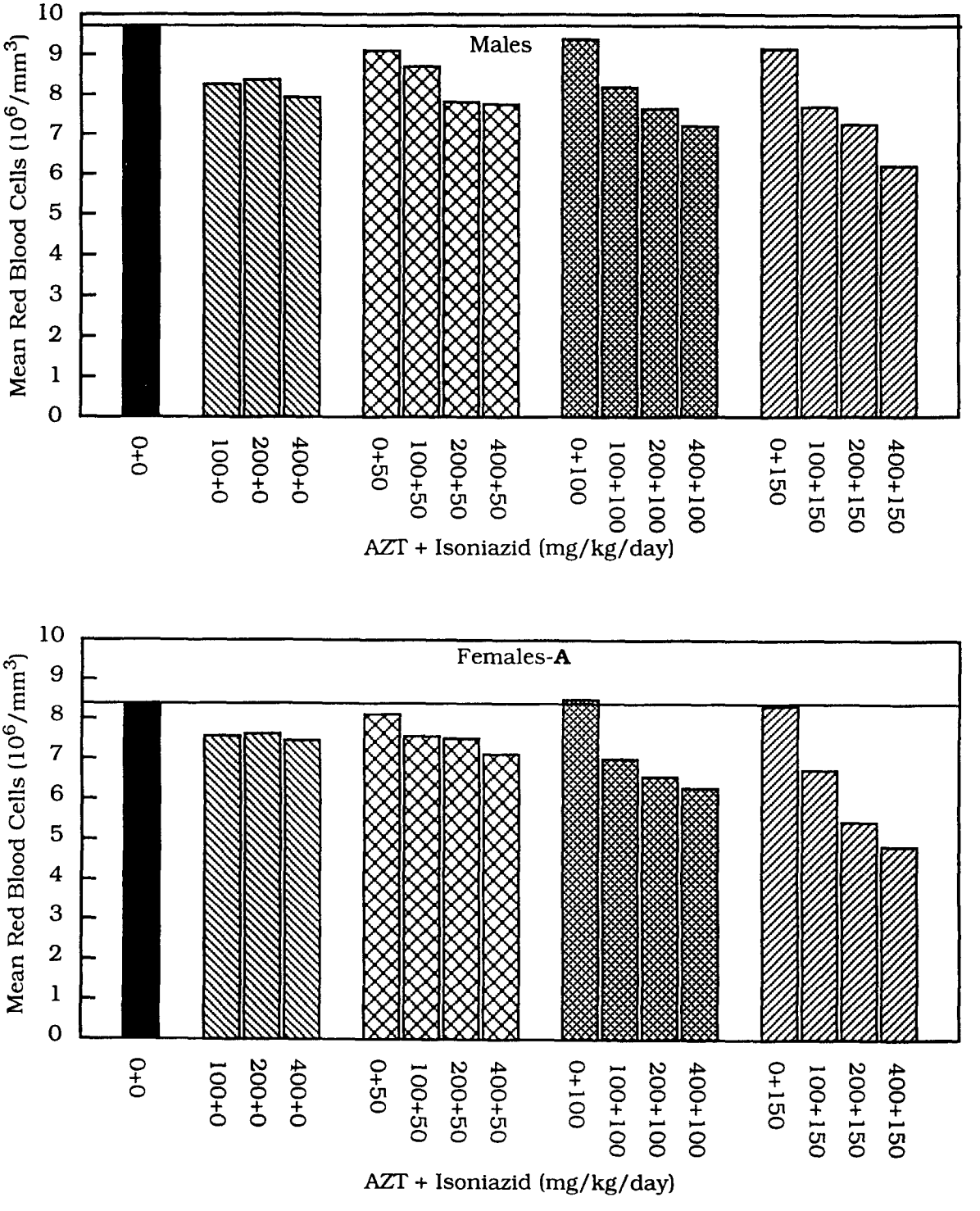


FIGURE 4
Mean Erythrocyte Values of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations (For statistically significant differences, see Tables A1 and A2.)

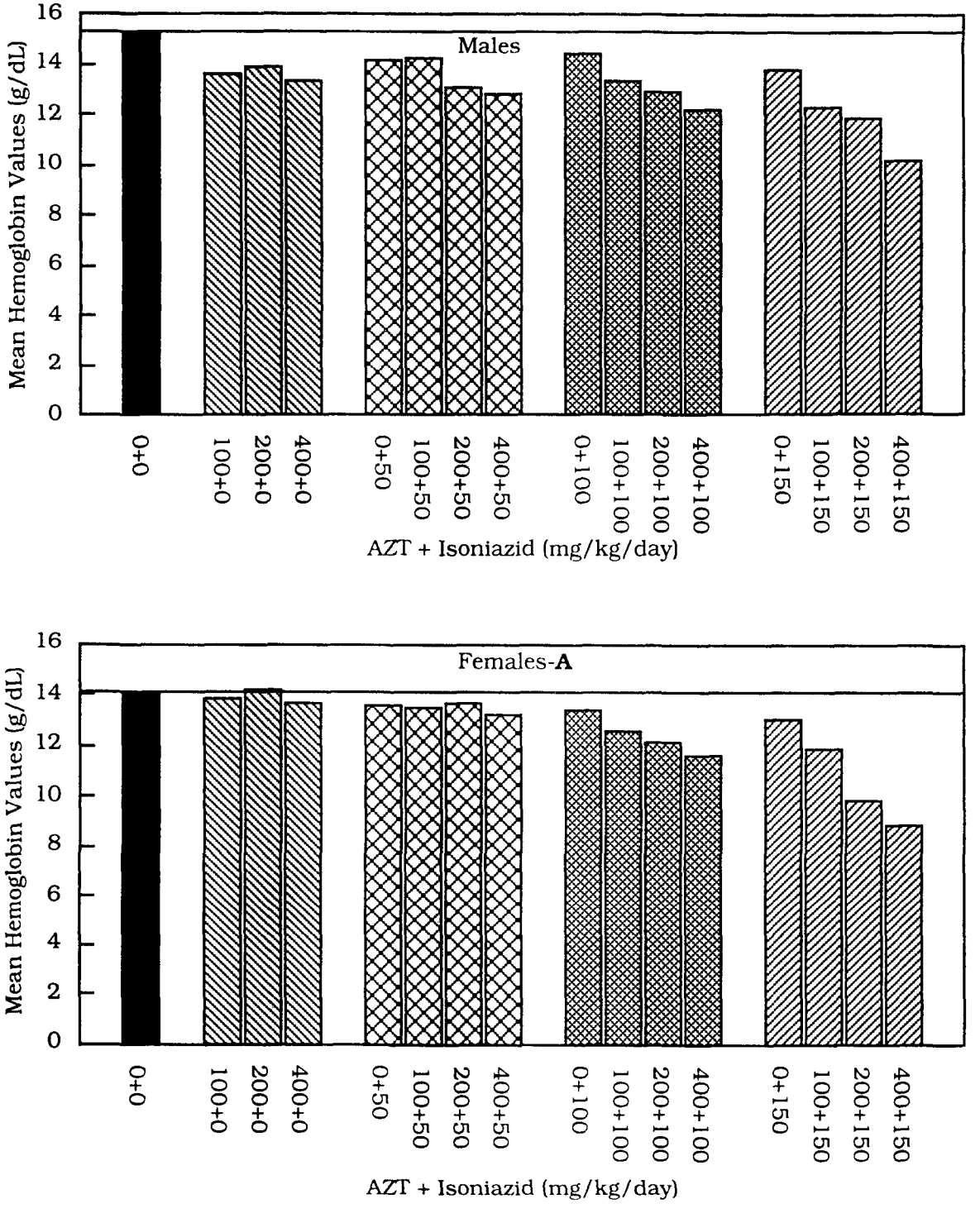


FIGURE 5
Mean Hemoglobin Values of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations (For statistically significant differences, see Tables A1 and A2.)

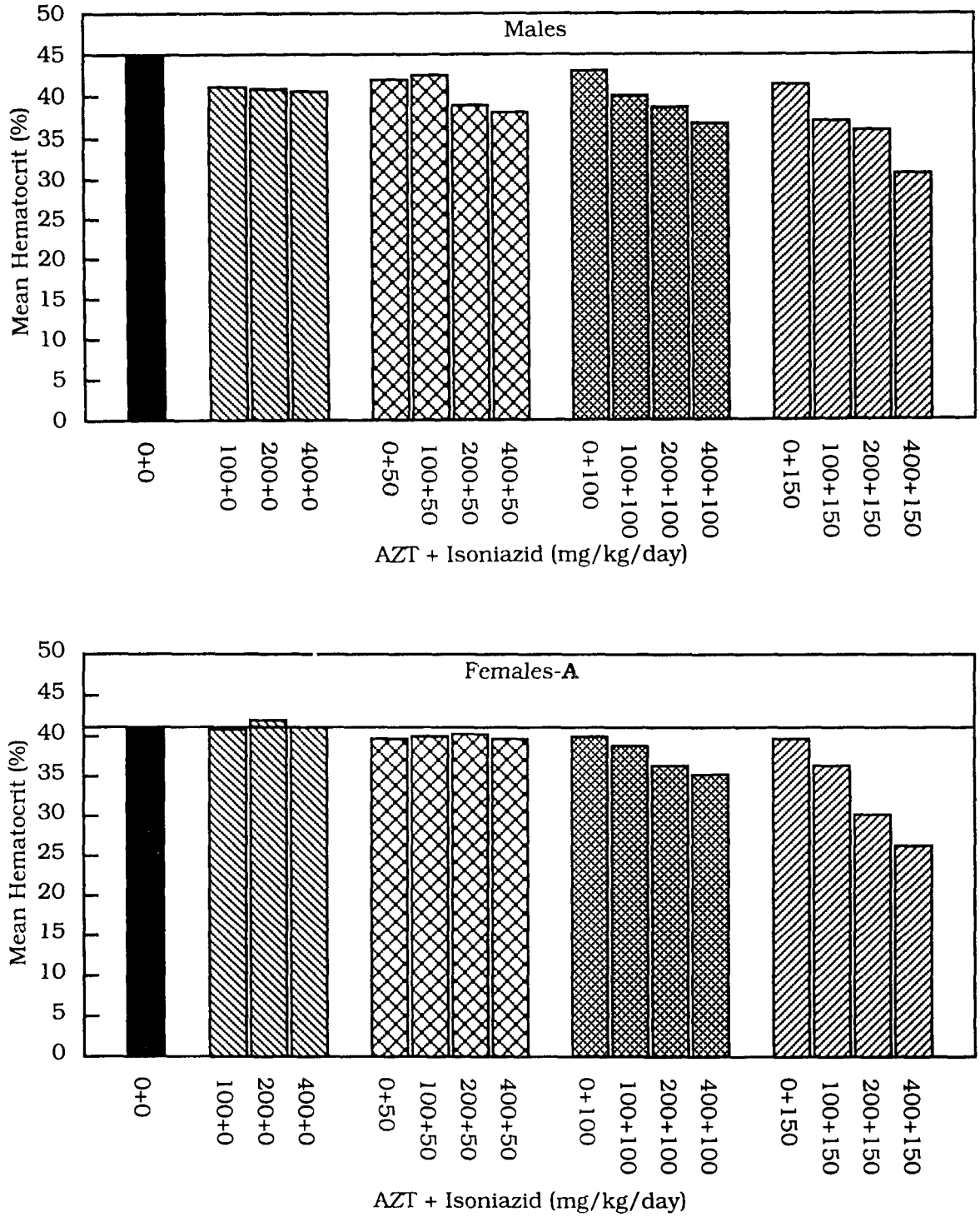


FIGURE 6
Mean Hematocrit Values of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations (For statistically significant differences, see Tables A1 and A2.)

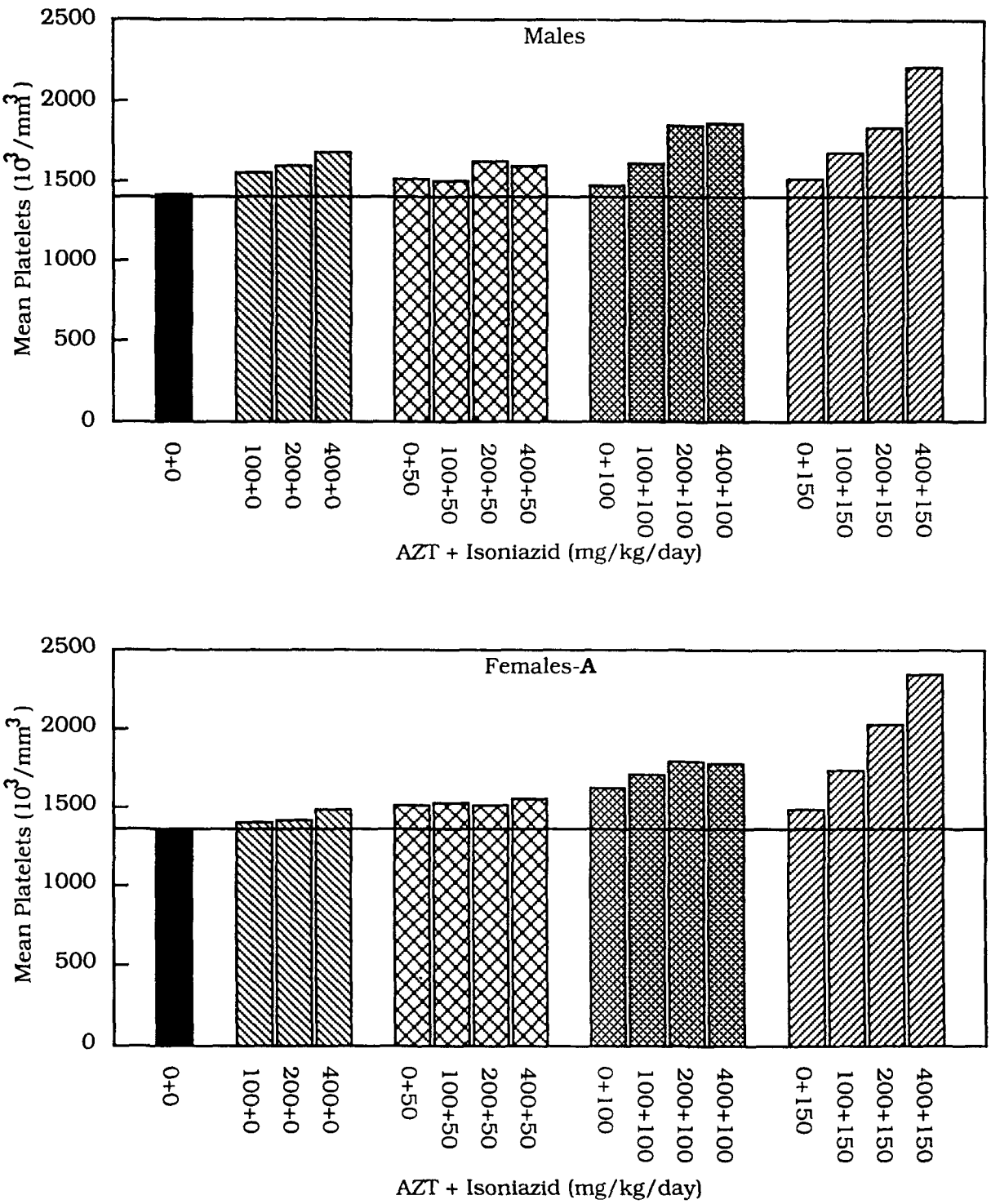


FIGURE 7
Mean Platelet Values of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations (For statistically significant differences, see Tables A1 and A2.)

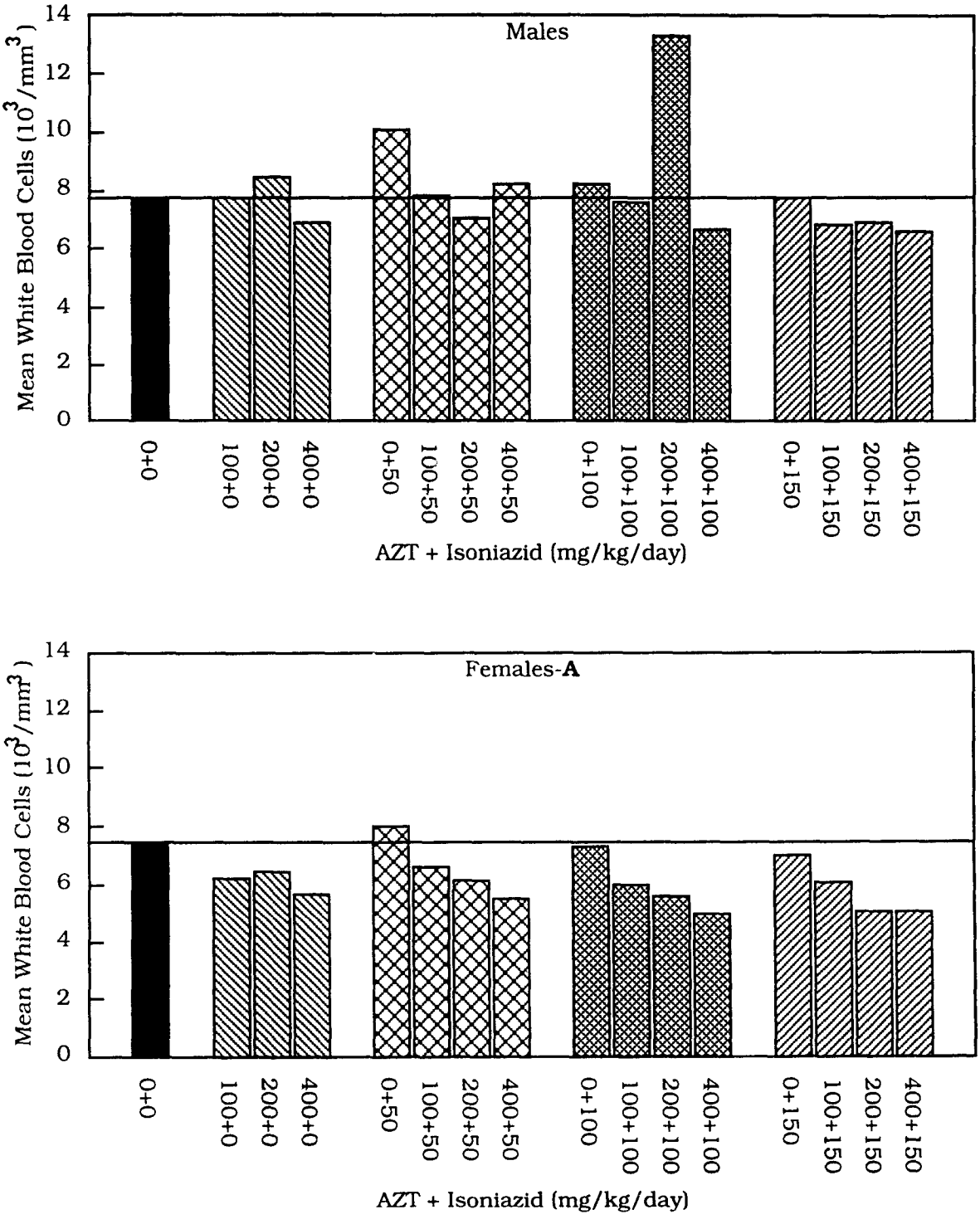


FIGURE 8
Mean Leukocyte Values of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations (For statistically significant differences, see Tables A1 and A2.)

In female-A mice administered AZT in combination with 150 mg/kg isoniazid, the effect on RBC counts was magnified. Respective RBC counts of the 100, 200, and 400 mg/kg AZT + 150 mg/kg isoniazid groups were approximately 20%, 34%, and 42% less ($P \leq 0.01$) than the RBC count of the vehicle controls. Hgb concentrations and Hct values were significantly reduced ($P \leq 0.01$) in the 200 and 400 mg/kg AZT + 150 mg/kg isoniazid groups (Table A2).

The mild leukopenia observed in female-A mice administered AZT alone was increased in groups administered AZT in combination with isoniazid; 400 + 150 mg/kg resulted in a 32% reduction ($P \leq 0.01$) in the WBC count compared to that of the vehicle control group (Figure 8 and Table A2). Evaluation of the corresponding WBC differentials revealed that the leukopenia was predominantly myelocytic in origin.

A prominent dose-related thrombocytosis was evident in the female-A mice administered both AZT and isoniazid. Respective platelet counts (Figure 7 and Table A2) of the 100, 200, and 400 mg/kg AZT + 150 mg/kg isoniazid groups were approximately 27% ($P \leq 0.05$), 49% ($P \leq 0.01$), and 72% ($P \leq 0.01$) greater than the mean platelet count of the vehicle control group. No biologically significant alterations were detected in any of the other parameters evaluated (Table A2).

Female-B Mice

Biologically significant alterations were not detected in any of the hematologic parameters evaluated in female-B mice treated with AZT alone, isoniazid alone, or combinations of AZT and isoniazid (Table A3).

Clinical Chemistry

Biologically significant alterations were not detected in any of the clinical chemistry parameters evaluated in male, female-A, or female-B mice treated with AZT alone, isoniazid alone, or combinations of AZT and isoniazid. AZT and isoniazid each appeared to cause a slight decrease in alanine aminotransferase in female-A mice. These changes were minimal, however, with most values within normal physiological ranges.

NECROPSY OBSERVATIONS

Splenic enlargement occurred in various treatment groups of males and females. This finding was observed in no more than one animal in any of the dosed groups with the exception of the female-A group administered 200 + 150 mg/kg; three incidences occurred in this group. Because splenic enlargement did not occur in the

vehicle control group, it may be associated with the administration of either AZT or isoniazid. Other gross findings were considered incidental in nature, and none were examined microscopically.

SPERM FUNCTION EVALUATIONS

AZT alone at 100 and 200 mg/kg and isoniazid alone at 50, 100, and 150 mg/kg did not affect epididymal sperm motility (Table 4). A significant ($P \leq 0.05$) decrease in epididymal sperm motility was caused by 400 mg/kg AZT + 100 mg/kg isoniazid. Slight decreases in sperm motility were caused by 400 mg/kg AZT alone and in combination with 50 or 150 mg/kg isoniazid, but these decreases were not significant ($P > 0.05$).

Other parameters, including left caudal, epididymal, and testicular weights; epididymal sperm counts; spermatid heads per testis; and total spermatid heads per gram testis, were not affected by AZT alone, isoniazid alone, or the combinations of AZT and isoniazid tested in this study.

TABLE 4
Epididymal Sperm Motility in Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

Dose[b]	Motility (%)
0 + 0	73.76 ± 5.26
100 + 0	72.88 ± 2.24
200 + 0	72.81 ± 6.78
400 + 0	58.36 ± 6.04
0 + 50	80.34 ± 2.14
100 + 50	75.41 ± 4.27
200 + 50	67.93 ± 4.07
400 + 50	62.53 ± 8.00
0 + 100	78.71 ± 2.03
100 + 100	79.64 ± 2.23[c]
200 + 100	70.87 ± 4.78
400 + 100	45.09 ± 8.21*
0 + 150	80.55 ± 1.54
100 + 150	69.46 ± 4.25
200 + 150	71.52 ± 4.08
400 + 150	53.59 ± 8.77

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Wilcoxon rank sum test
[a] Data are given as mean ± standard error; 10 animals per group were examined unless otherwise noted.
[b] Daily gavage doses of AZT + isoniazid (mg/kg per day)
[c] n=9

NATURAL DELIVERY DATA

Treatment with AZT and/or isoniazid had no significant effects on the pregnancy of female-B mice (Table 5). All of the pregnant mice delivered litters except one mouse in the 100 + 50 mg/kg group, two mice in the 400 + 100 mg/kg group, and four mice in the 400 + 150 mg/kg group.

TABLE 5
Occurrence of Pregnancy in Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

Dose[a]	Number per Group	Number Pregnant (%)	Number Delivered (%)
0 + 0	14	14 (100%)	14 (100%)
100 +0	13	12 (92.3%)	12 (100%)
200 + 0	14	11 (78.6%)	11 (100%)
400 + 0	14	12 (85.7%)	12 (100%)
0 + 50	13	13 (100%)	13 (100%)
100 + 50	14	14 (100%)	13 (92.8%)
200 + 50	14	13 (92.8%)	13 (100%)
400 + 50	14	14 (100%)	14 (100%)
0 + 100	14	14 (100%)	14 (100%)
100 + 100	14	13 (92.8%)	13 (100%)
200 + 100	14	13 (92.8%)	13 (100%)
400 + 100	14	13 (92.8%)	11 (84.6%)
0 + 150	13	12 (92.3%)	12 (100%)
100 + 150	13	11 (84.6%)	11 (100%)
200 + 150	14	11 (78.6%)	11 (100%)
400 + 150	13	13 (100%)	9 (69.2%)

[a] Daily gavage doses of AZT + isoniazid (mg/kg per day)

Among the dosed groups, the mean live litter size was similar to that of the vehicle control group except for significant reductions ($P \leq 0.05$) in the groups receiving 200 or 400 mg/kg AZT + 150 mg/kg isoniazid (Table 6). Administration of AZT alone, isoniazid alone, or AZT in combination with isoniazid produced no effect on any parameters evaluated except in the 400 + 150 mg/kg group for the following parameters: duration of gestation, mean live litter size, and cumulative litter survival. Mean live litter size was also decreased ($P \leq 0.05$) in the 200 + 150 mg/kg group.

TABLE 6
Summary of Natural Delivery Litter Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

Dose[a]	Duration of Gestation	Dams with Stillborn Pups (% of Dams that Delivered)	Dams with All Pups Dying on Days 0-4 (% of Dams with Liveborn Pups)	Mean Live Litter Size	Pups Dying on Days 1-4/Total Alive on Day 1 (%)	Survival/Live Litter Size[b] on Day 4 Postpartum
0 + 0	20.1	0 (0.0%)	0 (0.0%)	12.4	12/174 (6.9%)	11.6/11.6
100 + 0	20.2	1 (8.3%)	0 (0.0%)	12.5	1/138 (0.7%)	12.4/12.4
200 + 0	20.0	1 (9.1%)	0 (0.0%)	11.6	0/128 (0.0%)	11.36/11.6
400 + 0	20.0	1 (8.3%)	0 (0.0%)	12.7	2/152 (1.3%)	12.5/12.5
0 + 50	20.0	0 (0.0%)	0 (0.0%)	13.1	0/170 (0.0%)	13.0/13.0
100 + 50	20.2	0 (0.0%)	0 (0.0%)	12.1	1/157 (0.6%)	12.0/12.0
200 + 50	20.0	0 (0.0%)	0 (0.0%)	11.8	13/154 (8.4%)	10.6/11.5
400 + 50	20.4	0 (0.0%)	0 (0.0%)	11.5	6/149 (4.0%)	9.8/10.7
0 + 100	19.9	0 (0.0%)	0 (0.0%)	12.3	4/172 (2.3%)	12.0/12.0
100 + 100	19.9	0 (0.0%)	0 (0.0%)	12.2	0/158 (0.0%)	12.2/12.2
200 + 100	20.3	0 (0.0%)	0 (0.0%)	10.7	3/128 (2.4%)	10.3/10.3
400 + 100	20.5	1 (9.1%)	0 (0.0%)	10.1	3/91 (3.4%)	9.6/9.6
0 + 150	20.1	0 (0.0%)	0 (0.0%)	12.4	1/136 (0.7%)	12.3/12.3
100 + 150	20.0	0 (0.0%)	0 (0.0%)	11.3	2/124 (1.6%)	11.0/11.0
200 + 150	20.5	0 (0.0%)	1 (9.1%)	8.1*	0/89 (0.0%)	8.0/8.8
400 + 150	20.9*	0 (0.0%)	2 (22.2%)	5.2**	6/47 (13.0%)	4.4*/5.7

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + isoniazid (mg/kg per day)
[b] Excludes values for litters that had no surviving pups

CAESAREAN SECTION DATA

The administration of 200 or 400 mg/kg AZT alone or 150 mg/kg isoniazid alone, as well as several combination doses, resulted in reductions ($P \leq 0.05$) in pregnancy among female-A mice (Table 7). In addition, the groups that received 200 + 50 mg/kg, 200 + 100 mg/kg, or 400 + 100 mg/kg had significant ($P \leq 0.05$) decreases in the numbers of corpora lutea and implantations (Table 7).

TABLE 7
Occurrence of Pregnancy in Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

Dose[a]	Number Pregnant (%)[b]	Mean Corpora Lutea	Mean Implantations
0 + 0	17 (85.0%)	14.0	13.3
100 +0	12 (60.0%)	13.2	11.9
200 + 0	9 (45.0%)*	11.6	10.7
400 + 0	8 (40.0%)**	9.9	9.2
0 + 50	18 (90.0%)	14.8	13.7
100 + 50	11 (55.0%)	15.0	13.9
200 + 50	13 (65.0%)	8.3**	7.6*
400 + 50	6 (30.0%)**	10.8	10.5
0 + 100	13 (65.0%)	12.8	11.8
100 + 100	16 (80.0%)	11.2	10.2
200 + 100	8 (40.0%)**	10.1*	9.0*
400 + 100	8 (40.0%)**	11.1*	8.9*
0 + 150	10 (50.0%)*	13.0	12.2
100 + 150	11 (55.0%)	13.7	12.5
200 + 150	11 (55.0%)	12.7	9.9
400 + 150	6 (30.0%)**	11.5	9.7

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + isoniazid (mg/kg per day)
[b] Twenty mice were assigned to each group.

The administration of 400 mg/kg AZT alone as well as several combinations of AZT and isoniazid (including 200 or 400 mg/kg AZT + any dose of isoniazid) resulted in decreased litter sizes ($P \leq 0.05$), decreased live fetuses per litter ($P \leq 0.05$), and an increase ($P \leq 0.05$) in resorptions (Table 8). Most of the resorptions were early; late resorptions were significantly increased ($P \leq 0.05$) in the 200 + 150 mg/kg and 400 + 150 mg/kg groups only.

TABLE 8
Summary of Caesarean-Section Litter Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

Dose[a]	Litter Sizes	Live Fetuses		Dead Fetuses		Resorptions	
	Mean ± SD[b]	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
0 + 0	12.6 ± 2.2	212	12.5 ± 2.1	2	0.1 ± 0.3	12	0.7 ± 1.0
100 + 0	9.3 ± 3.9	112	9.3 ± 3.9	0	0.0 ± 0.0	31	2.1 ± 2.2
200 + 0	8.6 ± 4.6	77	8.6 ± 4.6	0	0.0 ± 0.0	19	2.1 ± 1.8
400 + 0	1.4 ± 1.5**	11	1.4 ± 1.5**	0	0.0 ± 0.0	63	7.9 ± 3.6**
0 + 50	12.6 ± 1.8	212	12.5 ± 1.7	3	0.2 ± 0.4	18	1.0 ± 1.0
100 + 50	10.4 ± 4.6	113	10.3 ± 4.8	1	0.1 ± 0.3	39	3.5 ± 3.8
200 + 50	4.2 ± 3.6**	53	4.1 ± 3.3**	2	0.2 ± 0.4	44	3.4 ± 3.1*
400 + 50	6.8 ± 2.8*	40	6.7 ± 2.9*	1	0.2 ± 0.4	22	3.7 ± 1.2**
0 + 100	10.2 ± 3.5	133	10.2 ± 3.5	0	0.0 ± 0.0	21	1.6 ± 1.4
100 + 100	7.4 ± 4.8*	118	7.4 ± 4.8*	0	0.0 ± 0.0	45	2.8 ± 3.4
200 + 100	4.1 ± 3.5**	32	4.0 ± 3.4**	1	0.1 ± 0.4	39	4.9 ± 2.7**
400 + 100	1.4 ± 2.2**	8	1.0 ± 2.1**	3	0.4 ± 1.1	60	7.5 ± 3.1**
0 + 150	10.8 ± 2.1	108	10.8 ± 2.1	0	0.0 ± 0.0	14	1.4 ± 1.7
100 + 150	8.9 ± 3.2	96	8.7 ± 3.3	2	0.2 ± 0.6	40	3.6 ± 3.8*
200 + 150	2.0 ± 3.3**	20	2.0 ± 3.3**	0	0.0 ± 0.0	79	7.9 ± 5.0**
400 + 150	1.5 ± 2.3**	9	1.5 ± 23.3**	0	0.0 ± 0.0	49	8.2 ± 4.7**

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Excludes dams that delivered or began to deliver litters before scheduled sacrifice; also excludes dams that were sacrificed prior to presumed day 18 of gestation and, due to gestational age of fetuses, gender and viability could not be determined; and excludes dams that were found dead.
[b] Daily gavage doses of AZT + isoniazid (mg/kg per day); SD=standard deviation

Significant reductions ($P \leq 0.05$) in the mean fetal weight per litter were noted for all doses of AZT alone, as well as the 200 + 50 mg/kg, 400 + 50 mg/kg, and 100 + 150 mg/kg groups (Table 9). Reductions in fetal weight were also noted in most other combination dose groups, although these were not statistically significant, probably due to limited numbers of live fetuses.

TABLE 9
Body Weights of Fetuses from Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

Dose[a]	Mean Fetal Weight[b] per Litter (g)
0 + 0	1.34
100 + 0	1.16**
200 + 0	1.07**
400 + 0	1.03**
0 + 50	1.36
100 + 50	1.22
200 + 50	1.09**
400 + 50	1.03*
0 + 100	1.33
100 + 100	1.18
200 + 100	1.16
400 + 100	0.94
0 + 150	1.40
100 + 150	1.07**
200 + 150	0.98
400 + 150	0.90

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's or Dunn's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + isoniazid (mg/kg per day)
[b] See Table 8 for litter size.

GROSS EXTERNAL ALTERATIONS (FEMALE-B LITTERS)

Compared to the control group which had no fetal gross abnormalities, treatment with AZT alone, isoniazid alone, and combinations of AZT and isoniazid caused some increases (not statistically significant) in the number of litters and fetuses with gross external alterations in some groups (Table 10). Although the percentage of fetuses per litter with any type of gross external alteration was increased ($P \leq 0.01$) in the group treated with 400 + 150 mg/kg, there were no individual gross alterations that were statistically significant. The alterations were observed in one to three fetuses in the affected groups and included exencephaly, meningocele, open eyes, gastroschisis, hyperflexed limbs, and curled or coiled tails; none of these specific alterations were increased significantly.

TABLE 10
Summary of Fetal Gross Alterations in Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

	0 + 0	100 + 0	0 + 50	0 + 100	200 + 100	0 + 150	400 + 150
Litters examined/ fetuses examined	17/212	12/112	17/212	13/133	6/32	10/108	2/9
Malformation[b]							
Exencephaly	—[c]	—	—	1 (7.7)/1 (0.8)	—	1 (10.0)/1 (0.9)	1 (50.0)/1 (11.1)
Meningocele	—	—	—	—	1 (16.7)/1 (3.1)	—	—
Open eyes	—	—	—	—	—	—	1 (50.0)/1 (11.1)
Gastroschisis	—	—	—	—	—	—	1 (50.0)/1 (11.1)
Hyperflexed limbs	—	1 (8.3)/1 (0.9)	1 (5.9)/1 (0.5)	1 (7.7)/1 (0.8)	—	2 (20.0)/2 (1.8)	—
Curled/coiled tail	—	1 (8.3)/1 (0.9)	—	—	—	—	1 (50.0)/1 (11.1)
% Fetuses with any alteration per litter[d]	—	1.98 ± 5.06	0.39 ± 1.62	3.42 ± 9.49	2.38 ± 5.84	2.97 ± 5.11	20.00 ± 28.28**

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
[a] Fetal gross alterations occurred only in the treatment groups listed; all other treatment groups had no fetal gross alterations. Doses are expressed as AZT + isoniazid (mg/kg per day).
[b] Data presented as the number (percentage of litters with the alteration over the number (percentage) of fetuses with the alteration
[c] Not observed in this treatment group
[d] Mean ± standard deviation

DISCUSSION

Administration of AZT alone resulted in mild hematologic toxicity in male mice. Administration of isoniazid alone did not produce toxicity in male mice. Treatment with combinations of AZT and isoniazid appeared to enhance the anemia produced by AZT. AZT alone caused slight decreases in epididymal sperm motility at all three doses tested (100, 200, and 400 mg/kg). Isoniazid alone or in combination with AZT did not have a significant affect on epididymal sperm motility.

In female-A mice, AZT alone caused anemia and leukopenia, but isoniazid alone did not cause hematologic toxicity. Treatment with combinations of AZT and isoniazid enhanced the anemia caused by AZT. In female-B mice, AZT alone or in combination with isoniazid did not cause hematologic toxicity because the duration of treatment was only 10 days whereas female-A mice were treated for 30 days. In pregnant female mice, AZT caused decreased body weights and body weight gains. These differences were probably related to reduced litter sizes, increased resorptions, and reduced pup weights that occurred in groups administered AZT, because the body weights corrected for gravid uterine weights were not reduced in these groups. Isoniazid alone at doses up to 150 mg/kg did not cause maternal toxicity. AZT and isoniazid each appeared to be more toxic to the developing fetus and pup than to adult mice. Doses of 100, 200, or 400 mg/kg AZT alone and 50, 100, or 150 mg/kg isoniazid alone produced developmental toxicity with increases in the incidences of resorptions and the percentage of dead or resorbed fetuses per litter. Administered in combination, AZT and isoniazid increased both maternal and developmental toxicity.

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APPENDIX A
HEMATOLOGY RESULTS

TABLE A1
Hematology Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

	Vehicle Control	100 + 0	200 + 0	400 + 0
n	10	10	10	10
Hematocrit (%)	45.1 ± 2.8	41.3 ± 3.3	40.9 ± 3.9[b]	40.7 ± 3.6
Hemoglobin (g/dL)	15.3 ± 0.8	13.6 ± 1.4*	13.9 ± 1.4[b]	13.4 ± 1.2**
Erythrocytes ($10^6/\mu L$)	9.77 ± 0.38	8.29 ± 0.76**	8.41 ± 0.66**[b]	7.99 ± 0.66**
Reticulocytes ($10^5/\mu L$)	3.3 ± 0.60	3.7 ± 0.85	3.7 ± 0.50	4.3 ± 0.53
Mean cell volume (fL)	46.2 ± 2.3	50.0 ± 2.7*	48.7 ± 3.6[b]	51.0 ± 3.1**
Mean cell hemoglobin (pg)	15.7 ± 0.6	16.4 ± 0.7	16.5 ± 1.3[b]	16.7 ± 0.9
Mean cell hemoglobin concentration (g/dL)	34.0 ± 1.0	32.8 ± 1.3	33.9 ± 0.7[b]	32.8 ± 0.6
Platelets ($10^3/\mu L$)	1,421 ± 290.4	1,567 ± 311.0	1,608 ± 442.2[b]	1,686 ± 501.2
Leukocytes ($10^3/\mu L$)[c]	7.74 ± 2.28	7.70 ± 2.46	8.44 ± 2.40[b]	6.84 ± 3.59
Segmented neutrophils ($10^3/\mu L$)	1.31 ± 0.67	0.98 ± 0.41	2.06 ± 2.53	1.87 ± 3.19
Lymphocytes ($10^3/\mu L$)	5.82 ± 0.36	6.11 ± 2.05	5.67 ± 1.54	4.42 ± 1.52
Monocytes ($10^3/\mu L$)	0.18 ± 0.09	0.19 ± 0.08	0.31 ± 0.21	0.21 ± 0.10
Basophils ($10^3/\mu L$)	0.02 ± 0.021	0.02 ± 0.018	0.02 ± 0.013	0.02 ± 0.014
Eosinophils ($10^3/\mu L$)	0.39 ± 0.19	0.40 ± 0.21	0.35 ± 0.22	0.25 ± 0.16
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01[d]	0.03 ± 0.02[e]	0.04 ± 0.04[d]	0.08 ± 0.17[b]
	0 + 50	**100 + 50**	**200 + 50**	**400 + 50**
n	10	10	10	10
Hematocrit (%)	42.2 ± 3.8	42.5 ± 2.7	38.9 ± 2.2**	38.2 ± 2.1**
Hemoglobin (g/dL)	14.2 ± 1.3	14.3 ± 0.8	13.1 ± 0.9**	12.8 ± 0.8**
Erythrocytes ($10^6/\mu L$)	9.15 ± 0.80	8.75 ± 0.38*	7.86 ± 0.68**	7.81 ± 0.61**
Reticulocytes ($10^5/\mu L$)	4.2 ± 1.61	4.1 ± 0.61	3.9 ± 0.43	4.3 ± 0.67
Mean cell volume (fL)	46.1 ± 1.3	48.5 ± 1.8	49.7 ± 2.7	49.0 ± 2.8
Mean cell hemoglobin (pg)	15.5 ± 0.5	16.3 ± 0.6	16.7 ± 0.6	16.4 ± 0.8
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.7	33.7 ± 0.6	33.6 ± 1.3	33.6 ± 0.7
Platelets ($10^3/\mu L$)	1,516 ± 269.6	1,502 ± 357.2	1,625 ± 223.0	1,599 ± 215.8
Leukocytes ($10^3/\mu L$)	10.03 ± 7.53	7.82 ± 2.28	7.05 ± 1.91	8.21 ± 1.41
Segmented neutrophils ($10^3/\mu L$)	3.35 ± 6.18	1.36 ± 1.07	1.19 ± 0.43	1.34 ± 0.49
Lymphocytes ($10^3/\mu L$)	5.80 ± 2.49	5.93 ± 1.40	5.20 ± 1.45	6.34 ± 1.43
Monocytes ($10^3/\mu L$)	0.34 ± 0.25	0.23 ± 0.11	0.26 ± 0.19	0.25 ± 0.16
Basophils ($10^3/\mu L$)	0.03 ± 0.027	0.03 ± 0.028	0.02 ± 0.013	0.01 ± 0.020
Eosinophils ($10^3/\mu L$)	0.41 ± 0.38	0.27 ± 0.19	0.35 ± 0.20	0.25 ± 0.15
Large unstained cells ($10^3/\mu L$)	0.10 ± 0.20[b]	0.03 ± 0.01[e]	0.03 ± 0.01[e]	0.03 ± 0.02[f]

TABLE A1
Hematology Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

	0 + 100	100 + 100	200 + 100	400 + 100
n	10	8	10	10
Hematocrit (%)	43.2 ± 2.7	40.2 ± 3.9	38.8 ± 7.2**	36.7 ± 3.7**
Hemoglobin (g/dL)	14.4 ± 0.7	13.4 ± 1.3*	12.9 ± 2.1**	12.2 ± 1.3**
Erythrocytes ($10^6/\mu L$)	9.44 ± 0.61	8.23 ± 0.68**	7.67 ± 0.97**	7.26 ± 0.70**
Reticulocytes ($10^5/\mu L$)	4.2 ± 0.67	4.5 ± 1.23*	4.5 ± 0.78*	4.5 ± 1.04*
Mean cell volume (fL)	45.8 ± 2.0	49.0 ± 3.8	50.1 ± 5.2*	50.5 ± 2.4*
Mean cell hemoglobin (pg)	15.3 ± 0.5	16.3 ± 1.3	16.7 ± 1.3	16.8 ± 0.7*
Mean cell hemoglobin concentration (g/dL)	33.4 ± 0.7	33.3 ± 0.7	33.5 ± 1.7	33.3 ± 1.1
Platelets ($10^3/\mu L$)	1,475 ± 313.5	1,612 ± 419.7	1,854 ± 769.9	1,863 ± 323.2
Leukocytes ($10^3/\mu L$)	8.18 ± 2.27	7.56 ± 2.52	13.37 ± 22.71	6.67 ± 1.74
Segmented neutrophils ($10^3/\mu L$)	1.49 ± 0.62	1.93 ± 1.16	7.17 ± 19.55	0.82 ± 0.24
Lymphocytes ($10^3/\mu L$)	5.92 ± 1.85	5.08 ± 1.44	6.01 ± 3.65	5.19 ± 1.35
Monocytes ($10^3/\mu L$)	0.34 ± 0.13	0.27 ± 0.24	0.31 ± 0.44	0.27 ± 0.14
Basophils ($10^3/\mu L$)	0.02 ± 0.013	0.01 ± 0.016	0.01 ± 0.013	0.02 ± 0.013
Eosinophils ($10^3/\mu L$)	0.39 ± 0.21	0.25 ± 0.10	0.24 ± 0.21	0.35 ± 0.21
Large unstained cells ($10^3/\mu L$)	0.03 ± 0.01[e]	0.04 ± 0.02[f]	0.03 ± 0.02[g]	0.03 ± 0.02[e]

	0 + 150	100 + 150	200 + 150	400 + 150
n	10	10	10	10
Hematocrit (%)	41.5 ± 2.7	37.0 ± 2.9**	35.9 ± 2.9**	30.8 ± 6.0**
Hemoglobin (g/dL)	13.8 ± 1.2	12.3 ± 1.0*	11.9 ± 1.1**	10.2 ± 1.9**
Erythrocytes ($10^6/\mu L$)	9.17 ± 0.62	7.74 ± 0.69**	7.32 ± 0.64**	6.23 ± 0.94**
Reticulocytes ($10^5/\mu L$)	4.9 ± 0.66**	4.5 ± 0.71*	5.2 ± 1.09**	3.9 ± 1.41
Mean cell volume (fL)	45.3 ± 2.4	48.0 ± 3.1	49.2 ± 3.1	49.2 ± 2.9
Mean cell hemoglobin (pg)	15.1 ± 0.8	15.9 ± 1.0	16.3 ± 0.7	16.4 ± 1.0
Mean cell hemoglobin concentration (g/dL)	33.3 ± 1.1	33.2 ± 0.9	33.2 ± 1.6	33.4 ± 1.0
Platelets ($10^3/\mu L$)	1,514 ± 409.7	1,689 ± 238.3	1,834 ± 172.3	2,214 ± 345.5**
Leukocytes ($10^3/\mu L$)	7.71 ± 2.58	6.83 ± 1.88	6.89 ± 2.02	6.53 ± 2.00
Segmented neutrophils ($10^3/\mu L$)	1.22 ± 0.62	0.93 ± 0.36	1.00 ± 0.47	1.15 ± 1.30
Lymphocytes ($10^3/\mu L$)	5.96 ± 2.38	5.40 ± 1.65	5.08 ± 2.09	5.32 ± 1.61
Monocytes ($10^3/\mu L$)	0.27 ± 0.12	0.23 ± 0.17	0.24 ± 0.09	0.22 ± 0.08
Basophils ($10^3/\mu L$)	0.02 ± 0.016	0.02 ± 0.012	0.02 ± 0.016	0.02 ± 0.010
Eosinophils ($10^3/\mu L$)	0.22 ± 0.14	0.22 ± 0.14	0.32 ± 0.11	0.36 ± 0.27
Large unstained cells ($10^3/\mu L$)	0.03 ± 0.02[h]	0.03 ± 0.01[e]	0.03 ± 0.01[h]	0.03 ± 0.02[e]

* 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 + isoniazid (mg/kg per day). Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] Leukocyte counts corrected for nucleated erythrocyte counts greater than 10 per 100 leukocytes
[d] n=6
[e] n=8
[f] n=4
[g] n=5
[h] n=7

TABLE A2
Hematology Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

	Vehicle Control	100 + 0	200 + 0	400 + 0
n	20	20	20	20
Hematocrit (%)	41.1 ± 3.0	40.8 ± 2.8	42.0 ± 2.9	41.0 ± 3.3
Hemoglobin (f/dL)	14.1 ± 1.1	13.9 ± 1.1	14.2 ± 1.1	13.7 ± 1.3
Erythrocytes ($10^6/\mu L$)	8.33 ± 0.68	7.56 ± 0.71	7.61 ± 0.73	7.45 ± 0.64
Reticulocytes ($10^5/\mu L$)	3.8 ± 1.19[b]	3.4 ± 0.76	3.8 ± 0.78	3.5 ± 0.73
Mean cell volume (fL)	49.5 ± 2.6	54.2 ± 2.4**	55.4 ± 2.5**	55.1 ± 2.8**
Mean cell hemoglobin (pg)	17.0 ± 1.0	18.4 ± 1.0**	18.7 ± 0.9**	18.5 ± 1.1**
Mean cell hemoglobin concentration (g/dL)	34.3 ± 1.2	34.0 ± 1.1	33.7 ± 1.2	33.5 ± 1.0
Platelets ($10^3/\mu L$)	1,363 ± 222.8	1,409 ± 303.3	1,422 ± 356.3	1,479 ± 415.1
Leukocytes ($10^3/\mu L$)[c]	7.49 ± 1.55	6.25 ± 1.43	6.43 ± 1.58	5.71 ± 1.60**
Segmented neutrophils ($10^3/\mu L$)	2.27 ± 0.58	1.41 ± 0.74**	1.26 ± 0.57**	0.76 ± 0.47**
Lymphocytes ($10^3/\mu L$)	4.62 ± 1.29	4.29 ± 1.07	4.72 ± 1.48	4.56 ± 1.47
Monocytes ($10^3/\mu L$)	0.31 ± 0.08	0.22 ± 0.08	0.18 ± 0.11**	0.13 ± 0.11**
Basophils ($10^3/\mu L$)	0.02 ± 0.009	0.01 ± 0.010	0.02 ± 0.010	0.01 ± 0.011
Eosinophils ($10^3/\mu L$)	0.25 ± 0.15	0.29 ± 0.20	0.23 ± 0.15	0.23 ± 0.14
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01	0.02 ± 0.01[b]	0.02 ± 0.01[d]	0.02 ± 0.01[e]
	0 + 50	**100 + 50**	**200 + 50**	**400 + 50**
n	20	20	20	20
Hematocrit (%)	39.8 ± 3.9	39.9 ± 2.3	40.4 ± 3.8	39.6 ± 2.8
Hemoglobin (g/dL)	13.6 ± 1.4	13.5 ± 0.9	13.7 ± 1.5	13.2 ± 1.0
Erythrocytes ($10^6/\mu L$)	8.11 ± 0.69	7.56 ± 0.60	7.48 ± 0.74	7.12 ± 0.55**
Reticulocytes ($10^5/\mu L$)	3.5 ± 1.17	3.5 ± 0.70[b]	3.6 ± 1.02	4.3 ± 1.23
Mean cell volume (fL)	49.0 ± 2.0	53.0 ± 3.2*	54.1 ± 2.0**	55.7 ± 2.2**
Mean cell hemoglobin (pg)	16.7 ± 0.7	17.9 ± 0.9	18.4 ± 1.0**	18.5 ± 0.9**
Mean cell hemoglobin concentration (g/dL)	34.1 ± 1.0	33.8 ± 1.1	33.9 ± 1.2	33.2 ± 0.8
Platelets ($10^3/\mu L$)	1,507 ± 276.0	1,528 ± 271.0	1,509 ± 398.7	1,549 ± 417.4
Leukocytes ($10^3/\mu L$)	8.03 ± 2.09	6.64 ± 2.14	6.16 ± 1.21	5.52 ± 1.96**
Segmented neutrophils ($10^3/\mu L$)	2.81 ± 1.36	1.52 ± 0.90*	1.19 ± 0.85**	0.83 ± 0.40**
Lymphocytes ($10^3/\mu L$)	4.64 ± 1.29	4.61 ± 1.67	4.56 ± 0.95	4.25 ± 1.72
Monocytes ($10^3/\mu L$)	0.33 ± 0.16	0.24 ± 0.16	0.17 ± 0.08**	0.17 ± 0.07**
Basophils ($10^3/\mu L$)	0.02 ± 0.010	0.02 ± 0.012	0.01 ± 0.009	0.01 ± 0.011
Eosinophils ($10^3/\mu L$)	0.22 ± 0.14	0.23 ± 0.16	0.21 ± 0.17	0.24 ± 0.18
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01[f]	0.02 ± 0.02[f]	0.02 ± 0.01[g]	0.02 ± 0.01[h]

TABLE A2
Hematology Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

	0 + 100	100 + 100	200 + 100	400 + 100
n	20	20	20	19
Hematocrit (%)	39.9 ± 3.1	38.8 ± 3.6	36.4 ± 8.3*	35.4 ± 10.1*
Hemoglobin (g/dL)	13.4 ± 1.1	12.6 ± 1.1*	12.2 ± 2.8**	11.6 ± 3.2**
Erythrocytes ($10^6/\mu L$)	8.48 ± 0.78	7.01 ± 0.79**	6.57 ± 1.36**	6.26 ± 1.60**
Reticulocytes ($10^5/\mu L$)	4.4 ± 1.27[b]	4.5 ± 1.94	4.5 ± 1.87	4.4 ± 2.42
Mean cell volume (fL)	47.1 ± 2.3	55.6 ± 3.5**	54.9 ± 4.5**	55.4 ± 5.7**
Mean cell hemoglobin (pg)	15.9 ± 0.9*	18.1 ± 1.2*	18.4 ± 1.6**	18.3 ± 1.5**
Mean cell hemoglobin concentration (g/dL)	33.7 ± 1.0	32.5 ± 1.0**	33.5 ± 1.0	33.2 ± 1.5
Platelets ($10^3/\mu L$)	1,631 ± 300.9	1,705 ± 216.6*	1,792 ± 425.5**	1,772 ± 575.1
Leukocytes ($10^3/\mu L$)	7.34 ± 1.63	5.95 ± 1.10*	5.60 ± 1.85**	5.00 ± 1.43**
Segmented neutrophils ($10^3/\mu L$)	2.13 ± 0.85	1.50 ± 0.62*	1.13 ± 1.26**	0.67 ± 0.40**
Lymphocytes ($10^3/\mu L$)	4.70 ± 1.24	4.01 ± 0.81	4.09 ± 0.85	4.00 ± 1.23
Monocytes ($10^3/\mu L$)	0.27 ± 0.11	0.22 ± 0.08	0.17 ± 0.15**	0.13 ± 0.08**
Basophils ($10^3/\mu L$)	0.02 ± 0.009	0.01 ± 0.007**	0.01 ± 0.008	0.01 ± 0.007**
Eosinophils ($10^3/\mu L$)	0.20 ± 0.18	0.20 ± 0.19	0.19 ± 0.15	0.19 ± 0.17
Large unstained cells ($10^3/\mu L$)	0.01 ± 0.01[b]	0.02 ± 0.01[g]	0.01 ± 0.01[d]	0.01 ± 0.01[i]
	0 + 150	**100 + 150**	**200 + 150**	**400 + 150**
n	20	20	17	18
Hematocrit (%)	39.6 ± 3.3	36.5 ± 5.0	30.2 ± 11.7**	26.4 ± 12.4**
Hemoglobin (g/dL)	13.1 ± 1.2	11.9 ± 1.6**	9.9 ± 3.7**	8.9 ± 3.9**
Erythrocytes ($10^6/\mu L$)	8.31 ± 0.90	6.70 ± 0.98**	5.46 ± 1.94**	4.82 ± 1.94**
Reticulocytes ($10^5/\mu L$)	5.2 ± 2.00	4.9 ± 1.88	4.8 ± 3.17	3.3 ± 2.22
Mean cell volume (fL)	47.8 ± 2.7	54.7 ± 3.8**	54.0 ± 7.4**	52.3 ± 7.2
Mean cell hemoglobin (pg)	15.8 ± 1.0*	17.8 ± 1.1	17.8 ± 1.8	17.9 ± 1.8
Mean cell hemoglobin concentration (g/dL)	33.0 ± 1.1**	32.6 ± 1.0**	33.1 ± 1.9*	34.4 ± 2.0
Platelets ($10^3/\mu L$)	1,482 ± 453.2	1,732 ± 421.4*	2,025 ± 455.3**	2,347 ± 731.8**
Leukocytes ($10^3/\mu L$)	7.01 ± 1.66	6.09 ± 1.43	5.07 ± 1.62**	5.06 ± 1.50**
Segmented neutrophils ($10^3/\mu L$)	1.75 ± 1.08	1.25 ± 0.65**	0.90 ± 0.79**	0.65 ± 0.34**
Lymphocytes ($10^3/\mu L$)	4.81 ± 1.30	4.43 ± 1.16	3.84 ± 1.07	4.10 ± 1.30
Monocytes ($10^3/\mu L$)	0.25 ± 0.11	0.20 ± 0.12*	0.16 ± 0.10**	0.14 ± 0.10**
Basophils ($10^3/\mu L$)	0.02 ± 0.010	0.01 ± 0.009	0.01 ± 0.007	0.01 ± 0.006*
Eosinophils ($10^3/\mu L$)	0.17 ± 0.13	0.19 ± 0.15	0.15 ± 0.11	0.15 ± 0.10
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01[f]	0.02 ± 0.01[d]	0.01 ± 0.01[d]	0.02 ± 0.01[i]

* 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 + isoniazid (mg/kg per day). Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=19
[c] Leukocyte counts corrected for nucleated erythrocyte counts greater than 10 per 100 leukocytes
[d] n=16
[e] n=11
[f] n=18
[g] n=15
[h] n=14
[i] n=13

TABLE A3
Hematology Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations[a]

	Vehicle Control	100 + 0	200 + 0	400 + 0
n	20	20	20	20
Hematocrit (%)	42.3 ± 2.8	42.0 ± 2.5	41.3 ± 5.7	40.9 ± 2.7
Hemoglobin (g/dL)	14.2 ± 0.8	14.1 ± 1.1	13.8 ± 2.2	13.7 ± 1.2
Erythrocytes ($10^6/\mu L$)	8.52 ± 0.60	8.08 ± 0.74	7.87 ± 1.52	7.75 ± 0.67
Reticulocytes ($10^5/\mu L$)[b]	4.9 ± 1.19	5.2 ± 2.12	5.9 ± 1.35	5.8 ± 1.77
Mean cell volume (fL)	49.7 ± 1.6	52.1 ± 2.2	53.2 ± 4.3	53.0 ± 4.0
Mean cell hemoglobin (pg)	16.7 ± 0.5	17.4 ± 0.8	17.6 ± 1.0	17.7 ± 1.1*
Mean cell hemoglobin concentration (g/dL)	33.6 ± 0.8	33.4 ± 1.1	33.2 ± 1.7	33.5 ± 1.9
Platelets ($10^3/\mu L$)	1,497 ± 351.0	1,413 ± 309.9	1,417 ± 360.9	1,380 ± 234.1
Leukocytes ($10^3/\mu L$)[c]	7.03 ± 1.78	6.99 ± 1.69	7.05 ± 0.85	7.33 ± 2.11
Segmented neutrophils ($10^3/\mu L$)[b]	1.77 ± 0.65	1.73 ± 0.51	1.73 ± 0.77	1.58 ± 0.54
Lymphocytes ($10^3/\mu L$)[b]	4.86 ± 1.14	4.69 ± 1.45	4.82 ± 1.06	5.23 ± 1.70
Monocytes ($10^3/\mu L$)[b]	0.20 ± 0.14	0.23 ± 0.08	0.22 ± 0.09	0.22 ± 0.12
Basophils ($10^3/\mu L$)[b]	0.01 ± 0.015	0.01 ± 0.012	0.01 ± 0.012	0.02 ± 0.016
Eosinophils ($10^3/\mu L$)[b]	0.20 ± 0.20	0.31 ± 0.22	0.27 ± 0.20	0.27 ± 0.18
Large unstained cells ($10^3/\mu L$)[b]	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02
	0 + 50	**100 + 50**	**200 + 50**	**400 + 50**
n	20	20	20	20
Hematocrit (%)	42.9 ± 2.7	41.1 ± 2.5	40.1 ± 3.9	41.9 ± 2.6
Hemoglobin (g/dL)	14.2 ± 1.0	13.6 ± 0.8	13.4 ± 1.1	13.8 ± 0.8
Erythrocytes ($10^6/\mu L$)	8.68 ± 0.74	7.87 ± 0.57	7.72 ± 0.74	7.76 ± 0.43
Reticulocytes ($10^5/\mu L$)	5.5 ± 1.94	5.6 ± 2.15	5.7 ± 1.56	5.4 ± 1.93
Mean cell volume (fL)	49.6 ± 2.5	52.3 ± 3.3	51.9 ± 2.5	54.0 ± 3.1
Mean cell hemoglobin (pg)	16.4 ± 0.8	17.3 ± 0.8	17.4 ± 0.6	17.8 ± 1.1*
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.7	33.1 ± 1.2	33.6 ± 1.4	32.9 ± 1.0
Platelets ($10^3/\mu L$)	1,583 ± 271.4	1,580 ± 330.1	1,572 ± 428.4	1,495 ± 347.1
Leukocytes ($10^3/\mu L$)	6.93 ± 1.97	7.54 ± 1.95	7.49 ± 2.80	6.44 ± 1.92
Segmented neutrophils ($10^3/\mu L$)	1.78 ± 0.67	1.49 ± 0.48	2.06 ± 1.00	1.74 ± 0.51
Lymphocytes ($10^3/\mu L$)	4.81 ± 1.58	5.63 ± 1.77	4.83 ± 1.85	4.19 ± 1.30
Monocytes ($10^3/\mu L$)	0.15 ± 0.14	0.23 ± 0.06	0.25 ± 0.19	0.23 ± 0.14
Basophils ($10^3/\mu L$)	0.01 ± 0.009	0.02 ± 0.019	0.02 ± 0.018	0.02 ± 0.017
Eosinophils ($10^3/\mu L$)	0.17 ± 0.22	0.16 ± 0.15	0.32± 0.37	0.25 ± 0.25
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.02	0.02 ± 0.01

TABLE A3
Hematology Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Isoniazid Combinations

	0 + 100	100 + 100	200 + 100	400 + 100
n	13 or 14	13 or 14	13 or 14	13 or 14
Hematocrit (%)	41.0 ± 4.6	40.7 ± 3.3	42.8 ± 3.1	42.5 ± 3.2
Hemoglobin (g/dL)	13.6 ± 1.6	13.5 ± 1.2	14.1 ± 0.9	14.2 ± 1.0
Erythrocytes ($10^6/\mu L$)	8.23 ± 1.00	7.81 ± 0.53	8.15 ± 0.77	8.02 ± 0.73
Reticulocytes ($10^5/\mu L$)	5.2 ± 1.68	6.0 ± 1.34	5.4 ± 2.21	6.7 ± 5.23
Mean cell volume (fL)	49.9 ± 2.2	52.1 ± 3.1	52.7 ± 3.2	53.2 ± 4.5
Mean cell hemoglobin (pg)	16.6 ± 0.9	17.3 ± 0.8	17.3 ± 1.1	17.7 ± 1.1*
Mean cell hemoglobin concentration (g/dL)	33.2 ± 1.0	32.2 ± 1.0	32.9 ± 0.5	33.4 ± 1.0
Platelets ($10^3/\mu L$)	1,458 ± 327.8	1,438 ± 234.0	1,461 ± 227.3	1,500 ± 212.7
Leukocytes ($10^3/\mu L$)	6.86 ± 1.42	6.01 ± 0.86	7.52 ± 2.21	6.39 ± 1.40
Segmented neutrophils ($10^3/\mu L$)	1.94 ± 0.77	1.70 ± 0.90	1.92 ± 0.86	1.63 ± 0.56
Lymphocytes ($10^3/\mu L$)	4.38 ± 0.84	3.94 ± 0.66	5.09 ± 1.80	4.28 ± 1.48
Monocytes ($10^3/\mu L$)	0.23 ± 0.12	0.20 ± 0.10	0.23 ± 0.24	0.19 ± 0.14
Basophils ($10^3/\mu L$)	0.02 ± 0.012	0.01 ± 0.009	0.01 ± 0.015	0.01 ± 0.012
Eosinophils ($10^3/\mu L$)	0.27 ± 0.25	0.16 ± 0.17	0.26 ± 0.21	0.26 ± 0.26
Large unstained cells ($10^3/\mu L$)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
	0 + 150	**100 + 150**	**200 + 150**	**400 + 150**
n	13 or 14	13 or 14	13 or 14	13 or 14
Hematocrit (%)	42.4 ± 3.5	43.7 ± 3.7	42.6 ± 2.8	44.6 ± 3.3
Hemoglobin (g/dL)	14.1 ± 1.3	14.3 ± 1.3	14.1 ± 1.2	14.9 ± 1.2
Erythrocytes ($10^6/\mu L$)	8.60 ± 0.86	8.37 ± 1.01	8.06 ± 0.74	8.49 ± 0.71
Reticulocytes ($10^5/\mu L$)	4.8 ± 1.46	6.0 ± 1.80	6.2 ± 4.09	3.2 ± 1.97
Mean cell volume (fL)	49.5 ± 2.2	52.5 ± 4.1	53.1 ± 4.4	52.6 ± 2.6
Mean cell hemoglobin (pg)	16.4 ± 0.7	17.2 ± 1.4	17.5 ± 1.2	17.6 ± 0.9
Mean cell hemoglobin concentration (g/dL)	33.2 ± 1.0	32.7 ± 1.2	33.0 ± 1.3	33.5 ± 1.0
Platelets ($10^3/\mu L$)	1,557 ± 304.2	1,435 ± 405.9	1,450 ± 313.9	1,483 ± 228.9
Leukocytes ($10^3/\mu L$)	7.19 ± 1.74	6.64 ± 2.08	6.58 ± 1.68	7.01 ± 1.72
Segmented neutrophils ($10^3/\mu L$)	1.90 ± 0.76	1.73 ± 0.75	1.29 ± 0.45	1.63 ± 0.70
Lymphocytes ($10^3/\mu L$)	4.79 ± 1.01	4.41 ± 1.66	4.74 ± 1.59	4.75 ± 1.54
Monocytes ($10^3/\mu L$)	0.19 ± 0.12	0.21 ± 0.13	0.23 ± 0.09	0.24 ± 0.11
Basophils ($10^3/\mu L$)	0.02 ± 0.020	0.01 ± 0.015	0.02 ± 0.014	0.02 ± 0.015
Eosinophils ($10^3/\mu L$)	0.28 ± 0.35	0.28 ± 0.33	0.28 ± 0.17	0.35 ± 0.11
Large unstained cells ($10^3/\mu L$)	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
[a] Daily gavage doses of AZT + isoniazid (mg/kg per day). Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=13 or 14
[c] Leukocyte counts corrected for nucleated erythrocyte counts greater than 10 per 100 leukocytes