National Institute of
Environmental Health Sciences

NIEHS Technical Report on the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide (CAS No. 98-96-4) Administered by Gavage to Swiss (CD-1®) Mice

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FOREWORD

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

CONTRIBUTORS

This report on the reproductive, developmental, and general toxicity studies of pyrazinamide is based primarily on 32-day studies that began in April 1993, and ended in May 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 pyrazinamide was evaluated by the 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 received and reviewed prior to the finalization of this document. Changes have been made such that the concerns of the reviewers have been addressed to the extent possible. The findings in this study suggest that higher doses could have been tolerated, therefore, future studies with pyrazinamide in this series will utilize higher doses.

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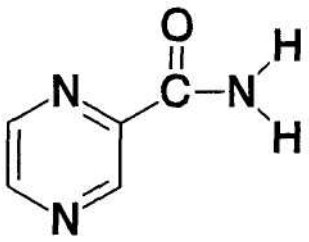
ABSTRACT

Pyrazinamide



Molecular Formula	$C_5H_5N_30$
CAS Number	98-96-4
Molecular Weight	123.12

Pyrazinamide is a synthetic pyrazine analogue of nicotinamide used in the treatment of tuberculosis, which is an opportunistic infection in the human immunodeficiency virus (HIV)-positive population. The reproductive and developmental toxicities of pyrazinamide were evaluated in male and female Swiss (CD-1®) mice by administering daily doses of 0, 400, 800, or 1,200 mg/kg of pyrazinamide in 0.5 % methyl cellulose in deionized water by gavage. Male mice (10 per group) were dosed on days 5 to 25 and sacrificed on day 25. Females were divided into two groups designated females-A and females-B. The females-A (20 per group) were dosed from day 0 to sacrifice and caesarean-sectioned on days 28 to 32 and were cohabited with dosed males on days 9 to 13 to test for effects on mating behavior, fertilization, and implantation. The females designated as females-B (20 per group) were cohabited with males on days 0 to 4, before the males began receiving pyrazinamide. Sperm-negative females-B were sacrificed after the cohabitation period; sperm-positive females-B were dosed during organogenesis on days 6 through 15 of presumed gestation. For females-B that delivered, both dams and pups were sacrificed on day 4 or 5 of lactation. Sperm-positive females-B that did not deliver pups were sacrificed on days 24 to 27 of presumed gestation. Adult mice were evaluated for clinical signs, body weights, pathologic findings at necropsy, and clinical pathology parameters. Sperm function was evaluated in males. From delivery until postnatal day 4, offspring from females-B were evaluated for viability, external anomalies, and body weight.

No apparent clinical, reproductive, or developmental toxicity was detected at the doses employed in this study. A reduction occurred in female fetal body weight in females-A in the 800 and 1,200 mg/kg groups. Since this reduction was not highly significant ($P \leq 0.05$) in the 1,200 mg/kg group, it is uncertain whether this finding is related to treatment with pyrazinamide. The highest dose administered in female Swiss (CD-1®) mice, 1,200 mg/kg per day, is approximately 8 times the therapeutic dose and resulted in a C_{max} 9 to 12 times the C_{max} caused by the therapeutic dose in humans. However, results of this study indicated that higher doses could have been tolerated.

INTRODUCTION

Because acquired immunodeficiency syndrome (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 3 '-azido-3 '-deoxythymidine (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/day), rifampin (600 mg/day or 450 mg/day for persons weighing less than 50 kg), and pyrazinamide (20 to 30 mg/kg per day) 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). The inclusion of pyrazinamide in treatment regimens has made possible the shortening of therapy to 6 months (East and Central African/British Medical Research Council, 1986). Pyrazinamide is the synthetic pyrazine analogue of nicotinamide. Its molecular formula is $C_5H_5N_3O$, and it has a molecular weight of 123.12. The daily dose for adults is 20 to 35 mg/kg orally, given in three or four equally spaced doses. The maximum quantity to be administered is 3 g per day, regardless of body weight.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Pyrazinamide is well absorbed from the gastrointestinal tract, and it is distributed throughout the body. The oral administration of 1 g produces plasma concentrations of about 45 µg/mL at 2 hours and 10 /µg/mL at 15 hours. The drug is excreted primarily by renal glomerular filtration; urinary concentrations are 50 to 100 *µg/mL* for several hours after a single dose. Pyrazinamide is hydrolyzed to pyrazinoic acid and subsequently hydroxylated to 5-hydroxypyrazinoic acid, the major excretory product (Weiner and Tinker, 1972). Grossett *et al.* (1992) reported that 150 mg/kg pyrazinamide (considered to be a therapeutic dose) administered by gavage to female Swiss (CD-1®) mice resulted in serum concentrations similar to those of humans receiving pyrazinamide therapy.

TOXICITY

Injury to the liver is the most common and serious side effect of pyrazinamide. When a dose of 3 g per day (40 to 50 mg/kg body weight) is orally administered, signs and symptoms of hepatic disease appear in about

15% of patients, with jaundice appearing in 2% to 3% of patients, and death due to hepatic necrosis occurring in rare instances (Mandell and Sande, 1990). Mild and transient elevations of the plasma alanine and aspartate aminotransferases are the earliest abnormalities produced by the drug and occur approximately 2 weeks after initiation of therapy (Ramakrishnan *et al.*, 1968). Baron and Bell (1974) have pointed out that such transient asymptomatic increases in the serum hepatic enzyme concentrations are also common during the early weeks of antituberculosis chemotherapy with other drugs, and these usually return to normal without interrupting or altering the regimen and are not clinically important.

Hepatotoxicity and nephrotoxicity occurred in male Wistar rats following the administration of 2.5 g/kg of pyrazinamide by gavage in 10% gum arabic (Zitkova *et al.*, 1983). Bederka *et al.* (1975) have reported a LD_{25} for pyrazinamide in young adult female Swiss albino and Charles River mice (20 to 32 g) of 705 mg/kg of body weight following intraperitoneal administration in dimethyl sulfoxide. The LD_{50} data for pyrazinamide reported in the *Registry of Toxic Effects of Chemical Substances* are as follows: mouse, intraperitoneal, 1,680 mg/kg; mouse, subcutaneous, 2,793 mg/kg (RTECS, 1983).

GENETIC TOXICOLOGY

Roman and Georgian (1977) studied the comparative cytogenetic effects of para-aminosalicylic acid sodium salt, pyrazinamide, and rifampin in human peripheral blood cultures. The structural chromosomal lesions were randomly distributed between the different chromosome groups of the human karyotype; nearly 20% to 30% of affected cells had more than one lesion per metaphase. In the treatments with sodium para-aminosalicylate and pyrazinamide, the analysis of the frequencies of the cells carrying chromosomal aberrations and of the chromosomal lesion types indicated a dose-response correlation. The *in vitro* studies indicate a potential genetic hazard in the use of these drugs.

STUDY RATIONALE

This study of pyrazinamide was conducted by the NIEHS as part of its program to evaluate the safety, in pregnant women and in the developing conceptus, of drugs used in the treatment of AIDS or the opportunistic infections accompanying AIDS. In the present study, effects on the motility and density of sperm were also evaluated. Tuberculosis is a frequent complication in AIDS and is commonly treated with pyrazinamide. Animal reproduction studies have not been conducted with pyrazinamide. Also, it is not known whether pyrazinamide can cause harm to the conceptus when administered to pregnant women or can affect reproductive capacity (PDR, 1994). To facilitate the screening of chemicals for reproductive and developmental toxicity, the National Toxicology Program has developed a protocol that combines aspects of other studies designed for this purpose (Morrissey *et al.*, 1989; Harris *et al.*, 1992). This protocol is intended

to provide results that may be used to set dose levels for a definitive developmental toxicity study, if necessary. Since the liver has been shown to be a target organ of pyrazinamide in humans, liver enzyme determinations were included depending on sample availability.

Based on mouse and human therapeutic doses and mouse toxic doses reported in the literature (Bederka *et al.*, 1975), the high dose selected for this study was approximately 8 times the mouse therapeutic dose and 50 times the human therapeutic dose. However, blood levels indicated the high dose was close to 10 times the mouse therapeutic dose (Grossett *et al.*, 1992). The mid and low doses were spaced at 400 mg/kg intervals from the high dose.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF PYRAZINAMIDE

Pyrazinamide (Lot 00909PJ) was manufactured by Aldrich Chemical Company (Milwaukee, WI). The material, a fine white powder, was identified as pyrazinamide by nuclear magnetic resonance and infrared spectroscopy. The purity of the compound was determined to be greater than 99% by high-performance liquid chromatography (HPLC).

DOSE FORMULATIONS

Suspensions of pyrazinamide were prepared by weighing the required amount of the drug into a volumetric flask and adding the appropriate amount of 0.5% methyl cellulose in deionized water. Each formulation was then stirred and sonicated until a homogeneous suspension was obtained. Homogeneity analyses were conducted on triplicate samples from the top, middle, and bottom of the suspension using HPLC. Results indicated that the suspension was slightly lower in concentration at the top and slightly higher in concentration at the bottom, but the differences were not significant. The range of dose concentrations among the three sampling locations was ± 2.5% of the target concentrations. Stability studies conducted on a formulation containing pyrazinamide at 3.75 mg/mL (equivalent in mice to 75 mg/kg body weight) in 0.5% aqueous methyl cellulose indicated that formulations were stable for 30 days in sealed glass bottles at refrigerated and room temperatures, and for 3 hours under simulated dosing conditions. After 3 hours, formulations showed slightly elevated concentrations (11%) of pyrazinamide. Dosing formulations of pyrazinamide used in this study were stored under refrigeration in the dark for a maximum of 29 days after mixing.

Samples of formulations from each dose level from the first mix showed that all formulations analyzed prior to dosing were within the acceptable range (± 10% of theoretical values). Animal room samples contained 105 % to 111 % of the target pyrazinamide concentrations; these values were consistent with the slight elevation of concentration seen in the dosage stability study. Since only the 400 mg/kg dose formulation exceeded the acceptable limit of ± 10% of theoretical values (111%), the elevated concentrations of the animal room samples were considered acceptable.

STUDY DESIGN

Male and female Swiss (CD-1®) mice were obtained from Charles River Laboratories (Raleigh, NC) and were placed on study at about 12 weeks of age. During the acclimation period of 17 days, five males and five

females were examined for parasites at four time points; all parasite examinations were negative. At terminal sacrifice, blood samples were collected from five male and five female sentinel animals for testing as part of the animal disease screening program. Results indicated that all animals were free of viral antibodies. The mice were housed five per sex per cage during quarantine before randomization and were individually housed after randomization, except during cohabitation. Polycarbonate cages were used. Animal rooms were maintained at an average temperature of 70.4° F and an average relative humidity of 57.8% with 12 hours of fluorescent light per day and a minimum of 10 room air changes per hour.

The design of this study is a modification of a design published elsewhere (Harris *et al.*, 1992). The oral route of administration was selected because it is the route used in humans. The study was conducted on Swiss (CD-1®) mice because this strain is routinely used for reproductive and developmental toxicity evaluations. Pyrazinamide was administered in a 0.5% (w/v) methyl cellulose suspension at concentrations of 0, 400, 800, and 1,200 mg/kg by gavage. Total daily doses of 20 mL/kg were divided into two equal doses of 10 mL/kg given approximately 6 hours apart. There were 10 males and 40 females per dose group. Each female dose group was further divided into groups of 20 females-A and 20 females-B. Males were dosed during study days 5 through 25, females-A were dosed during study days 0 through the day prior to sacrifice. Males were cohabited with females-A on study days 9 to 13 to identify any effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. The group females-B cohabited with males on study days 0 through 4, before the males began receiving pyrazinamide, and were dosed on gestation days 6 through 15 during organogenesis to identify effects on fetal development. Residual effects on parturition and the beginning of lactation were also evaluated. All animals were observed daily for clinical signs of toxicity.

During cohabitation periods, 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 each cohabitation period, all animals were housed individually.

On study day 25, all male mice were weighed, dosed, and retroorbital blood samples were obtained for hematology evaluation. Samples were also obtained from nine males per group, divided over three time points after dosing, for evaluation of plasma pyrazinamide concentrations. Sperm count, function, and motility were assessed, the males were euthanized with CO_2, and a gross necropsy was conducted.

On study days 28 to 32 (day 18 of presumed gestation) all female-A mice were weighed, and blood samples were collected for hematology and clinical chemistry evaluations. Samples were also obtained from nine animals per group, divided over three time points after dosing, for plasma pyrazinamide concentration

evaluations. The females-A were then euthanized with CO_2 and gross 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-A were examined for evidence of unsuccessful pregnancy, then "press-plated" between two heavy plates of glass to visualize early implantation sites. Additional endpoints for all females-A included gravid uterine weight, number of implantation sites, resorptions, corpora lutea, and dead and live fetuses.

Sperm-negative females-B were euthanized with CO_2 and discarded without necropsy. The sperm-positive females-B were assigned evenly across dose groups prior to gestation day 6. Beginning on gestation day 16 for females-B, the bedding material and feeders in the cages were no longer changed. From gestation day 17 until the litters were delivered, females-B were observed twice daily for evidence of labor or delivery. The day of delivery was determined to the nearest day and was designated as postnatal day 0. On postnatal days 0 and 1, dam weights were recorded, along with the number of live and dead pups, the number of male and female pups, any gross malformations, and live pup weights. Dead pups were discarded. On postnatal day 4, females-B, including any which did not deliver, were weighed and blood samples were collected for hematology and clinical chemistry determinations. These mice were then euthanized with CO_2 and a complete gross necropsy was performed. The uterus was removed and "press-plated." All pups were weighed, given a thorough external examination for lesions or malformations, and the sex was recorded. The pups were then euthanized with CO_2 and saved in Bouin's fixative.

A gross necropsy was performed on all animals except sperm-negative females-B, which were euthanized after cohabitation. 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 at 4 to 6 /am thickness, stained with hematoxylin and eosin, and examined by light microscopy. The right testis from all males was saved in Bouin's fixative.

Clinical Pathology

All blood samples for hematology and clinical chemistry were taken from the retroorbital sinus under CO_2/O_2 (70/30) anesthesia. 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 volume (MCV); mean cell hemoglobin concentration (MCHC); leukocyte differentials; and erythrocyte and platelet morphologies were determined on whole blood using a Technicon H-l automated hematology analyzer. Reticulocyte counts were conducted using a Coulter Model Elite Flow Cytometer. Blood smears were prepared to manually verify reticulocyte counts, leukocyte differentials, and morphologies, if necessary.

Values for alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, sorbitol dehydrogenase, and bile acids were determined using a Roche Cobas Fara automated analyzer. Priority for clinical chemistry tests was assigned in the order listed above. Determination of plasma concentrations of pyrazinamide was considered the highest priority. Due to the limited number (10) of male mice per group, all the plasma samples available from male mice were used for pyrazinamide determination, and serum chemistry determinations were not performed.

Plasma Pyrazinamide Concentrations

All blood samples were taken from the retroorbital sinus of control and dosed mice under CO_2/O_2 anesthesia at 30, 90, and 120 minutes after gavage administration. Samples from three males and three females-A per group were collected into a tube containing EDTA. Plasma was obtained and stored at approximately -70° C until the analyses were performed. Plasma samples were diluted with distilled water and an internal standard solution was added (1 mg benzamide/mL water). Samples were mixed and analyzed on an HPLC equipped with a Zorbax C8 column and UV detector (267 nm). Chromatography was achieved by gradient elution with a mobile phase of acetonitrile:0.01 heptanesulfonic acid in water (pH 3.5) in the following proportions: 4:96 for 11 minutes, 4:96 to 15:85 in 3 minutes, 15:85 for five minutes, 15:85 to 4:96 in 3 minutes, and 4:96 for 18 minutes with a flow rate of 1 mL/minute. Retention times were approximately 10 minutes for pyrazinamide and 25 minutes for the internal standard. The minimum detection limit was 1 fig/mL. To characterize the internal dose, the toxicokinetic parameters C^, T^, and $t_{,A}$ were estimated from the data.

Sperm Function Evaluation

Sperm motility was evaluated at necropsy. 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 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.

STATISTICAL METHODS

Paternal and maternal body weight data were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance (Snedecor and Cochran, 1967). If Bartlett's test was not significant (P>0.05) and the analysis of variance was significant (P^0.05), then Dunnett's test (Dunnett, 1955) was used to identify the statistical significance of individual groups. If Bartlett's test was significant ($P < 0.05$), the Kruskal-Wallis test (Hollander and Wolfe, 1973) was used; in cases where the Kruskal-Wallis test was significant ($P \leq 0.05$), Dunn's (Dunn, 1964) method of multiple comparisons was used to identify the statistical significance of individual groups. These methods were also used to analyze fetal body weight and pup body weight (per litter), as well as all other evaluations involving continuous data. For F_0 generation sires and dams, analysis of covariance (Snedecor and Cochran, 1967) was used to evaluate average body weight changes and average maternal body weight changes. Observations for delivered and dead conceptuses of the female-A dams, and 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 terminal body and epididymis weights. Epididymis/body weight ratios were also calculated. Mean terminal body weights of males and females and mean epididymis weights and epididymis/body weight ratios of males in each dose group were compared to those of the control group by a two-tailed Student's Mest. The standard deviations used in the f-tests were obtained by pooling the individual values for the control and dose groups. Hematology and clinical chemistry data were evaluated using Dunnett's test.

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

RESULTS AND DISCUSSION

MORTALITY

All male and female mice survived to the end of the study with the exception of one female-A control which died on day 29 after retroorbital bleeding. No clinical signs of toxicity were observed in any of the groups.

BODY AND ORGAN WEIGHTS

A significant reduction in body weight occurred in the male 1,200 mg/kg group during the first 4 days of treatment (study days 5 to 9). Otherwise, mean body weights of dosed males and females-A were comparable to those of the control group during the course of the study and at termination (Figures 1 and 2). Gravid uterine weights of dosed females-A were also similar to those of the control group.

Weight gain was reduced in the 800 and 1,200 mg/kg groups of females-B during days 8 to 15 of gestation and the mean body weights of these groups were significantly lower ($P \leq 0.05$) than that of the control group on gestation day 15 (Figure 3, Table 1). However, this does not appear to be a true reduction in weight gain but rather the result of the lower number of pups *in utero* in the 800 and 1,200 mg/kg groups: 127 and 100 pups respectively, versus 139 in the control group (Table 8). The postpartum mean body weights of all female-B dose groups were nearly identical to that of the control group as were the mean pup weights (Figure 4).

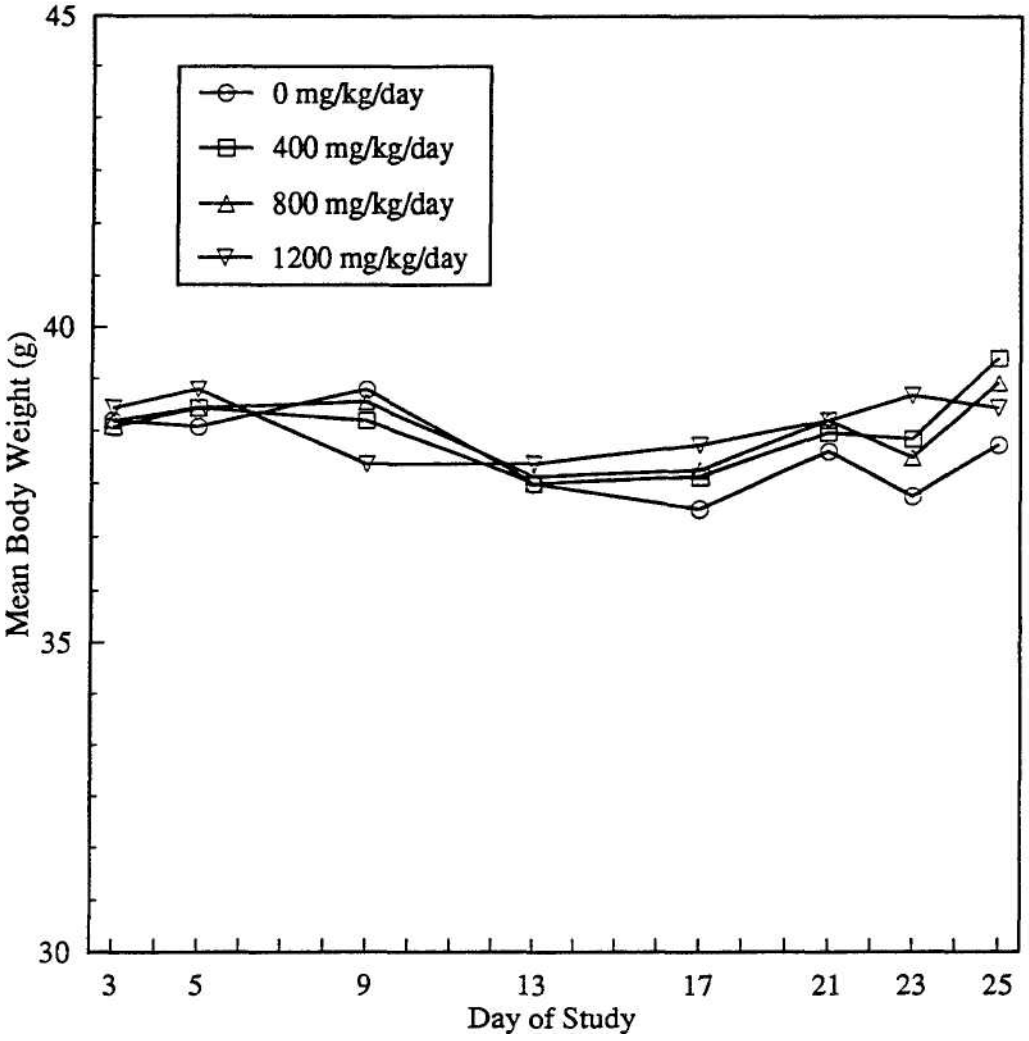


FIGURE 1 Mean Body Weights of Males in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice

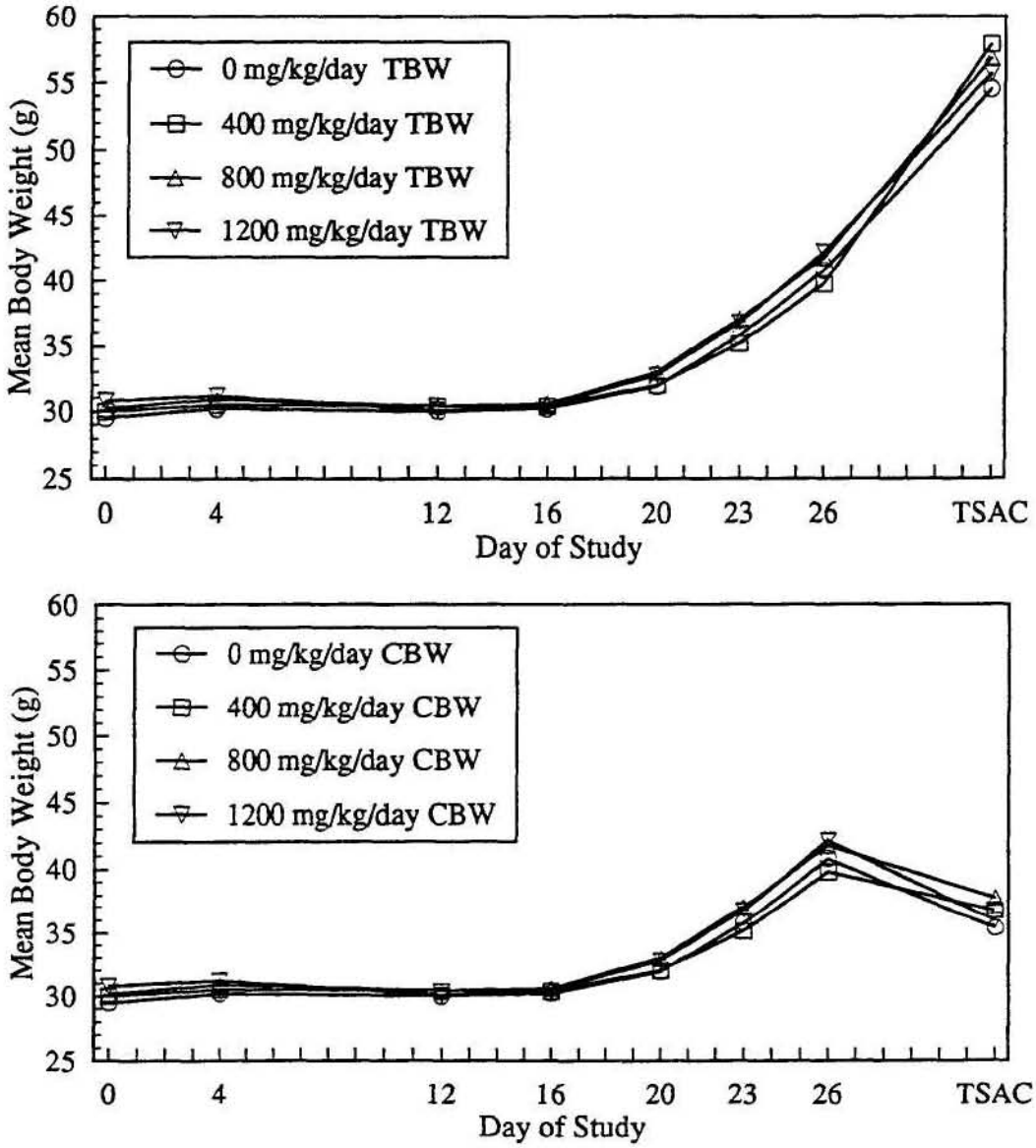


FIGURE 2 Mean Body Weights of Females-A in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice [TBW = Terminal Body Weight prior to Caesarean-Section; CBW = Corrected Body Weight (TBW - gravid uterine weight); TSAC = Terminal Sacrifice]

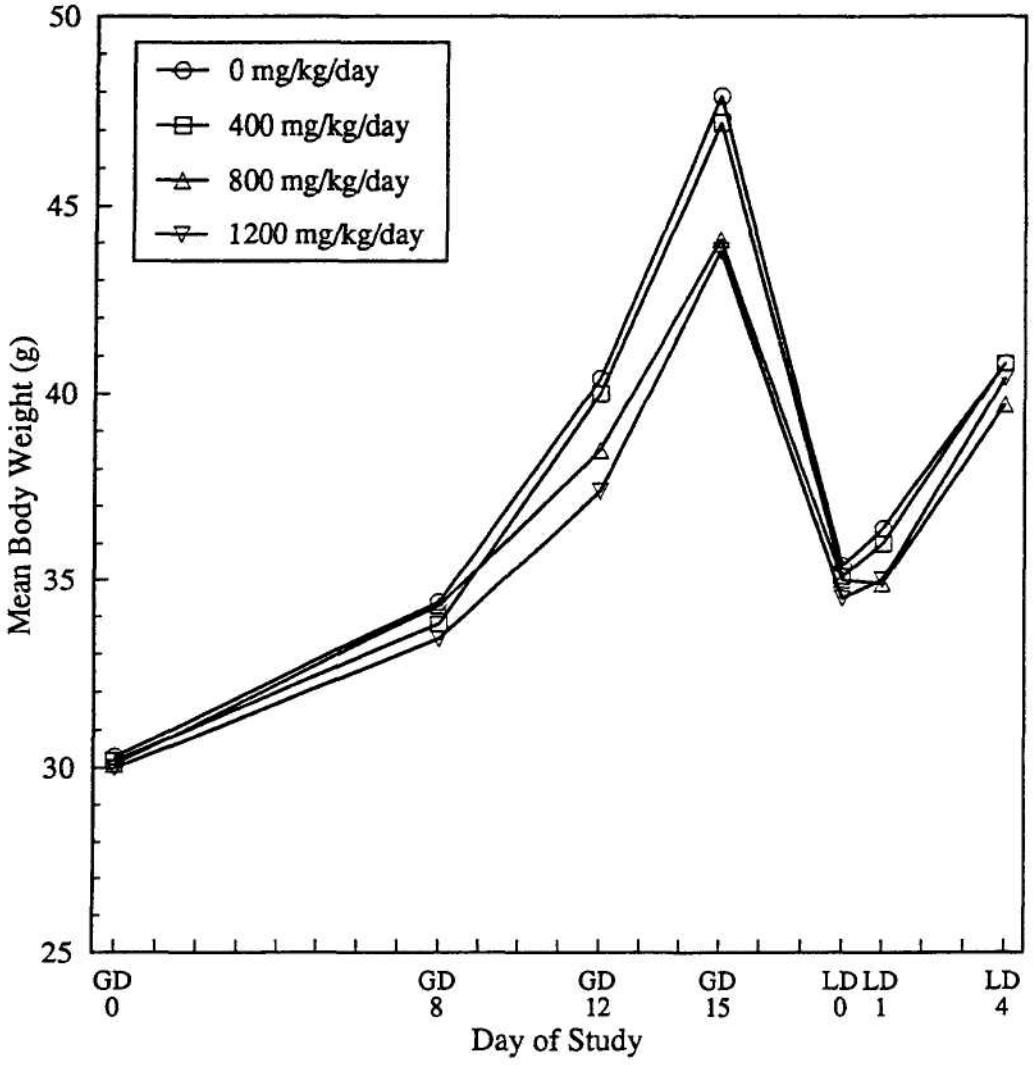


FIGURE 3 Mean Body Weights of Females-B in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-I[8]) Mice [GD = Gestation Day; LD = Lactation Day]

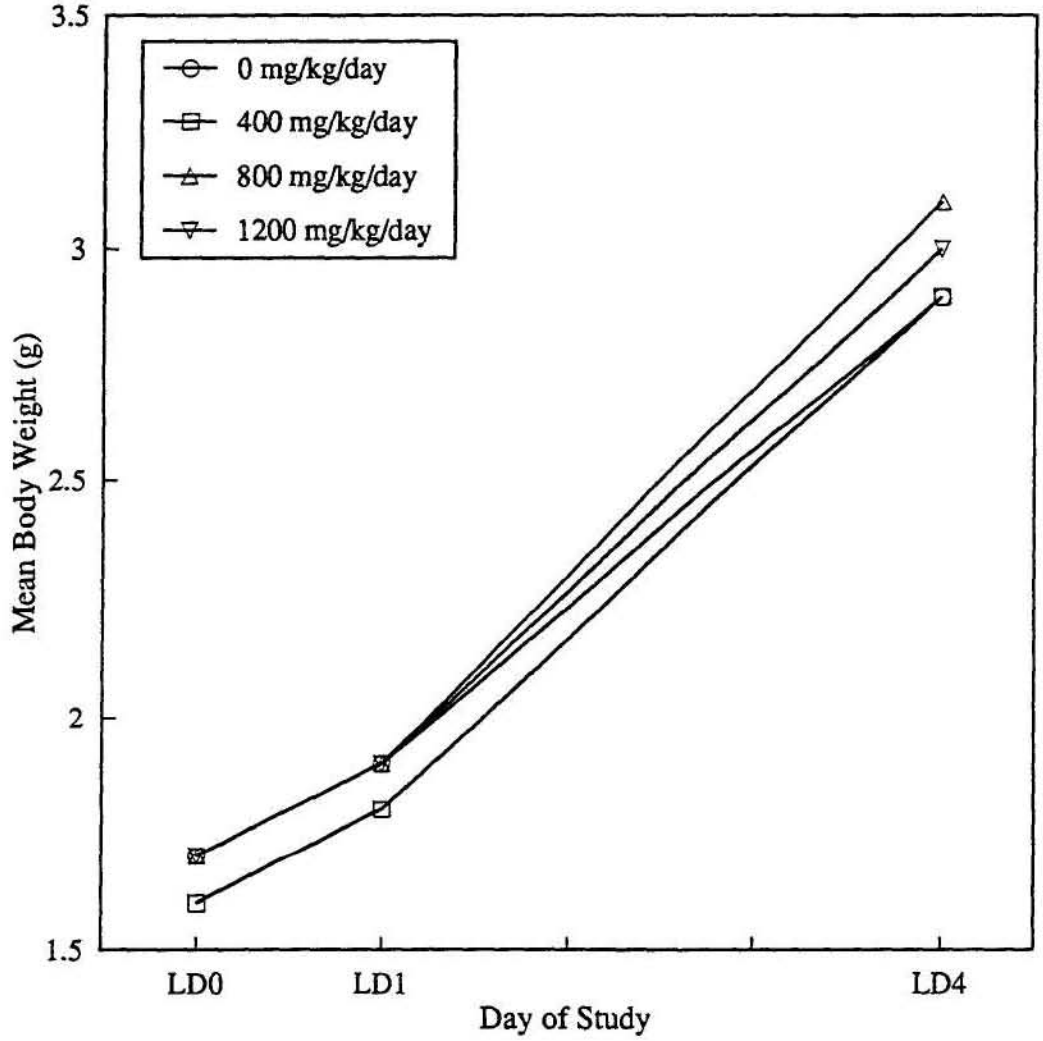


FIGURE 4 Mean Body Weights of Female-B Pups in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice [LD = Lactation Day]

TABLE 1 Body Weights of Females-B in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-I®) Mice[a]

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
Mice Assigned to				
Natural Delivery (n)	17	15	15	14
Pregnant (n)	11	12	14	11
GD 0	30.3 ± 0.9	30.2 ± 1.4	30.1 ± 1.0	30.0 ± 1.5
GD 8	**34.4 ± 1.5**	**33.8 ± 2.4**	**34.3 ± 1.2**	33.4 ± 1.4
GD 12	40.4 ± 2.4[7][b]	40.0 ± 3.4[9][b]	38.5 ± 2.1[ll][b]	37.4 ± 2.0[8][b]
GD15	47.9 ± 2.5	47.2 ± 4.5	44.1 ± 4.8*	**43.8 ± 3.9***
LD 0	35.4 ± 1.1	35.1 ± 2.5	35.0 ± 2.1[10][b]	**34.5 ± 1.5**
LD 1	36.4 ± 1.6	36.0 ± 2.8	34.9 ± 2.3	35.0 ± 2.0[9][c]
LD 4	40.8 ± 2.1	40.8 ± 3.4	39.7 ± 3.8	40.4 ± 3.0[9][c]

* Significantly different from the vehicle control group value (PsO.05) by Dunnett's or Dunn's test
GD=Gestation day
LD = Lactation day
[a] Data are presented as mean ± standard deviation
[b] Number of values averaged (due to error some values were not recorded at this time point)
[c] Excludes values for a dam with no surviving pups

During lactation, mean pup weights of dosed groups continued to be similar to the control group (Table 8). Although the reduction in the number of pups delivered occurred in the 800 and 1,200 mg/kg groups, it appears to be the result of biological variation rather than the result of the administration of pyrazinamide, since: a) the females-B were mated with untreated males, and b) the females-B were not treated until after implantation (day 6 of gestation). Also, the rate of resorption in the two highest dose groups was similar to the rate in the control group (Table 9).

Mean right epididymis weights were slightly decreased in all treated groups relative to the control group. Respective mean epididymis weights for the 400, 800, and 1,200 mg/kg groups were approximately 1.8% (47.75 mg), 10.0% (43.77 mg), and 2.6% (47.37 mg) lower than the mean body weight (48.61 mg) for the control group. The statistically significant ($P < 0.05$) decline in the 800 mg/kg group was not believed to be biologically significant, as a dose-related effect was not evident. Although Morrissey *et al.* (1989) found that reduced epididymal weights correlated well with reduced fertility in mouse continuous breeding studies, fertility was apparently unimpaired in the present study.

CLINICAL PATHOLOGY

Administration of pyrazinamide to male mice at 400, 800, and 1,200 mg/kg did not produce any biologically significant hematologic alterations. However, there were slight but statistically significant increases in MCH in 1,200 mg/kg males, and there were statistically significant increases in MCHC in 800 and 1,200 mg/kg males (Table 2).

Hematology values for dosed females-A and -B were generally similar to those of the control groups (Table 3). Reticulocyte counts in females-B were slightly reduced in the 800 and 1,200 mg/kg groups, and these reductions were statistically significant but not considered to be biologically significant. No significant effects occurred in any of the clinical chemistry parameters evaluated in this study (Table 4).

PLASMA PYRAZINAMIDE CONCENTRATIONS

The internal exposure of pyrazinamide appeared to be dose proportional based on C_{max} values measured at the end of the study (Table 5). No sex differences were apparent. The estimates for T_{max} and $t_{1/2}$ were consistent for all doses for males and females. The 90-minute T_{max} value for the 1,200 mg/kg dose in female mice resulted from the limited number of time points taken; the actual value is between 30 and 90 minutes. The estimates for $t_{\%}$ tend to support the conclusion that the doses given fall within the linear range. The variations seen in the estimates for $t_{1/2}$ showed no apparent dose dependency and may be accounted for by

interanimal variability and the uncertainty in the estimates, which were based on a small number of time points.

TABLE 2 Selected Hematology Parameters in Males in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice[a]

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
n	10	10	10	10
Hematocrit (%)	49.1 ± 3.2	47.9 ± 2.3	48.3 ± 4.0	48.3 ± 3.4
Hemoglobin (g/dL)	16.0 ± 1.1	15.6 ± 0.7	16.1 ± 1.5	16.0 ± 1.2
Erythrocytes (10^6/MM^3)	10.17 ± 0.72	9.87 ± 0.60	10.01 ± 0.90	9.89 ± 0.71
Reticulocytes (K^/MM^3)	3.9 ± 0.65	3.7 ± 0.42	3.6 ± 0.64	3.7 ± 0.74
Mean cell volume (fL)	48.4 ± 1.3	48.6 ± 1.9	48.3 ± 1.2	48.8 ± 1.2
Mean cell hemoglobin (pg)	15.7 ± 0.3	15.8 ± 0.5	16.0 ± 0.3	16.2 ± 0.4*
Mean cell hemoglobin concentration (g/dL)	32.6 ± 0.51	32.6 ± 0.54	33.2 ± 0.69*	33.2 ± 0.63*
Platelets (10^3/MM^3)	1,188 ±211.0	1,257 ± 225.3	1,250 ±351.7	1,236 ± 265.5
Leukocytes (10^3/MM^3)	7.95 ± 1.99	7.30 ± 3.24	7.09 ± 2.23	8.61 ± 2.69

* Significantly different from control group ($P \leq 0.05$) by Dunnett's test
[a]Data are presented as mean ± standard deviation

TABLE 3 Selected Hematology Parameters in Females in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice[a]

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
Females-A				
n	20	19	20	20
Hematocrit (%)	42.0 ± 2.6	42.1 ± 3.3	43.4 ± 4.1	42.6 ± 2.8
Hemoglobin (g/dL)	14.0 ± 1.1	13.8 ± 0.9	14.1 ± 1.5	13.9 ± 0.9
Erythrocytes (10^6/MM^3)	8.78 ± 0.71	8.75 ± 0.59	8.90 ± 0.93	8.59 ± 0.60
Reticulocytes (10^5/MM^3)	3.4 ± 1.19[b]	3.6 ± 1.54	3.5 ± 0.68	4.0 ± 1.96[C]
Mean cell volume (fL)	48.0 ± 2.5	48.1 ± 1.6	48.9 ± 1.7	49.6 ± 2.5
Mean cell hemoglobin (pg)	16.0 ± 0.8	15.8 ± 0.7	15.9 ± 0.4	16.2 ± 0.8
Mean cell hemoglobin concentration (g/dL)	33.3 ± 1.4	32.9 ± 1.4	32.5 ± 0.8	32.6 ± 1.2
Platelets (10^3/MM^3)	1,268 ± 322.2	1,305 ± 360.8	1,434 ± 241.0	1,453 ± 197.5
Leukocytes (10^3/MM^3)	7.86 ± 1.87	7.73 ± 2.09	7.11 ± 1.63	7.68 ± 1.45
n	16	15	14	14
Hematocrit (%)	43.3 ± 4.6	41.8 ± 2.5	42.2 ± 2.2	43.0 ± 4.4
Hemoglobin (g/dL)	14.1 ± 1.7	13.8 ± 0.9	14.0 ± 0.9	14.1 ± 1.6
Erythrocytes (10^6/MM^3)	8.89 ± 1.13	8.52 ± 0.69	8.59 ± 0.65	8.84 ± 1.12
Reticulocytes (10^5/MM^3)	4.6 ± 1.23	4.3 ± 0.81	3.3 ± 1.04**	3.4 ± 1.64*
Mean cell volume (fL)	48.9 ± 2.7	49.1 ± 2.4	49.2 ± 1.8	48.9 ± 3.1
Mean cell hemoglobin (pg)	15.9 ± 0.9	16.2 ± 0.7	16.3 ± 0.6	16.0 ± 0.8
(g/dL)	**32.6 ± 1.2**	32.9 ± 1.3	33.1 ± 0.9	32.8 ± 0.9
Platelets (1O^3/MM^3)	1,264 ± 267.0	1,313 ± 329.0	1,268 ± 264.7	1,300 ± 338.5
Leukocytes (10^3/MM^3)	7.6 ± 3.40	6.36 ± 1.20	7.19 ± 3.13	7.78 ± 1.66

* Significantly different from control group ($P \leq 0.05$) by Dunnett's test
** ($P \leq 0.01$)
[a] Data are presented as mean ± standard deviation
[b] n=19
[c] n=17

TABLE 4 Selected Clinical Chemistry Parameters in Females in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice[a]

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
Females-A				
n	11	11	11	11
Alanine aminotransferase (IU/L)	44 ± 22	38 ± 10	44 ± 11	40 ± 14
Alkaline phosphatase (IU/L)	56 ± 8	55 ± 15	56 ± 16	45 ± 12
Aspartate aminotransferase (IU/L)	88 ± 24	71 ± 21	89 ± 16	79 ± 19
Sorbitol dehydrogenase (IU/L)	27 ± 13[b]	21 ±4[C]	29 ±3[d]	25 ±5°
Bile acids (µmol/L)	20 ± U.5[e]	12 ± 7.2[f]	11 ± 9.7[f]	14 ± 7.9[f]
n	17	15	15	14
Alanine aminotransferase (IU/L)	45 ± 17	40 ± 11	47 ± 15	43 ± 11
Alkaline phosphatase (IU/L)	55 ± 18	44 ± 18	49 ± 20	47 ± 25
Aspartate aminotransferase (IU/L)	85 ± 23	77 ± 15	85 ± 28[g]	83 ±31
Sorbitol dehydrogenase (IU/L)	20 ± 8[h]	17 ±5[h]	15 ± 10[i]	17 ±8[i]
Bile acids (µmol/L)	16 ± 5.3[J]	18 ± 10.4[j]	14 ± 7.7[b]	15 ±6.1[e]

[a] Data are presented as mean ± standard deviation
[b] n=7
[c] n=3
[d] n=4
[e] n=8
[f] n=6
[s] n=14
[h] n=U
[1] n=9
[j] n=10

TABLE 5 Toxicokinetic Parameters of Pyrazinamide in Swiss (CD-1®) Mice[a]

Parameter	Males (mg/kg)			Females (mg/kg)		
	400	800	1,200	400	800	1,200
C_{max} (μg/mL)	143	315	539	113	222	408
T_{max} (min)	30	30	30	30	30	90
$T_{1/2}$ (min)	58.3	99.2	102	74.8	61.5	47.7[b]

[a] Except as noted, parameters were estimated from 30-, 90-, and 120-minute time points
[b] Based on 90- and 120-minute time points

GROSS NECROPSY FINDINGS AND HISTOPATHOLOGY

Microscopic evaluation of a gross lesion observed in the lung of one female-B in the 1,200 mg/kg group revealed the presence of a nodule composed of slightly anaplastic alveolar/bronchiolar epithelial cells. The lesion was well delineated and mitotic figures were not abundant. Slight compression of the adjacent pulmonary parenchyma was evident. These morphologic findings were compatible with a diagnosis of an alveolar/bronchiolar adenoma. This lesion was considered incidental and unrelated to chemical treatment. No other gross lesions of any consequence were observed.

SPERM EVALUATION

Male reproductive parameters were not significantly affected by the administration of pyrazinamide (Table 6).

TABLE 6 Reproductive Parameters in Males in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-I®) Mice[a]

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
n	10	10	10	10
Body weight (g)	37.94 ± 0. 80	39.54 ± 0. 80	39.18 ± 0. 54	38.94 ± 0. 61
Left caudal weight (mg)	18. 1 ± 1. 1	21. 3 ± 1. 0	18.8 ± 0 . 9	19. 6 ± 0 .8
Left epididymal weight (mg)	47. 2 ± 1. 9	50. 8 ± 1. 8	48. 2 ± 1. 0	47. 2 ± 1. 2
Left testicular weight (mg)	120. 8 ± 6 .2	125. 9 ± 3 .3	116.7 ± 5.9	123. 4 ± 4. 0
Epididymal sperm motility (%)	83. 40 ± 0 .42[d]	76. 36 ± 3. 37	76.76 ± 5. 72	75. 54 ± 3 .23
Epididymal sperm density[13] (x 10^6)	1,383 ± 90[d]	1,085 ± 39	1,229 ± 87	1,160 ± 65
Spermatid heads/testis (x 10^7)	2. 16 ± 0 .71	2. 32 ± 0 .07	2 .26 ± 0. 06	2. 25 ± 0. 05
Spermatid heads/g testis[c] (x 10^7)	17. 73 ± 1 .22	18. 48 ± 0. 45	19. 70 ± 0. 84	18. 32 ± 0. 42

[a] Data are presented as mean ± standard error
[b] Per gram of caudal tissue
[c] Total spermatid heads per testis/testicular weight in grams
[d] n=9

PREGNANCY AND FETAL PARAMETERS

In the female-B group, pregnancy occurred in 11/17 (64.7%), 12/15 (80.0%), 14/15 (93.3%), and 11/14 (78.6%) sperm-positive mice assigned to natural delivery in the groups administered pyrazinamide at 0, 400, 800, and 1,200 mg/kg, respectively. No significant differences were found between the dosed females-B group dams or their pups in comparison to the control group (Tables 7 and 8). The total number of pups delivered in the 800 and 1,200 mg/kg groups was lower than that in the control group, but as indicated earlier, this appears to be the result of biological variation as does the lower number of implantation sites in the 1,200 mg/kg group. One control dam and one dam in the 400 mg/kg group each had one stillborn pup; all others had only liveborn pups, and nearly all liveborn pups from all groups survived through postpartum day 4.

In the female-A group, pregnancy occurred in 16 (80.0%), 12 (60.0%), 13 (65.0%), and 17 (85.0%) of the 20 mice per group administered 0, 400, 800, and 1,200 mg/kg, respectively (Table 9). In the 800 mg/kg group, one mouse delivered a litter before sacrifice and one, two, three, and two mice from the 0, 400, 800, and 1,200 mg/kg groups, respectively, were sacrificed on the estimated day 14 of gestation due to technical error. Data from the dam that delivered early were excluded from averages, and fetal weight data were excluded from averages for the eight litters caesarean-sectioned on the estimated day 14 of gestation (two dams, one from the 400 mg/kg group and one from the 1,200 mg/kg group, had fetuses at such an early gestational age that viability and sexes could not be determined; all data were excluded for these two dams and litters).

TABLE 7 Natural Delivery Observations for Females-B in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-I®) Mice

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
Females Mated				
n	17	15	15	14
n	11	12	14	11
%	64.7	80.0	93.3	78.6
n	11	12	13	10
%	64.7	80.0	86.7	71.4
mean ± standard deviation	20.0 ± 0.0	19.8 ± 0.4	20.1 ± 0.3	20.4 ± 0.7
n	145	168	142	109
mean ± standard deviation	13.2 ± 2.1	14.0 ± 1.6	10.9 ± 3.0	10.9 ± 4.2
n	1	1	0	0
%	9.1	8.3	0.0	0.0
n	0	0	0	0

TABLE 8 Litter Observations and Mean Pup Weights for Females-B in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
n	11	12	13	9[a]
n	139 12.6±2.0[b]	150 12.5 ± 1.9	127 9.8 ± 3.1	100 11.1 ± 3.0
n	138	149	127	100
n %	0.7	1 0.7	0 0.0	0 0.0
	0.1 ± 0.3[b]	0.1 ±0.3	0.0 ±0.0	0.0 ±0.0
Day 0 n %	0 0.0	0 0.0	0 0.0	1 1.0
n %	1 0.7	3 2.0	3 2.4	0 0.0
Live Pups/Litter at Weighing				
Day 0	12.5 ± 2.1°	12.4 ± 1.9	9.8 ± 3.1	11.0 ± 3.1
Day 1	12.5 ±2.1	12.3 ± 1.9	9.6 ± 3.4	11.0 ± 3.1
Day 4	12.4 ± 2.0	12.3 ± 1.9	9.5 ± 3.5	11.0 ± 3.1
Day 0	1.7 ±0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
Day 4	2.9 ± 0.3	2.9 ± 0.2	3.1 ± 0.4	3.0 ± 0.4

[a] Excludes one litter that consisted of two pups of undetermined viability

[b] Data are presented as mean number of pups/litter ± standard deviation

[c] Data are presented as mean ± standard deviation

TABLE 9 Caesarean-Section Observations and Fetal Data for Females-A in the Reproductive, Developmental, and General Toxicity Studies of Pyrazinamide in Swiss (CD-1®) Mice

	Vehicle Control	400 mg/kg	800 mg/kg	1,200 mg/kg
Mice Tested				
n	20	20	20	20
n %	16 80 0	12 600	13 65 0	17 85 0
n	0	0	1	0
-				
n	16	ll[a]	12	16[a]
Corpora Lutea	13 7 ± 1 6[b]	*HA- ± 2 6*	14 1 ± 3 8	14 1 ± 2 9
Implantations	12 8 ± 1 5	134 ± 22	12 8 ± 40	13 1 ± 22
Litter Sizes	11 4 ± 1 6	124 ± 2 6	11 3 ± 44	12 4 ± 2 2
n	181 113 ± 15	134 122 ± 25	134 11 2 ± 43	199 12 4 ± 2 2
n	1 0 1 ± 02	2 02 ± 04	2 02 ± 04	0 00 ± 00
Resorptions	1 4 ± 1 2	1 1 ± 1 8	1 5 ± 1 5	07 ± 09
n	17 1 1 ± 0 9	10 0 9 ± 1 8	18 1 5 ± 1 5	9 0 6 ± 0 9
n	6 04 ± 06	2 02 ± 04	0 00 ± 0 0	2 0 1 ± 03
n %	12 75 0	6 54 5	9 75 0	8 500
n	0	0	0	0
n %	16 100 0	11 100 0	12 100 0	16 100 0

[a] Excludes two dams (400 and 1,200 mg/kg group) that were sacrificed by error on the estimated day 14 of gestation Due to gestational age of fetuses, sexes and viability could not be determined

[b] Data are presented as mean ± standard deviation

Necropsy observations were based on 16, 11, 12, and 16 litters in the 0, 400, 800 and 1,200 mg/kg groups, respectively. There were no effects on any of the parameters evaluated in females-A that could clearly be attributed to the administration of pyrazinamide (Table 9). All values were comparable to the control group values with the exception of lower female fetal body weights in the 800 and 1,200 mg/kg groups which were significant ($P \leq 0.05$) in the 1,200 mg/kg group (1.26 g and 1.25 g, respectively, vs. 1.36 g for the control group). It is uncertain whether these differences are related to the test article because: 1) there was no clear dose-response relationship, 2) the 1,200 mg/kg group had the largest live litter size (12.4 vs. 11.4 for the control group) and the lowest incidence of resorptions (0.7 vs. 1.4 for the control group) of any group, 3) there was no effect on body weight of dams or gravid uterine weight, and 4) no difference in pup weight was observed in the female-B litters (this group received pyrazinamide during days 6 through 15 of presumed gestation and was mated with undosed males). Future studies in this series using higher doses of pyrazinamide may help to resolve this question.

No gross external fetal malformations or alterations were observed as a consequence of administering pyrazinamide to pregnant mice.

In summary, with the exception of the equivocal findings in regard to the lower female fetal body weights in females-A, no apparent reproductive or developmental toxic effects of pyrazinamide were detected in male or female Swiss (CD-1®) mice. However, results of this study indicated that higher doses could have been tolerated, and future studies for toxicity of combination therapies may include higher doses of pyrazinamide. The dose of 1,200 mg/kg body weight employed in this study is equivalent to approximately eight times the therapeutic dose in female Swiss (CD-1®) mice (Grossett *et al.*, 1992) and resulted in a C_{max} 9 to 12 times the C_{max} caused by the therapeutic dose.

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