NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF MIXTURES OF 3′-AZIDO-3′-DEOXYTHYMIDINE (AZT), LAMIVUDINE (3TC), NEVIRAPINE (NVP), AND NELFINAVIR MESYLATE (NFV)

(CAS NOS. 30516-87-1, 134678-17-4, 129618-40-2, 159989-65-8)

IN B6C3F1 MICE

(TRANSPLACENTAL EXPOSURE STUDIES)

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

January 2013

NTP TR 569

NIH Publication No. 13-5911

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

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The study on 3′-azido-3′-deoxythymidine (AZT), lamivudine (3TC), nevirapine (NVP), and nelfinavir mesylate (NFV) was conducted at the Food and Drug Administration's (FDA) National Center for Toxicological Research (NCTR) under an interagency agreement between the FDA and the National Institute of Environmental Health Sciences (NIEHS). The studies were monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA centers, NIEHS, and other *ad hoc* members from other governmental agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers with information for hazard identification and risk assessment.

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SUMMARY

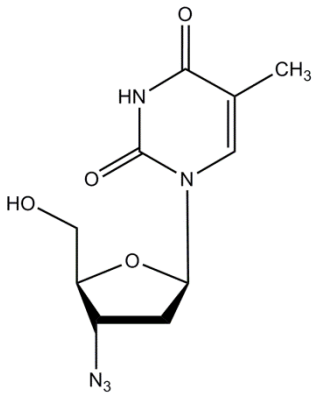
Background
Antiretroviral drugs are used to treat patients positive for the human immunovirus HIV-1, and increasingly treatments include a combination of such drugs. The noninfected children of women who are pregnant and receiving such treatment may also be exposed to the drugs by transplacental exposure. We studied the long-term effects of such transplacental exposure in mice by exposing pregnant mice to combinations of four such antiretroviral drugs for seven days and then observing their pups for two years following birth. The four drugs studied were 3′-azido-3′-deoxythymidine (AZT), lamivudine (3TC), nevirapine (NVP), and nelfinavir mesylate (NFV).

Methods
Four different sets of exposure studies were performed: exposure to AZT; to AZT plus 3TC; to AZT, 3TC, and NVP; or to AZT, 3TC, and NFV. In each of these studies, groups of pregnant females were given one of three concentrations of the drug combinations seven times though a tube directly into their stomachs, and after birth their pups were maintained with no further exposure for two years. The offspring of another group of pregnant females not treated with the drugs served as controls. At the end of the study, tissues from more than 40 sites were examined for every animal.

Results
Survival of pups whose mothers were exposed to AZT or AZT plus 3TC was similar to their controls, while the survival rates for offspring of mice exposed to AZT, 3TC, and NVP or AZT, 3TC, and NFP were lower than for controls. In most cases the body weights of pups from mothers exposed were slightly less than those of the controls. There were slight increases in the incidences of thyroid gland tumors and skin tumors in the female pups of mothers exposed to AZT alone and of lung tumors in female pups of mothers exposed to AZT plus 3TC. For offspring of mothers exposed to AZT, 3TC, and NVP there were increased incidences of skin tumors in both male and female pups, and more so in the males.

Conclusions
We conclude that exposure to the combination of AZT, 3TC, and NVP during pregnancy caused an increase in skin tumors in the male offspring and possibly also to the female offspring. Exposure to AZT alone during pregnancy may have been related to thyroid gland or skin tumors in female offspring, and exposure to AZT plus 3TC may have been related to lung tumors in female offspring.

ABSTRACT

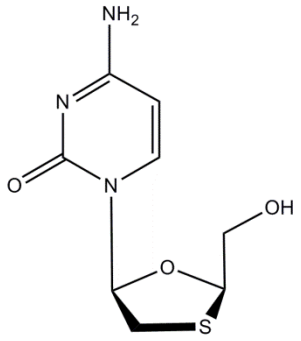


3′-AZIDO-3′-DEOXYTHYMIDINE

CAS No. 30516-87-1

Chemical Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24

Synonyms: AZT; zidovudine; 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; 3N-deoxy-3′-azidothymidine; 3′-deoxy-(8CI)(9CI); BW A509U; Compound S; ZDV
Trade name: Retrovir®

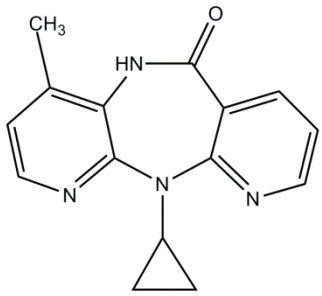


LAMIVUDINE

CAS No. 134678-17-4

Chemical Formula: $C_8H_{11}N_3O_3S$
Molecular Weight: 229.26

Synonyms: 3TC; (-)2′,3′-dideoxy-3′-thiacytidine; (2*R*-*cis*)-4-amino-1-[2-(hydroxymethyl)-; 1,3-oxathiolan-5-yl]-2(1*H*)-pyrimidinone; (-)-BCH-189; GR-109714X
Trade names: Epivir®, Zeffix®

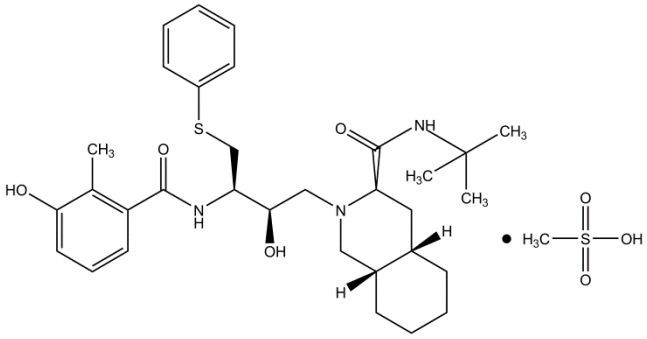


NEVIRAPINE

CAS No. 129618-40-2

Chemical Formula: $C_{15}H_{14}N_4O$
Molecular Weight: 266.30

Synonyms: NVP; BIRG-587; 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido-[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one
Trade name: Viramune®



NELFINAVIR MESYLATE

CAS No. 159989-65-8

Chemical Formula: $C_{32}H_{45}N_3O_4S{\bullet}CH_3SO_3H$
Molecular Weight: 663.89

Synonyms: NFV; AG1343; (*3S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)decahydro-2-[(2*R*,3*R*)-2-hydroxy-3-isoquinoline carboxamide] methane sulfonate
Trade name: Viracept®

With the increased administration of multidrug regimens to pregnant women who are human immunodeficiency virus type-1 (HIV-1) positive, along with the increased efficacy of these combinations, determining the long-term consequences of the antiretroviral agents in noninfected children becomes important. The goal of the current study was to determine the carcinogenicity of combinations of antiretroviral drugs in male and female B6C3F1 mouse pups exposed transplacentally and monitored for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*.

AZT

3′-Azido-3′-deoxythymidine (AZT) was synthesized initially for use as an anticancer agent and was later reported to block the infectivity and cytopathic effects, *in vitro*, of HIV-1, due to the inhibition (by AZT 5′-triphosphate) of viral reverse transcriptase. Pregnant women who are positive for HIV-1 are given AZT to manage the infection and to prevent maternal-to-fetal transmission of the virus.

3TC

Lamivudine (3TC) was synthesized initially as a racemate and then in enantiomerically pure forms. 3TC (as 3TC 5′-triphosphate) is thought to inhibit viral reverse transcriptase by competing with deoxycytidine 5′-triphosphate for incorporation into HIV-1 DNA. When used for the management of HIV-1 infections, 3TC is always used in combination with another nucleoside reverse transcriptase inhibitor (e.g., AZT) and either a protease inhibitor (e.g., nelfinavir mesylate, NFV) or a nonnucleoside reverse transcriptase inhibitor (e.g., nevirapine, NVP).

NVP

NVP, a nonnucleoside reverse transcriptase inhibitor, was first synthesized in 1991. NVP inhibits HIV-1 reverse transcriptase noncompetitively by binding to an allosteric site on the enzyme; this action is specific for HIV-1 reverse transcriptase. NVP is usually given as part of a three-drug regimen. Typical regimens in adults and adolescents include NVP and 3TC or emtricitabine and AZT or tenofovir.

NFV

The synthesis of NFV was reported in 1997. NFV acts by inhibiting HIV-1 protease, the enzyme responsible for cleavage of the polyprotein resulting from the *gag* and *gag-pol* genes of HIV-1. This inhibition results in an immature, noninfectious virus. NFV is always used in combination with other antiretroviral agents, typically two nucleoside reverse transcriptase inhibitors (e.g., AZT and 3TC).

2-YEAR TRANSPLACENTAL STUDY IN MICE

Female C57Bl/6N mice were bred to male C3H/HeNMTV mice, and from gestation day 12 until gestation day 18 (or until they littered), the pregnant dams were treated by gavage with AZT or mixtures of AZT and 3TC; AZT, 3TC, and NVP; or AZT, 3TC, and NFV. The high dose of each drug was 240 mg/kg body weight per day for AZT, 120 mg/kg body weight per day for 3TC, 168 mg/kg body weight per day for NVP, and 1,008 mg/kg body weight per day for NFV (ratio 1.0:0.5:0.7:4.2, respectively). The mid and low doses were 66% and 33% of these values, respectively, and maintained the same ratio among the drugs. The drugs were administered in a 0.2% methylcellulose and 0.1% Tween® 80 vehicle at a dosing volume of 20 mL/kg body weight. Control dams were administered the vehicle only. The tumor incidence in the male and female B6C3F1 offspring was monitored for 2 years after birth. The group sizes varied between 15 and 65 male or female mice per treatment.

Compared to the vehicle control group, none of the treatments affected the body weights of the pregnant dams. Likewise, none of the treatments affected the number of pups per litter or the ratio of male to female pups. Combinations of AZT/3TC/NVP and AZT/3TC/NFV caused dose-related decreases in body weights of male and female B6C3F1 offspring. Transplacental exposure to AZT/3TC/NVP and AZT/3TC/NFV caused dose-related decreases in survival of the B6C3F1 mice from birth until weaning at postnatal day 21.

Postweaning survival of transplacentally exposed groups of female mice was similar to that of the control group for each drug combination. Survival of all groups of male mice transplacentally exposed to AZT or AZT/3TC was similar to that of the control group; survival of male mice transplacentally exposed to AZT/3TC/NVP or AZT/3TC/NFV was decreased in a dose-related manner that was significant in the high-dose group for each of the drug combinations, relative to controls.

Mean body weights of female mice transplacentally exposed to AZT or the combination of AZT/3TC were similar to those of the controls during the 2-year transplacental exposure study. Transplacental exposure to the combination of AZT/3TC/NVP resulted in dose-related decreases in body weights in female mice; the high-dose group was significantly different from the control group at all time points, with the average decrease in weight being 18%; the low- and mid-dose combinations were significantly different from the control group at most time points, with the average decreases in weight being 8% and 5%, respectively. In female mice exposed to the combination AZT/3TC/NFV, the high-dose group was significantly different from the control group at all time points, with the average decrease in weight being 13%; the low- and mid-dose groups were significantly different from the control group at most time points, with the average decreases in weight being 5% and 6%, respectively.

Male mice exposed transplacentally to AZT showed dose-related decreases in body weight, with the differences being significant in all exposed groups at all time points. Compared to the control group, the average decrease in body weight was 9% in the high-dose group, 6% in the mid-dose group, and 5% in the low-dose group. Transplacental exposure to the combination of AZT/3TC caused dose-related decreases in body weight in male mice, with the differences being significant at all time points in the high- and mid-dose groups, and at nearly all time points in the low-dose group. The average decrease in body weight was 7% in the high-dose group, 5% in the mid-dose group, and 3% in the low-dose group. Male mice exposed transplacentally to the combination of AZT/3TC/NVP or the combination of AZT/3TC/NFV showed dose-related decreases in body weight, with the differences being significant in all exposed groups at all time points. For the AZT/3TC/NVP combination, the average decrease in body weight was 18% in the high-dose group, 9% in the mid-dose group, and 7% in the low-dose group. For the AZT/3TC/NFV combination, the average decrease in body weight was 11% in the high-dose group, 7% in the mid-dose group, and 4% in the low-dose group.

Transplacental exposure to AZT caused positive trends in the incidences of follicular cell adenoma of the thyroid gland, follicular cell adenoma or carcinoma (combined), and subcutaneous fibrosarcoma or sarcoma (combined) of the skin in female mice. The incidences of follicular cell adenoma of the thyroid gland (after adjusting for possible dam or sire effects) and follicular cell adenoma or carcinoma (combined) of the thyroid gland were significantly increased in female mice exposed to 240 mg/kg AZT.

Transplacental exposure to mixtures of AZT/3TC resulted in a positive trend in the incidences of alveolar/bronchiolar adenoma of the lung in female mice.

Transplacental exposure to mixtures of AZT/3TC/NVP caused positive trends in the incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma (combined) of the skin; and subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma (combined) of the skin in male mice. The incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma of the skin (combined); and subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma of the skin (combined) were significantly increased in the group of males exposed transplacentally to 240 mg/kg AZT, 120 mg/kg 3TC, and 168 mg/kg NVP. After adjusting for possible dam or sire effects, the incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma of the skin (combined); and subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma of the skin (combined) were significantly increased in the group of males transplacentally exposed to 160 mg/kg AZT, 80 mg/kg 3TC, and 112 mg/kg NVP. The incidence of subcutaneous skin fibrosarcoma was significantly increased in female mice in the same exposed group.

GENETIC TOXICOLOGY

AZT, 3TC, NVP, and NFV (the same lots that were used in the 2-year animal studies) were tested for bacterial mutagenicity in *S. typhimurium* strains TA98 and TA100 and in *E. coli* strain WP2 *uvrA*/pKM101. Only AZT was found to be mutagenic; the other three compounds showed no evidence of mutagenicity in bacteria. With AZT, significant increases in mutant colonies were seen in the *E. coli* strain, with and without induced rat liver metabolic activation enzymes. No evidence of mutagenicity was seen with AZT in *S. typhimurium* strains TA98 or TA100.

CONCLUSIONS

AZT

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity** of AZT in male B6C3F1 mice whose dams were exposed to 80, 160, or 240 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of AZT in female B6C3F1 mice based on increased incidences of thyroid gland neoplasms (primarily adenoma) and subcutaneous skin fibrosarcoma or sarcoma.

AZT and 3TC

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT and 3TC in male B6C3F1 mice whose dams were exposed to 80/40, 160/80, or 240/120 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of mixtures of AZT and 3TC in female B6C3F1 mice based on increased incidences of lung alveolar/bronchiolar adenoma.

AZT, 3TC, and NVP

Under the conditions of this transplacental exposure study, there was *some evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in male B6C3F1 mice whose dams were exposed to these chemicals by gavage based on increased incidences of subcutaneous skin neoplasms (fibroma, fibrous histiocytoma, or fibrosarcoma). There was *equivocal evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in female B6C3F1 mice based on an increased incidence of subcutaneous skin fibrosarcoma.

AZT, 3TC, and NFV

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NFV in male or female B6C3F1 mice whose dams were exposed to 80/40/336, 160/80/672, or 240/120/1,008 mg/kg by gavage.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 15.

Summary of the 2-Year Transplacental Carcinogenesis and Genetic Toxicology Studies of AZT, 3TC, NVP, and NFV

	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations *in utero*		
AZT	0, 80, 160, or 240 mg/kg	0, 80, 160, or 240 mg/kg
AZT/3TC	0, 80/40, 160/80, or 240/120 mg/kg	0, 80/40, 160/80, or 240/120 mg/kg
AZT/3TC/NVP	0, 80/40/56, 160/80/112, or 240/120/168 mg/kg	0, 80/40/56, 160/80/112, or 240/120/168 mg/kg
AZT/3TC/NFV	0, 80/40/336, 160/80/672, or 240/120/1,008 mg/kg	0, 80/40/336, 160/80/672, or 240/120/1,008 mg/kg
Body weights		
AZT	80 mg/kg group 5% less, 160 mg/kg group 6% less, and 240 mg/kg group 9% less than the control group	Exposed groups similar to the control group
AZT/3TC	160/80 mg/kg group 5% less, 240/120 mg/kg group 7% less than the control group	Exposed groups similar to the control group
AZT/3TC/NVP	80/40/56 mg/kg group 7% less, 160/80/112 mg/kg group 9% less, and 240/120/168 mg/kg group 18% less than the control group	80/40/56 mg/kg group 8% less, 160/80/112 mg/kg group 5% less, and 240/120/168 mg/kg group 18% less than the control group
AZT/3TC/NFV	160/80/672 mg/kg group 7% less, 240/120/1,008 mg/kg group 11% less than the control group	80/40/336 mg/kg group 5% less, 160/80/672 mg/kg group 6% less, and 240/120/1,008 mg/kg group 13% less than the control group
Survival rates		
AZT	46/65, 39/48, 38/48, 35/48	45/64, 38/48, 28/47, 37/48
AZT/3TC	46/65, 39/51, 35/48, 34/48	45/64, 32/48, 35/51, 35/48
AZT/3TC/NVP	46/65, 37/48, 35/48, 25/50	45/64, 31/48, 34/48, 39/49
AZT/3TC/NFV	46/65, 37/48, 36/51, 6/15	45/64, 30/50, 37/49, 16/26
Nonneoplastic effects		
AZT	None	None
AZT/3TC	None	None
AZT/3TC/NVP	None	None
AZT/3TC/NFV	None	None
Neoplastic effects		
AZT	None	None
AZT/3TC	None	None
AZT/3TC/NVP	Skin (subcutaneous tissue): fibroma, fibrous histiocytoma, or fibrosarcoma (2/65, 2/47, 7/48, 12/48)	None
AZT/3TC/NFV	None	None
Equivocal findings		
AZT	None	Thyroid gland (follicular cell): adenoma (0/59, 1/46, 0/46, 3/47); adenoma or carcinoma (0/59, 1/46, 0/46, 4/47) Skin (subcutaneous tissue): fibrosarcoma or sarcoma (2/63, 0/46, 4/47, 5/48)
AZT/3TC	None	Lung: alveolar/bronchiolar adenoma (2/62, 1/48, 3/50, 6/48)
AZT/3TC/NVP	None	Skin (subcutaneous tissue): fibrosarcoma (1/63, 0/47, 7/47, 0/49)
AZT/3TC/NFV	None	None
Level of evidence of carcinogenic activity		
AZT	No evidence	Equivocal evidence
AZT/3TC	No evidence	Equivocal evidence
AZT/3TC/NVP	Some evidence	Equivocal evidence
AZT/3TC/NFV	No evidence	No evidence

Summary of the 2-Year Transplacental Carcinogenesis and Genetic Toxicology Studies of AZT, 3TC, NVP, and NFV

Genetic toxicology	
Bacterial gene mutations:	
AZT	Negative in *S. typhimurium* strains TA98 and TA100, with and without S9; positive in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9
3TC	Negative in *S. typhimurium* strains TA98 and TA100, with and without S9; negative in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9
NVP	Negative in *S. typhimurium* strains TA98 and TA100, with and without S9; negative in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9
NFV	Negative in *S. typhimurium* strains TA98 and TA100, with and without S9; negative in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on mixtures of AZT, 3TC, NVP, and NFV on April 5, 2011, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW PANEL COMMENTS

On April 5, 2011, the draft Technical Report on the toxicology and carcinogenesis studies of mixtures of 3′-azido-3′-deoxythymidine (AZT), lamivudine (3TC), nevirapine (NVP), and nelfinavir mesylate (NFV) received public review by the National Toxicology Program's Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. F.A. Beland, NCTR, briefed the panel on the transplacental exposure studies of AIDS therapeutics. He noted that 40 million adults are infected with HIV worldwide, and 50% of them are women of childbearing age. In the absence of medical intervention, 25% of children born to HIV-positive women will become infected with the virus. Increasingly, multidrug antiretroviral regimens are being used by HIV-positive pregnant women, and although AZT is a known transplacental carcinogen in mice, there are limited data regarding the safety during pregnancy of other antiretroviral drugs or combinations. Thus, he stated that determining the long-term consequences of antiretroviral agents in non-infected children is important.

The proposed conclusions were: *No evidence of carcinogenic activity* of AZT in male B6C3F1 mice whose mothers were exposed to 80, 160, or 240 mg/kg by gavage, *equivocal evidence of carcinogenic activity* of AZT in female B6C3F1 mice, *no evidence of carcinogenic activity* of mixtures of AZT and 3TC in male B6C3F1 mice whose mothers were exposed to 80/40, 160/80, or 240/120 mg/kg by gavage, *equivocal evidence of carcinogenic activity* of mixtures of AZT and 3TC in female mice, *some evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in male B6C3F1 mice whose mothers were exposed to these chemicals by gavage, *equivocal evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in female B6C3F1 mice, and *no evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NFV in male or female B6C3F1 mice whose mothers were exposed to 80/40/336, 160/80/672, or 240/120/1,008 mg/kg by gavage.

Dr. Miller, the first primary reviewer, found the report to be very well written, but suggested photographs would augment the pathology information. He had several specific editorial comments and questions for Dr. Beland. His first comment was that it was not clear what criteria were used to judge whether body weight differences were considered biologically relevant, as both males and females treated with the combination of AZT/3TC/NVP had decreases in body weight of 18% in the high dose groups.

Dr. Miller asked that a primer on statistical methods used be included in the report, particularly the Poly-3 analysis and in terms of how various elements were weighted. He noted that in the AZT and 3TC regimens, there were some tumors not seen in the triple combinations, implying that there was some level of tumor suppression occurring, and noted that there should have been some elaboration on that element, potentially from the literature. Dr. Beland said that the incorrect body weight statement will be corrected and noted that the Poly-3 analysis is in fact survival-adjusted and corrects for animals that die early. Dr. J.R. Bucher, NIEHS, provided more details about the Poly-3 test. Based on the trend in the increased incidence of Harderian gland neoplasms in the male mice in the AZT/3TC/NFV groups, that it may be appropriate to change the call from *no evidence* to *equivocal evidence*.

Dr. Barlow, the second primary reviewer, agreed that further discussion was called for regarding the Harderian gland data. He felt that the study did not mimic what was happening in the real world, where exposures continue after birth, and was concerned that effects may have been missed by not dosing the pups long enough. He was also concerned about the lack of clear evidence of carcinogenesis shown for AZT, as had been previously established in other studies—in this study, it was listed as *equivocal*. With that in mind, with AZT as basically a "quasi-positive control," he questioned whether the study was valid at all, or whether at least it would have been more appropriate to compare results to the control group itself exclusively. Dr. Beland said there is a study in progress carrying the exposures out to 8 days after birth. He said the positive AZT studies had been conducted in CD1 mice, which he felt were more responsive than the B6C3F1 model.

The third primary reviewer, Dr. Anderson, said she was looking forward to seeing the results of the neonatal mouse studies, and felt that the CD1 mouse was probably a better model to use in this type of bioassay. She expressed concern that the *some* call in the draft report on the AZT/3TC/NVP combination may need to be upgraded to *clear* evidence, because there was a clear dose response, as well as several other reasons. Dr. Beland and Dr. N.J. Walker, NIEHS, responded, elaborating on the rationale for the *some* evidence call. Dr. Anderson agreed that there was enough uncertainty here to stay with *some* evidence.

Dr. Rogers felt that the impact of body size on tumor risk should be addressed in the report. He also cautioned against drawing too much comparison with previous studies in CD1 mice, in that the absorption,

distribution, metabolism, and excretion was different in those animals, as was the genotype of the pups. Dr. Anderson felt that the B6C3F1 model was not sensitive enough, and recommended that NTP consider switching to another genetic model. Dr. Beland acknowledged that there probably would have been a better response if the study had used CD1 mice, but stopped short of recommending a switch. Dr. Bucher said NTP had had meetings to discuss the strains used in its bioassays, and that despite its drawbacks the B6C3F1 model was still considered to be "the mouse of choice." The panel further debated the issue of which mouse model was most appropriate.

Dr. Miller moved that the conclusions on AZT be accepted as written. Dr. Rogers seconded. The panel voted unanimously in favor of the motion (five yes zero no).

Dr. Miller moved that the conclusions on AZT and 3TC be accepted as written. Dr. Anderson seconded. The panel voted unanimously in favor of the motion (five yes zero no).

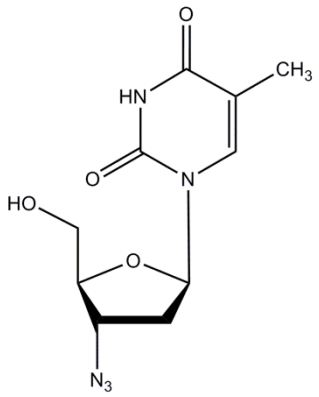
Dr. Rogers moved that in all of the conclusions, the word "mothers" be replaced with the word "dams." The motion was adopted by consensus.

Dr. Miller moved that the conclusions on AZT, 3TC and NVP be accepted as written. Dr. Barlow seconded. The panel voted unanimously in favor of the motion (five yes zero no).

Regarding the conclusions on AZT, 3TC and NFV, Dr. Miller moved that the call be changed to *equivocal* in the male mice. Thus the overall call would change from *no evidence* to *equivocal evidence* under the proposed change. Dr. Walker pointed out that the change would actually be split according to the sexes, as in the AZT conclusions. He also elaborated on why that call had been made for the combination including NFV. Dr. Birt called for a second of Dr. Miller's motion, which Dr. Barlow provided. Dr. Rogers suggested voting first on the amended language. The vote was taken, and there were two panel members in favor and two opposed to the motion. Dr. Birt as chair broke the tie, voting against the motion, which as a result failed.

Dr. Rogers moved to accept the language as written. Dr. Anderson seconded. There were two votes in favor, two opposed, and Dr. Birt as chair voted in favor. Thus the motion carried. Dr. Heiger-Bernays abstained from both votes, explaining that she did not feel qualified to comment on those particular issues.

INTRODUCTION

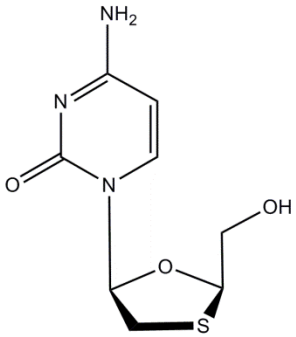


3′-AZIDO-3′-DEOXYTHYMIDINE

CAS No. 30516-87-1

Chemical Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24

Synonyms: AZT; zidovudine; 3′-azido-2′,3′-dideoxythymidine; azidodeoxythymidine; azidothymidine; 3′-azidothymidine; 3N-deoxy-3′-azidothymidine; 3′-deoxy-(8CI)(9CI); BW A509U; Compound S; ZDV
Trade name: Retrovir®

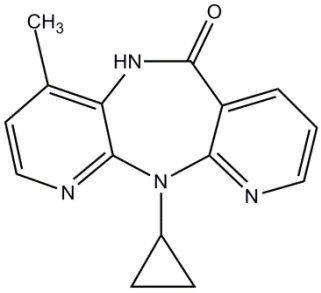


LAMIVUDINE

CAS No. 134678-17-4

Chemical Formula: $C_8H_{11}N_3O_3S$
Molecular Weight: 229.26

Synonyms: 3TC; (-)2′,3′-dideoxy-3′-thiacytidine; (2*R*-*cis*)-4-amino-1-[2-(hydroxymethyl)-; 1,3-oxathiolan-5-yl]-2(1*H*)-pyrimidinone; (-)-BCH-189; GR-109714X
Trade names: Epivir®, Zeffix®

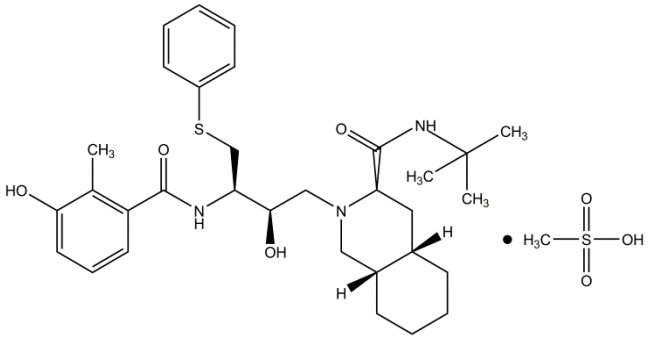


NEVIRAPINE

CAS No. 129618-40-2

Chemical Formula: $C_{15}H_{14}N_4O$
Molecular Weight: 266.30

Synonyms: NVP; BIRG-587; 11-cyclopropyl-5,11-dihydro-4-methyl-6*H*-dipyrido-[3,2-*b*:2′,3′-*e*][1,4]diazepin-6-one
Trade name: Viramune®



NELFINAVIR MESYLATE

CAS No. 159989-65-8

Chemical Formula: $C_{32}H_{45}N_3O_4S{\bullet}CH_3SO_3H$
Molecular Weight: 663.89

Synonyms: NFV; AG1343; (*3S*,4a*S*,8a*S*)-*N*-(1,1-dimethylethyl)decahydro-2-[(2*R*,3*R*)-2-hydroxy-3-isoquinoline carboxamide] methane sulfonate
Trade name: Viracept®

CHEMICAL AND PHYSICAL PROPERTIES

AZT

3′-Azido-3′-deoxythymidine (AZT) is an analogue of 2′-deoxythymidine in which the 3′-hydroxyl group is replaced by an azido function. AZT is an odorless, white-to-beige crystalline solid, with a solubility of 20.1 g/mL in water and 71 mg/mL in alcohol at 25° C (AHFS, 2007a). It has a melting point of 106° to 112° C when recrystallized from petroleum ether and 120° to 122° C when recrystallized from water, and an absorbance maximum of 266.5 nm (ε=11,650 $M^{-1}cm^{-1}$; solvent not specified) (*Merck*, 2006a).

3TC

Lamivudine (3TC) is an (−)enantiomer analogue of cytidine. 3TC is a white-to-off-white crystalline solid, with a solubility of approximately 70 mg/mL in water at 20° C (*PDR*, 2007a). It has a melting point of 160° to 162° C after recrystallization from ethanol (*Merck*, 2006b).

NVP

Nevirapine (NVP) is a white-to-off-white crystalline powder (*PDR*, 2007b). At neutral pH, NVP has a solubility in water of approximately 100 µg/mL; it is highly soluble in water at pHs less than 3. NVP has a melting point of 247° to 249° C after recrystallization from aqueous pyridine (*Merck*, 2006c) or ethyl acetate (Hargrave *et al.*, 1991).

NFV

Nelfinavir mesylate (NFV) is a white-to-off-white amorphous powder (*PDR*, 2007c). It has solubilities of 4.5 mg/mL in water, 2.6 mg/mL in 0.1 N HCl, 70 mg/g in glycerine, greater than 100 mg/g in propylene glycol, and greater than 200 mg/g in PEG 400. Aqueous solutions of NFV have a pH of approximately 2.6; at pHs greater than 4, the solubility decreases markedly (AHFS, 2007b). NFV is very soluble in methanol, ethanol, 2-propanol, propylene glycol, and acetonitrile, and is practically insoluble in soy bean oil and mineral oil (*Merck*, 2006d; *PDR*, 2007c).

PRODUCTION, USE, AND HUMAN EXPOSURE

AZT

AZT was synthesized initially in 1964 (Horwitz *et al*., 1964) for use as an anticancer agent (IARC, 2000). In 1985, AZT was reported to block the infectivity and cytopathic effects, *in vitro*, of human immunodeficiency virus type-1 (HIV-1), due to the inhibition (by AZT 5′-triphosphate) of viral reverse transcriptase (Mitsuya *et al*., 1985). Shortly thereafter, AZT was shown to reduce the morbidity and mortality associated with HIV-1 infection (Yarchoan *et al.*, 1986, 1987; Fischl *et al*., 1987), which led to it being the first anti-HIV-1 agent approved by the United States Food and Drug Administration (Brown, 1987).

AZT is typically given in combination with other antiretroviral agents to treat HIV-1 infections in adults, adolescents, and pediatric patients (AHFS, 2007a). In adults, the recommended oral dose is 600 mg/day, in divided doses, administered in combination with other antiretroviral agents (*PDR*, 2007d). Pediatric patients from the age of 6 weeks through 12 years of age receive 160 mg/m^2 every 8 hours, in combination with other antiretroviral agents (*PDR*, 2007d). Pregnant women who are positive for HIV-1 are given AZT to manage the infection and to prevent maternal-to-fetal transmission of the virus. The recommended maternal dose is 100 mg orally, five times per day, beginning after 14 weeks of pregnancy through the start of delivery, and then intravenous administration at 2 mg/kg body weight during labor and delivery (*PDR*, 2007d). For newborn infants of HIV-1-positive women, the recommended dose is 2 mg/kg body weight orally, every 6 hours, beginning within 12 hours of birth and continuing for 6 weeks. AZT is also used in combination with the antiretroviral agents 3TC or emtricitabine for postexposure prophylaxis of HIV-1 infection in individuals who are exposed to HIV-1 either occupationally or nonoccupationally (AHFS, 2007a).

3TC

3TC was synthesized initially as a racemate in 1991 (Soudeyns *et al*., 1991) and then in enantiomerically pure forms in 1992 (Beach *et al*., 1992; Humber *et al*., 1992). 3TC (as 3TC 5′-triphosphate) is thought to inhibit viral reverse transcriptase by competing with deoxycytidine 5′-triphosphate for incorporation into HIV-1 DNA (Perry and Faulds, 1997).

When used for the management of HIV-1 infections, 3TC is always used in combination with another nucleoside reverse transcriptase inhibitor (e.g., AZT) and either a protease inhibitor (e.g., NFV) or a non-nucleoside reverse transcriptase inhibitor (e.g., NVP) (AHFS, 2007c). In adults, the recommended daily dose is 300 mg, in either one or two doses (*PDR*, 2007a). Pediatric patients older than 3 months are given 4 mg 3TC/kg body weight, twice daily, up to a maximum daily dose of 300 mg. HIV-1-positive pregnant women are administered 3TC (150 mg twice daily) in combination with AZT beginning at 32 weeks of gestation; their offspring receive 2 mg 3TC, twice daily, until 6 weeks of age (AHFS, 2007c). 3TC is also administered in combination with AZT, tenofovir, stavudine, or didanosine for postexposure prophylaxis of HIV-1 infection in individuals who are exposed to HIV-1 either occupationally or nonoccupationally (AHFS, 2007c). These regimens can be expanded by the inclusion of a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor. 3TC is also used for the management of chronic hepatitis B virus; clinical trials indicate that 100 mg daily is more efficacious than 20 mg daily (AHFS, 2007c).

NVP

NVP, a nonnucleoside reverse transcriptase inhibitor (Merluzzi *et al*., 1990), was first synthesized in 1991 (Hargrave *et al*., 1991). NVP inhibits HIV-1 reverse transcriptase noncompetitively by binding to an allosteric site on the enzyme (Cohen *et al*., 1991; Wu *et al*., 1991); this action is specific for HIV-1 reverse transcriptase (Merluzzi *et al*., 1990; Koup *et al*., 1991; Richman *et al*., 1991).

NVP is usually given as part of a three-drug regimen. Typical regimens in adults and adolescents include NVP and 3TC or emtricitabine and AZT or tenofovir (AHFS, 2007d). The recommended initial dose of NVP is 200 mg daily for the first 14 days and then 200 mg twice daily (*PDR*, 2007b). In pediatric patients, the recommended dose is 4 mg/kg body weight daily for the first 14 days and then 7 mg/kg body weight twice daily for children less than 8 years old and 4 mg/kg body weight twice daily for children 8 years of age and older, with the total dose not to exceed 400 mg/day (*PDR*, 2007b). NVP is also given to prevent mother-to-child transmission of HIV-1. In pregnant women who have not received prior antiretroviral therapy, this typically involves a single 200 mg dose at the onset of labor followed by a single 2 mg/kg body weight dose to the infant (AHFS, 2007d). NVP is also used as part of the three-drug AZT regimen to prevent mother-to-child transmission of HIV-1.

NFV

The synthesis of nelfinavir (NFV) was reported by Kaldor *et al*. (1997). NFV acts by inhibiting HIV-1 protease, the enzyme responsible for cleavage of the polyprotein resulting from the *gag* and *gag-pol* genes of HIV-1 (Patick *et al*., 1996; Shetty *et al*., 1996). This

inhibition results in an immature, noninfectious virus (*PDR*, 2007c).

NFV is always used in combination with other antiretroviral agents, typically two nucleoside reverse transcriptase inhibitors (e.g., AZT and 3TC) (AHFS, 2007b). In adults, the recommended dose is 1,250 mg twice daily or 750 mg three times daily (*PDR*, 2007c). The recommended dose in pediatric patients is 45 to 55 mg/kg body weight twice daily or 25 to 35 mg/kg body weight three times daily (*PDR*, 2007c). NFV, in combination with two nucleoside reverse transcriptase inhibitors, is also used for postexposure prophylaxis following occupational or nonoccupational exposure to HIV-1 (AHFS, 2007b).

PHARMACOLOGY

AZT

The antiretroviral activity of AZT is dependent upon its conversion to 3′-azido-3′-deoxythymidine 5′-triphosphate (AZT 5′-triphosphate; Figure 1). The pathway involves a thymidine kinase-catalyzed formation of AZT 5′-phosphate followed by subsequent phosphorylation to AZT 5′-diphosphate and AZT 5′-triphosphate by thymidylate kinase and pyrimidine nucleoside diphosphate kinase, respectively (Yarchoan *et al*., 1989). AZT 5′-triphosphate is thought to inhibit HIV-1 by two mechanisms; first, by competing (K_i=0.01 to 0.03 µM) with the natural substrate deoxythymidine 5′-triphosphate for the active site of HIV-1 reverse transcriptase (Furman *et al.*, 1986; St. Clair *et al*., 1987; Heidenreich *et al*., 1990; Reardon and Miller, 1990; Hart *et al*., 1992; Nickel *et al*., 1992), and second, by acting as a chain terminator during the synthesis of the proviral DNA (Yarchoan *et al*., 1989). AZT 5′-triphosphate is also a substrate for mammalian DNA polymerases α, β, γ, δ, and ε, but with reduced K_is (45 to greater than 1,000, 0.67 to 810, 0.23 to 26, 0.36 to 230, and 320 to 400 µM, respectively) compared to that observed for HIV-1 reverse transcriptase (Furman *et al*., 1986; St. Clair *et al*., 1987; Cheng *et al*., 1990; Vazquez-Padua *et al*., 1990; Izuta *et al*., 1991; Parker *et al*., 1991; Copeland *et al*., 1992; Nickel *et al*., 1992; Cherrington *et al*., 1994; Lewis *et al*., 1994; Martin *et al*., 1994; Naviaux *et al.*, 1999; Kakuda, 2000).

3TC

3TC is converted to an active antiretroviral agent by sequential 5′-phosphorylation to 3TC 5′-phosphate (catalyzed by deoxycytidine kinase; Shewach *et al*., 1993), 3TC 5′-diphosphate, and 3TC 5′-triphosphate (catalyzed by unspecified kinases; Cammack *et al*., 1992; Hart *et al*., 1992; Figure 2).

In a manner similar to AZT, 3TC 5′-triphosphate is thought to inhibit HIV-1 by acting as a competitive inhibitor for HIV-1 reverse transcriptase (K_i=0.57 to 12 µM; Hart *et al*., 1992; Schinazi *et al*., 2002) and by causing chain termination upon incorporation into proviral DNA (Perry and Faulds, 1997). 3TC 5′-triphosphate is also a substrate for mammalian DNA polymerases α, β, γ, and ε with K_is of 110 to 175, 10 to 25, 4 to 44, and 120 µM, respectively (Hart *et al*., 1992; Martin *et al*., 1994; Kakuda, 2000; Schinazi *et al*., 2002).

NVP

In contrast to AZT, 3TC, and other nucleoside analogue reverse transcriptase inhibitors that require metabolic conversion to triphosphate derivatives in order to inhibit HIV-1 reverse transcriptase, NVP binds directly to the enzyme. This interaction is not through the reverse transcriptase catalytic site, but rather through an adjacent pocket that appears to involve two lysine residues. The interaction, which is noncompetitive in nature, does not prevent the binding of nucleoside triphosphate substrates, but rather prevents the formation of a productive complex (Cohen *et al*., 1991; Wu *et al*., 1991; Kohlstaedt *et al*., 1992; Smerdon *et al*., 1994; Spence *et al*., 1995). The K_i of NVP for HIV-1 reverse transcriptase is 200 nM, and it shows no inhibitory activity against mammalian DNA polymerases α, β, γ, or δ (Merluzzi *et al*., 1990).

NFV

The anti-HIV-1 activity of NFV is dependent upon its interaction with a viral-encoded aspartic protease that is responsible for cleavage of the polypeptides resulting from the *gag* (p55) and *gag-pol* (p160) genes (Patick *et al*., 1996; Shetty *et al*., 1996). Cleavage of these polypeptides yields structural proteins (p7, p9, p17, and p24) and enzymes (reverse transcriptase, integrase, and protease) necessary for viral activity. By inhibiting the protease, NFV blocks the maturation of the virus from a noninfectious form to an infectious form. NFV is a very potent inhibitor of HIV-1 protease, with a K_i of 1.7 to 2.0 nM (Patick *et al*., 1996; Kaldor *et al*., 1997) and it shows no inhibitory activity against human aspartic proteases (Patick *et al*., 1996).

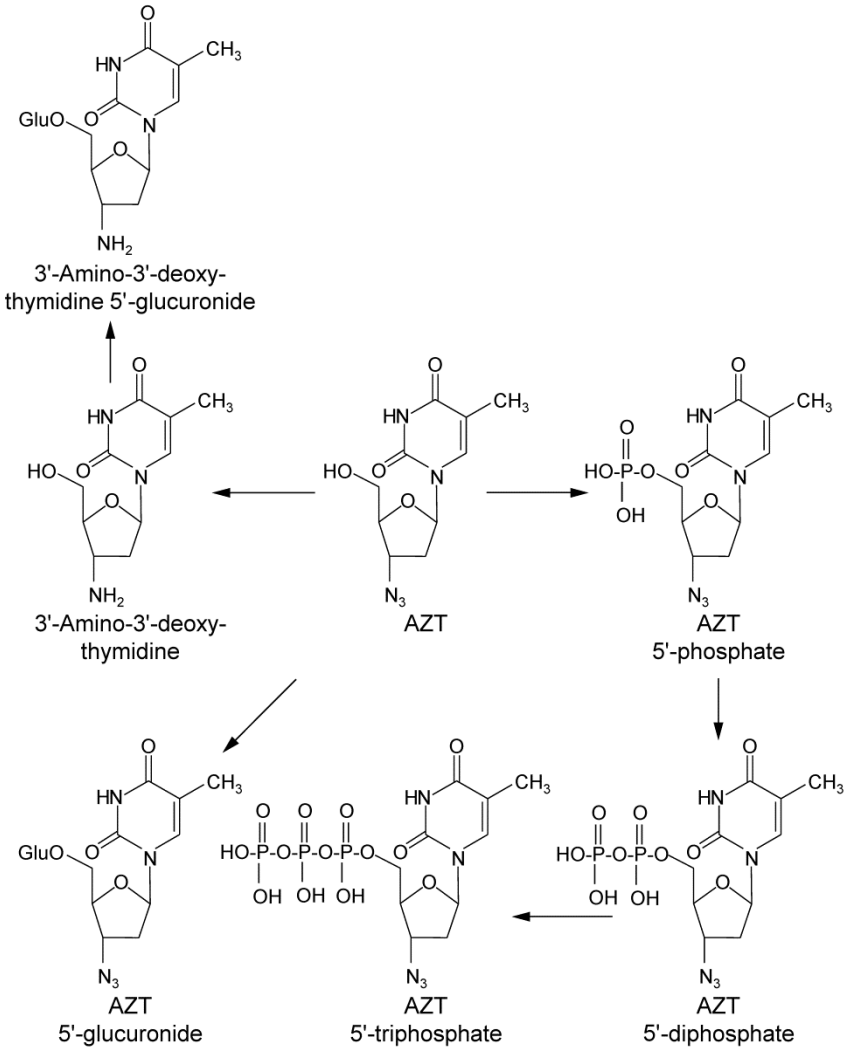


FIGURE 1
Structures of AZT Metabolites (Glu=glucuronyl)

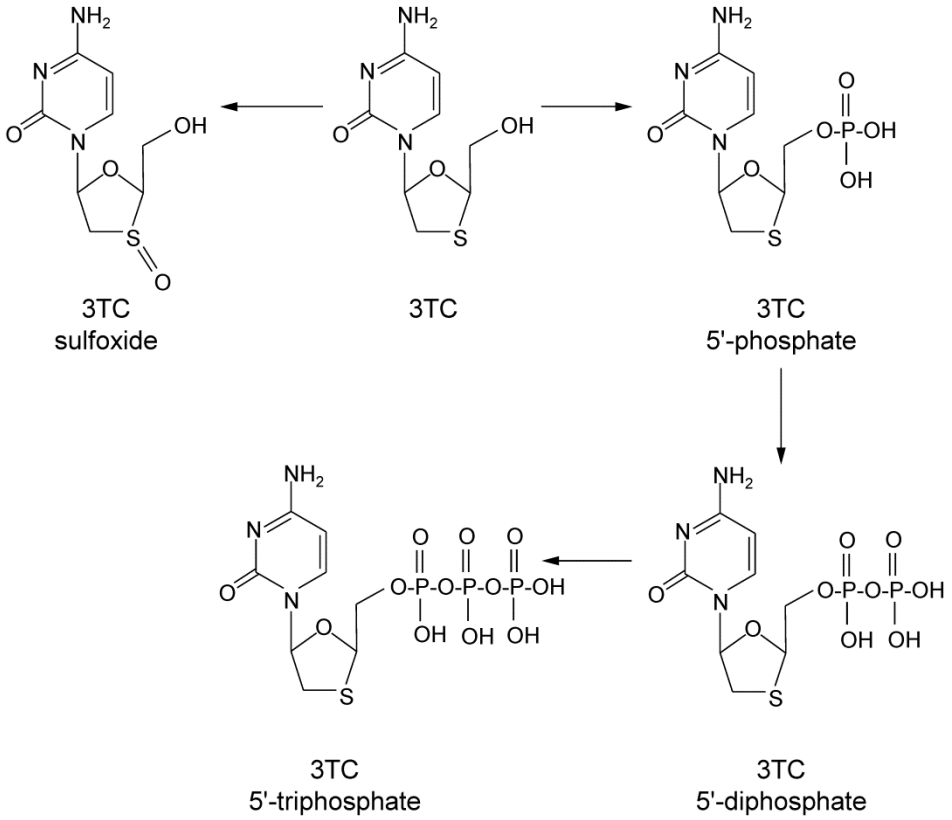


FIGURE 2
Structures of 3TC Metabolites

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

AZT

AZT is rapidly absorbed and distributed. In mice treated orally, AZT has a T_{max} of 5 to 22 minutes, a $t_{1/2}$ of 16 to 44 minutes, and a bioavailability of 82% to 93% (Trang *et al*., 1993; Manouilov *et al*., 1995; Williams *et al*., 2003; Von Tungeln *et al*., 2007); comparable $t_{1/2}$ values are obtained after intravenous administration (Doshi *et al*., 1989; Trang *et al*., 1993; Manouilov *et al*., 1995; Williams *et al*., 2003). In mouse fetuses exposed transplacentally, AZT has a T_{max} of 30 minutes, a $t_{1/2}$ of 40 minutes, and a C_{max} similar to that observed in the dams (Von Tungeln *et al*., 2007).

In rats treated orally, AZT has a T_{max} of 15 minutes, a $t_{1/2}$ of 54 minutes, and a rapid systemic distribution (de Miranda *et al*., 1990); $t_{1/2}$ values after intravenous administration are 26 to 95 minutes (Patel *et al*., 1989; Mays *et al*., 1991; Wientjes and Au, 1992; Huang *et al*., 1995; Brown *et al*., 2003; Alnouti *et al*., 2005). Rat fetuses exposed transplacentally have C_{max} and AUC values that are appreciably lower than those observed in the dams (Brown *et al*., 2003; Alnouti *et al*., 2005), but appear to eliminate AZT at a rate similar to the dams (Huang *et al*., 1996).

Domestic cats dosed intravenously with AZT have a $t_{1/2}$ of 90 minutes; the comparable values after intragastric or oral dosing are 84 minutes with T_{max} values of 13 and 45 minutes, respectively (Zhang *et al*., 2004a). The oral bioavailability of AZT in cats is 95%.

In rhesus monkeys (*Macaca mulatta*) dosed subcutaneously, AZT has a T_{max} of 42 minutes and a $t_{1/2}$ of 48 minutes (Cretton *et al*., 1991); after oral administration, AZT has a bioavailability of 45% to

92% and a $t_{1/2}$ of 83 minutes (Boudinot *et al*., 1990). *Macaca fascicularis* monkeys dosed intravenously have a $t_{1/2}$ value of 65 to 68 minutes (Qian *et al*., 1991; Gallo *et al*., 1993); oral treatment results in a T_{max} of 56 to 101 minutes, a $t_{1/2}$ of 77 to 99 minutes and a bioavailability of 53% (Qian *et al*., 1991, 1992; Gallo *et al*., 1992). In pregnant and nonpregnant macaques (*Macaca nemestrina*) treated intravenously, AZT has a $t_{1/2}$ of 38 to 40 minutes (Lopez-Anaya *et al*., 1990a, 1991); the $t_{1/2}$ in infant macaques is approximately twice this value (Lopez-Anaya *et al*., 1990a). The T_{max} and $t_{1/2}$ of AZT in patas monkeys (*Erythrocebus patas*) dosed orally with a mixture of AZT and 3TC are 50 and 61 minutes, respectively (Divi *et al*., 2008).

In humans, the oral bioavailability of AZT is 42% to 95%, the T_{max} is 30 to 60 minutes, and the $t_{1/2}$ is 60 minutes (reviewed in IARC, 2000; NTP, 2006; AHFS, 2007a); a similar $t_{1/2}$ is observed after intravenous administration. The oral bioavailability of AZT in children is similar to adults (AHFS, 2007a); however, the $t_{1/2}$ of AZT in children and, in particular, infants appears to be substantially longer than that observed in adults (Dudley, 1995; Mirochnick *et al*., 1999; King *et al*., 2002).

In mice, the major AZT "metabolite" detected in plasma is the parent drug, followed by lesser quantities of AZT 5′-glucuronide and 3′-amino-3′-deoxythymidine, and much smaller quantities of AZT 5′-monophosphate, AZT 5′-diphosphate, and AZT 5′-triphosphate (Chow *et al*., 1997; Williams *et al*., 2003; Von Tungeln *et al*., 2007; Figure 1). AZT 5′-glucuronide has a $t_{1/2}$ similar to AZT, whereas the $t_{1/2}$ for 3′-amino-3′-deoxythymidine is appreciably shorter and the $t_{1/2}$ for AZT 5′-phosphate is much longer (Williams *et al*., 2003; Von Tungeln *et al*., 2007). In rats, AZT is excreted primarily in the urine as unchanged drug, accompanied by small amounts of AZT 5′-glucuronide and 3′-amino-3′-deoxythymidine (de Miranda *et al*., 1990; Mays *et al*., 1991).

AZT and AZT 5′-glucuronide are the major plasma metabolites in macaque fetuses exposed transplacentally, with the concentration being approximately 80% of that detected in the maternal plasma (Lopez-Anaya *et al*., 1990b). AZT and AZT 5′-glucuronide are the major plasma metabolites in neonatal (2-day-old) and infant (4-month-old) macaques treated intravenously (Lopez-Anaya *et al*., 1990a); these metabolites are cleared from plasma at similar rates, with the rates being appreciably slower in the neonates compared to the infants. In macaques, the major route of excretion is the urine, with AZT 5′-glucuronide accounting for 86% of the administered dose. AZT 5′-glucuronide is also the major urinary metabolite of AZT in *M. fascicularis* monkeys (Qian *et al*., 1991, 1992; Gallo *et al*., 1992, 1993).

AZT 5′-glucuronide, AZT, and 3′-amino-3′-deoxythymidine are found in plasma of rhesus monkeys dosed subcutaneously (Cretton *et al*., 1991) and patas monkeys treated orally (Divi *et al*., 2008). AZT 5′-glucuronide is the major metabolite in both species and both AZT 5′-glucuronide and AZT are cleared at similar rates that are faster than that of 3′-amino-3′-deoxythymidine. As with macaques, the major route of excretion is in the urine. In rhesus monkeys, 3′-amino-3′-deoxythymidine 5′-glucuronide (Figure 1) is also detected as a urinary metabolite (Cretton *et al*., 1991).

Humans metabolize AZT in a manner similar to nonhuman primates: AZT 5′-glucuronide is the major plasma metabolite, followed by smaller quantities of AZT and 3′-amino-3′-deoxythymidine; AZT 5′-glucuronide and AZT are cleared at similar rates that are faster than that of 3 -amino-3′-deoxythymidine; and urine is the primary route of excretion (reviewed in IARC, 2000; NTP, 2006). As noted above, there is a slower rate of elimination of AZT in children and infants, which has been attributed to a decreased ability to form AZT 5′-glucuronide (King *et al*., 2002).

3TC

3TC is rapidly absorbed and distributed. In mice treated orally, 3TC has a T_{max} of 30 minutes and a $t_{1/2}$ of 110 minutes (Williams *et al*., 2003), values that are much greater than those observed with AZT. Comparable values after intravenous administration are 5 minutes (T_{max}) and 96 minutes ($t_{1/2}$) (Williams *et al*., 2003). In mouse fetuses exposed transplacentally, 3TC has a T_{max} of 60 minutes and a $t_{1/2}$ of 161 minutes, the latter being considerably greater than the $t_{1/2}$ of 44 minutes observed in the dams (Von Tungeln *et al*., 2007). In addition, the C_{max} is substantially lower in the fetuses as compared to the dams (Von Tungeln *et al*., 2007).

3TC has a $t_{1/2}$ of 105 minutes in rats treated intravenously (Alnouti *et al*., 2005). Rat fetuses exposed transplacentally to 3TC have C_{max} and AUC values that are appreciably lower than those observed in the dams (Alnouti *et al*., 2005). Domestic cats dosed intravenously with 3TC have a $t_{1/2}$ of 114 minutes; the comparable values after intragastric and oral dosing are 150 and 138 minutes, with T_{max} values of 30 and 66 minutes, respectively (Zhang *et al*., 2004b). The oral bioavailability of 3TC in cats is 80%. Woodchucks (*Marmota monax*) treated orally or intravenously with 3TC have a $t_{1/2}$ of 170 minutes; the oral bioavailability is 18% to 54% (Rajagopalan *et al*., 1996).

In rhesus monkeys dosed intravenously, 3TC has a $t_{1/2}$ of 84 minutes (Blaney *et al*., 1995). The T_{max} and $t_{1/2}$ of 3TC in patas monkeys given an oral mixture of AZT and 3TC are 50 and 136 minutes, respectively (Divi *et al*., 2008).

In humans administered 3TC orally, the T_{max} is approximately 1 hour, the $t_{1/2}$ is 3.5 to 11.5 hours, and the bioavailability is 86% (reviewed in Perry and Faulds, 1997; King *et al*., 2002; *PDR*, 2007a). The $t_{1/2}$ for 3TC in infants and children appears to be slightly less than in adults (Perry and Faulds, 1997; King *et al*., 2002).

In humans and experimental animals, the majority of 3TC is excreted unchanged, primarily in the urine. The percent excreted as 3TC varies across species, with 75% being reported in rats (Rajagopalan *et al*., 1996), 26% in woodchucks (Rajagopalan *et al*., 1996), 32% to 59% in rhesus monkeys (Blaney *et al*., 1995), and 68% to 71% in humans (reviewed in Dudley, 1995; *PDR*, 2007a). Other than 5′-phosphate derivatives, the only reported metabolite of 3TC is 3TC sulfoxide (Figure 2), which has been detected in the urine of dogs and humans (Plumb *et al*., 1996; *PDR*, 2007a).

NVP

NVP is readily absorbed following oral dosing. In chimpanzees, greater than 64% is bioavailable (Cheeseman *et al*., 1993); the corresponding value in humans is greater than 90% (Lamson *et al*., 1999a; *PDR*, 2007b), with a T_{max} occurring 1.3 to 4.6 hours after dosing (Cheeseman *et al*., 1995; Lamson *et al*., 1999a; *PDR*, 2007b). Compared to AZT and 3TC, NVP is eliminated very slowly. In chimpanzees, the $t_{1/2}$ is 11 to 24 hours (Cheeseman *et al*., 1993), while the value in humans after a single oral dose is 40 to 51 hours (Cheeseman *et al*., 1993; Lamson *et al*., 1999a; Riska *et al*., 1999a). A similar $t_{1/2}$ is obtained following intravenous dosing (Lamson *et al*., 1999a). Repeated administration of NVP to humans results in a decrease in $t_{1/2}$ (Riska *et al*., 1999b), which has been attributed to the autoinduction of cytochrome P450 (CYP) enzymes, in particular CYP3A4 and CYP2B6 (Lamson *et al*., 1999b). The $t_{1/2}$ in infants appears to be greater than that in adults (Luzuriaga *et al*., 1996; Mirochnick *et al*., 1998). The induction of CYP3A also occurs in rats exposed to NVP (Walubo *et al*., 2006).

The disposition, biotransformation, and elimination of NVP have been reported in mice, rats, rabbits, dogs, monkeys (cynomolgus), chimpanzees, and humans (Riska *et al*., 1999a,b). In mice, rabbits, monkeys, and humans, urinary excretion is approximately twice that found in feces. The distribution is approximately equal in rats, and in dogs fecal excretion predominates due to poor absorption of the drug. NVP is extensively metabolized. Among the identified metabolites are 3- and 8-hydroxy-NVP, 4-hydroxymethyl-NVP (12-hydroxy-NVP), 4-carboxy-NVP, and 2-, 3-, 8-, and 12-hydroxy-NVP glucuronide (Figure 3).

The major urinary metabolites in dogs, monkeys, chimpanzees, and humans are glucuronides, primarily of 2-, 3-, and 12-hydroxy-NVP. In rats and mice 12-carboxy-NVP is the predominant urinary metabolite. In dogs, unchanged NVP is the primary "metabolite" found in the feces. In the other species, the major fecal metabolite is 4-carboxy-NVP or 3-hydroxy-NVP.

In humans, the formation of 2-hydroxy-NVP is attributed to the CYP3A subfamily, 3-hydroxy-NVP to CYP2B6, 8-hydroxy-NVP to CYP3A4, CYP2B6, and CYP2D6, and 12-hydroxy-NVP to CYP3A4 and possibly CYP2D6 and CYP2C9 (Erickson *et al*., 1999). Recently, a NVP-glutathione conjugate has been detected upon the incubation of NVP with human liver microsomes in the presence of glutathione (Wen *et al*., 2009). The NVP-glutathione conjugate formation was catalyzed primarily by CYP3A4 and to a lesser extent by CYP2D6, CYP2C19, and CYP2A6. The oxidation of NVP by CYP3A4 also caused mechanism-based inactivation of the enzyme.

NFV

In rats treated orally, NFV has a bioavailability of 43% and a T_{max} of 169 minutes, which decrease to 29% and 83 minutes upon fasting (Shetty *et al*., 1996). The oral bioavailability of NFV in dogs, monkeys (cynomolgus), and marmosets is 40%, 26%, and 17%, respectively, with T_{max} values of 105, 150, and 45 minutes, respectively (Shetty *et al*., 1996). In humans, the oral bioavailability of NFV is 70% to 80% when administered with food as compared to 27% to 50% when given to fasted individuals (Pai and Nahata, 1999; Bardsley-Elliot and Plosker, 2000). Infants appear to have a reduced bioavailability compared to children and adults (Hirt *et al*., 2006). The T_{max} in adults occurs at 2.2 to 6.8 hours, with a shift toward longer times in children (Moyle *et al*., 1998; Barry *et al*., 1999; Pai and Nahata, 1999; Ford *et al*., 2004; Payen *et al*., 2005; Regazzi *et al*., 2005; Bryson *et al*., 2008).

When given intravenously to rats, NFV has a $t_{1/2}$ of 77 minutes (Shetty *et al*., 1996). The comparable values in dogs, monkeys, and marmosets are 45, 86, and 63 minutes, respectively. In humans treated orally, NFV has a $t_{1/2}$ of 180 to 300 minutes (Barry *et al*., 1999; Bardsley-Elliot and Plosker, 2000; Villani *et al*., 2006; Bryson *et al*., 2008); similar values have been reported in children and infants (Payen *et al*., 2005).

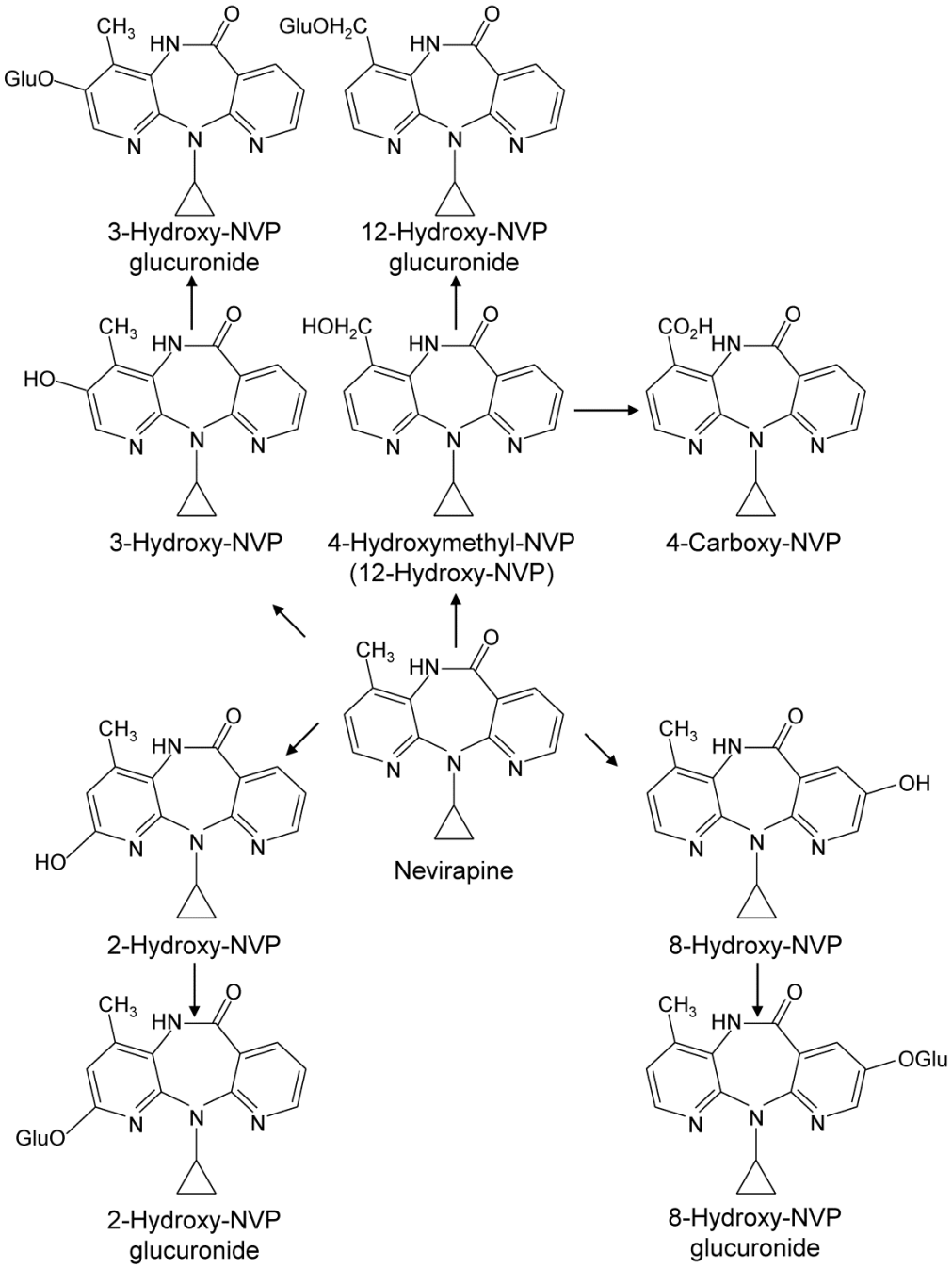


FIGURE 3
Structures of NVP Metabolites (Glu=glucuronyl)

In rats, nearly all of a NFV dose is excreted in the feces; less than 0.5% is found in the urine (Shetty *et al*., 1996). A similar pattern exists in humans: the majority (87%) of a NFV dose is excreted in the feces, with only 1% to 2% being found in the urine (Bardsley-Elliot and Plosker, 2000; *PDR*, 2007c).

NFV is extensively metabolized *in vivo*. In humans, only 22% of the fecal metabolites are present as the unchanged drug. Among the identified plasma metabolites are a hydroxy-*tert*-butylamide, designated M8, that results from CYP2C19-catalyzed oxidation of the tertiary butyl moiety; a catechol, designated M3, that results from CYP3A4-catalyzed oxidation of the hydroxyltoluene substituent; a methoxycatechol, designated M1, that results from methylation of the catechol metabolite; and two diastereomers, designated M10 and M11, that result from the oxidation of the sulfur atom (Lillibridge *et al*., 1998; Zhang *et al*., 2001; Figure 4). The plasma levels of the hydroxy-*tert*-butylamide metabolite, M8, are approximately 20% those of NFV (Payen *et al*., 2005; Regazzi *et al*., 2005). The levels of the other metabolites do not appear to have been determined. Both M8 and the methoxycatechol metabolite, M1, show activity against HIV-1; M8 has activity similar to the parent drug, whereas M1 shows substantially lower activity (Zhang *et al*., 2001). The $t_{1/2}$ of M8 is comparable to that of NFV (Litalien *et al*., 2003; Ford *et al*., 2004; Payen *et al*., 2005), and both NFV and M8 undergo transplacental transfer (Hirt *et al*., 2007; Bryson *et al*., 2008; Bennetto-Hood *et al*., 2009).

TOXICITY

Experimental Animals

AZT

In experimental animals, the administration of AZT is associated with hematologic toxicities and cardiac and skeletal muscle myopathies. Hematologic abnormalities, including thrombocytopenia, myelodysplasia, and/or macrocytic normochromic anemia, are observed in mice, rats, dogs, cats, and cynomolgus monkeys. Cardiac and/or skeletal muscle abnormalities are found in mice and rats (reviewed in IARC, 2000; NTP, 2006; also see Lewis *et al*., 2006). These toxicities are attributed to mitochondrial dysfunction, possibly as a consequence of the incorporation of AZT into mitochondrial DNA by the action of DNA polymerase γ (Kakuda, 2000; Lewis *et al.,* 2003; Kohler and Lewis, 2007).

3TC

Transgenic mice expressing the mitochondrial deoxynucleotide carrier do not show any indication of cardiac damage when treated with 3TC under conditions where AZT causes decreases in left ventricular mass and mitochondrial ultrastructure defects (Lewis *et al*., 2006).

NVP

NVP causes an idiosyncratic skin rash in rats (Shenton *et al*., 2003) through a process mediated by $CD4^+$ T-cells (Shenton *et al*., 2005; Popovic *et al*., 2006). Female Brown Norway rats are the most sensitive to this response followed by female Sprague-Dawley rats (Shenton *et al*., 2003). Higher concentrations of the drug induce the idiosyncratic response in male Brown Norway rats and female Lewis rats (Shenton *et al*., 2004). Male Sprague-Dawley rats and female Stevens-Johnson syndrome mice appear to be resistant to the induction of the rash (Shenton *et al*., 2003). Both NVP and the NVP metabolite 12-hydroxy-NVP induce the rash (Popovic *et al*., 2006; Chen *et al*., 2008), and it has been suggested that 12-hydroxy-NVP is the metabolite responsible for the rash as a result of subsequent metabolism to a quinone methide (Chen *et al*., 2008). Rats treated orally with NVP do not have elevated serum levels of alanine transferase, aspartate transferase, or alkaline phosphatase, but histological examination of the livers indicates hepatocellular hypertrophy, nuclear degranulation, disintegration, and vacuolation (Walubo *et al*., 2006).

Oral or intraperitoneal treatment of mice with NVP causes a systemic sensitization to a subsensitizing dose of trinitrophenyl-ovalbumin (Nierkens *et al*., 2005). Mice dosed orally with NVP show decreased creatine kinase activity in the cerebellum, hippocampus, striatum, and cortex of the brain (Streck *et al*., 2008).

NFV

Oral administration of NFV to male Sprague-Dawley rats for 4 weeks results in an increase in circulating thyroid stimulating hormone, which is accompanied by an increase in the severity of thyroid gland follicular cell hypertrophy (Burns-Naas *et al*., 2005a). The circulating levels of triiodothyronine and thyroxine are not affected; however, there is an increased rate of elimination of [^{125}I]-thyroxine. In addition, there is a slight increase in the incidence of hepatocellular hypertrophy.

NFV is not immunosuppressive in rats treated orally for a period of 1 or 6 months (Burns-Naas *et al*., 2005b).

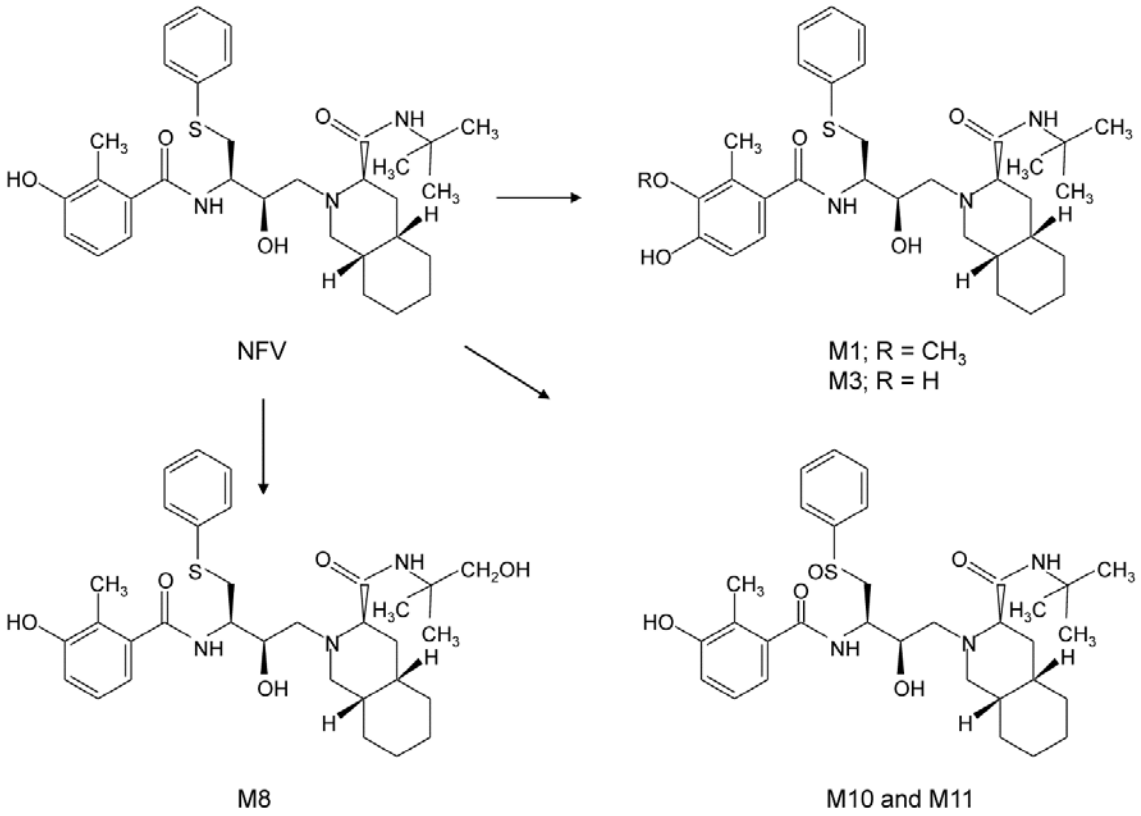


FIGURE 4
Structures of NFV Metabolites

Humans

AZT

The toxicity of AZT in humans has been reviewed (IARC, 2000; NTP, 2006; AHFS, 2007a). The major dose-limiting effect of AZT in humans is bone marrow toxicity resulting in severe anemia, neutropenia, or both. AZT treatment is also associated with lactic acidosis and severe hepatomegaly with steatosis, which can result in death. Other toxicities occurring from AZT treatment include skeletal muscle myopathy, cardiomyopathy, severe headaches, seizures, gastrointestinal effects, and lipodystrophy. Some of these adverse events appear to be the consequence of mitochondrial toxicity (Estanislao *et al*., 2004; Lewis, 2004; McComsey and Leonard, 2004; McComsey and Lonergan, 2004).

3TC

The toxicity of 3TC in humans has been reviewed (Perry and Faulds, 1997; AHFS, 2007c). When used as monotherapy in adults and children for the treatment of HIV-1 or chronic hepatitis B virus infection, 3TC treatment results (in some instances) in neutropenia, thrombocytopenia, peripheral neuropathy, headaches, gastrointestinal effects, and lactic acidosis.

NVP

The toxicity of NVP in humans has been reviewed (Pollard *et al*., 1998; Mirochnick *et al*., 2000; Murphy, 2003; AHFS, 2007d; Waters *et al*., 2007). The most severe toxicity associated with NVP is hepatotoxity, which in some instances is fatal. The most common side effect is a rash consisting of maculopapular erythematous cutaneous eruptions. This occurs in children and adults (including pregnant women), at times is life threatening, and can lead to discontinuation of the drug. Whether or not the rash in humans is due to 12-hydroxy-NVP is currently uncertain (Hall and MacGregor, 2007). Other reported side effects are gastrointestinal disturbances and lipodystrophy.

NFV
The toxicity of NFV in humans has been reviewed (Pai and Nahata, 1999; Bardsley-Elliot and Plosker, 2000; AHFS, 2007b). The most frequent complication reported with NFV in adults, children, and infants, is mild to moderate diarrhea. Other potential complications include hyperglycemia, new-onset diabetes mellitus, exacerbation of preexisting diabetes mellitus, and lipodystrophy.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

AZT
Transplacental treatment of mice, rats, and rabbits with AZT can result in increased numbers of fetal resorptions and decreased fetal weights, but there is not an increase in the frequency of malformations (reviewed in IARC, 2000; NTP, 2006).

Mice transplacentally exposed to AZT weighed significantly less at birth and throughout their lives compared to control mice (Walker *et al*., 2004). These mice also showed enlarged hearts, atypical heart mitochondria, and increased cardiac cytochrome c oxidase activity. Patas monkeys exposed perinatally to AZT showed morphological damage in cardiac and skeletal muscle mitochondria and altered levels of mitochondrial DNA (Divi *et al*., 2005, 2007a).

3TC
Transplacental treatment of rabbits with 3TC results in some evidence of embryolethality (AHFS, 2007c). The effect is not observed in rats treated similarly; likewise, there is no indication of teratogenicity in either species (AHFS, 2007c).

Perinatal exposure to 3TC is associated with mitochondrial toxicity in mice as indicated by a decrease in mitochondrial DNA (Chan *et al*., 2007). Patas monkeys transplacentally exposed to 3TC show evidence of morphological damage to umbilical cord artery endothelial cell mitochondria (Divi *et al*., 2007b) but no evidence of skeletal muscle mitochondrial morphologic damage at birth (Divi *et al*., 2007a).

Perinatal administration of 3TC and AZT to patas monkeys, a model that mimics a dosing regimen used with pregnant women and their infants, induces cardiac and skeletal muscle mitochondrial damage to an extent that is equal to or only slightly greater than that of AZT by itself (Divi *et al*., 2005, 2007a). Infant patas monkeys exposed transplacentally to 3TC and AZT have substantial depletion of mitochondrial oxidative phosphorylation in heart and skeletal muscle (Gerschenson *et al*., 2004). CD-1 mice treated perinatally with mixtures of AZT and 3TC show significant decreases in the mean number and area of cardiomyocytic mitochondria (Bishop *et al.*, 2004); however, it is unclear if this is due to AZT, 3TC, or a combination of the two.

NVP
Transplacental treatment of rats with NVP causes significant decreases in fetal body weight (AHFS, 2007d). There is no indication of teratogenicity with NVP in either rats or rabbits (AHFS, 2007d).

NFV
Transplacental treatment of rats or rabbits with NFV does not cause embryo-fetal toxicity (Burns-Naas *et al*., 2003a). NFV does not produce adverse effects on fertility, pregnancy, embryo-fetal development, parturition, or lactation in pregnant rats treated on gestation day 6 through lactation day 20 (Burns-Naas *et al*., 2003b). Likewise, the male and female offspring from this treatment show no signs of reproductive impairment.

Humans

AZT
Infants exposed perinatally to AZT present (in some instances) with seizures, lactic acidosis, anemia, altered cerebral pathology (based upon magnetic resonance imaging), impaired skeletal muscle, heart, and/or liver oxidative phosphorylation, skeletal muscle mitochondrial abnormalities, and cardiomyopathy (Blanche *et al*., 1999; Barret *et al*., 2003; Tardieu *et al*., 2005; Tovo *et al*., 2005). Perinatal exposure to AZT is also associated with a decrease in mitochondrial DNA in leukocytes obtained from the infants (Poirier *et al*., 2003).

3TC
Infants exposed *in utero*, during the third trimester, to 3TC or a combination of 3TC and AZT have (in some instances) mitochondrial dysfunction (Brogly *et al*., 2007). In combination with AZT, perinatal treatments with 3TC cause seizures, lactic acidosis, anemia, altered cerebral pathology (based upon magnetic resonance imaging), impaired skeletal muscle, heart, and/or liver oxidative phosphorylation, skeletal muscle mitochondrial abnormalities, and cardiomyopathy (Blanche *et al*., 1999; Barret *et al*., 2003; Tardieu *et al*., 2005). Transplacental exposure to 3TC and AZT results in morphologic damage to mitochondria of umbilical cord artery endothelium and a decrease in mitochondrial DNA copy number in cord blood mononuclear cells and in umbilical cord tissue (Divi *et al*., 2004, 2007b).

NVP and NFV
There are no adequate studies to assess the reproductive toxicity and teratogenicity of NVP or NFV in humans (AHFS, 2007b,d).

CARCINOGENICITY
Experimental Animals
AZT
Male and female CD-1 mice were treated daily by gavage with 0, 30, 60, or 120 mg AZT/kg body weight, which was reduced to 20, 30, or 40 mg/kg per day after 90 days of treatment due to anemia. At 22 months, there was a low (8%) incidence of vaginal squamous cell carcinoma in the high-dose group of female mice compared to no occurrences of the neoplasm in the vehicle control group or other treated groups (Ayers *et al*., 1996).

Male and female CD rats were treated daily by gavage with 0, 80, 220, or 600 mg AZT/kg body weight, which, for the high-dose group, was reduced to 450 mg/kg per day after 90 days of treatment, and then to 300 mg/kg per day after 278 days of treatment due to anemia. At 24 months, there was a low (3%) incidence of vaginal squamous cell carcinoma in the high-dose group of female rats compared to no occurrences of the neoplasm in the vehicle control group or other treated groups (Ayers *et al*., 1996).

Female CD-1 mice were treated twice daily intravaginally with 0, 1, or 4 mg AZT per treatment. At 24 months, the incidences of vaginal squamous cell carcinoma were 0%, 3%, and 19% in the 0, 1, and 4 mg AZT treatment groups, respectively (Ayers *et al*., 1996).

Pregnant CD-1 mice were treated once daily by gavage from gestation day 10 through lactation day 21 with 0, 20, or 40 mg AZT/kg body weight. The offspring were then administered 0, 20, or 40 mg/kg in the drinking water for 0 days, 90 days, or 24 months. The only treatment-related neoplasm was vaginal squamous cell carcinoma, which occurred in 3% and 16% of the female mice treated for 24 months after weaning with 20 or 40 mg/kg, respectively (Ayers *et al*., 1997).

Pregnant CD-1 mice were dosed daily by gavage on gestation days 12 to 18 with 0, 12.5, or 25 mg AZT (corresponding to approximately 0, 225, and 450 mg/kg, respectively) (Olivero *et al*., 1997; Diwan *et al*., 1999). One year after treatment, the offspring had dose-dependent, statistically significant increases in the incidences and multiplicities of lung, liver, skin, and female reproductive tract tumors (Olivero *et al*., 1997). Two years after treatment, there were statistically significant increased incidences of lung, mammary gland, and ovarian tumors and histiocytic sarcomas in female offspring, and seminal vesicle tumors in male offspring (Diwan *et al*., 1999). As part of this study, CD-1 mice were treated daily for the first 8 days after birth by subcutaneous injection with 0, 25, 50, 100, or 200 mg AZT/kg body weight. When assessed at 2 years of age, the female mice had an increased multiplicity of lung and liver tumors. In a separate experiment, pregnant CD-1 mice were dosed daily by gavage on gestation days 12 to 18 with 0 or 25 mg AZT and, beginning at 5 weeks of age, the offspring received topical applications of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) for 30 weeks (Zhang *et al*., 1998). At 1 year of age, the mice treated with AZT and TPA had an incidence of skin papilloma that was twofold greater than that in mice given TPA alone.

Male and female B6C3F1 mice were administered doses of 0, 15, 30, or 60 mg AZT/kg body weight by gavage, twice daily at 6-hour intervals, 5 days per week (NTP, 1999). In female mice, there were statistically significant increased incidences of squamous cell carcinoma of the vagina in the 30 and 60 mg/kg groups; in male mice, AZT caused marginal increases in the incidences of renal tubule and Harderian gland neoplasms. In a subsequent transplacental study, female CD-1 mice were dosed orally with 0, 25, 50, 100, or 150 mg/kg, twice daily at 6-hour intervals (NTP, 2006). On days 9 through 13 after the initiation of dosing, the female mice were cohabitated with male CD-1 mice. Dosing continued through the cohabitation period and until the female mice gave birth to their pups, which occurred on dose day 39. When assessed 2 years after birth, AZT caused significant increases in the incidences of lung alveolar/bronchiolar carcinoma and combined alveolar/bronchiolar adenoma or carcinoma in the male mice.

Female C57Bl/6 mice were bred with male C3H mice and on days 12 to 18 of gestation, the dams were treated by gavage with 0, 80, 240, or 480 mg AZT/kg body weight (Walker *et al*., 2007). When assessed 2 years after birth, AZT caused significant increases in the incidences of hepatic carcinoma and hemangiosarcoma in the male B6C3F1 offspring. In an experiment of similar design, female F344 rats were treated by gavage with 0, 80, 240, or 480 mg/kg on days 15 through 21 of gestation (Walker *et al*., 2007). When assessed 2 years after birth, AZT caused a significant increase in the incidence of mononuclear cell leukemia in the female F344 offspring.

3TC
The carcinogenicity of 3TC has been assessed following long-term administration to mice and rats (*PDR*,

2007a). There was no evidence of carcinogenicity in mice given 10 times the recommended therapeutic dose of 3TC for treating HIV-1 infection or in rats given 58 times the recommended therapeutic dose of 3TC.

NVP

The carcinogenicity of NVP has been assessed following long-term administration to mice and rats (*PDR*, 2007b). In mice administered 0, 50, 375, or 750 mg NVP/kg body weight per day, there were increased incidences of hepatocellular adenoma and carcinoma at all doses of NVP in male mice and at the two highest doses in female mice. In rats administered 0, 3.5, 17.5, or 35 mg/kg per day, there were increased incidences of hepatocellular adenoma at all doses of NVP in male rats and at the highest dose in female rats.

NFV

The carcinogenicity of NFV has been assessed following long-term oral administration to mice and rats (Burns-Naas *et al*., 2005a; *PDR*, 2007c). Sprague-Dawley rats were administered 0, 100, 300, or 1,000 mg NFV/kg body weight per day for 2 years. At the end of the treatment period, there were increased incidences of combined thyroid gland follicular cell adenoma or carcinoma in the male rats treated with 300 or 1,000 mg/kg (12% and 17%, respectively) compared to the vehicle control rats (2%); in female rats, there was an increased incidence of combined thyroid gland follicular cell adenoma or carcinoma in the group treated with 1,000 mg/kg (23%) compared to the vehicle control rats (0% to 2%). In mice, there was no evidence of carcinogenicity at systemic exposures of NFV up to nine times the levels measured in humans receiving recommended therapeutic doses of NFV.

Humans

AZT

The carcinogenicity of AZT in humans was reviewed by the International Agency for Research on Cancer (2000), which concluded there was "inadequate evidence" for the carcinogenicity of AZT in humans.

3TC, NVP, and NFV

There have been no studies reported in the literature on any association between 3TC, NVP, or NFV and the development of cancer in humans.

GENETIC TOXICITY

AZT

The genotoxicity of AZT has been reviewed (IARC, 2000; Poirier *et al*., 2004; NTP, 2006). These reviews concluded that: AZT induces mutations in bacterial and mammalian cells; the mechanism of mutation induction typically involves large deletions, which is consistent with the chain-terminating properties of the drug; AZT is clastogenic in mammalian cells, both *in vitro* and *in vivo*; and AZT can be incorporated into nuclear and mitochondrial DNA of cultured cells, experimental animals, and humans. Studies published since these reviews are summarized below.

***In Vitro* Studies**

TK6 human lymphoblastoid cells were incubated for 3 days with 0, 33, 100, or 300 μM AZT, at which time the mutant frequencies at the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) and thymidine kinase (*Tk*) genes were assessed (Torres *et al*., 2007). Compared to control cultures, incubation with 300 μM AZT caused a significant increase in the *Hprt* mutant frequency, while 100 and 300 μM AZT caused a significant increase in the *Tk* mutant frequency.

Incubation of L5178Y mouse lymphoma cells with 0, 374, 1,233, 2,245, 2,994, or 3,742 μM AZT for 24 hours resulted in dose-dependent increases in cytotoxicity and mutagenicity (Wang *et al*., 2007). Analysis of DNA from cultures conducted with 3,742 μM AZT indicated that the mutations resulted primarily from loss of heterozygosity, with the majority of loss of heterozygosity mutations being deletions.

Normal human mammary gland epithelial cells from 19 individuals were incubated with 200 μM AZT for 24 hours (Olivero *et al*., 2008). AZT binding to genomic DNA was assessed by radioimmunoassay, which indicated the incorporation of AZT into the DNA from 12 of the samples (range=16 to 259 AZT molecules/10^6 nucleotides). Higher levels of incorporation of AZT into the DNA were associated with higher protein levels of thymidine kinase 1.

Experimental Animal Studies

Neonatal B6C3F1/$Tk^{+/-}$ mice were treated intraperitoneally on postnatal days 1 to 8 with 200 mg AZT/kg body weight per day (Von Tungeln *et al*., 2002). When assessed on postnatal days 9 and 10, AZT caused a significant increase in polychromatic erythrocytes containing micronuclei. AZT treatment also caused a significant increase in the mutant frequency at the *Tk* gene but not the *Hprt* gene of spleen T-lymphocytes. Subsequent analysis indicated that these mutations were due primarily to deletions and recombinations (Mittelstaedt *et al*., 2004). In a further study, female C57Bl/6N and female C57Bl/6N/$Tk^{+/-}$ mice were bred to male C3H/HeNMTV mice and then were treated by gavage on gestation days 12 to 17 with 0, 80, 160, or 240 mg AZT/kg body weight per day (Von Tungeln *et al*., 2007). As with the neonatal-only exposure, treatment with AZT resulted in an increase in

micronucleated reticulocytes and micronucleated normochromatic erythrocytes and an increase in the *Tk* mutant frequency (males only), which was associated with loss of heterozygosity.

C57Bl/6N $Tk^{+/+}$, $Tk^{+/-}$, and $Tk^{-/-}$ mice were treated intraperitoneally on postnatal days 1 to 8 with 0 or 200 mg AZT/kg body weight per day (Dobrovolsky *et al*., 2005). When assessed 1 day after the last dose, AZT-treated mice with $Tk^{+/+}$ and $Tk^{+/-}$ genotypes had an increase in micronucleated reticulocytes and micronucleated normochromatic erythrocytes. This did not occur with $Tk^{-/-}$ mice, which indicates the importance of thymidine kinase in the metabolic activation of AZT.

Pregnant CD-1 mice were given 0 or 200 mg AZT/kg body weight per day for the last 7 days of gestation (Torres *et al*., 2007). When assessed on postnatal day 13, AZT increased the mutant frequency of the *Hprt* gene in spleen T-lymphocytes. An increase in mutant frequency was not detected at postnatal days 15 or 21.

Female C3H/HeN ($p53^{+/+}$) mice were bred to $p53^{+/+}$ or $p53^{+/-}$ male mice, and the pregnant female mice were treated by gavage on gestation days 12 to 18 with 0, 40, 80, or 160 mg AZT/kg body weight/day (Dobrovolsky *et al*., 2007). After delivery, the $p53^{+/+}$ and $p53^{+/-}$ pups were treated by gavage on postnatal days 1 to 10 with 0, 20, 40, or 80 mg/kg per day and on postnatal days 11 to 28 with 0, 40, 80, and 160 mg/kg per day. When assessed on postnatal days 1, 10, and 28, there were dose-dependent increases in micronucleated reticulocytes and micronucleated normochromatic erythrocytes that were independent of genotype. AZT treatment also increased the mutant frequency at the *Hprt* gene of spleen lymphocytes in $p53^{+/-}$ mice but not in $p53^{+/+}$ mice.

Human Studies

Umbilical cord blood was obtained from infants whose HIV-1-positive mothers had been treated with AZT during pregnancy (Meng *et al*., 2007). When assessed by radioimmunoassay, the incorporation of AZT was detected in DNA isolated from mononuclear cells (mean=14.6 AZT molecules/10^6 nucleotides; range=0 to 34.2 AZT molecules/10^6 nucleotides; n=6). AZT incorporation was also detected in mononuclear cell DNA from maternal blood samples (mean=37.4 AZT molecules/10^6 nucleotides; range=0 to 100.4 AZT molecules/10^6 nucleotides; n=9). In an extension of these studies, the presence of mutations in glycophorin A was assessed in maternal and umbilical cord blood (Escobar *et al*., 2007; also see Meng *et al*., 2007). Compared to infants whose mothers had not received AZT, the frequency of glycophorin A variants was elevated in the infants whose mothers had received AZT. The difference was not statistically significant, but this may be a consequence of the limited number of samples (n=4) available from AZT-exposed infants.

DNA was isolated from umbilical cord tissue of infants whose mothers had been treated during pregnancy with AZT (Torres *et al*., 2009). The DNA was then analyzed by density gradient gel electrophoresis for sequence variations in mitochondrial DNA that were indicative of mutations. Mitochondrial sequence variations occurred at a threefold greater frequency in infants whose mothers had been administered AZT.

3TC

***In Vitro* Studies**

TK6 human lymphoblastoid cells were incubated for 3 days with 0, 33, 100, or 300 µM 3TC by itself (Carter *et al.*, 2007; Torres *et al*., 2007) or in the presence of an equimolar quantity of AZT (Torres *et al*., 2007). Compared to control cultures, incubation with 300 µM 3TC caused a significant increase in the *Hprt* and *Tk* mutant frequencies, while all three levels of the combined drugs caused significant increases in the *Hprt* and *Tk* mutant frequencies.

Experimental Animal Studies

Neonatal B6C3F1/$Tk^{+/-}$ mice were treated intraperitoneally on postnatal days 1 to 8 with 200 mg 3TC/kg body weight per day or a mixture of 200 mg/kg 3TC and 200 mg/kg AZT per day (Von Tungeln *et al*., 2002). When assessed on postnatal days 9 and 10, 3TC did not increase the frequency of polychromatic erythrocytes containing micronuclei. The percentage of polychromatic erythrocytes containing micronuclei was increased by the mixture of 3TC and AZT, but the response did not differ from that observed with AZT alone. Treatment with 3TC did not affect the mutant frequencies at either the *Tk* or *Hprt* genes of spleen T-lymphocytes. The combined treatment of 3TC and AZT did increase the *Tk* but not the *Hprt* mutant frequency; however, the response did not differ from treatment with AZT alone. The increase in the *Tk* mutant frequency was attributed to loss of heterozygosity.

Female C57Bl/6N and female C57Bl/6N/$Tk^{+/-}$ mice were bred to male C3H/HeNMTV mice and then were treated by gavage on gestation days 12 to 17 with 0 or 120 mg 3TC/kg body weight per day or a mixture of either 40 mg/kg 3TC and 80 mg/kg AZT per day, 80 mg/kg 3TC and 160 mg/kg AZT per day, or 120 mg/kg 3TC and 240 mg/kg AZT per day (Von Tungeln *et al*., 2007). When assessed 1 day after birth, there were no increases in micronucleated reticulocytes or micronucleated normochromatic erythrocytes in mice that had been exposed to 3TC alone, but there were

dose-dependent increases in mice that had been exposed to the mixtures of 3TC and AZT. Treatment with 3TC resulted in an increase in the *Tk* mutant frequency when assessed 5 weeks after treatment, whereas the mixture of 3TC and AZT resulted in an increased *Tk* mutant frequency at 3 weeks after treatment.

Pregnant CD-1 mice were given 100 mg 3TC/kg body weight per day or a mixture of 100 mg/kg 3TC and 200 mg/kg AZT per day for the last 7 days of gestation (Torres *et al*., 2007). When assessed on postnatal day 13, the mixture of 3TC and AZT, but not 3TC by itself, increased the mutant frequency of the *Hprt* gene in spleen T-lymphocytes. An increase in mutant frequency was not detected at postnatal days 15 or 21 with either treatment.

Female C3H/HeN ($p53^{+/+}$) mice were bred to $p53^{+/+}$ or $p53^{+/-}$ male mice and the pregnant female mice were treated by gavage on gestation days 12 to 18 with a mixture of 100 mg 3TC and 160 mg AZT/kg body weight per day (Dobrovolsky *et al*., 2007). After delivery, the $p53^{+/+}$ and $p53^{+/-}$ pups were treated by gavage on postnatal days 1 to 10 with 50 mg/kg 3TC and 80 mg/kg AZT per day and on postnatal days 11 to 28 with 100 mg/kg 3TC and 160 mg/kg AZT per day. When assessed on postnatal days 1, 10, and 28, the mixture caused increases in micronucleated reticulocytes and micronucleated normochromatic erythrocytes that were independent of genotype. The mixture of 3TC and AZT also increased the mutant frequency at the *Hprt* gene of spleen lymphocytes in $p53^{+/-}$ mice but not in $p53^{+/+}$ mice.

Human Studies

Umbilical cord blood was obtained from infants whose HIV-1-positive mothers had received antiretroviral therapy during pregnancy (Witt *et al*., 2007). Infants whose mothers had received regimens containing 3TC and AZT plus at least one additional antiretroviral drug had significant increases in micronucleated reticulocytes compared to infants whose mothers had either not been treated or had received regimens that did not contain 3TC and AZT. Likewise, venous blood from mothers given regimens containing 3TC and AZT had significant increases in micronucleated reticulocytes compared to mothers administered regimens that did not contain 3TC and AZT or compared to typical values measured in "control" adults.

DNA was isolated from mononuclear cells of umbilical cord blood obtained from infants whose HIV-1-positive mothers had been treated with 3TC and AZT during pregnancy (Meng *et al*., 2007). When assessed by radioimmunoassay, AZT incorporation was detected (mean=51.6 AZT molecules/10^6 nucleotides; range=3 to 151.5 AZT molecules/10^6 nucleotides; n=21). These levels of AZT incorporation were significantly greater than in infants treated with AZT alone. The levels of 3TC incorporation were not measured. AZT incorporation was also detected in mononuclear cell DNA from maternal blood samples (mean=52.8 AZT molecules/10^6 nucleotides; range=0 to 241.7 AZT molecules/10^6 nucleotides; n=9). In further work, the presence of mutations in glycophorin A was assessed in maternal and umbilical cord blood (Escobar *et al*., 2007; also see Meng *et al*., 2007). Compared to infants whose mothers had not been treated, the frequency of glycophorin A variants was elevated in the infants whose mothers had received mixtures of 3TC and AZT.

Umbilical cord tissue DNA of infants whose mothers had been treated during pregnancy with mixtures of AZT and 3TC was examined for sequence variations in mitochondrial DNA (Torres *et al*., 2009). Density gradient gel electrophoresis indicated the presence of a shift in the mutation spectrum.

NVP

NVP is not mutagenic or clastogenic in a variety of assays, including microbial and mammalian gene mutation tests and micronucleus tests (*PDR*, 2007b). Synthetic esters of the NVP metabolite 12-hydroxynevirapine have been shown to react with DNA to give a number of DNA adducts (Antunes *et al*., 2008). Whether or not these DNA adducts are formed *in vivo* is currently not known.

NFV

NFV is not mutagenic or clastogenic in a variety of assays, including microbial and mammalian gene mutation tests and micronucleus tests (Burns-Naas *et al*., 2005a; *PDR*, 2007c).

STUDY RATIONALE

Data regarding the safety of antiretroviral drugs (other than AZT) administered during pregnancy are limited. With the increased administration of multidrug regimens to pregnant women who are HIV-1 positive, along with the increased efficacy of these combinations, determining the long-term consequences of the antiretroviral agents in noninfected children becomes important. The goal of the current study was to determine the cacinogenicity of combinations of antiretroviral drugs administered transplacentally to pregnant mice.

A study conducted within the Pediatric AIDS Clinical Trial Group (Shapiro *et al*., 2000) showed that of HIV-1-positive pregnant women treated in 1998 and 1999 with anti-retroviral therapy, 25% received AZT

alone, 29% were given AZT and 3TC, 36% were administered two nucleoside analogues with a protease inhibitor (mostly NFV), and 5% were given two nucleoside analogues and a nonnucleoside reverse transcriptase inhibitor (mostly NVP). Since the transplacental carcinogenicity of AZT had been investigated in mice, we proposed to focus the current study on combination treatments of AZT and 3TC; AZT, 3TC, and NVP; and AZT, 3TC, and NFV, and compare the tumor incidences obtained with the mixtures to those obtained in vehicle control mice. The study was conducted by breeding male C3H/HeNMTV mice to female C57Bl/6N mice, and then treating the pregnant females with the antiretroviral drugs by gavage once daily on gestation days 12 to 18. The transplacental exposure, which encompasses the last third of gestation, was modeled after transplacental tumorigenesis bioassays previously conducted with mice that were dosed once daily with AZT (Olivero *et al*., 1997; Zhang *et al*., 1998; Diwan *et al*., 1999; Walker *et al*., 2007). The compounds were administered orally because this is the typical route of administration for pregnant women. Male and female B6C3F1 mice were chosen as the test animal to provide continuity with our previous mutagenesis and pharmacokinetic studies (Von Tungeln *et al*., 2002, 2007; Williams *et al*., 2003; Mittelstaedt *et al*., 2004; Dobrovolsky *et al*., 2005) and to allow comparisons to the tumorigenicity data for AZT reported by Walker *et al*. (2007).

At the initiation of this study, the routine doses of AZT, 3TC, and NVP given to adult humans were 300, 150, and 200 mg *bid*, respectively; the daily dose for NFV was 2,500 mg (DHHS, 2000). For a woman weighing 70 kg, these doses would be equivalent to 8.6 mg AZT, 4.3 mg 3TC, 5.7 mg NVP, and 35.7 mg NFV/kg body weight per day. This ratio (AZT:3TC:NVP:NFV, 1:0.5:0.7:4.2) was maintained for the transplacental dosing of mice in the current study.

In the study protocol for the current transplacental bioassay, a range-finding study was outlined in which the highest doses of AZT, 3TC, NVP, and NFV would be 400, 200, 266, and 1,660 mg/kg body weight per day, respectively. Before conducting the range-finding study, a preliminary range-finding study was performed at the NCTR in which mice were exposed transplacentally to mixtures of AZT, 3TC, and NVP (400, 200, and 266 mg/kg body weight per day, respectively) or AZT, 3TC, and NFV (400, 200, and 1,660 mg/kg per day, respectively). These treatments produced unacceptable toxicities, as indicated by maternal and infant mortality and depressed infant weights (data not presented). Because of these toxicities, the highest doses for the range-finding study were adjusted to 240, 120, 168, and 1,008 mg/kg body weight per day for AZT, 3TC, NVP, and NFV, respectively. The mid and low doses were selected after consideration of preliminary data from a study conducted by Walker and colleagues (personal communication) in which B6C3F1 mice were exposed to AZT transplacentally at 0, 80, 240, or 400 mg/kg per day for the last 7 days of gestation.

In the range-finding study conducted at the NCTR, there were dose- and treatment-related decreases in the number of live births and in the body weights of the offspring, with the maximum body weight decrement being approximately 10% (data not presented). For most combinations, there were significant decreasing trends in neutrophils and platelets that were indicative of a mild bone marrow suppression (data not presented). With AZT by itself, there was a significant increase in lactic acid, which is consistent with a mild mitochondrial impairment (data not presented). There were no histopathologic changes that were considered to be related to the treatment (data not presented). In view of the limited toxicities observed in the range-finding study, the same doses were used in the transplacental bioassay (Table 1).

TABLE 1
Summary of Doses Used in the 2-Year Transplacental Exposure Study of AZT, 3TC, NVP, and NFV[a]

Treatment	Dose Level	AZT	3TC	NVP	NFV
Vehicle control		0	0	0	0
AZT	Low	80	0	0	0
	Mid	160	0	0	0
	High	240	0	0	0
AZT and 3TC	Low	80	40	0	0
	Mid	160	80	0	0
	High	240	120	0	0
AZT, 3TC, and NVP	Low	80	40	56	0
	Mid	160	80	112	0
	High	240	120	168	0
AZT, 3TC, and NFV	Low	80	40	0	336
	Mid	160	80	0	672
	High	240	120	0	1,008

[a] Doses are given in mg compound/kg body weight per day.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

AZT, 3TC, NVP, and NFV were obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in single lots F00573, B10250, FX1009, and HX1292, respectively. Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR) and Galbraith Laboratories, Inc. (Knoxville, TN) (Appendix D). To ensure stability, the bulk chemicals were stored in the original cardboard containers at room temperature protected from light inside multiple, high-density polyethylene bags. Reports on analyses performed in support of the AZT, 3TC, NVP, and NFV transplacental study are on file at the NCTR.

AZT

The chemical, a white-to-beige crystalline solid, was identified as AZT by proton nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe/electron ionization (DEP/EI) mass spectrometry (MS), liquid chromatography combined with mass spectrometry (LC-MS), and melting point analysis. Purity of lot F00573 was determined by elemental analyses, proton NMR spectroscopy, and high-performance liquid chromatography (HPLC) with photodiode array (PDA) detection.

Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for AZT. Total impurity was estimated at 0.3% to 0.4% by proton NMR. HPLC-PDA detected no impurities with peak areas exceeding 0.1% of the total peak area and estimated a purity of approximately 99.9%. The overall purity of lot F00573 was determined to be 99% or greater.

3TC

The chemical, a white-to-off-white crystalline solid, was identified as 3TC by proton NMR spectroscopy, DEP/EI-MS, and LC-MS. Purity of lot B10250 was determined by elemental analyses, proton NMR spectroscopy, and HPLC-PDA.

Karl Fischer titration indicated less than 0.097% water. Elemental analyses for carbon, hydrogen, nitrogen, and sulfur were in agreement with the theoretical values for 3TC. Total impurity was estimated at 0.5% by proton NMR spectroscopy. HPLC-PDA detected one impurity with a peak area of 1.1% of the total peak area and estimated a purity of approximately 98.9%. The overall purity of lot B10250 was estimated to be 99%.

NVP

The chemical, a white-to-off-white crystalline powder, was identified as NVP by proton NMR spectroscopy, DEP/EI-MS, gas chromatography/electron ionization (GC/EI) MS, and LC-MS. Purity of lot FX1009 was determined by elemental analyses, proton NMR spectroscopy, and HPLC-PDA.

Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for NVP. Total impurity was estimated at 0.2% by proton NMR spectroscopy. HPLC-PDA detected a single peak, indicating that the test article was 100% pure. The overall purity of lot FX1009 was estimated to be at least 99.5%.

NFV

The chemical, a white-to-off-white amorphous powder, was identified as NFV by proton and carbon-13 NMR spectroscopy, DEP/EI-MS, LC-MS, and melting point analysis. Purity of lot HX1292 was determined by elemental analyses, proton NMR spectroscopy, GC with flame ionization detection (GC-FID), and HPLC-PDA.

Karl Fischer titration indicated 2.92% water. Elemental analyses for carbon, hydrogen, nitrogen, and sulfur were in agreement with the theoretical values for NFV. Proton NMR spectroscopy data suggested that the lot was contaminated with approximately 2.1% tetrahydrofuran, 0.7% diethyl ether, and 0.1% to 0.2% impurities structurally related to NFV, indicating a total of approximately 3% organic impurities. The presence of tetrahydrofuran in lot HX1292 was corroborated by GC-FID. HPLC-PDA detected one impurity peak with an area of 0.20% of the total peak area.

Subsequent experiments were conducted to determine a method for removal of tetrahydrofuran and diethyl ether from lot HX1292, and a procedure was developed for drying the test article for 24 hours at 60° C under 30 inches of mercury vacuum. Characterization of the dried test article by proton NMR spectroscopy, HPLC-MS, and HPLC-PDA indicated that it was not significantly altered by the purification steps and that the concentrations of tetrahydrofuran and diethyl ether were reduced to 0.64% and 0.16%, respectively. Because the total impurities were reduced to approximately 1% by weight, the organic purity of the dried test article was estimated to be approximately 99%. HPLC-PDA of the dried test article detected one impurity with a peak area of 0.7% of the total peak area and estimated a purity of 99.3%. The overall purity of the dried sample of lot HX1292 was determined to be approximately 99%. Only dried samples of lot HX1292 were used in the dose formulations for the animal studies.

Methylcellulose/Tween® 80 Vehicle

The vehicle used for dose formulations in this study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. This vehicle was selected based upon preliminary experiments to find a vehicle that gave suitable suspensions with the drug combinations. Methylcellulose was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in one batch (062K0144-1) and Tween® 80 was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) in one lot (13127CA-1). Proton and carbon-13 NMR analyses of both chemicals were performed by the study laboratory. For methylcellulose, proton and carbon-13 NMR spectra of batch 062K0144-1 were similar to those of a methylcellulose sample obtained from Fischer Scientific (Fair Lawn, NJ), and no resonances from small molecule impurities were detected. For Tween® 80, the proton NMR spectrum of lot 13127CA-1 was consistent with the structure of the chemical, and the carbon-13 NMR spectrum of this lot was consistent with a literature spectrum (Bugay and Findlay, 1999); both spectra of lot 13127CA-1 showed smaller resonances indicative of minor impurities.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the test chemicals with an aqueous solution of 0.2% methylcellulose/0.1% Tween® 80 (Table D1). Homogeneity and stability studies of high-dose and low-dose suspensions of AZT, 3TC, and NVP, and AZT, 3TC, and NFV in the methylcellulose/Tween® 80 vehicle were conducted by the study laboratory using HPLC. Homogeneity was confirmed, and stability was confirmed for 21 days for dose formulations stored in capped glass vials at room temperature.

At four time points during the transplacental dosing period, analyses of the dose formulations of the antiretroviral drugs were conducted by the study laboratory using HPLC-PDA. Of the 43 samples measured for concentration of the test chemical, 38 were within 10% of the target concentration, and all were within 15% of the target concentration (Table D2).

TRANSPLACENTAL STUDY

Study Design

Female C57Bl/6N mice were bred to male C3H/HeNMTV mice, and from gestation day 12 until gestation day 18 (or until they littered), the pregnant dams were treated by gavage with AZT or mixtures of AZT and 3TC; AZT, 3TC, and NVP; or AZT, 3TC, and NFV (Table 1). The high dose of each drug was 240 mg/kg body weight per day for AZT, 120 mg/kg per day for 3TC, 168 mg/kg per day for NVP, and 1,008 mg/kg per day for NFV (ratio 1.0:0.5:0.7:4.2, respectively). The mid and low doses were 66% and 33% of these values, respectively, and maintained the same ratio. The drugs were administered in 0.2% methylcellulose and 0.1% Tween® 80 at a dosing volume of 20 mL/kg body weight. Control dams were administered the vehicle only. The neoplasm and nonneoplastic lesion incidences in the male and female B6C3F1 offspring were monitored for 2 years after birth. The group sizes varied between 15 and 65 mice per treatment.

The study was conducted in three staggered loads, with the initiation of mating beginning on July 9, 2003 (load 1), July 16, 2003 (load 2), and April 29, 2004 (load 3). A target was set of 48 mice per sex per treatment group, and load 3 was conducted to reach this number. Due to the extensive mortality caused by the high-dose combination of AZT, 3TC, and NFV in loads 1 and 2, this treatment group was eliminated from load 3. Litter information for each of the loads is presented in Tables E1 through E4.

Source and Specification of Animals

Male C3H/HeNMTV mice and female C57B1/6N mice were obtained from the National Center for Toxicological Research (NCTR) (Jefferson, AR) for use in the 2-year transplacental exposure study. Male mice were 21 days old and female mice were 21 to 22 days old upon receipt. Males and females were mated to produce B6C3F1 offspring. The health of the mice was

monitored during the study according to the protocols of the NCTR Sentinel Animal Program (Appendix G).

Animal Maintenance

Prior to mating, female mice were housed two per cage, with one mouse being tail-tattooed. Males were housed one per cage. Mating began when the breeders were approximately 8 weeks old. Issue numbers were maintained with the animals to allow littermates of the dams to be identified.

At the initiation of mating, two females were moved from their home cage to a cage containing one male. Plug checks were performed daily throughout the mating session. When a plug was detected, the dam was weighed and then moved to a treatment cage assigned to avoid having littermates of a dam in the same treatment. Body weights were collected daily on all dams from the time a plug was detected until they gave birth, and once again when their pups were 1 day old.

Litter checks were performed twice daily, beginning on gestation day 17. Litters were not disturbed at first observation (postnatal day 0), but the cage was tagged to indicate litter date and the number of live and dead pups observed in the cage. On postnatal day 1, the litter information was entered into the Multigeneration Support System. Litters were adjusted to six (load 3) or eight (loads 1 and 2) pups, with an attempt to return equal numbers of male and female pups back into the original litter. Litters having less than the desired six or eight pups were adjusted by placing fosters culled from other litters from the same treatment into the cage.

Pups were weaned on postnatal day 21 and four of the same sex were assigned per cage in polycarbonate cages with polycarbonate filter tops and hardwood chip bedding. Animals were identified by a tail tattoo consisting of a three-digit cage number and a single digit from 1 to 4. The animals were also ear-clipped to aid in identification. Feed and water were available *ad libitum*, except mice were fasted overnight prior to the day of necropsy. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix F.

Clinical Examinations and Pathology

All animals were observed twice daily, and clinical findings were recorded weekly. Pups from the original litter were grouped by sex and weighed on postnatal days 1 to 8, 14, and 21. Fostered pups were excluded from the daily body weight collections. After weaning, 15 to 65 male and 26 to 64 female pups were kept on study for up to 104 weeks of age. Body weights were recorded weekly and at the end of the study. Animal data were collected using an Inlife Interactive Data Collection System.

Complete necropsies and microscopic examinations were performed on all pups assigned to the study after weaning. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in Tissue-Prep II, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. Tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by the study pathologist, and the pathology data were entered into the Laboratory Data Acquisition System II and subsequently uploaded to the TDMSE database on the TDMSE computer at NIEHS. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the Block and Slide Laboratory for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment group. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. Four quality assessment pathologists evaluated slides of all proliferative lesions from the liver, lung, and pituitary gland of male and female mice and from the thyroid gland of control and AZT-treated female mice. In addition, the lymph nodes, thymus, and spleen were reviewed for the presence of lymphoma. All tumors diagnosed by the study pathologist from all tissues from all animals were also reviewed by the quality assessment pathologists. Differences of opinion were reconciled between the study pathologist and the quality assessment pathologists.

The quality assessment pathologist served as the NTP Pathology Working Group (PWG) coordinator and presented histopathology slides containing the diagnoses made by the study pathologist and herself. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist,

the diagnoses was changed. Final diagnoses for reviewed lesions represent a consensus between the study pathologist, reviewing pathologists, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 2
Experimental Design and Materials and Methods in the Transplacental Studies of AZT, 3TC, NVP, and NFV

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

Strain and Species
C3H/HeNMTV male mice (sires)
C57Bl/6N female mice (dams)
B6C3F1 mice (pups)

Animal Source
National Center for Toxicological Research (Jefferson, AR)

Time Held Before Studies
Approximately 5 weeks

Average Age at Mating
8 weeks (dams and sires)

Date of First Dose
July 22, 2003; July 29, 2003; or May 11, 2004

Duration of Dosing
Gestation days 12 to 18

Date of Last Dose
August 3, 2003, August 10, 2003, or May 23, 2004

Date of Last Necropsy
May 10, 2006

Average Age at Necropsy
104 weeks

Size of Study Groups
Dams: 14 to 21 per treatment group
Male pups: 15 to 65 per treatment group
Female pups: 26 to 64 per treatment group

Method of Distribution
F_0 mice: Pregnant dams were moved to a treatment cage with randomization to avoid same littermates per same treatment.
F_1 mice: On postnatal day 21, weaned pups were assigned four of the same sex per cage. Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
Dams were housed two per cage prior to mating, and sires were individually housed except during mating. During cohabitation, one male and two females were housed together. After cohabitation, each female was housed alone.
Pups were housed with dams until weaning at postnatal day 21. On postnatal day 1, litters were adjusted to six or eight pups, using foster pups from other litters of the same treatment to equalize the sex ratio when necessary. After weaning, four pups of the same sex were housed together.

TABLE 2
Experimental Design and Materials and Methods in the Transplacental Studies of AZT, 3TC, NVP, and NFV

Method of Animal Identification
Ear punch and tail tattoo

Diet
Autoclaved NIH-31 pelleted diet (Purina Mills, Richmond, IN), available *ad libitum* until the day before necropsy

Water
Millipore-filtered tap water (Jefferson, AR, municipal supply) via water bottles, available *ad libitum*

Cages
Polycarbonate cages, changed twice weekly

Bedding
Hardwood chips (Northeastern Products Corp., Warrensburg, NY)

Animal Room Environment
Temperature: 22° ± 4° C
Relative humidity: 40% - 70%
Room fluorescent light: 12 hours/day
Room air changes: 10 - 15/hour

Doses
80 (low), 160 (mid), or 240 (high) mg AZT/kg body weight; 80/40 (low), 160/80 (mid), or 240/120 (high) mg AZT/3TC/kg body weight; 80/40/56 (low), 160/80/112 (mid), or 240/120/168 (high) mg AZT/3TC/NVP/kg body weight; 80/40/336 (low), 160/80/672 (mid), or 240/120/1,008 (high) mg AZT/3TC/NFV/kg body weight per day by gavage in 0.2% methylcellulose and 0.1% Tween® 80 (dosing volume 20 mL/kg body weight).

Type and Frequency of Observation
Breeder mice were weighed one day prior to the scheduled mating session. When a vaginal plug was detected, the dam was weighed and moved to a treatment cage; daily body weights were determined during treatment.
Litters were observed twice daily beginning on gestation day 17. Pups from the original litter were grouped by sex and weighed on postnatal days 1- 8, 14, and 21. Pups were weaned on day 21 and weighed weekly and at the end of the study; clinical findings were recorded weekly.

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all animals.

Histopathology
Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, blood vessel, bone marrow, brain, clitoral gland, esophagus, eye, gallbladder, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, pancreatic islets, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

STATISTICAL METHODS

Survival Analysis

Kaplan-Meier estimates (Kaplan and Meier, 1958) of mean survival times were calculated for each sex-by-treatment group. For each sex, a proportional hazards model (Cox, 1972) was used to test the effect of the dose (linear trend and comparison to control) within drug combination and the difference between drug combinations within dose level. All survival analysis P values are two sided. Weaned pups reaching terminal sacrifice were considered to be censored.

Litter Analysis

The effect of dose on the number of mice per litter and the distribution of male/female mice in litters were analyzed using one-way ANOVA within drug combinations. In instances where the data showed a skewed distribution, unequal variance, or both, the data were analyzed using the method of Kruskal-Wallis.

Where calculations indicated a significant overall dose effect ($P<0.05$), pairwise comparisons to the appropriate control group were conducted using Dunnett's

test (for endpoints where ANOVA was used; Dunnett, 1955) or Dunn's test (for endpoints where Kruskal-Wallis was used; Dunn, 1964). All analysis P values are two sided.

Body Weight Analysis

The body weight data for each animal were rasterized to evenly-spaced time points (every 4 weeks) via locally weighted scatterplot smoothing scoring (Cleveland, 1979; Cleveland, *et al*., 1988). This process reduces the number of time points for the mixed-effects model, reduces the effects of outliers, and creates a grid of regularly spaced time points. Since several drug combinations exhibited high mortality in the higher dose groups, the time points chosen ranged from ages 6 to 78 weeks. The scored data were then treated as primary data for the repeated measures mixed effects models. These models were run separately for each sex. The model treated body weight as a function of treatment group and age. Repeated observations within each animal as it aged were presumed to be correlated, and the variance was allowed to change with age.

Dunnett's method was used to compare dose levels to control within each drug combination at each age. A polynomial contrast was used to test for linear trend with dose at each age. Contrasts were used to compare drug combinations within dose levels at each age. Since this results in a very large number of comparisons, additional contrasts among ages were used to summarize the data as "IR" (average initial growth rate: 6 to 14 weeks), "LR" (average late growth rate: 46 to 58 weeks), and "AS" (asymptotic average late body weight: 46 to 58 weeks). These contrasts were also compared among dose levels within drug and drug combinations and were designed to capture the essential features of the growth.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, and B4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of neoplasms (Tables A2 and B2) and nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. Tables A2 and B2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice. For dam- and sire-adjusted correlation models, the Poly-3 weighted generalized linear model (GLIM) is used to generate estimated correlation-adjusted incidences and these are given along with the relevant test P value. The multiplicity of neoplasms within specific organs (e.g., liver and lung) was low in all experimental groups; as such, statistical analyses of neoplasm multiplicities were not conducted.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) is typically used to assess treatment effects on neoplastic and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. The variance correction of Bieler and Williams (1993) is usually used to account for the extra-binomial variability induced by using a stochastic denominator in the Cochran-Armitage test. Pairwise comparisons in this test are accomplished by reanalyzing the treatment groups in pairs. This framework limits the Poly-k test to one-way designs with no correlation.

Bieler and Williams (1993), in the derivation of their variance correction, used the fact that the Cochran-Armitage test can be envisioned as a binomial-weighted regression. If we begin with a weighted regression paradigm with binomial weights, we can generalize this framework and view the Cochran-Armitage test as a generalized linear model with binomial variation and an identity link function. If this analysis is performed with the Poly-k weights then the resulting analysis can be used with more complex designs, including litter correlations and factorial effects as well as alternative link functions.

Correlation among littermates (dam-adjusted) was achieved by using the generalized linear model described above with estimation using generalized estimating equations (Liang and Zeger, 1986) and an exchangeable correlation among littermates. Sire-adjusted analyses were generated in the same manner differing only in the specification of the correlation group variable.

It should be noted that the implementation details of this method are different from the Bieler and Williams variance-adjusted Poly-k test (Bieler and Williams, 1993). Particularly, the variance is not quantal-adjusted and all comparisons are estimated within a single analysis of variance model rather than multiple regression models. Suitable contrasts were used to test the

relevant hypotheses. One-sided results were generated and, per NTP custom, an "N" was suffixed to indicate negative trends. Since the variance structure is group specific rather than estimated from the null hypothesis, uniform treatment groups were dealt with by adding an uncorrelated dummy lesion observation to all groups (if necessary for any group) with value=0.005 and Poly-3 weight=0.005.

The presented results include the usual unadjusted Bieler and Williams adjusted Poly-3, Poly-3 weighted binomial/identity-link GLIM with dam-adjusted GEE correlation, and Poly-3 weighted binomial/identity-link GLIM with sire-adjusted GEE correlation.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. The historical database used for this study consisted of studies conducted by the NCTR using B6C3F1 mice.

QUALITY ASSURANCE METHODS

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of the NCTR performed audits and inspections of protocols, procedures, data, and reports throughout the course of the study. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this Technical Report were conducted. Audit procedures and findings are on file at the NCTR. The audit findings were reviewed and assessed by the NCTR staff, and all comments were resolved or otherwise addressed either before or during preparation of the Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of AZT, 3TC, NVP, and NFV was assessed by testing the ability of the chemicals to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*. The protocol for these studies and the results are given in Appendix C.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

RESULTS

BODY WEIGHTS OF PREGNANT DAMS

Female C57Bl/6N mice were dosed by gavage daily beginning on gestation day 12 until gestation day 18 (or until they littered) with the treatments indicated in Table 1. Daily maternal body weights are presented in Table 3. Compared to the vehicle control group, none of the treatments affected the body weights of the pregnant dams.

TABLE 3
Mean Maternal Body Weights of C57B1/6N Mice Administered AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV by Gavage on Gestation Days 12 to 18

Gestation Day	Body Wt. (g)	Body Wt. (g)	Body Wt. (g)	Body Wt. (g)
AZT	**Vehicle Control**	**80 mg/kg**	**160 mg/kg**	**240 mg/kg**
n	20	19	20	14
12	29.13	29.19	29.36	30.82
13	30.74	30.85	30.82	32.11
14	32.39	32.54	32.32	33.74
15	34.19	34.23	34.21	35.66
16	36.27	35.87	36.03	37.76
17	38.06	37.56	37.81	39.74
18	39.59	39.41	39.55	41.49
AZT/3TC		**80/40 mg/kg**	**160/80 mg/kg**	**240/120 mg/kg**
n		18	19	15
12		29.22	29.84	30.09
13		30.69	31.43	31.79
14		32.14	32.98	33.19
15		34.12	34.83	35.21
16		35.98	36.64	37.25
17		37.54	38.38	38.84
18		39.29	40.27	40.70
AZT/3TC/NVP		**80/40/56 mg/kg**	**160/80/112 mg/kg**	**240/120/168 mg/kg**
n		20	17	18
12		29.13	29.94	28.96
13		30.48	30.98	30.12
14		31.76	32.20	31.38
15		33.78	34.14	33.46
16		35.64	36.61	35.62
17		37.65	38.06	37.40
18		39.42	40.09	39.01
AZT/3TC/NFV		**80/40/336 mg/kg**	**160/80/672 mg/kg**	**240/120/1,008 mg/kg**
n		20	20	21
12		28.95	28.63	30.25
13		29.96	29.71	30.98
14		31.41	30.52	31.59
15		32.93	31.51	33.12
16		34.63	33.55	34.75
17		36.33	35.10	36.30
18		38.05	36.46	38.03

LITTER EFFECTS

The number of pups per litter and the distribution of male and female pups within the litters are presented in Table 4. Compared to the vehicle control group, none of the treatments affected the number of pups or the ratio of male to female pups.

Body weights of the litters were obtained on postnatal days 1 through 8 and 14. Compared to the control groups, there were significant reductions in the body weights of male and female pups (Table 5) at nearly all time points with the high-dose combinations of AZT/3TC/NVP and AZT/3TC/NFV, with the decreases being up to 40%. Significant reductions (approximately 15%) in body weights also occurred in the mid-dose combination of AZT/3TC/NVP at later time points (Table 5).

Transplacental exposure to the combination of AZT/3TC/NFV caused dose-related reductions in survival between postnatal day 1 and weaning at postnatal day 21 (Table 6), with only 33% of the males and 51% of the females in the high-dose group surviving. A decrease in survival also occurred with the high-dose combination of AZT/3TC/NVP.

TABLE 4
Litter Parameters for C57B1/6N Mouse Dams Administered AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV by Gavage on Gestation Days 12 to 18

Treatment	Dose (mg/kg/day)	Number of Litters	Pups per Litter (Postnatal Day 0)	Males per Litter (Postnatal Day 1)	Females per Litter (Postnatal Day 1)
Vehicle Control	0	20	8.7 ± 0.3	4.1 ± 0.4	4.6 ± 0.2
AZT	80	19	8.2 ± 0.4	4.6 ± 0.4	3.6 ± 0.4
	160	20	8.4 ± 0.3	3.9 ± 0.4	4.6 ± 0.4
	240	14	9.6 ± 0.4	4.9 ± 0.4	4.6 ± 0.3
AZT/3TC	80/40	18	8.3 ± 0.3	4.3 ± 0.4	4.0 ± 0.4
	160/80	19	8.4 ± 0.3	3.8 ± 0.3	4.5 ± 0.4
	240/120	15	8.7 ± 0.6	4.1 ± 0.4	4.7 ± 0.5
AZT/3TC/NVP	80/40/56	20	8.4 ± 0.4	4.2 ± 0.3	4.2 ± 0.3
	160/80/112	17	8.4 ± 0.6	4.2 ± 0.5	4.1 ± 0.3
	240/120/168	18	8.9 ± 0.3	4.1 ± 0.4	4.8 ± 0.4
AZT/3TC/NFV	80/40/336	20	8.1 ± 0.4	4.4 ± 0.4	3.6 ± 0.3
	160/80/672	20	7.6 ± 0.7	3.7 ± 0.4	4.0 ± 0.4
	240/120/1,008	21	7.4 ± 0.6	2.8 ± 0.4	3.8 ± 0.5

TABLE 5
Mean Body Weights of B6C3F1 Mice Transplacentally Exposed to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV[a]

Postnatal Day	Body Wt.	Body Wt.	Body Wt.	Body Wt.
Male				
AZT	**Control**	**80 mg/kg**	**160 mg/kg**	**240 mg/kg**
1	1.50 [12/12]	1.44 [13/14]	1.47 [17/17]	1.40 [13/14]
2	1.68 (112) [12/12]	1.57 (109) [14/14]	1.64 (112) [16/17]	1.56 (111) [14/14]
3	2.07 (138) [12/12]	1.86 (129) [14/14]	1.96 (133) [16/16]	1.80 (129) [14/14]
4	2.52 (168) [12/12]	2.26 (157) [14/14]	2.23 (152) [15/15]	2.27 (162) [14/14]
5	3.08 (205) [12/12]	2.69 (187) [14/14]	2.87 (195) [15/15]	2.72 (194) [14/14]
6	3.62 (241) [12/12]	3.19 (222) [14/14]	3.05 (207) [14/15]	3.16 (226) [14/14]
7	4.15 (277) [12/12]	3.82 (265) [14/14]	3.82 (260) [14/15]	3.60 (257) [14/14]
8	4.65 (310) [12/12]	4.16 (289) [14/14]	4.30 (293) [13/15]	3.44 (246) [12/14]
14	7.01 (467) [12/12]	6.69 (465) [14/14]	6.31 (429) [15/15]	6.63 (474) [14/14]
AZT/3TC		**80/40 mg/kg**	**160/80 mg/kg**	**240/120 mg/kg**
1		1.51 [14/14]	1.46 [15/15]	1.43 [15/15]
2		1.66 (110) [14/14]	1.61 (110) [15/15]	1.59 (111) [15/15]
3		2.01 (133) [14/14]	1.91 (131) [15/15]	1.83 (128) [15/15]
4		2.41 (160) [14/14]	2.34 (160) [15/15]	2.23 (156) [15/15]
5		2.89 (191) [13/13]	2.83 (194) [15/15]	2.67 (187) [15/15]
6		3.33 (221) [13/13]	3.39 (232) [15/15]	3.13 (219) [15/15]
7		3.89 (258) [13/13]	3.87 (265) [15/15]	3.55 (248) [15/15]
8		4.24 (281) [13/13]	4.37 (299) [15/15]	4.06 (284) [14/15]
14		6.79 (450) [13/13]	6.67 (457) [15/15]	6.36 (445) [14/15]
AZT/3TC/NVP		**80/40/56 mg/kg**	**160/80/112 mg/kg**	**240/120/168 mg/kg**
1		1.42 [17/17]	1.39 [16/16]	1.22[b] [15/15]
2		1.49 (105) [17/17]	1.51 (109) [16/16]	1.30[b] (107) [15/15]
3		1.81 (127) [16/17]	1.82 (131) [16/16]	1.41[b] (116) [14/14]
4		2.16 (152) [15/17]	2.13 (153) [16/16]	1.62[b] (133) [12/13]
5		2.61 (184) [17/17]	2.63 (189) [16/16]	1.87[b] (153) [13/13]
6		3.07 (216) [17/17]	3.03[b] (218) [16/16]	2.20[b] (180) [12/12]
7		3.57 (251) [17/17]	3.53[b] (254) [16/16]	2.60[b] (213) [12/12]
8		4.00 (282) [17/17]	4.06[b] (292) [16/16]	3.03[b] (248) [11/12]
14		6.27 (442) [17/17]	6.39[b] (460) [16/16]	5.51[b] (452) [12/12]
AZT/3TC/NFV		**80/40/336 mg/kg**	**160/80/672 mg/kg**	**240/120/1,008 mg/kg**
1		1.39 [14/14]	1.33[b] [10/10]	1.23[b] [14/14]
2		1.61 (116) [12/13]	1.52 (114) [9/9]	1.31[b] (107) [7/7]
3		1.90 (137) [13/13]	1.81 (136) [8/8]	1.51[b] (123) [6/6]
4		2.29 (165) [11/13]	2.26 (170) [8/8]	1.73[b] (141) [6/6]
5		2.77 (199) [12/13]	2.67 (201) [8/8]	2.06[b] (167) [6/6]
6		3.30 (237) [13/13]	3.14 (236) [8/8]	2.38[b] (193) [6/6]
7		3.79 (273) [13/13]	3.62 (272) [8/8]	2.73[b] (222) [5/6]
8		4.31 (310) [13/13]	4.04 (304) [8/8]	3.23[b] (263) [5/6]
14		6.79 (488) [13/13]	6.44 (484) [8/8]	6.10 (496) [6/6]

TABLE 5
Mean Body Weights of B6C3F1 Mice Transplacentally Exposed to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV

Postnatal Day	Body Wt.	Body Wt.	Body Wt.	Body Wt.
Female				
AZT	**Control**	**80 mg/kg**	**160 mg/kg**	**240 mg/kg**
1	1.44 [12/12]	1.40 [13/14]	1.41 [17/17]	1.34 [13/14]
2	1.64 (114) [12/12]	1.58 (113) [13/13]	1.56 (111) [16/17]	1.58 (118) [14/14]
3	1.97 (137) [12/12]	1.88 (134) [13/13]	1.83 (130) [15/15]	1.82 (136) [13/14]
4	2.40 (167) [12/12]	2.31 (165) [13/13]	2.30 (163) [14/15]	2.24 (167) [14/14]
5	2.94 (204) [12/12]	2.85 (204) [13/13]	2.71 (192) [15/15]	2.66 (199) [14/14]
6	3.49 (242) [11/12]	3.30 (236) [13/13]	3.15 (223) [15/15]	3.13 (234) [14/14]
7	4.02 (279) [12/12]	3.79 (271) [13/13]	3.78 (268) [15/15]	3.61 (269) [14/14]
8	4.51 (313) [12/12]	4.25 (304) [13/13]	3.57 (253) [13/15]	4.02 (300) [12/14]
14	6.98 (485) [12/12]	6.71 (479) [13/13]	6.94 (492) [15/15]	6.39 (477) [14/14]
AZT/3TC		**80/40 mg/kg**	**160/80 mg/kg**	**240/120 mg/kg**
1		1.45 [14/14]	1.45 [15/15]	1.40 [14/15]
2		1.64 (113) [13/13]	1.58 (109) [15/15]	1.53 (109) [15/15]
3		1.95 (134) [13/13]	1.90 (131) [15/15]	1.83 (131) [15/15]
4		2.34 (161) [13/13]	2.29 (158) [15/15]	2.17 (155) [15/15]
5		2.81 (194) [13/13]	2.79 (192) [15/15]	2.61 (186) [15/15]
6		3.30 (228) [13/13]	3.34 (230) [15/15]	3.03 (216) [15/15]
7		3.81 (263) [13/13]	3.80 (262) [15/15]	3.55 (254) [15/15]
8		4.46 (308) [13/13]	4.31 (297) [15/15]	4.01 (286) [15/15]
14		6.77 (467) [13/13]	6.66 (459) [15/15]	6.47 (462) [15/15]
AZT/3TC/NVP		**80/40/56 mg/kg**	**160/80/112 mg/kg**	**240/120/168 mg/kg**
1		1.39 [17/17]	1.45 [16/16]	1.21[b] [15/15]
2		1.56 (112) [17/17]	1.50 (103) [15/16]	1.30[b] (107) [15/15]
3		1.82 (131) [16/17]	1.74 (120) [16/16]	1.54[b] (127) [14/14]
4		2.20 (158) [16/16]	2.12 (146) [16/16]	1.61[b] (133) [13/13]
5		2.62 (188) [16/16]	2.54 (175) [16/16]	1.85[b] (153) [13/13]
6		3.10 (223) [16/16]	2.97[b] (205) [16/16]	2.17[b] (179) [12/12]
7		3.59 (258) [16/16]	3.43[b] (237) [16/16]	2.62[b] (217) [12/12]
8		4.04 (291) [16/16]	3.90[b] (269) [16/16]	3.04[b] (251) [11/12]
14		6.36[b] (458) [16/16]	6.34[b] (437) [16/16]	5.57[b] (460) [12/12]
AZT/3TC/NFV		**80/40/336 mg/kg**	**160/80/672 mg/kg**	**240/120/1,008 mg/kg**
1		1.34 [13/13]	1.30[b] [10/10]	1.12[b] [14/14]
2		1.45 (108) [12/12]	1.48 (114) [8/8]	1.20[b] (107) [9/9]
3		1.73 (129) [12/12]	1.80 (138) [8/8]	1.34[b] (120) [7/7]
4		2.07 (154) [11/12]	2.16 (166) [8/8]	1.60[b] (143) [6/6]
5		2.51 (187) [12/12]	2.65 (204) [8/8]	1.82[b] (163) [6/6]
6		2.99 (223) [12/12]	3.12 (240) [8/8]	2.11[b] (188) [6/6]
7		3.43[b] (256) [12/12]	3.58 (275) [8/8]	2.49[b] (222) [6/6]
8		3.96 (296) [12/12]	4.09 (315) [8/8]	2.94[b] (263) [5/6]
14		6.44 (481) [12/12]	6.40 (492) [8/8]	5.75[b] (513) [6/6]

[a] Female C57Bl/6N mice were administered AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV by gavage on gestational days 12 to 18. Body weights of transplacentally exposed pups (by litter) were obtained on postnatal days 1 through 8 and 14 and are given in grams with the percentage change from postnatal day 1 given in parentheses and the number of litters weighed/total number of litters given in brackets.

[b] Significantly different ($P \leq 0.05$) from the control group.

TABLE 6
Survival From Birth Until Weaning of Mice Transplacentally Exposed to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV[a]

Treatment	Dose (mg/kg)	% Survival[b]	
		Male	Female
Control	0	100 (67)[12/12]	100 (75)[12/12]
AZT	80	94 (78) [14/14]	96 (56) [14/13]
	160	88 (69) [17/15]	88 (80) [17/15]
	240	98 (56) [14/14]	98 (54) [14/14]
AZT/3TC	80/40	87 (68) [14/13]	89 (62) [14/13]
	160/80	97 (66) [15/15]	100 (73)[15/15]
	240/120	100 (54)[15/15]	97 (58) [15/15]
AZT/3TC/NVP	80/40/56	96 (74) [17/17]	97 (74) [17/16]
	160/80/112	95 (61) [16/16]	98 (62) [16/16]
	240/120/168	76 (66) [15/12]	79 (70) [15/12]
AZT/3TC/NFV	80/40/336	88 (72) [14/13]	87 (62) [13/12]
	160/80/672	92 (61) [10/8]	83 (66) [10/8]
	240/120/1,008	33 (45) [14/6]	51 (51) [14/6]

[a] Female C57Bl/6N mice were administered AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV by gavage on gestational days 12 to 18.
[b] Percentage of pups alive at weaning on postnatal day 21, with the number of pups at postnatal day 1 given in parentheses and the number of litters at postnatal day 1/number of litters at postnatal day 21 given in brackets.

BODY WEIGHT CHANGES

After weaning, body weights of the mice exposed transplacentally to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV were recorded weekly until the end of the study, but only data for weeks 6 through 78 were considered for statistical evaluations, since after week 78 the mice began to lose weight rapidly and die.

Transplacental exposure to AZT (Figure 5A) or the combination of AZT/3TC (Figure 5C) caused only minor effects on the body weights of female mice. The average body weights for each of the exposed groups were greater than or equal to 96% of those of the control group. Exposure to the combination of AZT/3TC/NVP (Figure 5E) or the combination of AZT/3TC/NFV (Figure 5G) resulted in dose-related decreases in body weights in female mice. In female mice treated with the combination containing NVP, the high-dose group body weight was significantly less than that of the control group at all time points with the average decrease being 18% (Figure 5E); the low- and mid-dose combinations were significantly less than the control group at most time points with the average decreases being 8% and 5%, respectively. In female mice exposed to the combination containing NFV, the high-dose group was significantly less than the control group at all time points with the average decrease being 13% (Figure 5G); the low- and mid-dose groups were significantly less than the control group at most time points with the average decreases being 5% and 6%, respectively.

Male mice exposed transplacentally to AZT showed dose-related decreases in body weight (Figure 5B), with the decrease being significant in all exposed groups at all time points. Compared to the control group, the average decrease in body weight was 9% in the high-dose group, 6% in the mid-dose group, and 5% in the low-dose group. Transplacental exposure to the combination of AZT/3TC caused dose-related decreases in body weight in male mice (Figure 5D), with the decreases being significant at all time points in the high- and mid-dose groups, and at nearly all time points in the low-dose group. The average decrease in body weight was 7% in the high-dose group, 5% in the mid-dose group, and 3% in the low-dose group. Male mice exposed transplacentally to the combination of AZT/3TC/NVP (Figure 5F) or the combination of AZT/3TC/NFV (Figure 5H) showed dose-related decreases in body weight, with the differences being significant in all exposed groups at all time points. For

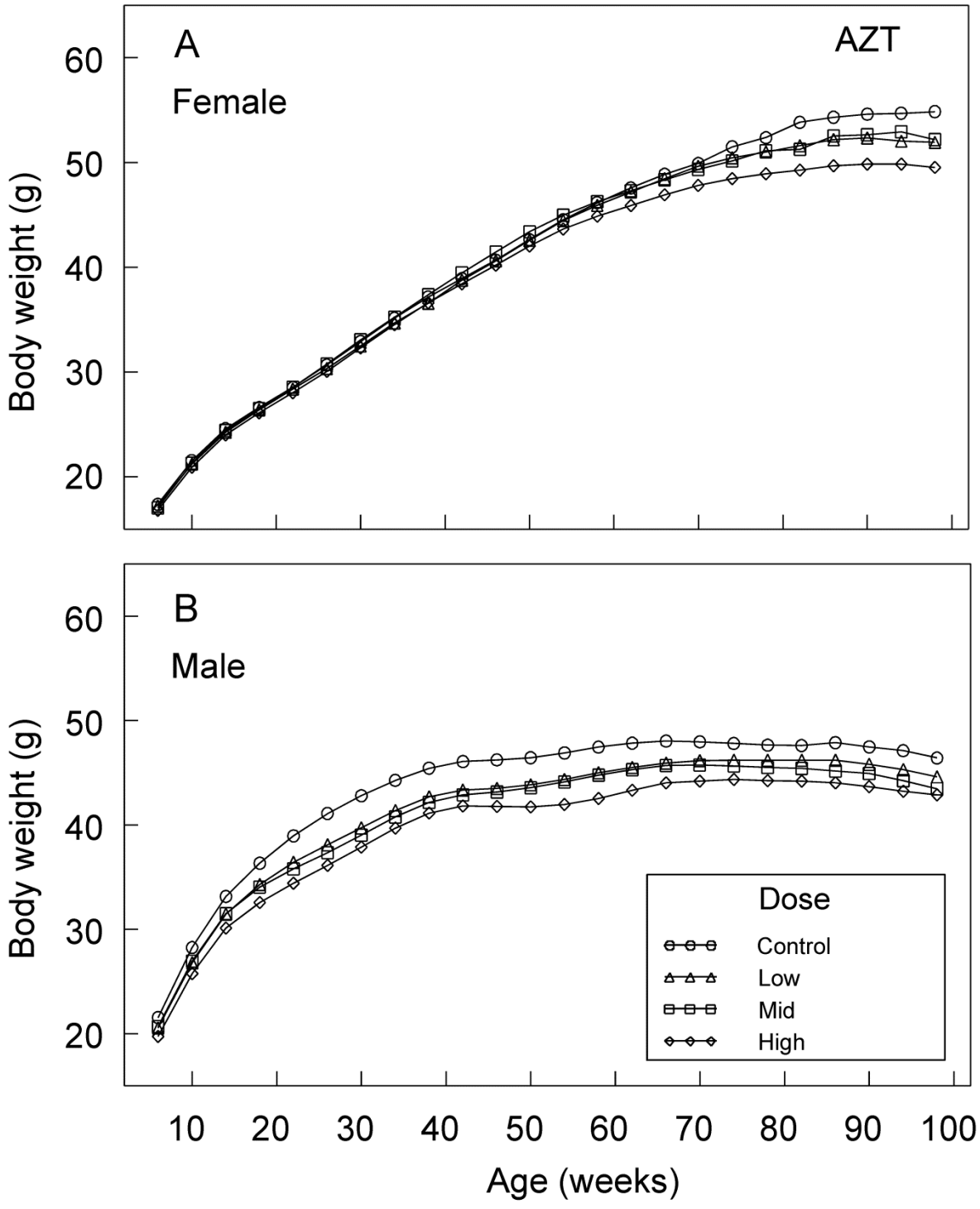


FIGURE 5 (A and B)
Growth Curves for B6C3F1 Mice Transplacentally Exposed to Antiretroviral Drugs

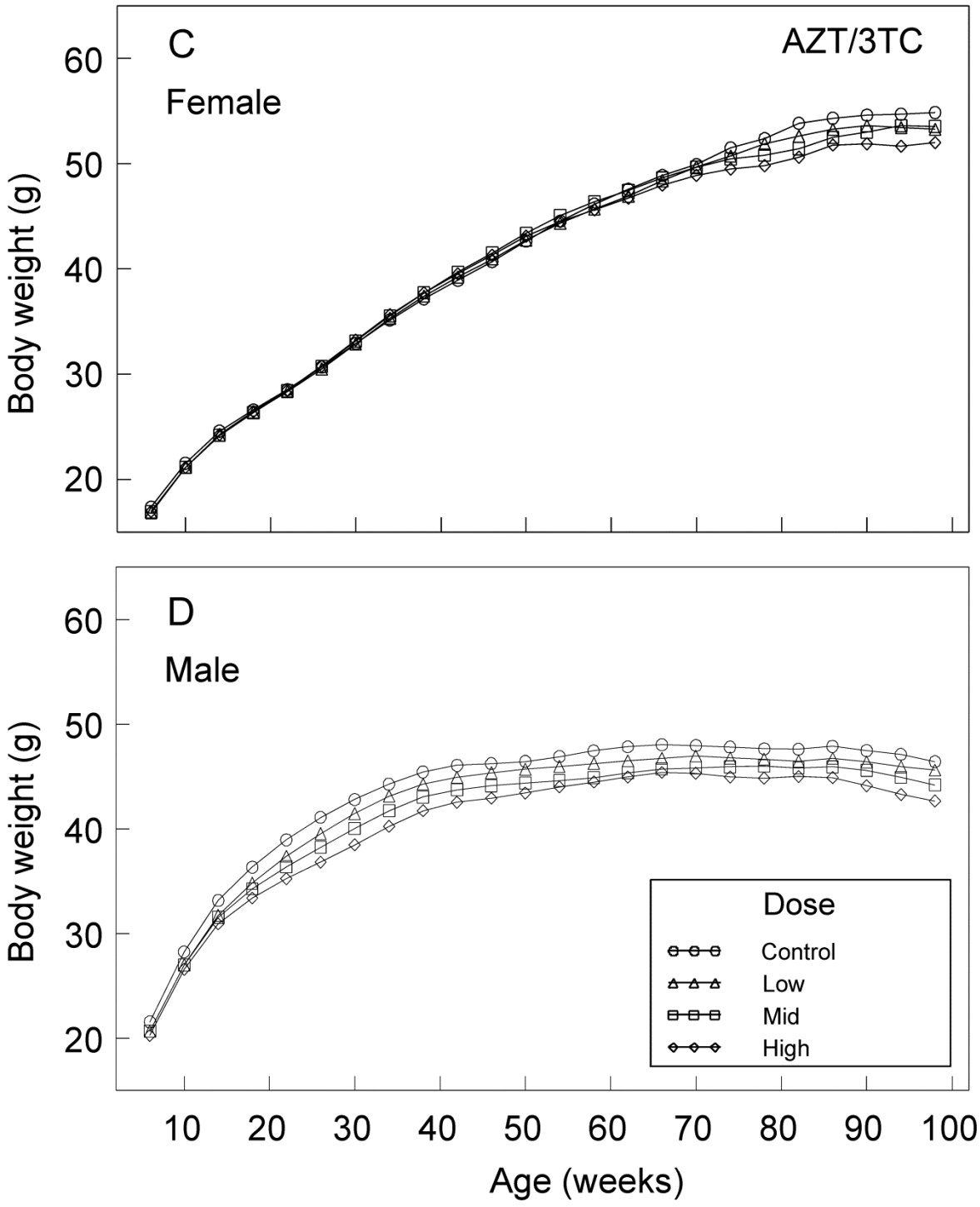


FIGURE 5 (C and D)
Growth Curves for B6C3F1 Mice Transplacentally Exposed to Antiretroviral Drugs

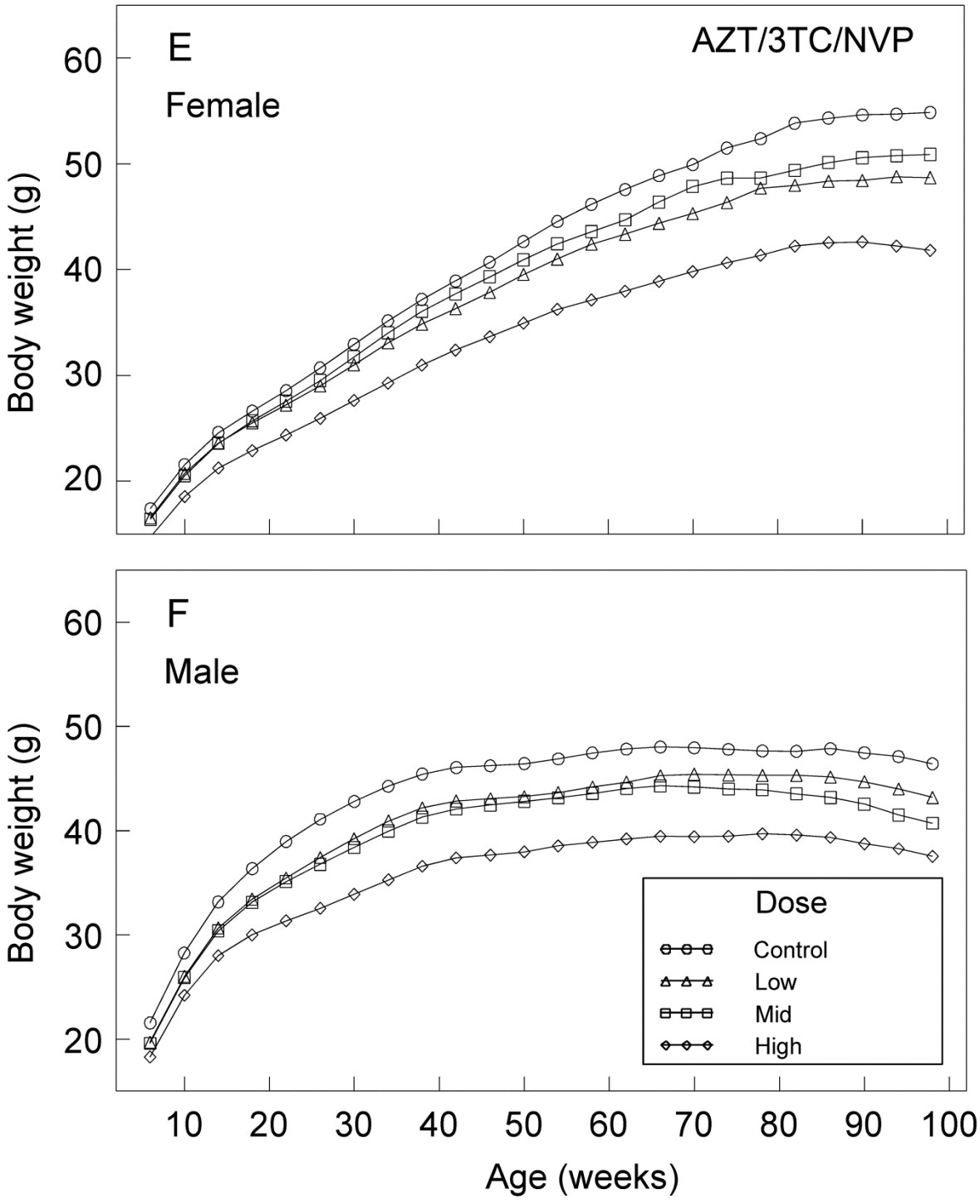


FIGURE 5 (E and F)
Growth Curves for B6C3F1 Mice Transplacentally Exposed to Antiretroviral Drugs

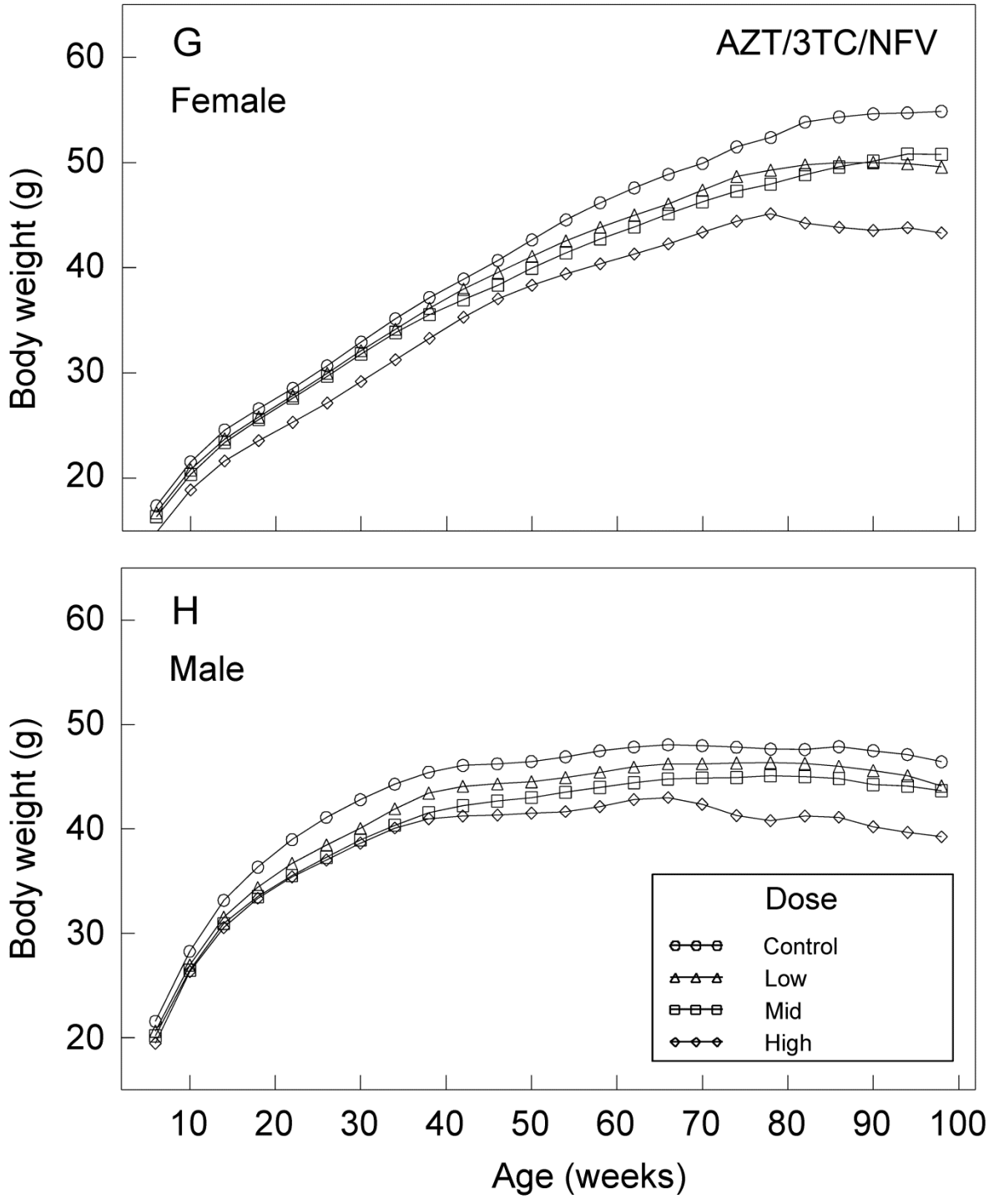


FIGURE 5 (G and H)
Growth Curves for B6C3F1 Mice Transplacentally Exposed to Antiretroviral Drugs

the AZT/3TC/NVP combination, the average decrease in body weight was 18% in the high-dose group, 9% in the mid-dose group, and 7% in the low-dse group. For the AZT/3TC/NFV combination, the average decrease in body weight was 11% in the high-dose group, 7% in the mid-dose group, and 4% in the low-dose group. With the exception of male and female of mice treated with the high-dose combination of AZT/3TC/NVP, all changes in body weight were considered to have little biological importance.

SURVIVAL

The effect of transplacental exposure to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV upon the survival of the mice at 2 years is presented in this section (Figure 6).

Transplacental exposure to AZT (Figure 6A), AZT/3TC (Figure 6C), AZT/3TC/NVP (Figure 6E), or AZT/3TC/NFV (Figure 6G) had no effect upon the survival of female mice compared to control female mice. Transplacental exposure to AZT (Figure 6B) or AZT/3TC (Figure 6D) had no effect upon the survival of male mice compared to control male mice, whereas exposure to AZT/3TC/NVP (Figure 6F) or AZT/3TC/NFV (Figure 6H) caused a dose-related decrease in survival of males, with the difference being significant in the high-dose group of each combination. The major cause of death in male mice exposed to AZT/3TC/NVP was liver hepatocellular adenoma or carcinoma, or fibrosarcoma or fibrous histiocytoma of the skin. The major cause of death in male mice exposed to AZT/3TC/NFV was liver hepatocellular carcinoma or fibrosarcoma of the skin.

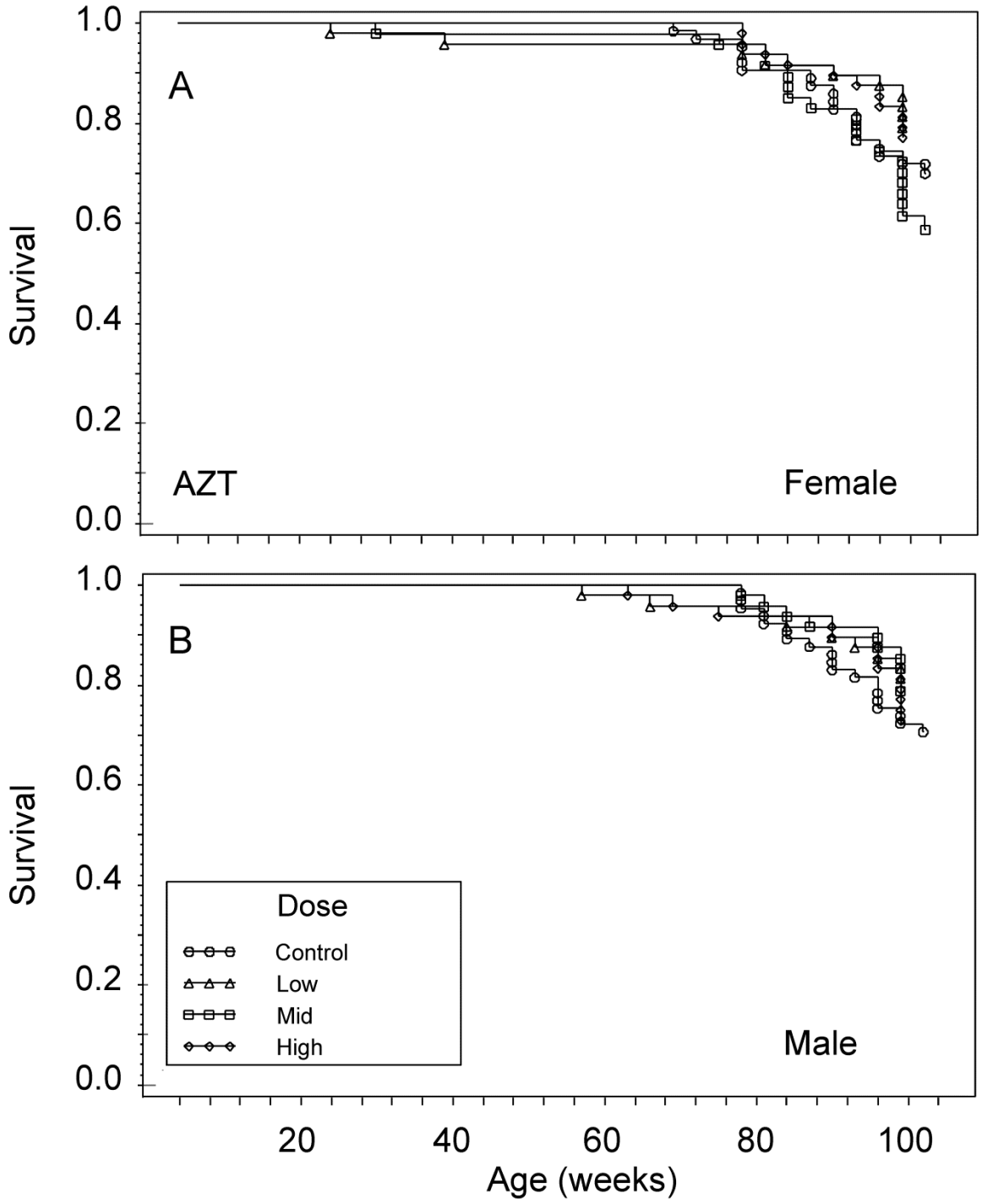


FIGURE 6 (A and B)
Survival Curves for B6C3F1 Mice Transplacentally Exposed to Combinations of Antiretroviral Drugs

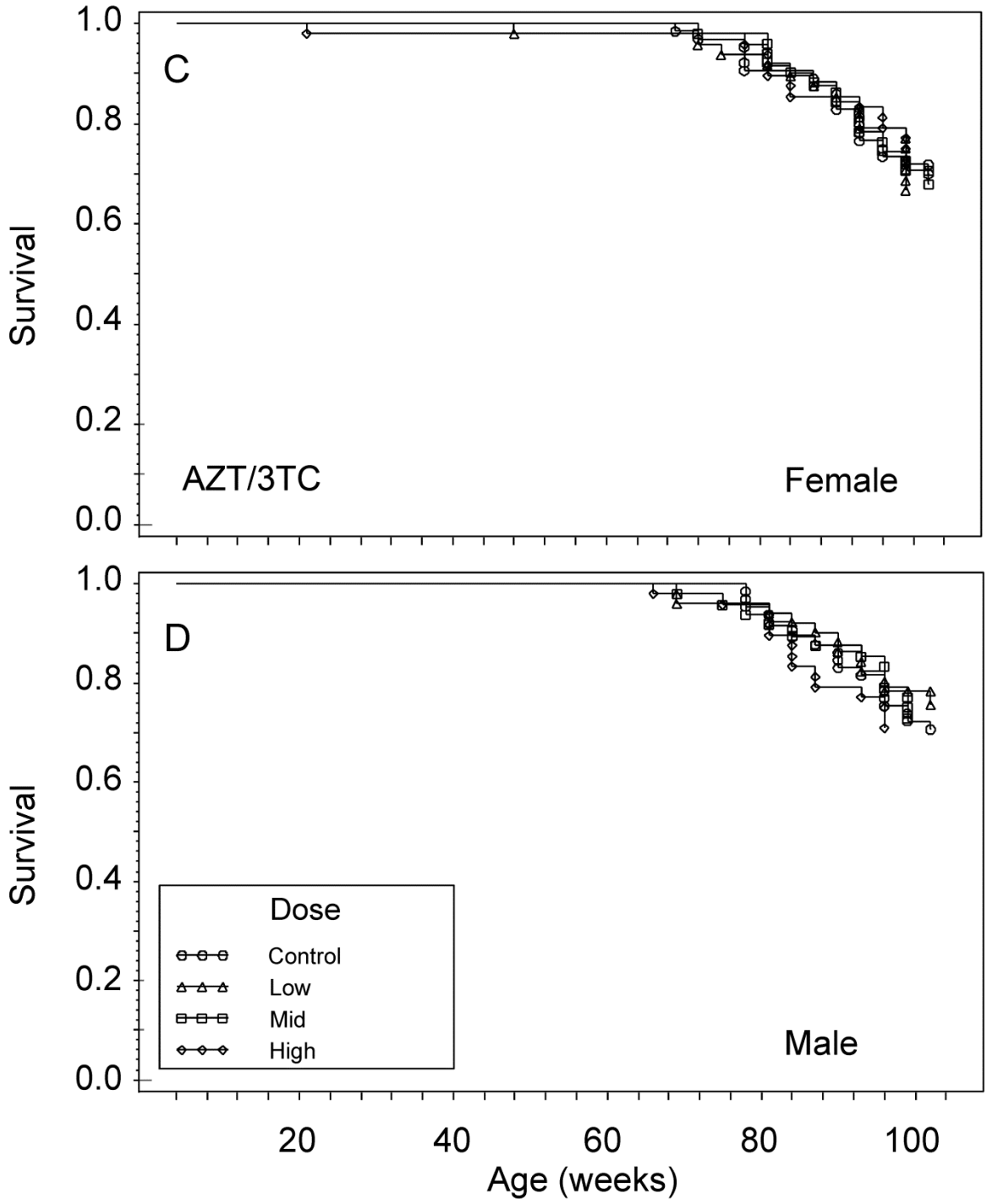


FIGURE 6 (C and D)
Survival Curves for B6C3F1 Mice Transplacentally Exposed to Combinations of Antiretroviral Drugs

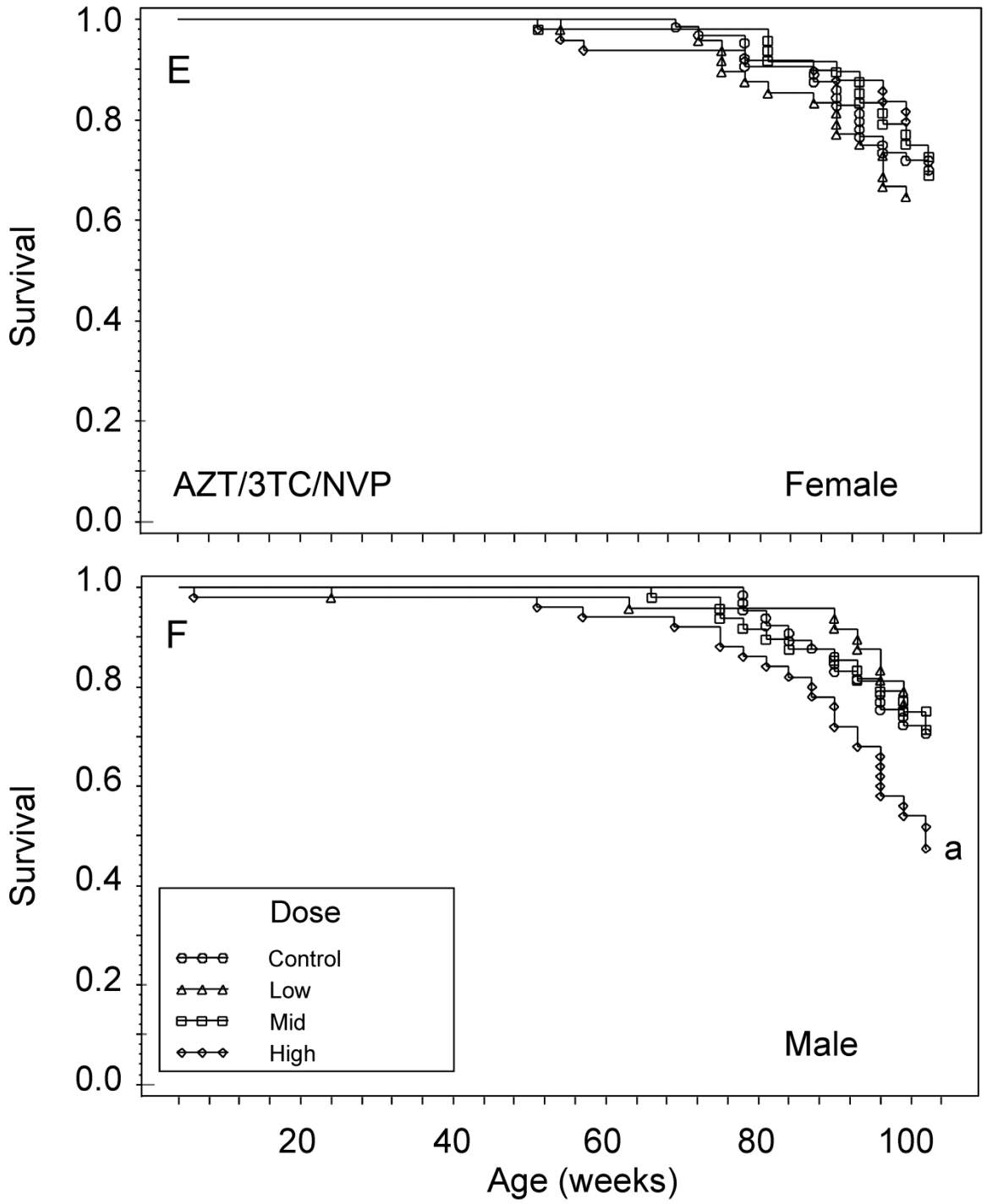


FIGURE 6 (E and F)
Survival Curves for B6C3F1 Mice Transplacentally Exposed to Combinations of Antiretroviral Drugs
a=Significantly different (P≤0.05) from the control group

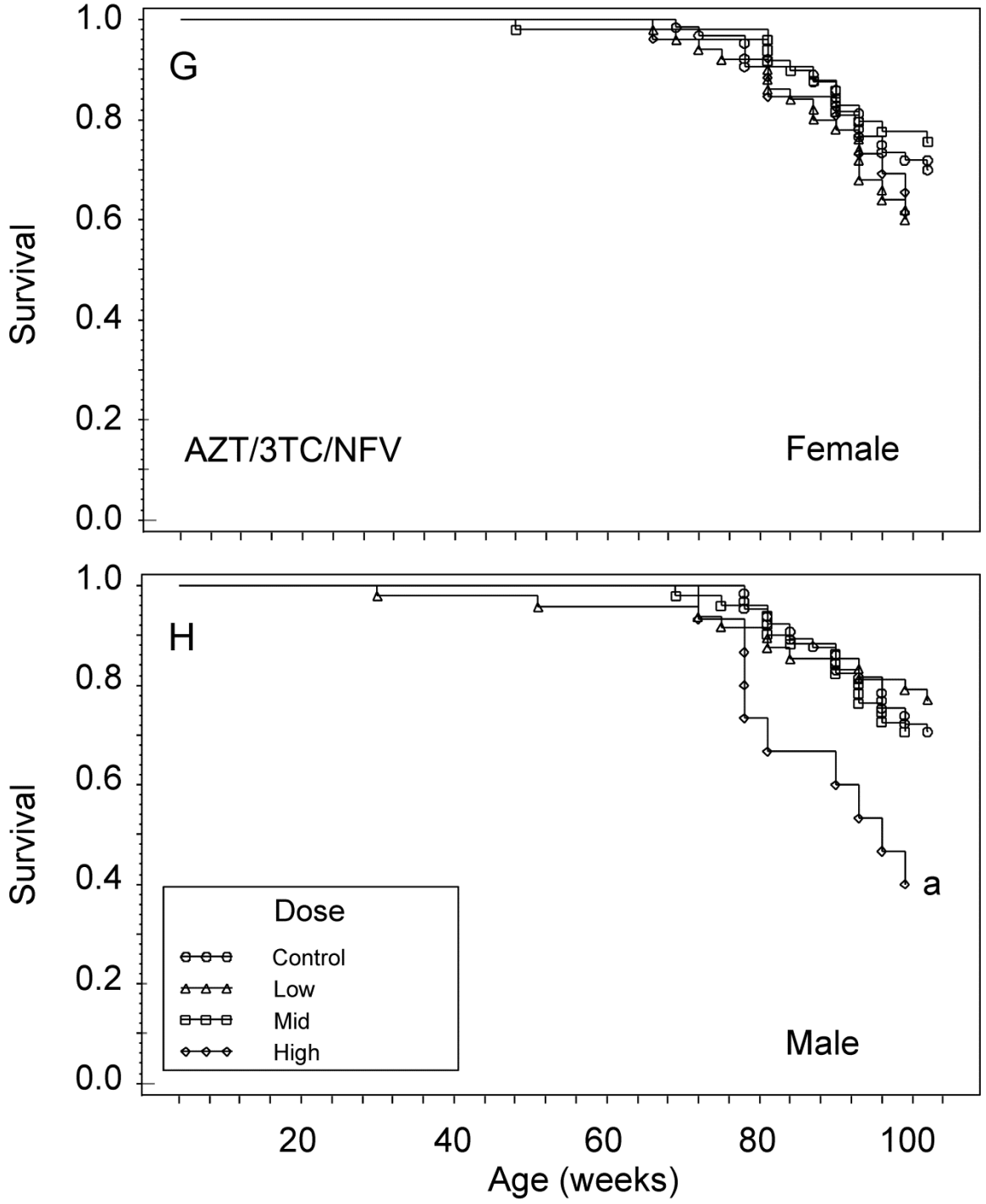


FIGURE 6 (G and H)
Survival Curves for B6C3F1 Mice Transplacentally Exposed to Combinations of Antiretroviral Drugs
a=Significantly different ($P \leq 0.05$) from the control group

NEOPLASTIC CHANGES

The effect of transplacental exposure to AZT, AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV upon the induction of neoplasms is presented in this section and in Tables A1 and A2 for male mice and B1 and B2 for female mice. Historical incidences for the neoplasms mentioned in this section are presented in Tables A3 and B3 for male and female mice, respectively.

AZT

Dose-related positive trends were seen in the incidences of follicular cell adenoma of the thyroid gland, follicular cell adenoma or carcinoma (combined) of the thyroid gland, and subcutaneous fibrosarcoma or sarcoma (combined) of the skin in female mice exposed transplacentally to AZT (Tables 7, B1a, and B2a). Compared to the control group, the incidences of follicular cell adenoma of the thyroid gland (after adjusting for possible dam or sire effects) and follicular cell adenoma or carcinoma (combined) of the thyroid gland were significantly increased in female mice exposed to 240 mg AZT/kg body weight per day.

There were no dose-related positive trends in the incidences of neoplasms in male mice exposed transplacentally to AZT (Table A2a).

AZT and 3TC

A dose-related positive trend in the incidences of alveolar/bronchiolar adenoma of the lung was seen in female mice transplacentally exposed to mixtures of AZT/3TC (Tables 8, B1b, and B2b).

TABLE 7
Incidences of Neoplasms in Female B6C3F1 Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Thyroid Gland (Follicular Cell): Adenoma[a]				
Number of litters	20	19	20	14
Overall rate[b]	0/59 (0.0%)	1/46 (2.2%)	0/46 (0.0%)	3/47 (6.4%)
Adjusted rate[c]	0.0%	2.3%	0.0%	6.8%
Terminal rate[d]	0/45 (0.0%)	1/38 (2.6%)	0/27 (0.0%)	3/37 (8.1%)
First incidence (days)	—[e]	733 (T)	—	734 (T)
Poly-3 test[f]	P=0.041	P=0.455	—[g]	P=0.083
Dam-adjusted Poly-3 test	P=0.044	P=0.146	—	P=0.025
Sire-adjusted Poly-3 test	P=0.044	P=0.148	—	P=0.025
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma[a]				
Number of litters	20	19	20	14
Overall rate	0/59 (0.0%)	1/46 (2.2%)	0/46 (0.0%)	4/47 (8.5%)
Adjusted rate	0.0%	2.3%	0.0%	9.1%
Terminal rate	0/45 (0.0%)	1/38 (2.6%)	0/27 (0.0%)	4/37 (10.8%)
First incidence (days)	—	733 (T)	—	734 (T)
Poly-3 test	P=0.013	P=0.455	—	P=0.036
Dam-adjusted Poly-3 test	P=0.015	P=0.147	—	P=0.008
Sire-adjusted Poly-3 test	P=0.015	P=0.148	—	P=0.008
Skin (Subcutaneous Tissue): Fibrosarcoma				
Number of litters	20	19	20	14
Overall rate	1/63 (1.6%)	0/46 (0.0%)	2/47 (4.3%)	3/48 (6.3%)
Adjusted rate	1.8%	0.0%	4.8%	6.6%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	0/28 (0.0%)	0/37 (0.0%)
First incidence (days)	739 (T)	—	633	663
Poly-3 test	P=0.070	P=0.553N	0.393	P=0.228
Dam-adjusted Poly-3 test	P=0.054	P=0.144N	0.207	P=0.105
Sire-adjusted Poly-3 test	P=0.059	P=0.134N	0.205	P=0.118
Skin (Subcutaneous Tissue): Sarcoma				
Number of litters	20	19	20	14
Overall rate	2/63 (3.2%)	0/46 (0.0%)	2/47 (4.3%)	3/48 (6.3%)
Adjusted rate	3.5%	0.0%	4.8%	6.6%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	735	—	707	598
Poly-3 test	P=0.184	P=0.298N	0.574	P=0.400
Dam-adjusted Poly-3 test	P=0.181	P=0.059N	0.361	P=0.269
Sire-adjusted Poly-3 test	P=0.173	P=0.055N	0.361	P=0.260
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma[h]				
Number of litters	20	19	20	14
Overall rate	2/63 (3.2%)	0/46 (0.0%)	4/47 (8.5%)	5/48 (10.4%)
Adjusted rate	3.5%	0.0%	9.5%	10.9%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	735	—	633	598
Poly-3 test	P=0.028	P=0.298N	0.207	P=0.138
Dam-adjusted Poly-3 test	P=0.029	P=0.058N	0.091	P=0.088
Sire-adjusted Poly-3 test	P=0.032	P=0.057N	0.084	P=0.097

(T) Terminal sacrifice

[a] Historical incidence for control groups in 2-year NCTR studies (mean): 10/643 (1.6%), range 0.0%-2.8%

[b] Number of animals with neoplasm per number of animals with tissue examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Not applicable; no neoplasms in animal group

[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

[g] Value of statistic cannot be computed.

[h] Historical incidence for skin mesenchymal tumors (fibrous histoma, fibrosarcoma, sarcoma, or myxosarcoma) in control groups in 2-year NCTR studies (mean): 8/651 (1.6%), range 0.0%-8.3%

TABLE 8
Incidences of Alveolar/bronchiolar Adenoma in Female B6C3F1 Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Alveolar/bronchiolar Adenoma[a]				
Number of litters	20	18	19	15
Overall rate[b]	2/62 (3.2%)	1/48 (2.1%)	3/50 (6.0%)	6/48 (12.5%)
Adjusted rate[c]	3.5%	2.3%	6.5%	13.7%
Terminal rate[d]	2/45 (4.4%)	0/32 (0.0%)	1/35 (2.9%)	5/35 (14.3%)
First incidence (days)	737 (T)	608	587	585
Poly-3 test[e]	P=0.022	P=0.592N	P=0.405	P=0.065
Dam-adjusted Poly-3 test	P=0.082	P=0.327N	P=0.254	P=0.108
Sire-adjusted Poly-3 test	P=0.076	P=0.366N	P=0.216	P=0.104

(T) Terminal sacrifice
[a] Historical incidence for control groups in 2-year NCTR studies (mean): 33/658 (5.0%), range 2.1%-8.3%
[b] Number of animals with neoplasm per number of animals with lung examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

AZT, 3TC, and NVP

Transplacental exposure to mixtures of AZT/3TC/NVP caused dose-related positive trends in the incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma (combined) of the skin; and subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma (combined) of the skin in male mice (Tables 9, A1c, and A2c). The incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma of the skin (combined); and subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma of the skin (combined) were significantly increased in the group exposed transplacentally to 240 mg AZT, 120 mg 3TC, and 168 mg NVP/kg body weight per day compared to the control group.

The incidences of subcutaneous fibrosarcoma of the skin; subcutaneous fibrous histiocytoma or fibrosarcoma of the skin (combined); and of subcutaneous fibroma, fibrous histiocytoma, or fibrosarcoma of the skin (combined) were significantly increased in the group transplacentally exposed to 160 mg AZT, 80 mg 3TC, and 112 mg NVP/kg body weight per day compared to the control group and after adjusting for possible dam or sire effects.

Female mice exposed transplacentally to 160 mg AZT, 80 mg 3TC, and 112 mg NVP/kg body weight per day had an increased incidence of skin fibrosarcoma (Tables 9, B1c, and B2c).

TABLE 9
Incidences of Neoplasms of the Skin (Subcutaneous Tissue) in B6C3F1 Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Male				
Number examined microscopically	65	47	48	48
Fibroma[a]	0	1	0	2
Fibrous Histiocytoma	0	0	1	2
Fibrosarcoma, Multiple	0	0	1	1
Fibrosarcoma (includes multiple)				
Number of litters	20	20	17	18
Overall rate[b]	2/65 (3.1%)	1/47 (2.1%)	6/48 (12.5%)	8/48 (16.7%)
Adjusted rate[c]	3.4%	2.3%	13.1%	18.9%
Terminal rate[d]	2/46 (4.3%)	0/37 (0.0%)	1/35 (2.9%)	0/25 (0.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test[e]	P=0.002	P=0.601N	P=0.066	P=0.011
Dam-adjusted Poly-3 test	P<0.001	P=0.366N	P=0.039	P=0.004
Sire-adjusted Poly-3 test	P=0.001	P=0.362N	P=0.041	P=0.004
Fibrous Histiocytoma or Fibrosarcoma				
Number of litters	20	20	17	18
Overall rate	2/65 (3.1%)	1/47 (2.1%)	7/48 (14.6%)	10/48 (20.8%)
Adjusted rate	3.4%	2.3%	15.3%	23.5%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	1/35 (2.9%)	0/25 (0.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test	P<0.001	P=0.601N	P=0.033	P=0.002
Dam-adjusted Poly-3 test	P<0.001	P=0.361N	P=0.030	P=0.001
Sire-adjusted Poly-3 test	P<0.001	P=0.363N	P=0.030	P=0.001
Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Number of litters	20	20	17	18
Overall rate	2/65 (3.1%)	2/47 (4.3%)	7/48 (14.6%)	12/48 (25.0%)
Adjusted rate	3.4%	4.5%	15.3%	28.2%
Terminal rate	2/46 (4.3%)	1/37 (2.7%)	1/35 (2.9%)	2/25 (8.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test	P<0.001	P=0.585	P=0.033	P<0.001
Dam-adjusted Poly-3 test	P<0.001	P=0.380	P=0.029	P<0.001
Sire-adjusted Poly-3 test	P<0.001	P=0.379	P=0.029	P<0.001
Female				
Skin (Subcutaneous Tissue): Fibrosarcoma[f]				
Number of litters	20	20	17	18
Overall rate	1/63 (1.6%)	0/47 (0.0%)	7/47 (14.9%)	0/49 (0.0%)
Adjusted rate	1.8%	0.0%	15.8%	0.0%
Terminal rate	1/45 (2.2%)	0/31 (0.0%)	2/34 (5.9%)	0/39 (0.0%)
First incidence (days)	739 (T)	—	595	—
Poly-3 test	P=0.228	P=0.565N	0.011	P=0.549N
Dam-adjusted Poly-3 test	P=0.079	P=0.145N	0.007	P=0.145N
Sire-adjusted Poly-3 test	P=0.065	P=0.146N	0.006	P=0.145N

(T) Terminal sacrifice

[a] Number of animals with neoplasm

[b] Number of animals with neoplasm per number of animals with skin examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

[f] Historical incidence for skin mesenchymal tumors (fibrous histoma, fibrosarcoma, sarcoma, or myxosarcoma) in control groups in 2-year NCTR studies (mean): 8/651 (1.6%), range 0.0%-8.3%

AZT, 3TC, and NFV

A dose-related positive trend (P=0.048) was seen in the incidences of Harderian gland adenoma in male mice exposed transplacentally to mixtures of AZT/3TC/NFV (Tables 10, A1d, and A2d); however, in none of the dosed groups was the incidence significantly greater than in the control.

There were no dose-related positive trends in the incidences of neoplasms in female mice exposed transplacentally to mixtures of AZT/3TC/NFV (Table B2d).

NONNEOPLASTIC CHANGES

A dose-related positive trend (P=0.013) in the incidences of liver basophilic foci (severity not indicated) occurred in female mice exposed to mixtures of AZT/3TC, with the increase being significant (P=0.034) in the 240/120 mg/kg group compared to the control group (Table B4b).

Dose-related positive trends in the incidences of liver basophilic foci (P=0.021; severity not indicated) and pituitary gland (pars distalis) hyperplasia (P=0.037; minimal to moderate severity) were observed in female mice exposed transplacentally to mixtures of AZT/3TC/NVP, with the increases being significant (P=0.036 and P=0.028, respectively) in the 240/120/168 mg/kg group compared to the control group (Table B4c).

There were no dose-related positive trends in the incidences of nonneoplastic lesions in female mice exposed transplacentally to AZT (Table B4a) or mixtures of AZT/3TC/NFV (Table B4d).

Dose-related positive trends (P=0.020 and P=0.035, respectively) in the incidences of liver necrosis (minimal to marked severity) occurred in male mice transplacentally exposed to AZT and mixtures of AZT/3TC, with the increase being significant (P=0.028) in the high-dose (240 mg/kg) AZT group compared to the control group (Tables A4a and A4b). Mixtures of AZT/3TC also resulted in a dose-related positive trend (P<0.001) in the incidences of pituitary gland (pars distalis) cyst (minimal to mild severity), with the increase being significant (P=0.002) in the 240/120 mg/kg group compared to the control group.

Dose-related positive trends in the incidences of skin ulceration (P<0.001; mild to marked severity) and inflammation (P=0.040; mild to moderate severity) were observed in male mice transplacentally exposed to AZT/3TC/NVP with the increase in skin ulceration being significant (P=0.010) in the 240/120/168 mg/kg group compared to the control group (Table A4c).

TABLE 10
Incidences of Harderian Gland Adenoma in Male B6C3F1 Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Adenoma[a]				
Number of litters	20	20	20	21
Overall rate[b]	5/64 (7.8%)	2/45 (4.4%)	7/50 (14.0%)	3/14 (21.4%)
Adjusted rate[c]	8.5%	4.8%	15.5%	27.5%
Terminal rate[d]	4/46 (8.7%)	1/37 (2.7%)	5/36 (13.9%)	2/6 (33.3%)
First incidence (days)	643	609	694	663
Poly-3 test[e]	P=0.048	P=0.374N	P=0.213	P=0.108
Dam adjusted Poly-3 test	P=0.072	P=0.202N	P=0.150	P=0.105
Sire adjusted Poly-3 test	P=0.075	P=0.221N	P=0.154	P=0.107

[a] Historical incidence for control groups in 2-year NCTR studies (mean): 28/372 (7.5%), range 2.2%-10.6%.
[b] Number of animals with neoplasm per number of animals with Harderian gland examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.

A dose-related positive trend (P=0.011) was seen in the incidences of spleen hematopoietic cell proliferation (mild to marked severity) in male mice exposed to mixtures of AZT/3TC/NFV, with the increase being significant (P=0.003) in the 240/120/1,008 mg/kg group compared to the control group (Table A4d).

GENETIC TOXICOLOGY

The same lots of AZT, 3TC, NVP, and NFV that were used in the 2-year animal studies were tested for bacterial mutagenicity in *Salmonella typhimurium* and *Escherichia coli* (Tables C1 through C4). The highest concentrations tested with AZT and NFV were limited by toxicity. 3TC and NVP showed no evidence of toxicity and were therefore tested up to 6,000 µg/plate, the limit concentration established by the assay protocol.

Of the four compounds tested, only AZT (0.03 to 6.0 µg/plate) was found to be mutagenic; significant increases in revertant colonies were seen in the *E. coli* strain WP2 *uvrA*/pKM101, with and without induced rat liver metabolic activation enzymes (S9), suggesting that the observed mutagenic activity did not require metabolic transformation of the parent compound. The highest number of mutant colonies was seen at AZT concentrations of 0.25 to 0.5 µg/plate, with and without S9. AZT was not mutagenic in *S. typhimurium* strains TA98 or TA100.

DISCUSSION AND CONCLUSIONS

In this study, male and female B6C3F1 mice were exposed transplacentally to AZT or mixtures of AZT/3TC, AZT/3TC/NVP, or AZT/3TC/NFV. In female B6C3F1 mice treated with AZT, there were positive trends in the incidences of thyroid gland follicular cell adenoma or carcinoma (primarily adenoma) and subcutaneous skin fibrosarcoma or sarcoma; in female B6C3F1 mice exposed to mixtures of AZT/3TC, there was a positive trend in the incidences of alveolar/bronchiolar adenoma; in male B6C3F1 mice treated with mixtures of AZT/3TC/NVP, there was a positive trend in the incidences of subcutaneous skin neoplasms (fibroma, fibrous histiocytoma, or fibrosarcoma); and in male B6C3F1 mice exposed to mixtures of AZT/3TC/NFV, there was a positive trend in the incidences of Harderian gland adenoma. With each of the treatments, the increase in tumor incidence was modest and only reached statistical significance with the mid- and high-dose combinations of AZT/3TC/NVP.

AZT

This bioassay was modeled after the study of Walker *et al*. (2007) in which female C57Bl/6 mice were treated daily on gestation days 12 to 18 with 0, 80, 240, or 480 mg AZT/kg body weight. When assessed 2 years after birth, the male B6C3F1 offspring in the Walker *et al*. (2007) bioassay had a dose-related increase in the incidences of hemangioma or hemangiosarcoma (primarily hemangiosarcoma) in all organs, with the incidences being significantly increased at each dose level of AZT compared to the controls. The male mice also had a dose-related increase in the incidences of hepatocellular carcinoma, with the incidence being significantly increased in the 480 mg AZT/kg body weight group. Using a similar treatment model, we demonstrated that male and female B6C3F1 mice exposed transplacentally to AZT had dose-related increases in micronucleated reticulocytes and micronucleated normochromatic erythrocytes and that male B6C3F1/*Tk*$^{+/-}$ mice exposed transplacentally to AZT had an increased mutant frequency in the *Tk* gene of spleen T-lymphocytes that was associated with a loss of heterozygosity (Von Tungeln *et al*., 2007). These data suggest that transplacental exposure of B6C3F1 mice to AZT can result in the activation of AZT to a genotoxic metabolite (e.g., AZT 5′-triphosphate), with a resultant increase in neoplasia.

In the Walker *et al*. (2007) bioassay, male and female B6C3F1 mice exposed transplacentally to AZT showed dose-related decreases in body weight, with the decreases being statistically significant at all time points in the 480 mg AZT/kg body weight per day group, and at later time points in male mice that had been exposed to 240 mg AZT/kg body weight per day. In the current study, significant decreases in body weight were observed in male, but not female, mice treated with AZT; nonetheless, the magnitude of body weight changes was almost identical to that observed in the Walker *et al*. (2007) study, with male and female mice exposed to 240 mg AZT/kg body weight per day showing decreases of approximately 9% and 4%, respectively, compared to the control groups.

In the current bioassay, the incidences of hemangiosarcoma in all organs were 13.8%, 4.2%, 8.3%, and 8.3% in male B6C3F1 mice whose dams had been exposed to 0, 80, 160, or 240 mg AZT per kg body weight per day, respectively (Table A2a). With the exception of the control group, these values are similar to those observed by Walker *et al*. (2007), who reported incidences of hemangioma or hemangiosarcoma (primarily hemangiosarcoma) in all organs of 0%, 15.6%, 9.1%, and 13.3% in male B6C3F1 mice whose dams had been treated with 0, 80, 240, or 480 mg AZT per kg body weight per day. In experiments conducted at the NCTR, the incidence of spontaneous hemangiosarcoma in all organs in male B6C3F1 mice has been 2.1% (range 0.0% to 8.3%; Table A3). In the current bioassay, the incidence of spontaneous hemangiosarcoma in the control group exceeded the historical range; nonetheless, based upon the fact that the incidences of hemangiosarcoma in all the groups exposed to AZT were within the historical control range, there was no evidence for the induction of hemangiosarcoma in the current study upon exposure to AZT.

Walker *et al*. (2007) reported an incidence of hepatocellular carcinoma of 11.1%, 11.4%, or 22.2% in male B6C3F1 mice exposed to 80, 240, or 480 mg AZT/kg body weight per day, compared to 2.2% in the control group. In the current study, the incidences of hepatocellular carcinoma were 18.5%, 16.7 %, 17.0%, or 19.6% in male B6C3F1 mice whose dams had been exposed to 0, 80, 160, or 240 mg AZT/kg body weight

per day (Table A2a). A comparison of these studies indicates that the major difference lies in the spontaneous incidence of hepatocellular carcinoma in the control groups [2.2% in the Walker, *et al*. (2007) study versus 18.5% in the current study]. In studies conducted at the NCTR, the incidence of spontaneous hepatocellular carcinoma in male B6C3F1 mice has been 11.0% (range 6.5% to 20.8%; Table A3), and the range of hepatocellular carcinoma in male B6C3F1 mice from feed, drinking water, and water gavage studies in the NTP historical control database for the NIH-07 diet is 10% to 42%. Thus, the incidence of hepatocellular carcinoma in the control group of male B6C3F1 mice in the Walker *et al*. (2007) study was considerably lower than the range reported in the NCTR or NTP historical control databases.

In the current study, female B6C3F1 mice exposed transplacentally to AZT had a dose-related positive trend in the incidences of thyroid gland follicular cell adenoma or carcinoma (primarily adenoma) with the incidence in the 240 mg AZT/kg body weight group (8.5%; Tables 7, B1a, and B2a) being significantly increased compared to the control group (0.0%). In experiments conducted at the NCTR, the incidence of spontaneous thyroid gland follicular gland adenoma or carcinoma (exclusively due to adenoma) in female B6C3F1 mice has been 1.6% (range 0.0% to 2.8%; Tables 7 and B3). These data suggest that the induction of thyroid gland follicular cell neoplasms (primarily adenoma) may have been a result of transplacental exposure to AZT. Thyroid gland neoplasms occurred at only a very low frequency (≤2.2%) in the Walker *et al*. (2007) study, and they have not been reported in other bioassays conducted with AZT in mice (Ayers *et al*., 1996, 1997; Olivero *et al*., 1997; Zhang *et al*., 1998; Diwan *et al*., 1999; NTP, 1999, 2006). In addition to thyroid gland neoplasms, transplacental exposure to AZT resulted in a dose-related positive trend in the incidences of subcutaneous fibrosarcoma or sarcoma (combined) of the skin in female B6C3F1 mice (Tables 7, B1a, and B2a). The significance of this trend is uncertain. The incidence (10.4%) of these neoplasms in the high dose of AZT (240 mg AZT/kg body weight) does exceed the historical spontaneous incidence observed in other experiments conducted at the NCTR [mean 1.6%; range 0.0% to 8.3% (includes fibrous histiocytoma and myxosarcoma); Tables 7 and B3]; nonetheless, this type of neoplasm was not reported in the Walker *et al*. (2007) study.

AZT AND 3TC

In previous studies, B6C3F1 (Von Tungeln *et al*., 2007), *p53*$^{+/-}$ (Dobrovolsky *et al*., 2007), and *p53*$^{+/+}$ (Dobrovolsky *et al*., 2007) mice treated transplacentally with mixtures of AZT/3TC had dose-related increases in micronucleated reticulocytes and micronucleated normochromatic erythrocytes. Likewise, transplacental exposure to mixtures of AZT/3TC increased mutant frequency in the *Tk* gene of spleen T-lymphocytes of B6C3F1/*Tk*$^{+/-}$ mice (Von Tungeln *et al*., 2007) and the *Hprt* gene of spleen T-lymphocytes of CD-1 (Torres *et al*., 2007) and *p53*$^{+/-}$ (Dobrovolsky *et al*., 2007) mice. These results suggest that transplacental exposure of B6C3F1 mice to mixtures of AZT/3TC could result in the activation of AZT, 3TC, or both to genotoxic metabolites that could lead to an increase in neoplasia.

Female B6C3F1 mice exposed transplacentally to mixtures of AZT/3TC had a dose-related positive trend in the incidences of lung alveolar/bronchiolar adenoma (Tables 8, B1b, and B2b). Although the difference from the control group was not significant, the incidence in the high-dose group (12.5%) exceeded the historical control range (average, 5.0%; range 2.1% to 8.3%) for experiments conducted at the NCTR in female B6C3F1 mice (Tables 8 and B3). Thus, the occurrence of those tumors was considered equivocal evidence of carcinogenicity. The carcinogenicity of mixtures of AZT/3TC does not appear to have been assessed previously. In the Walker *et al*. (2007) study, female B6C3F1 mice exposed transplacentally to AZT alone had a lung alveolar/bronchiolar adenoma incidence as high as 11.1% compared to 8.9% in the control group, and in the current study, female B6C3F1 mice exposed transplacentally to AZT alone had a lung alveolar/bronchiolar adenoma incidence of 8.3% (Tables B1a and B2a). Lung neoplasms have also been detected in CD-1 mice exposed transplacentally to zidovudine alone (Olivero *et al*., 1997; Diwan *et al*., 1999; NTP, 2006).

AZT, 3TC, AND NVP

Male B6C3F1 mice treated transplacentally with mixtures of AZT/3TC/NVP had increased incidences of subcutaneous skin neoplasms (fibroma, fibrous histiocytoma, or fibrosarcoma) in the two highest dose groups (Tables 9, A1c, and A2c). The incidence of subcutaneous skin neoplasms from the high-dose mixture of AZT/3TC/NVP (20.8%) was significantly greater than that found from the high dose of AZT (4.3%; Table A1a; P=0.046) or the high-dose mixture of AZT/3TC (4.3%; Table A1b; P=0.020). A significant increase in the incidence of subcutaneous skin tumors (fibrosarcoma) was also observed in female B6C3F1 mice treated with the middle-dose mixture of AZT/3TC/NVP (Tables 9, B1c, and B2c), with the incidence exceeding the spontaneous historical range for other bioassays conducted at the NCTR [mean, 1.6%; range 0.0% to 8.3%; (includes fibrous histiocytoma, sarcoma, and myxosarcoma); Tables 9 and B3]. The fibrosarcomas were considered equivocal

evidence of carcinogenicity. Transplacental exposure to mixtures of AZT/3TC/NVP also caused nonneoplastic changes in the skin of male B6C3F1 mice, including inflammation and ulceration (Table A4c).

Nonneoplastic skin lesions have been observed in rats and humans exposed to NVP (Pollard *et al*., 1998; Mirochnick *et al*., 2000; Shenton *et al*., 2003, 2004, 2005; Popovic *et al*., 2006; AHFS, 2007d; Waters *et al*., 2007), although there is no indication that these lesions progress to neoplasms. NVP has been reported to induce hepatocellular adenoma and carcinoma in mice after long-term administration (*PDR*, 2007b); however, this response was not observed in the current experiment.

AZT, 3TC, AND NFV

Male B6C3F1 mice treated transplacentally with mixtures of AZT/3TC/NFV had a dose-related positive trend in the incidences of Harderian gland adenoma (Tables 10, A1d, and A2d). Although none of the individual exposed group incidences reached statistical significance, the incidences in the two highest dose groups (14.0% and 21.4%) exceeded the spontaneous historical range observed in other bioassays conducted at the NCTR (average, 7.5%; range 2.2% to 10.6%; Tables 10 and A3). The lack of statistical significance may be due in part to the small number of mice in the high-dose group as a result of the toxicity associated with administration of the AZT/3TC/NFV mixture. The incidence of Harderian gland adenoma from the high dose of AZT (8.9%; Tables A1a and A2a) was within the spontaneous historical range, while the incidence from the high-dose mixture of AZT/3TC (13.3%; Tables A1b and A2b) only slightly exceeded the spontaneous historical range (Tables 10 and A3). Harderian gland neoplasms in mice are typically associated with genotoxic carcinogens. Nothing in the structure of NFV suggests that it should be genotoxic and it is not mutagenic or clastogenic in a variety of assays, including microbial and mammalian gene mutation tests and micronucleus tests (Burns-Naas *et al*., 2005b; *PDR*, 2007c; Table C4). Therefore, the occurrence of the Harderian gland adenoma was not considered to be related to treatment.

CONCLUSIONS

AZT

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity** of AZT in male B6C3F1 mice whose dams were exposed to 80, 160, or 240 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of AZT in female B6C3F1 mice based on increased incidences of thyroid gland neoplasms (primarily adenoma) and subcutaneous skin fibrosarcoma or sarcoma.

AZT and 3TC

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT and 3TC in male B6C3F1 mice whose dams were exposed to 80/40, 160/80, or 240/120 mg/kg by gavage. There was *equivocal evidence of carcinogenic activity* of mixtures of AZT and 3TC in female B6C3F1 mice based on increased incidences of lung alveolar/bronchiolar adenomas.

AZT, 3TC, and NVP

Under the conditions of this transplacental exposure study, there was *some evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in male B6C3F1 mice whose dams were exposed to these chemicals by gavage based on increased incidences of subcutaneous skin neoplasms (fibroma, fibrous histiocytoma, or fibrosarcoma). There was *equivocal evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NVP in female B6C3F1 mice based on an increased incidence of subcutaneous skin fibrosarcoma.

AZT, 3TC, and NFV

Under the conditions of this transplacental exposure study, there was *no evidence of carcinogenic activity* of mixtures of AZT, 3TC, and NFV in male or female B6C3F1 mice whose dams were exposed to 80/40/336, 160/80/672, or 240/120/1,008 mg/kg by gavage.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 15.

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APPENDIX A
SUMMARY OF LESIONS IN MALE B6C3F1 MICE IN THE 2-YEAR TRANSPLACENTAL STUDY OF 3′-AZIDO-3′-DEOXYTHYMIDINE, LAMIVUDINE, NEVIRAPINE, AND NELFINAVIR MESYLATE

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TABLE A1a
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT[a]

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Disposition Summary				
Animals initially in study	65	48	48	48
Early deaths				
Moribund	4	8	5	8
Natural deaths	2		3	2
Survivors				
Moribund	10		1	1
Died last week of study	1		1	1
Terminal sacrifice	46	39	38	35
Harvest	2	1		1
Animals examined microscopically	65	48	48	48
Alimentary System				
Gallbladder	(59)	(48)	(44)	(45)
Intestine large, cecum	(63)	(48)	(45)	(45)
Intestine large, rectum	(63)	(48)	(45)	(45)
Intestine small, duodenum	(63)	(48)	(45)	(45)
Adenoma	1 (2%)			1 (2%)
Intestine small, ileum	(63)	(48)	(45)	(45)
Intestine small, jejunum	(62)	(48)	(44)	(45)
Liver	(65)	(48)	(47)	(46)
Hemangiosarcoma	5 (8%)	1 (2%)	1 (2%)	1 (2%)
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	15 (23%)	9 (19%)	9 (19%)	8 (17%)
Hepatocellular adenoma, multiple	2 (3%)	1 (2%)	1 (2%)	
Hepatocellular carcinoma	10 (15%)	6 (13%)	6 (13%)	9 (20%)
Hepatocellular carcinoma, multiple	2 (3%)	2 (4%)	2 (4%)	
Hepatocholangiocarcinoma			2 (4%)	2 (4%)
Liposarcoma, metastatic, skin		1 (2%)		
Mesentery	(4)	(2)	(1)	(3)
Hemangiosarcoma				1 (33%)
Hemangiosarcoma, metastatic, liver	1 (25%)			
Hepatocellular carcinoma, metastatic liver	1 (25%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Liposarcoma, metastatic, skin		1 (50%)		
Pancreas	(64)	(48)	(46)	(46)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)
Salivary glands	(64)	(48)	(46)	(46)
Stomach, forestomach	(64)	(48)	(46)	(46)
Squamous cell papilloma	1 (2%)	2 (4%)	3 (7%)	
Stomach, glandular	(63)	(48)	(44)	(45)
Cardiovascular System				
Blood vessel	(65)	(48)	(47)	(47)
Heart	(65)	(48)	(48)	(47)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)
Sarcoma, metastatic, lung			1 (2%)	
Sarcoma, metastatic, skeletal muscle			1 (2%)	

TABLE A1a
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Endocrine System				
Adrenal cortex	(63)	(48)	(45)	(47)
Subcapsular, adenoma	2 (3%)	1 (2%)	1 (2%)	2 (4%)
Subcapsular, carcinoma		1 (2%)		
Adrenal medulla	(63)	(46)	(45)	(45)
Pheochromocytoma benign			1 (2%)	
Pheochromocytoma malignant				1 (2%)
Islets, pancreatic	(65)	(48)	(48)	(47)
Adenoma	1 (2%)			
Parathyroid gland	(52)	(37)	(41)	(43)
Pituitary gland	(61)	(47)	(48)	(45)
Pars distalis, adenoma				1 (2%)
Thyroid gland	(64)	(48)	(45)	(46)
Follicular cell, adenoma		1 (2%)	1 (2%)	
General Body System				
Tissue NOS	(1)	(0)	(2)	(1)
Sarcoma, metastatic, skeletal muscle			1 (50%)	
Abdominal, hemangiosarcoma	1 (100%)			
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung				1 (100%)
Thoracic, hepatocholangiocarcinoma, metastatic, liver			1 (50%)	
Genital System				
Coagulating gland	(2)	(0)	(1)	(0)
Epididymis	(63)	(48)	(45)	(46)
Hemangioma		1 (2%)		
Preputial gland	(64)	(48)	(44)	(46)
Hemangiosarcoma	1 (2%)			
Prostate	(64)	(48)	(43)	(44)
Seminal vesicle	(63)	(48)	(46)	(46)
Testes	(64)	(48)	(45)	(45)
Hematopoietic System				
Bone marrow	(64)	(48)	(46)	(46)
Hemangiosarcoma, metastatic, mesentery				1 (2%)
Hemangiosarcoma, metastatic, uncertain primary site		1 (2%)		
Lymph node	(7)	(4)	(7)	(1)
Lumbar, hemangiosarcoma, metastatic, uncertain primary site		1 (25%)		
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (14%)			
Mediastinal, sarcoma, metastatic, lung			1 (14%)	
Mediastinal, sarcoma, metastatic, skeletal muscle			1 (14%)	
Renal, hemangiosarcoma, metastatic, uncertain primary site		1 (25%)		
Lymph node, mandibular	(63)	(46)	(45)	(43)

TABLE A1a
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(63)	(48)	(46)	(45)
Hemangiosarcoma	1 (2%)		1 (2%)	1 (2%)
Hemangiosarcoma, metastatic, uncertain primary site		1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Spleen	(63)	(48)	(45)	(46)
Hemangiosarcoma	2 (3%)	2 (4%)	2 (4%)	1 (2%)
Thymus	(51)	(43)	(39)	(37)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (3%)
Hepatocholangiocarcinoma, metastatic, liver			1 (3%)	1 (3%)
Sarcoma, metastatic, skeletal muscle			1 (3%)	
Integumentary System				
Skin	(65)	(48)	(48)	(46)
Squamous cell papilloma	3 (5%)			
Subcutaneous tissue, fibroma		1 (2%)		2 (4%)
Subcutaneous tissue, fibroma, multiple			1 (2%)	
Subcutaneous tissue, fibrosarcoma	2 (3%)	2 (4%)		2 (4%)
Subcutaneous tissue, liposarcoma		1 (2%)		
Subcutaneous tissue, sarcoma			1 (2%)	
Musculoskeletal System				
Skeletal muscle	(0)	(1)	(4)	(3)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (25%)	1 (33%)
Hepatocholangiocarcinoma, metastatic, liver			2 (50%)	2 (67%)
Sarcoma			1 (25%)	
Nervous System				
Brain, cerebrum	(64)	(48)	(46)	(46)
Respiratory System				
Lung	(64)	(48)	(46)	(47)
Alveolar/bronchiolar adenoma	6 (9%)	7 (15%)	7 (15%)	5 (11%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	7 (11%)	1 (2%)	3 (7%)	4 (9%)
Hepatocellular carcinoma, metastatic, liver	2 (3%)	1 (2%)		
Hepatocholangiocarcinoma, metastatic, liver			2 (4%)	2 (4%)
Liposarcoma, metastatic, skin		1 (2%)		
Sarcoma			1 (2%)	
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Nose	(65)	(48)	(48)	(47)

TABLE A1a
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Special Senses System				
Eye	(62)	(48)	(43)	(45)
Harderian gland	(64)	(48)	(45)	(45)
Adenocarcinoma	1 (2%)		1 (2%)	
Adenoma	5 (8%)	3 (6%)	1 (2%)	4 (9%)
Adenoma, multiple		1 (2%)		
Urinary System				
Kidney	(64)	(48)	(45)	(46)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Urethra	(1)	(0)	(1)	(1)
Urinary bladder	(65)	(48)	(45)	(46)
Systemic Lesions				
Multiple organs[b]	(65)	(48)	(48)	(48)
Histiocytic sarcoma	1 (2%)		2 (4%)	1 (2%)
Lymphoma malignant	9 (14%)	7 (15%)	9 (19%)	5 (10%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	31	36	37
Total primary neoplasms	79	51	57	52
Total animals with benign neoplasms	29	21	21	20
Total benign neoplasms	36	28	25	24
Total animals with malignant neoplasms	36	20	23	25
Total malignant neoplasms	43	23	32	28
Total animals with metastatic neoplasms	5	3	4	4
Total metastatic neoplasms	7	8	18	12
Total animals with malignant neoplasms of uncertain primary site		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1b
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC[a]

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Disposition Summary				
Animals initially in study	65	51	48	48
Early deaths				
Moribund	4	7	5	6
Natural deaths	2	1	4	5
Survivors				
Moribund	10	3	1	
Died last week of study	1			
Terminal sacrifice	46	39	35	34
Harvest	2	1	3	3
Animals examined microscopically	65	51	48	48
Alimentary System				
Gallbladder	(59)	(49)	(42)	(44)
Intestine large, cecum	(63)	(50)	(45)	(43)
Intestine large, colon	(63)	(50)	(45)	(44)
Intestine small, duodenum	(63)	(50)	(44)	(43)
Adenoma	1 (2%)	2 (4%)		
Fibrous histiocytoma		1 (2%)		
Intestine small, ileum	(63)	(50)	(45)	(43)
Intestine small, jejunum	(62)	(50)	(46)	(43)
Liver	(65)	(51)	(48)	(46)
Hemangioma			1 (2%)	
Hemangiosarcoma	5 (8%)	1 (2%)	1 (2%)	1 (2%)
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	15 (23%)	5 (10%)	5 (10%)	7 (15%)
Hepatocellular adenoma, multiple	2 (3%)	2 (4%)		2 (4%)
Hepatocellular carcinoma	10 (15%)	5 (10%)	9 (19%)	12 (26%)
Hepatocellular carcinoma, multiple	2 (3%)	2 (4%)	1 (2%)	1 (2%)
Hepatocholangiocarcinoma			1 (2%)	1 (2%)
Mesentery	(4)	(2)	(1)	(0)
Hemangiosarcoma, metastatic, liver	1 (25%)			
Hepatocellular carcinoma, metastatic, liver	1 (25%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Pancreas	(64)	(50)	(45)	(45)
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Salivary glands	(64)	(50)	(47)	(44)
Stomach, forestomach	(64)	(50)	(45)	(45)
Squamous cell papilloma	1 (2%)	2 (4%)		1 (2%)
Stomach, glandular	(63)	(50)	(45)	(43)
Tongue	(0)	(0)	(0)	(1)
Squamous cell carcinoma				1 (100%)
Cardiovascular System				
Blood vessel	(65)	(50)	(48)	(46)
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Heart	(65)	(50)	(48)	(46)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)

TABLE A1b
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Endocrine System				
Adrenal cortex	(63)	(50)	(46)	(45)
Subcapsular, adenoma	2 (3%)	2 (4%)	3 (7%)	
Adrenal medulla	(63)	(49)	(46)	(43)
Pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(65)	(50)	(44)	(45)
Adenoma	1 (2%)			
Parathyroid gland	(52)	(45)	(37)	(41)
Adenoma			1 (3%)	
Pituitary gland	(61)	(50)	(44)	(45)
Thyroid gland	(64)	(50)	(46)	(46)
Follicular cell, adenoma		1 (2%)		1 (2%)
General Body System				
Tissue NOS	(1)	(1)	(1)	(3)
Hepatocellular carcinoma, metastatic, liver				1 (33%)
Hepatocholangiocarcinoma, metastatic, liver				1 (33%)
Abdominal, fibrous histiocytoma		1 (100%)		
Abdominal, hemangiosarcoma	1 (100%)			
Genital System				
Coagulating gland	(2)	(2)	(0)	(1)
Epididymis	(63)	(50)	(46)	(45)
Hepatocholangiocarcinoma, metastatic, liver				1 (2%)
Preputial gland	(64)	(50)	(46)	(44)
Adenoma			1 (2%)	
Hemangiosarcoma	1 (2%)			
Sarcoma		1 (2%)		
Prostate	(64)	(50)	(46)	(44)
Sarcoma		1 (2%)		
Seminal vesicle	(63)	(50)	(45)	(45)
Testes	(64)	(50)	(45)	(44)
Hematopoietic System				
Bone marrow	(64)	(51)	(46)	(44)
Hemangiosarcoma		2 (4%)		1 (2%)
Lymph node	(7)	(3)	(4)	(3)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (14%)			
Lymph node, mandibular	(63)	(50)	(46)	(45)
Lymph node, mesenteric	(63)	(50)	(46)	(44)
Fibrous histiocytoma		1 (2%)		
Hemangiosarcoma	1 (2%)			
Spleen	(63)	(50)	(47)	(45)
Fibrous histiocytoma		1 (2%)		
Hemangiosarcoma	2 (3%)	1 (2%)	1 (2%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)
Thymus	(51)	(48)	(43)	(38)
Hemangiosarcoma			1 (2%)	
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (3%)

TABLE A1b
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Integumentary System				
Skin	(65)	(50)	(48)	(46)
Hemangiosarcoma			1 (2%)	
Squamous cell papilloma	3 (5%)			
Subcutaneous tissue, fibroma			1 (2%)	1 (2%)
Subcutaneous tissue, fibrosarcoma	2 (3%)		1 (2%)	2 (4%)
Musculoskeletal System				
Skeletal muscle	(0)	(0)	(1)	(0)
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Nervous System				
Brain, cerebellum	(64)	(50)	(45)	(46)
Brain, cerebrum	(64)	(50)	(45)	(45)
Respiratory System				
Lung	(64)	(50)	(47)	(48)
Alveolar/bronchiolar adenoma	6 (9%)	4 (8%)	5 (11%)	8 (17%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)		
Alveolar/bronchiolar carcinoma	7 (11%)	6 (12%)	3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (3%)	1 (2%)	2 (4%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)
Nose	(65)	(51)	(46)	(46)
Special Senses System				
Eye	(62)	(50)	(45)	(43)
Harderian gland	(64)	(50)	(45)	(45)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	4 (8%)	5 (11%)	6 (13%)
Urinary System				
Kidney	(64)	(50)	(46)	(44)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	1 (2%)
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(65)	(50)	(46)	(45)
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(65)	(51)	(48)	(48)
Histiocytic sarcoma	1 (2%)	1 (2%)		3 (6%)
Lymphoma malignant	9 (14%)	5 (10%)	14 (29%)	3 (6%)

TABLE A1b
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	33	40	40
Total primary neoplasms	79	53	55	57
Total animals with benign neoplasms	29	19	19	23
Total benign neoplasms	36	24	22	27
Total animals with malignant neoplasms	36	21	27	27
Total malignant neoplasms	43	29	33	30
Total animals with metastatic neoplasms	5	1	3	2
Total metastatic neoplasms	7	1	9	11

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1c
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP[a]

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Disposition Summary				
Animals initially in study	65	48	48	50
Early deaths				
Moribund	4	8	10	18
Natural deaths	2	1	2	2
Survivors				
Moribund	10			5
Died last week of study	1	1	1	
Terminal sacrifice	46	37	35	25
Harvest	2	1		
Animals examined microscopically	65	48	48	50
Alimentary System				
Gallbladder	(59)	(45)	(44)	(47)
Intestine large, cecum	(63)	(47)	(45)	(48)
Intestine large, rectum	(63)	(47)	(45)	(47)
Anus, fibrosarcoma, metastatic, skin		1 (2%)		
Intestine small, duodenum	(63)	(47)	(45)	(48)
Adenoma	1 (2%)	2 (4%)	1 (2%)	
Intestine small, ileum	(63)	(47)	(45)	(48)
Intestine small, jejunum	(62)	(47)	(45)	(48)
Liver	(65)	(48)	(47)	(48)
Hemangiosarcoma	5 (8%)	1 (2%)	1 (2%)	
Hepatoblastoma	1 (2%)	1 (2%)		
Hepatocellular adenoma	15 (23%)	7 (15%)	7 (15%)	9 (19%)
Hepatocellular adenoma, multiple	2 (3%)	2 (4%)		3 (6%)
Hepatocellular carcinoma	10 (15%)	5 (10%)	8 (17%)	8 (17%)
Hepatocellular carcinoma, multiple	2 (3%)	4 (8%)	3 (6%)	
Mesentery	(4)	(1)	(0)	(1)
Fibrosarcoma		1 (100%)		
Hemangiosarcoma, metastatic, liver	1 (25%)			
Hepatocellular carcinoma, metastatic, liver	1 (25%)			
Pancreas	(64)	(47)	(45)	(48)
Fibrous histiocytoma		1 (2%)		
Salivary glands	(64)	(47)	(45)	(48)
Stomach, forestomach	(64)	(48)	(45)	(48)
Squamous cell papilloma	1 (2%)		1 (2%)	1 (2%)
Stomach, glandular	(63)	(47)	(45)	(47)
Cardiovascular System				
Blood vessel	(65)	(47)	(47)	(48)
Fibrous histiocytoma		1 (2%)		
Heart	(65)	(47)	(47)	(48)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Endocrine System				
Adrenal cortex	(63)	(47)	(45)	(47)
Fibrous histiocytoma		1 (2%)		
Subcapsular, adenoma	2 (3%)		1 (2%)	

TABLE A1c
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Endocrine System (continued)				
Adrenal medulla	(63)	(46)	(44)	(46)
Pheochromocytoma benign		1 (2%)		2 (4%)
Islets, pancreatic	(65)	(47)	(45)	(48)
Adenoma	1 (2%)		1 (2%)	
Fibrous histiocytoma		1 (2%)		
Parathyroid gland	(52)	(38)	(33)	(34)
Pituitary gland	(61)	(46)	(44)	(48)
Thyroid gland	(64)	(48)	(45)	(48)
Follicular cell, adenoma		1 (2%)	1 (2%)	
General Body System				
Tissue NOS	(1)	(2)	(0)	(0)
Abdominal, fibrous histiocytoma		1 (50%)		
Abdominal, hemangiosarcoma	1 (100%)			
Genital System				
Coagulating gland	(2)	(1)	(1)	(0)
Epididymis	(63)	(47)	(45)	(48)
Fibrosarcoma, metastatic, skin		1 (2%)		
Fibrous histiocytoma		1 (2%)		
Preputial gland	(64)	(48)	(44)	(48)
Hemangiosarcoma	1 (2%)	3 (6%)		
Prostate	(64)	(47)	(43)	(48)
Fibrous histiocytoma		1 (2%)		
Seminal vesicle	(63)	(48)	(45)	(49)
Fibrous histiocytoma		1 (2%)		
Testes	(64)	(47)	(45)	(49)
Fibrous histiocytoma		1 (2%)		
Lipoma				1 (2%)
Hematopoietic System				
Bone marrow	(64)	(48)	(45)	(48)
Hemangiosarcoma				1 (2%)
Hemangiosarcoma, metastatic, preputial gland		1 (2%)		
Lymph node	(7)	(3)	(2)	(6)
Axillary, fibrous histiocytoma, metastatic, skin				1 (17%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (14%)			
Lymph node, mandibular	(63)	(46)	(45)	(47)
Lymph node, mesenteric	(63)	(46)	(45)	(48)
Fibrous histiocytoma		1 (2%)		
Hemangiosarcoma	1 (2%)			
Spleen	(63)	(47)	(45)	(48)
Hemangiosarcoma	2 (3%)	1 (2%)	2 (4%)	2 (4%)
Thymus	(51)	(39)	(37)	(38)
Fibrous histiocytoma		1 (3%)		

TABLE A1c
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Integumentary System				
Skin	(65)	(47)	(48)	(48)
Hemangiosarcoma		1 (2%)		
Squamous cell papilloma	3 (5%)			
Subcutaneous tissue, fibroma		1 (2%)		2 (4%)
Subcutaneous tissue, fibrosarcoma	2 (3%)	1 (2%)	5 (10%)	7 (15%)
Subcutaneous tissue, fibrosarcoma, multiple			1 (2%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	2 (4%)
Musculoskeletal System				
None				
Nervous System				
Brain, cerebrum	(64)	(47)	(47)	(48)
Respiratory System				
Lung	(64)	(47)	(45)	(48)
Alveolar/bronchiolar adenoma	6 (9%)	5 (11%)	3 (7%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma	7 (11%)	1 (2%)	2 (4%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Fibrous histiocytoma		1 (2%)		
Fibrous histiocytoma, metastatic, skin				1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (3%)			
Nose	(65)	(47)	(46)	(49)
Special Senses System				
Eye	(62)	(47)	(45)	(48)
Harderian gland	(64)	(47)	(45)	(48)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	5 (11%)		5 (10%)
Adenoma, multiple			1 (2%)	
Urinary System				
Kidney	(64)	(47)	(45)	(48)
Fibrous histiocytoma		1 (2%)		
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(65)	(47)	(46)	(48)
Fibrous histiocytoma		1 (2%)		

TABLE A1c
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Systemic Lesions				
Multiple organs[b]	(65)	(48)	(48)	(50)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	9 (14%)	8 (17%)	4 (8%)	4 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	34	30	33
Total primary neoplasms	79	66	44	54
Total animals with benign neoplasms	29	20	13	19
Total benign neoplasms	36	24	17	28
Total animals with malignant neoplasms	36	23	23	22
Total malignant neoplasms	43	42	27	26
Total animals with metastatic neoplasms	5	2		2
Total metastatic neoplasms	7	3		2

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A1d
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV[a]

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Disposition Summary				
Animals initially in study	65	48	51	15
Early deaths				
Moribund	4	6	5	6
Natural deaths	2	3	2	1
Survivors				
Moribund	10	1	5	
Died last week of study	1		1	
Terminal sacrifice	46	37	36	6
Harvest	2	1	2	2
Animals examined microscopically	65	48	51	15
Alimentary System				
Gallbladder	(59)	(45)	(47)	(13)
Intestine large, cecum	(63)	(45)	(48)	(14)
Intestine small, duodenum	(63)	(45)	(48)	(14)
Adenoma	1 (2%)	1 (2%)	1 (2%)	
Intestine small, ileum	(63)	(45)	(48)	(14)
Intestine small, jejunum	(62)	(45)	(48)	(14)
Adenocarcinoma				1 (7%)
Liver	(65)	(48)	(50)	(15)
Fibrous histiocytoma		1 (2%)		1 (7%)
Hemangiosarcoma	5 (8%)	1 (2%)	2 (4%)	2 (13%)
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	15 (23%)	4 (8%)	5 (10%)	2 (13%)
Hepatocellular adenoma, multiple	2 (3%)	1 (2%)	2 (4%)	1 (7%)
Hepatocellular carcinoma	10 (15%)	8 (17%)	7 (14%)	2 (13%)
Hepatocellular carcinoma, multiple	2 (3%)		2 (4%)	2 (13%)
Hepatocholangiocarcinoma		1 (2%)	1 (2%)	
Mesentery	(4)	(0)	(2)	(0)
Hemangiosarcoma, metastatic, liver	1 (25%)			
Hepatocellular carcinoma, metastatic, liver	1 (25%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (50%)	
Pancreas	(64)	(45)	(49)	(15)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Salivary glands	(64)	(46)	(50)	(15)
Stomach, forestomach	(64)	(45)	(50)	(15)
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	
Stomach, glandular	(63)	(45)	(48)	(14)
Adenoma		1 (2%)		
Cardiovascular System				
Blood vessel	(65)	(48)	(50)	(15)
Heart	(65)	(48)	(50)	(15)
Hemangiosarcoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)		

TABLE A1d
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Endocrine System				
Adrenal cortex	(63)	(45)	(49)	(15)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Subcapsular, adenoma	2 (3%)		1 (2%)	
Adrenal medulla	(63)	(44)	(47)	(13)
Islets, pancreatic	(65)	(45)	(50)	(15)
Adenoma	1 (2%)			
Pituitary gland	(61)	(46)	(50)	(15)
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(64)	(46)	(50)	(15)
Follicular cell, carcinoma		1 (2%)		
General Body System				
Tissue NOS	(1)	(2)	(1)	(2)
Fibrous histiocytoma		1 (50%)		
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Abdominal, hemangiosarcoma	1 (100%)			
Thoracic, alveolar/bronchiolar carcinoma, metastatic, lung				1 (50%)
Thoracic, fibrous histiocytoma				1 (50%)
Thoracic, hepatocholangiocarcinoma, metastatic, liver		1 (50%)		
Genital System				
Coagulating gland	(2)	(1)	(0)	(0)
Epididymis	(63)	(45)	(50)	(15)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Preputial gland	(64)	(47)	(50)	(15)
Hemangiosarcoma	1 (2%)			
Prostate	(64)	(44)	(48)	(15)
Seminal vesicle	(63)	(46)	(49)	(15)
Testes	(64)	(45)	(49)	(15)
Sertoli cell tumor benign			1 (2%)	
Hematopoietic System				
Bone marrow	(64)	(45)	(50)	(15)
Lymph node	(7)	(4)	(3)	(3)
Lumbar, fibrous histiocytoma				1 (33%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung	1 (14%)			
Renal, fibrous histiocytoma				1 (33%)
Lymph node, mandibular	(63)	(46)	(49)	(14)
Lymph node, mesenteric	(63)	(46)	(48)	(14)
Fibrous histiocytoma		2 (4%)		1 (7%)
Hemangiosarcoma	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	

TABLE A1d
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Hematopoietic System (continued)				
Spleen	(63)	(45)	(49)	(15)
Fibrous histiocytoma		1 (2%)		1 (7%)
Hemangiosarcoma	2 (3%)	2 (4%)	5 (10%)	
Thymus	(51)	(35)	(44)	(12)
Hepatocholangiocarcinoma, metastatic, liver		1 (3%)	1 (2%)	
Integumentary System				
Skin	(65)	(48)	(51)	(15)
Squamous cell papilloma	3 (5%)			
Ear, squamous cell papilloma		1 (2%)		
Subcutaneous tissue, fibrosarcoma	2 (3%)	1 (2%)	3 (6%)	1 (7%)
Subcutaneous tissue, schwannoma malignant				1 (7%)
Musculoskeletal System				
Bone	(0)	(1)	(0)	(0)
Mandible, osteosarcoma		1 (100%)		
Skeletal muscle	(0)	(0)	(1)	(0)
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Nervous System				
Brain, cerebrum	(64)	(46)	(50)	(15)
Respiratory System				
Lung	(64)	(47)	(50)	(15)
Alveolar/bronchiolar adenoma	6 (9%)	3 (6%)	4 (8%)	
Alveolar/bronchiolar carcinoma	7 (11%)	3 (6%)	1 (2%)	1 (7%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	2 (3%)		1 (2%)	3 (20%)
Hepatocholangiocarcinoma, metastatic, liver		1 (2%)	1 (2%)	
Nose	(65)	(47)	(51)	(15)
Special Senses System				
Eye	(62)	(45)	(49)	(14)
Harderian gland	(64)	(45)	(50)	(14)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	2 (4%)	7 (14%)	3 (21%)

TABLE A1d
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Urinary System				
Kidney	(64)	(46)	(49)	(14)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Urethra	(1)	(2)	(0)	(0)
Urinary bladder	(65)	(46)	(50)	(15)
Transitional epithelium, papilloma		2 (4%)		
Systemic Lesions				
Multiple organs[b]	(65)	(48)	(51)	(15)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	9 (14%)	7 (15%)	5 (10%)	4 (27%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	29	32	11
Total primary neoplasms	79	46	50	26
Total animals with benign neoplasms	29	14	18	5
Total benign neoplasms	36	16	23	6
Total animals with malignant neoplasms	36	24	24	11
Total malignant neoplasms	43	30	27	20
Total animals with metastatic neoplasms	5	1	2	4
Total metastatic neoplasms	7	4	11	4

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/64 (7.8%)	4/48 (8.3%)	1/45 (2.2%)	4/45 (8.9%)
Adjusted rate[b]	8.5%	8.8%	2.3%	9.1%
Terminal rate[c]	4/46 (8.7%)	2/39 (5.1%)	1/38 (2.6%)	1/35 (2.9%)
First incidence (days)	643	616	737 (T)	520
Poly-3 test[d]	P=0.431N	P=0.615	P=0.186N	P=0.595N
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	5/64 (7.8%)	4/48 (8.3%)	2/45 (4.4%)	4/45 (8.9%)
Adjusted rate	8.5%	8.8%	4.6%	9.1%
Terminal rate	4/46 (8.7%)	2/39 (5.1%)	2/38 (5.3%)	1/35 (2.9%)
First incidence (days)	643	616	737 (T)	520
Poly-3 test	P=0.489N	P=0.615	P=0.355N	P=0.595N
Liver: Hemangiosarcoma				
Overall rate	5/65 (7.7%)	1/48 (2.1%)	1/47 (2.1%)	1/46 (2.2%)
Adjusted rate	8.4%	2.2%	2.2%	2.3%
Terminal rate	2/46 (4.3%)	1/39 (2.6%)	0/38 (0.0%)	1/35 (2.9%)
First incidence (days)	677	739 (T)	713	737 (T)
Poly-3 test	P=0.081N	P=0.180N	P=0.180N	P=0.194N
Liver: Hepatocellular Adenoma				
Overall rate	17/65 (26.2%)	10/48 (20.8%)	10/47 (21.3%)	8/46 (17.4%)
Adjusted rate	28.4%	22.2%	22.4%	18.6%
Terminal rate	12/46 (26.1%)	8/39 (20.5%)	10/38 (26.3%)	7/35 (20.0%)
First incidence (days)	658	672	732 (T)	698
Poly-3 test	P=0.151N	P=0.310N	P=0.323N	P=0.183N
Liver: Hepatocellular Carcinoma				
Overall rate	12/65 (18.5%)	8/48 (16.7%)	8/47 (17.0%)	9/46 (19.6%)
Adjusted rate	19.5%	17.5%	17.6%	20.6%
Terminal rate	5/46 (10.9%)	4/39 (10.3%)	5/38 (13.2%)	7/35 (20.0%)
First incidence (days)	572	616	593	463
Poly-3 test	P=0.497	P=0.496N	P=0.499N	P=0.542
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/65 (38.5%)	16/48 (33.3%)	16/47 (34.0%)	16/46 (34.8%)
Adjusted rate	40.4%	35.0%	35.1%	36.6%
Terminal rate	15/46 (32.6%)	12/39 (30.8%)	13/38 (34.2%)	14/35 (40.0%)
First incidence (days)	572	616	593	463
Poly-3 test	P=0.357N	P=0.357N	P=0.360N	P=0.425N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	13/65 (20.0%)	8/48 (16.7%)	8/47 (17.0%)	9/46 (19.6%)
Adjusted rate	20.9%	17.5%	17.6%	20.6%
Terminal rate	5/46 (10.9%)	4/39 (10.3%)	5/38 (13.2%)	7/35 (20.0%)
First incidence (days)	572	616	593	463
Poly-3 test	P=0.489N	P=0.421N	P=0.424N	P=0.579N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/65 (40.0%)	16/48 (33.3%)	16/47 (34.0%)	16/46 (34.8%)
Adjusted rate	41.7%	35.0%	35.1%	36.6%
Terminal rate	15/46 (32.6%)	12/39 (30.8%)	13/38 (34.2%)	14/35 (40.0%)
First incidence (days)	572	616	593	463
Poly-3 test	P=0.304N	P=0.307N	P=0.311N	P=0.374N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/64 (9.4%)	8/48 (16.7%)	7/46 (15.2%)	6/47 (12.8%)
Adjusted rate	10.0%	17.9%	15.5%	13.7%
Terminal rate	1/46 (2.2%)	8/39 (20.5%)	5/38 (13.2%)	6/35 (17.1%)
First incidence (days)	589	733 (T)	573	733 (T)
Poly-3 test	P=0.336	P=0.191	P=0.293	P=0.394
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/64 (10.9%)	1/48 (2.1%)	3/46 (6.5%)	4/47 (8.5%)
Adjusted rate	11.8%	2.2%	6.7%	9.1%
Terminal rate	5/46 (10.9%)	1/39 (2.6%)	2/38 (5.3%)	3/35 (8.6%)
First incidence (days)	579	733 (T)	733	664
Poly-3 test	P=0.391N	P=0.073N	P=0.298N	P=0.452N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	12/64 (18.8%)	8/48 (16.7%)	10/46 (21.7%)	10/47 (21.3%)
Adjusted rate	19.9%	17.9%	22.2%	22.7%
Terminal rate	5/46 (10.9%)	8/39 (20.5%)	7/38 (18.4%)	9/35 (25.7%)
First incidence (days)	579	733 (T)	573	664
Poly-3 test	P=0.350	P=0.498N	P=0.483	P=0.456
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	2/65 (3.1%)	3/48 (6.3%)	2/48 (4.2%)	4/46 (8.7%)
Adjusted rate	3.4%	6.7%	4.4%	9.4%
Terminal rate	2/46 (4.3%)	1/39 (2.6%)	1/38 (2.6%)	3/34 (8.8%)
First incidence (days)	733 (T)	665	733	723
Poly-3 test	P=0.182	P=0.379	P=0.598	P=0.201
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/64 (1.6%)	2/48 (4.2%)	3/46 (6.5%)	0/46 (0.0%)
Adjusted rate	1.7%	4.5%	6.8%	0.0%
Terminal rate	0/46 (0.0%)	2/39 (5.1%)	3/38 (7.9%)	0/35 (0.0%)
First incidence (days)	643	732 (T)	732 (T)	—[e]
Poly-3 test	P=0.545N	P=0.404	P=0.210	P=0.564N
All Organs: Hemangiosarcoma				
Overall rate	9/65 (13.8%)	2/48 (4.2%)	4/48 (8.3%)	4/48 (8.3%)
Adjusted rate	15.1%	4.5%	8.8%	9.0%
Terminal rate	6/46 (13.0%)	2/39 (5.1%)	3/38 (7.9%)	3/35 (8.6%)
First incidence (days)	589	738 (T)	713	728
Poly-3 test	P=0.211N	P=0.075	P=0.246N	P=0.261N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	9/65 (13.8%)	3/48 (6.3%)	4/48 (8.3%)	4/48 (8.3%)
Adjusted rate	15.1%	6.7%	8.8%	9.0%
Terminal rate	6/46 (13.0%)	3/39 (7.7%)	3/38 (7.9%)	3/35 (8.6%)
First incidence (days)	677	738 (T)	713	728
Poly-3 test	P=0.193N	P=0.153N	P=0.246N	P=0.261N
All Organs: Malignant Lymphoma				
Overall rate	9/65 (13.8%)	7/48 (14.6%)	9/48 (18.8%)	5/48 (10.4%)
Adjusted rate	15.0%	15.4%	19.7%	11.2%
Terminal rate	6/46 (13.0%)	5/39 (12.8%)	7/38 (18.4%)	4/35 (11.4%)
First incidence (days)	589	616	713	727
Poly-3 test	P=0.436N	P=0.585	P=0.354	P=0.393N

TABLE A2a
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
All Organs: Benign Neoplasms				
Overall rate	29/65 (44.6%)	21/48 (43.8%)	21/48 (43.8%)	20/48 (41.7%)
Adjusted rate	46.9%	45.4%	45.0%	43.4%
Terminal rate	18/46 (39.1%)	16/39 (41.0%)	18/38 (47.4%)	16/35 (45.7%)
First incidence (days)	579	616	573	520
Poly-3 test	P=0.386N	P=0.517N	P=0.500N	P=0.435N
All Organs: Malignant Neoplasms				
Overall rate	36/65 (55.4%)	20/48 (41.7%)	23/48 (47.9%)	25/48 (52.1%)
Adjusted rate	56.5%	42.4%	48.5%	53.7%
Terminal rate	22/46 (47.8%)	12/39 (30.8%)	14/38 (36.8%)	15/35 (42.9%)
First incidence (days)	572	502	593	463
Poly-3 test	P=0.424N	P=0.099N	P=0.258N	P=0.458N
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/65 (72.3%)	31/48 (64.6%)	36/48 (75.0%)	37/48 (77.1%)
Adjusted rate	73.0%	65.7%	75.0%	77.1%
Terminal rate	30/46 (65.2%)	23/39 (59.0%)	26/38 (68.4%)	24/35 (68.6%)
First incidence (days)	572	502	573	463
Poly-3 test	P=0.268	P=0.266N	P=0.493	P=0.394

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Adrenal Cortex (Subcapsular): Adenoma				
Overall rate[a]	2/63 (3.2%)	2/50 (4.0%)	3/46 (6.5%)	0/45 (0.0%)
Adjusted rate[b]	3.5%	4.3%	7.0%	0.0%
Terminal rate[c]	2/45 (4.4%)	2/39 (5.1%)	3/35 (8.6%)	0/34 (0.0%)
First incidence (days)	746 (T)	732 (T)	739 (T)	—[e]
Poly-3 test[d]	P=0.372N	P=0.613	P=0.366	P=0.316N
Harderian Gland: Adenoma				
Overall rate	5/64 (7.8%)[f]	4/50 (8.0%)	5/45 (11.1%)	6/45 (13.3%)
Adjusted rate	8.5%	8.5%	11.9%	14.6%
Terminal rate	4/46 (8.7%)	2/39 (5.1%)	5/35 (14.3%)	5/34 (14.7%)
First incidence (days)	643	663	733 (T)	698
Poly-3 test	P=0.171	P=0.633	P=0.408	P=0.263
Liver: Hemangiosarcoma				
Overall rate	5/65 (7.7%)	1/51 (2.0%)	1/48 (2.1%)	1/46 (2.2%)
Adjusted rate	8.4%	2.1%	2.3%	2.4%
Terminal rate	2/46 (4.3%)	0/39 (0.0%)	1/35 (2.9%)	0/34 (0.0%)
First incidence (days)	677	648	743 (T)	697
Poly-3 test	P=0.084N	P=0.163N	P=0.186N	P=0.203N
Liver: Hepatocellular Adenoma				
Overall rate	17/65 (26.2%)	7/51 (13.7%)	5/48 (10.4%)	9/46 (19.6%)
Adjusted rate	28.4%	14.9%	11.4%	21.4%
Terminal rate	12/46 (26.1%)	7/39 (17.9%)	4/35 (11.4%)	7/34 (20.6%)
First incidence (days)	658	732 (T)	719	617
Poly-3 test	P=0.131N	P=0.076N	P=0.030N	P=0.285N
Liver: Hepatocellular Carcinoma				
Overall rate	12/65 (18.5%)	7/51 (13.7%)	10/48 (20.8%)	13/46 (28.3%)
Adjusted rate	19.5%	14.3%	21.8%	30.5%
Terminal rate	5/46 (10.9%)	2/39 (5.1%)	4/35 (11.4%)	10/34 (29.4%)
First incidence (days)	572	510	519	617
Poly-3 test	P=0.089	P=0.322N	P=0.481	P=0.143
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/65 (38.5%)	14/51 (27.5%)	14/48 (29.2%)	19/46 (41.3%)
Adjusted rate	40.4%	28.6%	30.5%	44.6%
Terminal rate	15/46 (32.6%)	9/39 (23.1%)	8/35 (22.9%)	16/34 (47.1%)
First incidence (days)	572	510	519	617
Poly-3 test	P=0.430	P=0.136N	P=0.194N	P=0.409
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	13/65 (20.0%)	7/51 (13.7%)	10/48 (20.8%)	13/46 (28.3%)
Adjusted rate	20.9%	14.3%	21.8%	30.5%
Terminal rate	5/46 (10.9%)	2/39 (5.1%)	4/35 (11.4%)	10/34 (29.4%)
First incidence (days)	572	510	519	617
Poly-3 test	P=0.126	P=0.256N	P=0.554	P=0.188
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/65 (40.0%)	14/51 (27.5%)	14/48 (29.2%)	19/46 (41.3%)
Adjusted rate	41.7%	28.6%	30.5%	44.6%
Terminal rate	15/46 (32.6%)	9/39 (23.1%)	8/35 (22.9%)	16/34 (47.1%)
First incidence (days)	572	510	519	617
Poly-3 test	P=0.490	P=0.107N	P=0.158N	P=0.461

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/64 (9.4%)	5/50 (10.0%)	5/47 (10.6%)	8/48 (16.7%)
Adjusted rate	10.0%	10.8%	11.4%	18.4%
Terminal rate	1/46 (2.2%)	5/39 (12.8%)	4/35 (11.4%)	6/34 (17.6%)
First incidence (days)	589	732 (T)	692	495
Poly-3 test	P=0.142	P=0.577	P=0.538	P=0.176
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/64 (10.9%)	6/50 (12.0%)	3/47 (6.4%)	3/48 (6.3%)
Adjusted rate	11.8%	13.0%	6.9%	7.0%
Terminal rate	5/46 (10.9%)	6/39 (15.4%)	2/35 (5.7%)	2/34 (5.9%)
First incidence (days)	579	732 (T)	704	594
Poly-3 test	P=0.175N	P=0.549	P=0.308N	P=0.320N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	12/64 (18.8%)	10/50 (20.0%)	7/47 (14.9%)	10/48 (20.8%)
Adjusted rate	19.9%	21.6%	15.9%	22.7%
Terminal rate	5/46 (10.9%)	10/39 (25.6%)	5/35 (14.3%)	7/34 (20.6%)
First incidence (days)	579	732 (T)	692	495
Poly-3 test	P=0.493	P=0.510	P=0.398N	P=0.457
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	2/65 (3.1%)	0/50 (0.0%)	2/48 (4.2%)	3/46 (6.5%)
Adjusted rate	3.4%	0.0%	4.5%	7.1%
Terminal rate	2/46 (4.3%)	0/39 (0.0%)	1/35 (2.9%)	1/34 (2.9%)
First incidence (days)	733 (T)	—	705	635
Poly-3 test	P=0.156	P=0.293N	P=0.585	P=0.349
All Organs: Hemangiosarcoma				
Overall rate	9/65 (13.8%)	3/51 (5.9%)	4/48 (8.3%)	3/48 (6.3%)
Adjusted rate	15.1%	6.3%	9.1%	7.1%
Terminal rate	6/46 (13.0%)	2/39 (5.1%)	3/35 (8.6%)	2/34 (5.9%)
First incidence (days)	677	648	704	697
Poly-3 test	P=0.129N	P=0.130N	P=0.268N	P=0.175N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	9/65 (13.8%)	3/51 (5.9%)	5/48 (10.4%)	3/48 (6.3%)
Adjusted rate	15.4%	6.3%	11.4%	7.1%
Terminal rate	6/46 (13.0%)	2/39 (5.1%)	4/35 (11.4%)	2/34 (5.9%)
First incidence (days)	677	648	704	697
Poly-3 test	P=0.166N	P=0.130N	P=0.396N	P=0.175N
All Organs: Histiocytic Sarcoma				
Overall rate	1/65 (1.5%)	1/51 (2.0%)	0/48 (0.0%)	3/48 (6.3%)
Adjusted rate	1.7%	2.1%	0.0%	6.9%
Terminal rate	1/46 (2.2%)	1/39 (2.6%)	0/35 (0.0%)	0/34 (0.0%)
First incidence (days)	732 (T)	745 (T)	—	588
Poly-3 test	P=0.148	P=0.709	P=0.559N	P=0.203
All Organs: Malignant Lymphoma				
Overall rate	9/65 (13.8%)	5/51 (9.8%)	14/48 (29.2%)	3/48 (6.3%)
Adjusted rate	15.0%	10.5%	31.4%	7.1%
Terminal rate	6/46 (13.0%)	2/39 (5.1%)	9/35 (25.7%)	2/34 (5.9%)
First incidence (days)	589	663	692	697
Poly-3 test	P=0.520	P=0.343N	P=0.037	P=0.179N

TABLE A2b
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
All Organs: Benign Neoplasms				
Overall rate	29/65 (44.6%)	19/51 (37.3%)	19/48 (39.6%)	23/48 (47.9%)
Adjusted rate	46.9%	39.9%	43.0%	51.2%
Terminal rate	18/46 (39.1%)	16/39 (41.0%)	17/35 (48.6%)	16/34 (47.1%)
First incidence (days)	579	663	692	495
Poly-3 test	P=0.364	P=0.297N	P=0.422N	P=0.402
All Organs: Malignant Neoplasms				
Overall rate	36/65 (55.4%)	21/51 (41.2%)	27/48 (56.3%)	27/48 (56.3%)
Adjusted rate	56.5%	41.8%	57.3%	57.9%
Terminal rate	22/46 (47.8%)	11//39 (28.2%)	16/35 (45.7%)	16/34 (47.1%)
First incidence (days)	572	510	519	546
Poly-3 test	P=0.333	P=0.083N	P=0.546	P=0.519
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/65 (72.3%)	33/51 (64.7%)	40/48 (83.3%)	40/48 (83.3%)
Adjusted rate	73.0%	65.3%	84.8%	83.3%
Terminal rate	30/46 (65.2%)	22/39 (56.4%)	29/35 (82.9%)	26/34 (76.5%)
First incidence (days)	572	510	519	495
Poly-3 test	P=0.036	P=0.246N	P=0.101	P=0.142

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] One adenocarcinoma occurred in an animal that also had an adenoma.

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/64 (7.8%)[e]	5/47 (10.6%)	1/45 (2.2%)	5/48 (10.4%)
Adjusted rate[b]	8.5%	11.3%	2.4%	12.1%
Terminal rate[c]	4/46 (8.7%)	3/37 (8.1%)	1/35 (2.9%)	3/25 (12.0%)
First incidence (days)	643	666	737 (T)	591
Poly-3 test[d]	P=0.528	P=0.445	P=0.199N	P=0.397
Liver: Hemangiosarcoma				
Overall rate	5/65 (7.7%)	1/48 (2.1%)	1/47 (2.1%)	0/48 (0.0%)
Adjusted rate	8.4%	2.2%	2.3%	0.0%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	0/35 (0.0%)	0/25 (0.0%)
First incidence (days)	677	732	563	—[f]
Poly-3 test	P=0.023N	P=0.181N	P=0.187N	P=0.077N
Liver: Hepatocellular Adenoma				
Overall rate	17/65 (26.2%)	9/48 (18.8%)	7/47 (14.9%)	12/48 (25.0%)
Adjusted rate	28.4%	20.1%	16.0%	29.0%
Terminal rate	12/46 (26.1%)	7/37 (18.9%)	5/35 (14.3%)	7/25 (28.0%)
First incidence (days)	658	652	568	636
Poly-3 test	P=0.407N	P=0.227N	P=0.105N	P=0.561
Liver: Hepatocellular Carcinoma				
Overall rate	12/65 (18.5%)	9/48 (18.8%)	11/47 (23.4%)	8/48 (16.7%)
Adjusted rate	19.5%	19.9%	24.7%	18.9%
Terminal rate	5/46 (10.9%)	6/37 (16.2%)	6/35 (17.1%)	2/25 (8.0%)
First incidence (days)	572	672	553	555
Poly-3 test	P=0.463	P=0.574	P=0.344	P=0.568N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/65 (38.5%)	17/48 (35.4%)	18/47 (38.3%)	19/48 (39.6%)
Adjusted rate	40.4%	37.4%	39.8%	43.8%
Terminal rate	15/46 (32.6%)	12/37 (32.4%)	11/35 (31.4%)	8/25 (32.0%)
First incidence (days)	572	652	553	555
Poly-3 test	P=0.391	P=0.453N	P=0.556N	P=0.440
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	13/65 (20.0%)	10/48 (20.8%)	11/47 (23.4%)	8/48 (16.7%)
Adjusted rate	20.9%	22.2%	24.7%	18.9%
Terminal rate	5/46 (10.9%)	7/37 (18.9%)	6/35 (17.1%)	2/25 (8.0%)
First incidence (days)	572	672	553	555
Poly-3 test	P=0.505N	P=0.534	P=0.413	P=0.495N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/65 (40.0%)	18/48 (37.5%)	18/47 (38.3%)	19/48 (39.6%)
Adjusted rate	41.7%	39.6%	39.8%	43.8%
Terminal rate	15/46 (32.6%)	13/37 (35.1%)	11/35 (31.4%)	8/25 (32.0%)
First incidence (days)	572	652	553	555
Poly-3 test	P=0.463	P=0.490N	P=0.502N	P=0.492
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/64 (9.4%)	5/47 (10.6%)	4/45 (8.9%)	5/48 (10.4%)
Adjusted rate	10.0%	11.4%	9.5%	12.2%
Terminal rate	1/46 (2.2%)	4/37 (10.8%)	3/35 (8.6%)	2/25 (8.0%)
First incidence (days)	589	732	713	636
Poly-3 test	P=0.457	P=0.540	P=0.599N	P=0.496

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/64 (10.9%)	2/47 (4.3%)	2/45 (4.4%)	1/48 (2.1%)
Adjusted rate	11.8%	4.6%	4.8%	2.5%
Terminal rate	5/46 (10.9%)	2/37 (5.4%)	2/35 (5.7%)	0/25 (0.0%)
First incidence (days)	579	733 (T)	734 (T)	712
Poly-3 test	P=0.042N	P=0.174N	P=0.192N	P=0.094N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	12/64 (18.8%)	6/47 (12.8%)	6/45 (13.3%)	6/48 (12.5%)
Adjusted rate	19.9%	13.7%	14.3%	14.5%
Terminal rate	5/46 (10.9%)	5/37 (13.5%)	5/35 (14.3%)	2/25 (8.0%)
First incidence (days)	579	732	713	636
Poly-3 test	P=0.255N	P=0.286N	P=0.321N	P=0.336N
Preputial Gland: Hemangiosarcoma				
Overall rate	1/64 (1.6%)	3/48 (6.3%)	0/44 (0.0%)	0/48 (0.0%)
Adjusted rate	1.7%	6.7%	0.0%	0.0%
Terminal rate	1/46 (2.2%)	2/37 (5.4%)	0/34 (0.0%)	0/25 (0.0%)
First incidence (days)	739 (T)	652	—	—
Poly-3 test	P=0.210N	P=0.215	P=0.572N	P=0.573N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	2/65 (3.1%)	1/47 (2.1%)	6/48 (12.5%)	8/48 (16.7%)
Adjusted rate	3.4%	2.3%	13.1%	18.9%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	1/35 (2.9%)	0/25 (0.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test	P=0.002	P=0.601N	P=0.066	P=0.011
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Fibrosarcoma				
Overall rate	2/65 (3.1%)	1/47 (2.1%)	7/48 (14.6%)	10/48 (20.8%)
Adjusted rate	3.4%	2.3%	15.3%	23.5%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	1/35 (2.9%)	0/25 (0.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test	P<0.001	P=0.601N	P=0.033	P=0.002
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, or Fibrosarcoma				
Overall rate	2/65 (3.1%)	2/47 (4.3%)	7/48 (14.6%)	12/48 (25.0%)
Adjusted rate	3.4%	4.5%	15.3%	28.2%
Terminal rate	2/46 (4.3%)	1/37 (2.7%)	1/35 (2.9%)	2/25 (8.0%)
First incidence (days)	733 (T)	687	502	574
Poly-3 test	P<0.001	P=0.585	P=0.033	P<0.001
All Organs: Hemangiosarcoma				
Overall rate	9/65 (13.8%)	5/48 (10.4%)	2/48 (4.2%)	2/50 (4.0%)
Adjusted rate	15.1%	11.1%	4.5%	4.9%
Terminal rate	6/46 (13.0%)	3/37 (8.1%)	1/35 (2.9%)	1/25 (4.0%)
First incidence (days)	677	652	563	666
Poly-3 test	P=0.027N	P=0.381N	P=0.078N	P=0.098N
All Organs: Malignant Lymphoma				
Overall rate	9/65 (13.8%)	8/48 (16.7%)	4/48 (8.3%)	4/50 (8.0%)
Adjusted rate	15.0%	17.9%	9.1%	9.7%
Terminal rate	6/46 (13.0%)	7/37 (18.9%)	3/35 (8.6%)	3/25 (12.0%)
First incidence (days)	589	672	682	555
Poly-3 test	P=0.164N	P=0.450	P=0.278N	P=0.315N

TABLE A2c
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
All Organs: Benign Neoplasms				
Overall rate	29/65 (44.6%)	20/48 (41.7%)	13/48 (27.1%)	19/50 (38.0%)
Adjusted rate	46.9%	43.8%	29.2%	44.1%
Terminal rate	18/46 (39.1%)	15/37 (40.5%)	9/35 (25.7%)	10/25 (40.0%)
First incidence (days)	579	652	568	555
Poly-3 test	P=0.206N	P=0.454N	P=0.048N	P=0.468N
All Organs: Malignant Neoplasms				
Overall rate	36/65 (55.4%)	23/48 (47.9%)	23/48 (47.9%)	22/50 (44.0%)
Adjusted rate	56.5%	50.3%	48.4%	48.8%
Terminal rate	22/46 (47.8%)	17/37 (45.9%)	11/35 (31.4%)	6/25 (24.0%)
First incidence (days)	572	652	502	555
Poly-3 test	P=0.206N	P=0.327N	P=0.256N	P=0.273N
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/65 (72.3%)	34/48 (70.8%)	30/48 (62.5%)	33/50 (66.0%)
Adjusted rate	73.0%	73.6%	63.1%	71.2%
Terminal rate	30/46 (65.2%)	26/37 (70.3%)	18/35 (51.4%)	13/25 (52.0%)
First incidence (days)	572	652	502	555
Poly-3 test	P=0.293N	P=0.561	P=0.181N	P=0.501N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] One adenocarcinoma occurred in an animal that also had an adenoma.
[f] Not applicable; no neoplasms in animal group

TABLE A2d
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/64 (7.8%)[e]	2/45 (4.4%)	7/50 (14.0%)	3/14 (21.4%)
Adjusted rate[b]	8.5%	4.8%	15.5%	27.5%
Terminal rate[c]	4/46 (8.7%)	1/37 (2.7%)	5/36 (13.9%)	2/6 (33.3%)
First incidence (days)	643	609	694	663
Poly-3 test[d]	P=0.048	P=0.374N	P=0.213	P=0.108
Liver: Hemangiosarcoma				
Overall rate	5/65 (7.7%)	1/48 (2.1%)	2/50 (4.0%)	2/15 (13.3%)
Adjusted rate	8.4%	2.3%	4.5%	17.5%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	2/36 (5.6%)	1/6 (16.7%)
First incidence (days)	677	677	737 (T)	720
Poly-3 test	P=0.559	P=0.193N	P=0.344N	P=0.354
Liver: Hepatocellular Adenoma				
Overall rate	17/65 (26.2%)	5/48 (10.4%)	7/50 (14.0%)	3/15 (20.0%)
Adjusted rate	28.4%	11.7%	15.4%	26.2%
Terminal rate	12/46 (26.1%)	4/37 (10.8%)	5/36 (13.9%)	2/6 (33.3%)
First incidence (days)	658	713	589	720
Poly-3 test	P=0.122N	P=0.035N	P=0.087N	P=0.578N
Liver: Hepatocellular Carcinoma				
Overall rate	12/65 (18.5%)	8/48 (16.7%)	9/50 (18.0%)	4/15 (26.7%)
Adjusted rate	19.5%	18.3%	19.2%	32.2%
Terminal rate	5/46 (10.9%)	4/37 (10.8%)	3/36 (8.3%)	1/6 (16.7%)
First incidence (days)	572	609	520	544
Poly-3 test	P=0.334	P=0.540N	P=0.581N	P=0.279
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	25/65 (38.5%)	10/48 (20.8%)	14/50 (28.0%)	7/15 (46.7%)
Adjusted rate	40.4%	22.9%	29.8%	55.8%
Terminal rate	15/46 (32.6%)	6/37 (16.2%)	8/36 (22.2%)	3/6 (50.0%)
First incidence (days)	572	609	520	544
Poly-3 test	P=0.460N	P=0.046N	P=0.174N	P=0.252
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	13/65 (20.0%)	8/48 (16.7%)	9/50 (18.0%)	4/15 (26.7%)
Adjusted rate	20.9%	18.3%	19.2%	32.2%
Terminal rate	5/46 (10.9%)	4/37 (10.8%)	3/36 (8.3%)	1/6 (16.7%)
First incidence (days)	572	609	520	544
Poly-3 test	P=0.409	P=0.466N	P=0.506N	P=0.320
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	26/65 (40.0%)	10/48 (20.8%)	14/50 (28.0%)	7/15 (46.7%)
Adjusted rate	41.7%	22.9%	29.8%	55.8%
Terminal rate	15/46 (32.6%)	6/37 (16.2%)	8/36 (22.2%)	3/6 (50.0%)
First incidence (days)	572	609	520	544
Poly-3 test	P=0.399N	P=0.034N	P=0.141N	P=0.279
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/64 (9.4%)	3/47 (6.4%)	4/50 (8.0%)	0/15 (0.0%)
Adjusted rate	10.0%	7.1%	8.9%	0.0%
Terminal rate	1/46 (2.2%)	3/37 (8.1%)	3/36 (8.3%)	0/6 (0.0%)
First incidence (days)	589	740 (T)	684	—[f]
Poly-3 test	P=0.285N	P=0.440N	P=0.552N	P=0.310N

TABLE A2d
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/64 (10.9%)	3/47 (6.4%)	2/50 (4.0%)	1/15 (6.7%)
Adjusted rate	11.8%	7.1%	4.4%	8.4%
Terminal rate	5/46 (10.9%)	3/37 (8.1%)	1/36 (2.8%)	0/6 (0.0%)
First incidence (days)	579	739 (T)	684	567
Poly-3 test	P=0.158N	P=0.331N	P=0.163N	P=0.560N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	12/64 (18.8%)	6/47 (12.8%)	5/50 (10.0%)	1/15 (6.7%)
Adjusted rate	19.9%	14.3%	11.1%	8.4%
Terminal rate	5/46 (10.9%)	6/37 (16.2%)	4/36 (11.1%)	0/6 (0.0%)
First incidence (days)	579	739 (T)	684	567
Poly-3 test	P=0.096N	P=0.321N	P=0.171N	P=0.308N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	2/65 (3.1%)	1/48 (2.1%)	3/51 (5.9%)	1/15 (6.7%)
Adjusted rate	3.4%	2.3%	6.4%	8.4%
Terminal rate	2/46 (4.3%)	0/37 (0.0%)	0/36 (0.0%)	0/6 (0.0%)
First incidence (days)	733 (T)	532	545	579
Poly-3 test	P=0.223	P=0.606N	P=0.395	P=0.502
Spleen: Hemangiosarcoma				
Overall rate	2/63 (3.2%)	2/45 (4.4%)	5/49 (10.2%)	0/15 (0.0%)
Adjusted rate	3.5%	4.8%	11.2%	0.0%
Terminal rate	2/46 (4.3%)	2/37 (5.4%)	4/36 (11.1%)	0/6 (0.0%)
First incidence (days)	741 (T)	745 (T)	665	—
Poly-3 test	P=0.215	P=0.568	P=0.126	P=622N
All Organs: Fibrous Histiocytoma				
Overall rate	0/65 (0.0%)	2/48 (4.2%)	0/51 (0.0%)	1/15 (6.7%)
Adjusted rate	0.0%	4.7%	0.0%	8.8%
Terminal rate	0/46 (0.0%)	2/37 (5.4%)	0/36 (0.0%)	1/6 (16.7%)
First incidence (days)	—	744 (T)	—	745 (T)
Poly-3 test	P=0.248	P=0.169	—[g]	P=0.187
All Organs: Hemangiosarcoma				
Overall rate	9/65 (13.8%)	3/48 (6.3%)	7/51 (13.7%)	2/15 (13.3%)
Adjusted rate	15.1%	7.0%	15.3%	17.5%
Terminal rate	6/46 (13.0%)	2/37 (5.4%)	6/36 (16.7%)	1/6 (16.7%)
First incidence (days)	677	677	665	720
Poly-3 test	P=0.490	P=0.169N	P=0.600	P=0.591
All Organs: Malignant Lymphoma				
Overall rate	9/65 (13.8%)	7/48 (14.6%)	5/51 (9.8%)	4/15 (26.7%)
Adjusted rate	15.0%	16.2%	10.9%	33.0%
Terminal rate	6/46 (13.0%)	5/37 (13.5%)	3/36 (8.3%)	2/6 (33.3%)
First incidence (days)	589	594	678	567
Poly-3 test	P=0.369	P=0.546	P=0.371N	P=0.150
All Organs: Benign Neoplasms				
Overall rate	29/65 (44.6%)	14/48 (29.2%)	18/51 (35.3%)	5/15 (33.3%)
Adjusted rate	46.9%	31.8%	38.6%	42.5%
Terminal rate	18/46 (39.1%)	11/37 (29.7%)	14/36 (38.9%)	3/6 (50.0%)
First incidence (days)	579	546	589	663
Poly-3 test	P=0.248N	P=0.087N	P=0.251N	P=0.517N

TABLE A2d
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
All Organs: Malignant Neoplasms				
Overall rate	36/65 (55.4%)	24/48 (50.0%)	24/51 (47.1%)	11/15 (73.3%)
Adjusted rate	56.5%	51.6%	48.8%	78.8%
Terminal rate	22/46 (47.8%)	15/37 (40.5%)	13/36 (36.1%)	4/6 (66.7%)
First incidence (days)	572	244	520	544
Poly-3 test	P=0.384	P=0.374N	P=0.264N	P=0.107
All Organs: Benign or Malignant Neoplasms				
Overall rate	47/65 (72.3%)	29/48 (60.4%)	32/51 (62.7%)	11/15 (73.3%)
Adjusted rate	73.0%	61.5%	65.0%	78.8%
Terminal rate	30/46 (65.2%)	19/37 (51.4%)	21/36 (58.3%)	4/6 (66.7%)
First incidence (days)	572	244	520	544
Poly-3 test	P=0.410N	P=0.139N	P=0.236N	P=0.456

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] One adenocarcinoma occurred in an animal that also had an adenoma.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

TABLE A3
Historical Incidence of Neoplasms in Control Male B6C3F1/Nctr BR Mice[a]

Study	Harderian Gland Adenoma	Hepatocellular Carcinoma	Skin Fibrous Histiocytoma, Fibrosarcoma, Sarcoma, or Myxosarcoma	All Organs Hemangiosarcoma
Sulfamethazine	15/184 (8.2%)	20/185 (10.8%)	0/183 (0.0%)	3/187 (1.6%)
Doxylamine	—[b]	4/48 (8.3%)	1/47 (2.1%)	0/48 (0.0%)
Pyrilamine	—	3/46 (6.5%)	0/47 (0.0%)	0/47 (0.0%)
Triprolidine	—	5/48 (10.4%)	1/48 (2.1%)	1/48 (2.1%)
Fumonisin B_1	1/46 (2.2%)	4/47 (8.5%)	6/48 (12.5%)	0/48 (0.0%)
Chloral Hydrate	4/48 (8.3%)	10/48 (20.8%)	1/47 (2.1%)	2/48 (4.2%)
Chloral Hydrate	5/47 (10.6%)	4/48 (8.3%)	0/48 (0.0%)	1/48 (2.1%)
Urethane and Ethanol	3/47 (6.4%)	7/46 (15.2%)	10/47 (21.3%)	4/48 (8.3%)
Total	28/372 (7.5%)	57/516 (11.0%)	19/515 (3.7%)	11/522 (2.1%)
Range	2.2%-10.6%	6.5%-20.8%	0.0%-21.3%	0.0%-8.3%

[a] Data as of June 9, 2009. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed.
[b] Not examined.

TABLE A4a
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT[a]

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Disposition Summary				
Animals initially in study	65	48	48	48
Early deaths				
Moribund	4	8	5	8
Natural deaths	2		3	2
Survivors				
Moribund	10		1	1
Died last week of study	1		1	1
Terminal sacrifice	46	39	38	35
Harvest	2	1		1
Animals examined microscopically	65	48	48	48
Alimentary System				
Gallbladder	(59)	(48)	(44)	(45)
Vacuolization cytoplasmic	1 (2%)			
Intestine large, cecum	(63)	(48)	(45)	(45)
Hyperplasia, lymphoid	6 (10%)	2 (4%)	1 (2%)	
Intestine large, rectum	(63)	(48)	(45)	(45)
Anus, hemorrhage		1 (2%)		
Anus, inflammation, chronic		1 (2%)		
Anus, necrosis		1 (2%)		
Intestine small, duodenum	(63)	(48)	(45)	(45)
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Inflammation, chronic active		1 (2%)		
Intestine small, ileum	(63)	(48)	(45)	(45)
Intestine small, jejunum	(62)	(48)	(44)	(45)
Hyperplasia, lymphoid	2 (3%)			
Inflammation, chronic active				1 (2%)
Liver	(65)	(48)	(47)	(46)
Basophilic focus	7 (11%)	2 (4%)	6 (13%)	4 (9%)
Clear cell focus	1 (2%)			
Cyst	1 (2%)			
Eosinophilic focus	1 (2%)	4 (8%)		1 (2%)
Fatty change			1 (2%)	
Hematopoietic cell proliferation				1 (2%)
Infiltration cellular, lymphocyte	3 (5%)	4 (8%)	3 (6%)	1 (2%)
Inflammation, chronic			1 (2%)	
Inflammation, chronic active		2 (4%)		1 (2%)
Mineralization		1 (2%)		
Necrosis		2 (4%)	2 (4%)	4 (9%)
Tension lipidosis	12 (18%)	12 (25%)	11 (23%)	10 (22%)
Vacuolization cytoplasmic	2 (3%)	2 (4%)	1 (2%)	5 (11%)
Mesentery	(4)	(2)	(1)	(3)
Hemorrhage	1 (25%)			
Necrosis	1 (25%)			
Fat, necrosis	1 (25%)	1 (50%)		1 (33%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4a
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Alimentary System (continued)				
Pancreas	(64)	(48)	(46)	(46)
Cyst	2 (3%)	1 (2%)		
Infiltration cellular, lymphocyte	7 (11%)	6 (13%)	2 (4%)	4 (9%)
Acinus, degeneration	6 (9%)	7 (15%)	5 (11%)	1 (2%)
Salivary glands	(64)	(48)	(46)	(46)
Infiltration cellular, lymphocyte	54 (84%)	45 (94%)	38 (83%)	40 (87%)
Stomach, forestomach	(64)	(48)	(46)	(46)
Hyperkeratosis			1 (2%)	
Inflammation, chronic active			1 (2%)	
Ulcer	2 (3%)			
Epithelium, hyperplasia	2 (3%)		1 (2%)	1 (2%)
Stomach, glandular	(63)	(48)	(44)	(45)
Degeneration	1 (2%)		1 (2%)	
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active	2 (3%)			
Epithelium, hyperplasia	1 (2%)			
Cardiovascular System				
Blood vessel	(65)	(48)	(47)	(47)
Polyarteritis	1 (2%)			
Heart	(65)	(48)	(48)	(47)
Cardiomyopathy	1 (2%)	2 (4%)	1 (2%)	
Inflammation	1 (2%)			
Inflammation, chronic active			1 (2%)	
Polyarteritis	2 (3%)	1 (2%)		
Endocrine System				
Adrenal cortex	(63)	(48)	(45)	(47)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)		1 (2%)
Cyst	1 (2%)			
Hypertrophy	6 (10%)	4 (8%)		2 (4%)
Subcapsular, hyperplasia	47 (75%)	38 (79%)	33 (73%)	38 (81%)
Adrenal medulla	(63)	(46)	(45)	(45)
Islets, pancreatic	(65)	(48)	(48)	(47)
Hyperplasia	7 (11%)	5 (10%)	6 (13%)	5 (11%)
Parathyroid gland	(52)	(37)	(41)	(43)
Cyst		1 (3%)	1 (2%)	
Infiltration cellular, lymphocyte		2 (5%)		
Pituitary gland	(61)	(47)	(48)	(45)
Pars distalis, cyst		7 (15%)	3 (6%)	3 (7%)
Pars distalis, hyperplasia	2 (3%)	1 (2%)		1 (2%)
Thyroid gland	(64)	(48)	(45)	(46)
Depletion		1 (2%)		
Ectopic thymus				1 (2%)
Infiltration cellular, lymphocyte	3 (5%)	1 (2%)		1 (2%)
Follicle, cyst	1 (2%)		1 (2%)	
Follicle, degeneration	10 (16%)	7 (15%)	6 (13%)	5 (11%)

TABLE A4a
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
General Body System				
Tissue NOS	(1)	(0)	(2)	(1)
Genital System				
Coagulating gland	(2)	(0)	(1)	(0)
Lumen, dilatation	2 (100%)		1 (100%)	
Epididymis	(63)	(48)	(45)	(46)
Hypospermia	2 (3%)	1 (2%)		
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)	3 (7%)	
Inflammation, chronic active	1 (2%)	1 (2%)		
Spermatocele	1 (2%)	1 (2%)		
Duct, degeneration	1 (2%)			
Preputial gland	(64)	(48)	(44)	(46)
Cyst	4 (6%)	7 (15%)	10 (23%)	2 (4%)
Degeneration	32 (50%)	17 (35%)	19 (43%)	16 (35%)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)	1 (2%)	3 (7%)
Inflammation, suppurative		2 (4%)		
Inflammation, chronic active	6 (9%)	1 (2%)	7 (16%)	3 (7%)
Duct, dilatation			1 (2%)	
Fat, degeneration				1 (2%)
Fat, necrosis		1 (2%)		
Prostate	(64)	(48)	(43)	(44)
Infiltration cellular, lymphocyte	9 (14%)	6 (13%)	8 (19%)	5 (11%)
Polyarteritis	1 (2%)			
Seminal vesicle	(63)	(48)	(46)	(46)
Atrophy	1 (2%)			1 (2%)
Infiltration cellular, lymphocyte				1 (2%)
Lumen, dilatation	8 (13%)	1 (2%)	3 (7%)	3 (7%)
Testes	(64)	(48)	(45)	(45)
Seminiferous tubule, degeneration	7 (11%)	5 (10%)	6 (13%)	2 (4%)
Hematopoietic System				
Bone marrow	(64)	(48)	(46)	(46)
Hyperplasia	6 (9%)	1 (2%)	3 (7%)	5 (11%)
Lymph node	(7)	(4)	(7)	(1)
Axillary, hyperplasia, lymphoid		2 (50%)	1 (14%)	
Axillary, infiltration cellular, histiocyte				1 (100%)
Lumbar, hemorrhage	1 (14%)			
Lumbar, hyperplasia, lymphoid	3 (43%)		1 (14%)	
Mediastinal, hyperplasia, lymphoid	2 (29%)		1 (14%)	
Mediastinal, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, hyperplasia, lymphoid	1 (14%)	1 (25%)		
Pancreatic, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, sinus, dilatation	1 (14%)			
Renal, hemorrhage	1 (14%)			
Renal, hyperplasia, lymphoid	2 (29%)		1 (14%)	
Renal, infiltration cellular, histiocyte	1 (14%)			

TABLE A4a
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(63)	(46)	(45)	(43)
Hyperplasia, lymphoid	9 (14%)	9 (20%)	9 (20%)	6 (14%)
Hyperplasia, plasma cell	1 (2%)			
Infiltration cellular, plasma cell	1 (2%)			2 (5%)
Lymph node, mesenteric	(63)	(48)	(46)	(45)
Angiectasis	10 (16%)	8 (17%)	6 (13%)	4 (9%)
Hemorrhage	19 (30%)	16 (33%)	18 (39%)	10 (22%)
Hyperplasia, lymphoid	37 (59%)	29 (60%)	28 (61%)	24 (53%)
Infiltration cellular, histiocyte	4 (6%)	3 (6%)	4 (9%)	3 (7%)
Infiltration cellular, mast cell	1 (2%)	1 (2%)		
Infiltration cellular, plasma cell	2 (3%)		1 (2%)	
Infiltration cellular, polymorphonuclear	1 (2%)			
Necrosis			1 (2%)	
Polyarteritis				1 (2%)
Thrombosis	1 (2%)	1 (2%)		
Sinus, dilatation	8 (13%)	5 (10%)	5 (11%)	5 (11%)
Spleen	(63)	(48)	(45)	(46)
Angiectasis	1 (2%)			
Hematopoietic cell proliferation	11 (17%)	7 (15%)	6 (13%)	8 (17%)
Hyperplasia, lymphoid	30 (48%)	14 (29%)	16 (36%)	13 (28%)
Thymus	(51)	(43)	(39)	(37)
Atrophy	23 (45%)	18 (42%)	19 (49%)	13 (35%)
Hyperplasia, lymphoid		2 (5%)	1 (3%)	2 (5%)
Integumentary System				
Skin	(65)	(48)	(48)	(46)
Fibrosis		1 (2%)		
Hemorrhage			1 (2%)	
Hyperkeratosis	1 (2%)			
Inflammation, suppurative	1 (2%)			1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Mineralization	1 (2%)			1 (2%)
Necrosis		1 (2%)		
Ulcer				2 (4%)
Epithelium, hyperplasia		1 (2%)		1 (2%)
Musculoskeletal System				
Skeletal muscle	(0)	(1)	(4)	(3)
Nervous System				
Brain, cerebrum	(64)	(48)	(46)	(46)
Mineralization	35 (55%)	16 (33%)	25 (54%)	26 (57%)

TABLE A4a
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Respiratory System				
Lung	(64)	(48)	(46)	(47)
Congestion	1 (2%)			1 (2%)
Crystals	3 (5%)			3 (6%)
Hemorrhage			1 (2%)	
Infiltration cellular, histiocyte	3 (5%)	1 (2%)		3 (6%)
Infiltration cellular, lymphocyte	3 (5%)	1 (2%)	1 (2%)	
Inflammation, chronic active	1 (2%)			
Alveolar epithelium, hyperplasia	4 (6%)	4 (8%)	2 (4%)	1 (2%)
Nose	(65)	(48)	(48)	(47)
Posterior to upper incisor, dysplasia	2 (3%)	4 (8%)	1 (2%)	1 (2%)
Special Senses System				
Eye	(62)	(48)	(43)	(45)
Cataract	1 (2%)	2 (4%)		1 (2%)
Bilateral, cataract	1 (2%)			1 (2%)
Cornea, inflammation, chronic active				2 (4%)
Retina, degeneration				1 (2%)
Harderian gland	(64)	(48)	(45)	(45)
Infiltration cellular, lymphocyte	5 (8%)	8 (17%)	2 (4%)	3 (7%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Acinus, degeneration		2 (4%)		
Urinary System				
Kidney	(64)	(48)	(45)	(46)
Cyst	3 (5%)	1 (2%)	3 (7%)	1 (2%)
Hyaline droplet			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	6 (9%)	3 (6%)	7 (16%)	9 (20%)
Metaplasia, osseous		2 (4%)	1 (2%)	3 (7%)
Nephropathy	54 (84%)	39 (81%)	31 (69%)	34 (74%)
Polyarteritis	1 (2%)		1 (2%)	
Pelvis, dilatation		1 (2%)		
Urethra	(1)	(0)	(1)	(1)
Dilatation				1 (100%)
Bulbourethral gland, cyst	1 (100%)			
Bulbourethral gland, hemorrhage	1 (100%)		1 (100%)	
Bulbourethral gland, necrosis	1 (100%)			
Bulbourethral gland, epithelium, hyperplasia			1 (100%)	
Urinary bladder	(65)	(48)	(45)	(46)
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)	6 (13%)	3 (7%)
Lumen, dilatation	6 (9%)	2 (4%)	3 (7%)	1 (2%)

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC[a]

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Disposition Summary				
Animals initially in study	65	51	48	48
Early deaths				
Moribund	4	7	5	6
Natural deaths	2	1	4	5
Survivors				
Moribund	10	3	1	
Died last week of study	1			
Terminal sacrifice	46	39	35	34
Harvest	2	1	3	3
Animals examined microscopically	65	51	48	48
Alimentary System				
Gallbladder	(59)	(49)	(42)	(44)
Vacuolization cytoplasmic	1 (2%)			
Epithelium, hyperplasia				1 (2%)
Intestine large, cecum	(63)	(50)	(45)	(43)
Hyperplasia, lymphoid	6 (10%)	2 (4%)	5 (11%)	2 (5%)
Intestine large, colon	(63)	(50)	(45)	(44)
Hyperplasia, lymphoid				1 (2%)
Inflammation, chronic active			1 (2%)	
Ulcer			1 (2%)	
Intestine small, duodenum	(63)	(50)	(44)	(43)
Infiltration cellular, plasma cell		1 (2%)		
Epithelium, hyperplasia			1 (2%)	
Intestine small, ileum	(63)	(50)	(45)	(43)
Hyperplasia, lymphoid				1 (2%)
Intestine small, jejunum	(62)	(50)	(46)	(43)
Hyperplasia, lymphoid	2 (3%)			
Inflammation, chronic active		1 (2%)		
Liver	(65)	(51)	(48)	(46)
Angiectasis			1 (2%)	
Basophilic focus	7 (11%)	6 (12%)	5 (10%)	4 (9%)
Basophilic focus, multiple			1 (2%)	
Clear cell focus	1 (2%)	1 (2%)		1 (2%)
Cyst	1 (2%)	1 (2%)		
Eosinophilic focus	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Eosinophilic focus, multiple				1 (2%)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation		3 (6%)		
Infiltration cellular, lymphocyte	3 (5%)	4 (8%)	4 (8%)	3 (7%)
Inflammation, chronic active		1 (2%)	1 (2%)	1 (2%)
Mixed cell focus			2 (4%)	1 (2%)
Necrosis			2 (4%)	2 (4%)
Tension lipidosis	12 (18%)	8 (16%)	6 (13%)	10 (22%)
Vacuolization cytoplasmic	2 (3%)	4 (8%)		3 (7%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Alimentary System (continued)				
Mesentery	(4)	(2)	(1)	(0)
Hemorrhage	1 (25%)			
Necrosis	1 (25%)			
Fat, necrosis	1 (25%)	2 (100%)	1 (100%)	
Pancreas	(64)	(50)	(45)	(45)
Cyst	2 (3%)		1 (2%)	
Infiltration cellular, lymphocyte	7 (11%)	6 (12%)	5 (11%)	6 (13%)
Inflammation, chronic active			1 (2%)	
Acinus, degeneration	6 (9%)	6 (12%)	5 (11%)	4 (9%)
Salivary glands	(64)	(50)	(47)	(44)
Fibrosis			1 (2%)	
Infiltration cellular, lymphocyte	54 (84%)	44 (88%)	41 (87%)	38 (86%)
Inflammation, chronic active			1 (2%)	
Stomach, forestomach	(64)	(50)	(45)	(45)
Hyperplasia				1 (2%)
Ulcer	2 (3%)	1 (2%)		1 (2%)
Epithelium, hyperplasia	2 (3%)	2 (4%)		
Stomach, glandular	(63)	(50)	(45)	(43)
Degeneration	1 (2%)			
Inflammation, chronic active	2 (3%)		1 (2%)	
Epithelium, hyperplasia	1 (2%)	2 (4%)		
Tongue	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(65)	(50)	(48)	(46)
Hemorrhage				1 (2%)
Polyarteritis	1 (2%)			
Heart	(65)	(50)	(48)	(46)
Cardiomyopathy	1 (2%)	1 (2%)	2 (4%)	
Inflammation	1 (2%)			
Polyarteritis	2 (3%)			
Ventricle, dilatation		2 (4%)		
Endocrine System				
Adrenal cortex	(63)	(50)	(46)	(45)
Accessory adrenal cortical nodule	1 (2%)		2 (4%)	3 (7%)
Cyst	1 (2%)	1 (2%)		1 (2%)
Hyperplasia			2 (4%)	
Hypertrophy	6 (10%)	3 (6%)	4 (9%)	1 (2%)
Subcapsular, hyperplasia	47 (75%)	37 (74%)	36 (78%)	34 (76%)
Adrenal medulla	(63)	(49)	(46)	(43)
Islets, pancreatic	(65)	(50)	(44)	(45)
Hyperplasia	7 (11%)	12 (24%)	9 (20%)	8 (18%)
Parathyroid gland	(52)	(45)	(37)	(41)
Infiltration cellular, lymphocyte		1 (2%)	1 (3%)	
Pituitary gland	(61)	(50)	(44)	(45)
Pars distalis, cyst		1 (2%)	3 (7%)	7 (16%)
Pars distalis, hyperplasia	2 (3%)	3 (6%)	1 (2%)	

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Endocrine System (continued)				
Thyroid gland	(64)	(50)	(46)	(46)
Ectopic thymus			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	3 (5%)			
Inflammation, chronic active			1 (2%)	
Polyarteritis		1 (2%)		
Follicle, cyst	1 (2%)			
Follicle, degeneration	10 (16%)	4 (8%)	6 (13%)	3 (7%)
General Body System				
Tissue NOS	(1)	(1)	(1)	(3)
Genital System				
Coagulating gland	(2)	(2)	(0)	(1)
Lumen, dilatation	2 (100%)	2 (100%)		1 (100%)
Epididymis	(63)	(50)	(46)	(45)
Fibrosis		1 (2%)		
Hypospermia	2 (3%)	4 (8%)	1 (2%)	
Infiltration cellular, lymphocyte	3 (5%)	1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic active	1 (2%)		1 (2%)	
Polyarteritis				1 (2%)
Spermatocele	1 (2%)		1 (2%)	
Duct, degeneration	1 (2%)			
Preputial gland	(64)	(50)	(46)	(44)
Cyst	4 (6%)	5 (10%)	2 (4%)	1 (2%)
Degeneration	32 (50%)	27 (54%)	21 (46%)	19 (43%)
Infiltration cellular, lymphocyte	1 (2%)		1 (2%)	1 (2%)
Inflammation, suppurative				2 (5%)
Inflammation, chronic active	6 (9%)	1 (2%)	6 (13%)	4 (9%)
Bilateral, cyst			1 (2%)	
Duct, dilatation		1 (2%)		
Prostate	(64)	(50)	(46)	(44)
Infiltration cellular, lymphocyte	9 (14%)	9 (18%)	4 (9%)	7 (16%)
Inflammation, chronic active			1 (2%)	
Polyarteritis	1 (2%)			
Seminal vesicle	(63)	(50)	(45)	(45)
Atrophy	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic active			1 (2%)	
Lumen, dilatation	8 (13%)	2 (4%)	3 (7%)	5 (11%)
Testes	(64)	(50)	(45)	(44)
Seminiferous tubule, degeneration	7 (11%)	9 (18%)	5 (11%)	3 (7%)
Hematopoietic System				
Bone marrow	(64)	(51)	(46)	(44)
Fibrosis			1 (2%)	
Hyperplasia	6 (9%)	3 (6%)	2 (4%)	1 (2%)

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Hematopoietic System (continued)				
Lymph node	(7)	(3)	(4)	(3)
Lumbar, hemorrhage	1 (14%)			
Lumbar, hyperplasia, lymphoid	3 (43%)			1 (33%)
Mediastinal, hyperplasia, lymphoid	2 (29%)			
Mediastinal, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, hyperplasia, lymphoid	1 (14%)		1 (25%)	
Pancreatic, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, sinus, dilatation	1 (14%)			
Renal, hemorrhage	1 (14%)			
Renal, hyperplasia, lymphoid	2 (29%)			1 (33%)
Renal, infiltration cellular, histiocyte	1 (14%)			
Lymph node, mandibular	(63)	(50)	(46)	(45)
Hyperplasia, lymphoid	9 (14%)	9 (18%)	4 (9%)	9 (20%)
Hyperplasia, plasma cell	1 (2%)			
Infiltration cellular, mast cell			1 (2%)	
Infiltration cellular, plasma cell	1 (2%)			1 (2%)
Lymph node, mesenteric	(63)	(50)	(46)	(44)
Angiectasis	10 (16%)	5 (10%)	4 (9%)	7 (16%)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	19 (30%)	7 (14%)	13 (28%)	15 (34%)
Hyperplasia, lymphoid	37 (59%)	34 (68%)	23 (50%)	23 (52%)
Infiltration cellular, histiocyte	4 (6%)	4 (8%)	2 (4%)	3 (7%)
Infiltration cellular, mast cell	1 (2%)		1 (2%)	1 (2%)
Infiltration cellular, plasma cell	2 (3%)	1 (2%)	2 (4%)	2 (5%)
Infiltration cellular, polymorphonuclear	1 (2%)	1 (2%)	3 (7%)	1 (2%)
Thrombosis	1 (2%)			
Sinus, dilatation	8 (13%)	4 (8%)	4 (9%)	9 (20%)
Spleen	(63)	(50)	(47)	(45)
Angiectasis	1 (2%)			
Congestion				1 (2%)
Hematopoietic cell proliferation	11 (17%)	11 (22%)	6 (13%)	9 (20%)
Hyperplasia, lymphoid	30 (48%)	19 (38%)	17 (36%)	20 (44%)
Inflammation, chronic active			1 (2%)	
Thymus	(51)	(48)	(43)	(38)
Atrophy	23 (45%)	25 (52%)	20 (47%)	21 (55%)
Cyst		1 (2%)		
Hyperplasia, lymphoid		3 (6%)		2 (5%)
Integumentary System				
Skin	(65)	(50)	(48)	(46)
Fibrosis		1 (2%)	1 (2%)	
Hyperkeratosis	1 (2%)			
Inflammation, suppurative	1 (2%)			1 (2%)
Inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Metaplasia, osseous			1 (2%)	
Mineralization	1 (2%)			1 (2%)
Ulcer			1 (2%)	2 (4%)
Epithelium, hyperplasia				1 (2%)

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Musculoskeletal System				
Skeletal muscle	(0)	(0)	(1)	(0)
Nervous System				
Brain, cerebellum	(64)	(50)	(45)	(46)
Autolysis				1 (2%)
Hemorrhage				1 (2%)
Brain, cerebrum	(64)	(50)	(45)	(45)
Degeneration			1 (2%)	
Gliosis			1 (2%)	
Mineralization	35 (55%)	29 (58%)	22 (49%)	18 (40%)
Respiratory System				
Lung	(64)	(50)	(47)	(48)
Autolysis				1 (2%)
Congestion	1 (2%)			
Crystals	3 (5%)	4 (8%)	3 (6%)	
Hemorrhage		1 (2%)		
Infiltration cellular, histiocyte	3 (5%)	6 (12%)	5 (11%)	
Infiltration cellular, lymphocyte	3 (5%)	3 (6%)	5 (11%)	4 (8%)
Inflammation, chronic active	1 (2%)		1 (2%)	
Pigmentation		1 (2%)		
Alveolar epithelium, hyperplasia	4 (6%)	4 (8%)	2 (4%)	
Nose	(65)	(51)	(46)	(46)
Inflammation, chronic active			1 (2%)	
Mucosa, dysplasia			1 (2%)	
Posterior to upper incisor, dysplasia	2 (3%)	6 (12%)	1 (2%)	
Special Senses System				
Eye	(62)	(50)	(45)	(43)
Cataract	1 (2%)	2 (4%)	1 (2%)	
Bilateral, cataract	1 (2%)	1 (2%)	1 (2%)	
Cornea, inflammation, suppurative		1 (2%)		
Cornea, ulcer		1 (2%)		
Retina, degeneration		1 (2%)		
Harderian gland	(64)	(50)	(45)	(45)
Cyst		1 (2%)		
Hyperplasia			1 (2%)	
Infiltration cellular, lymphocyte	5 (8%)	3 (6%)	5 (11%)	3 (7%)
Infiltration cellular, polymorphonuclear			1 (2%)	
Inflammation, chronic active	1 (2%)		1 (2%)	
Acinus, degeneration		1 (2%)	1 (2%)	

TABLE A4b
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Urinary System				
Kidney	(64)	(50)	(46)	(44)
Amyloid deposition		1 (2%)		
Cyst	3 (5%)		3 (7%)	2 (5%)
Fibrosis			1 (2%)	
Infiltration cellular, lymphocyte	6 (9%)	3 (6%)	6 (13%)	5 (11%)
Inflammation, suppurative				1 (2%)
Inflammation, chronic active			1 (2%)	
Necrosis				1 (2%)
Nephropathy	54 (84%)	42 (84%)	32 (70%)	33 (75%)
Polyarteritis	1 (2%)			1 (2%)
Pelvis, dilatation			1 (2%)	
Urethra	(1)	(0)	(0)	(0)
Bulbourethral gland, cyst	1 (100%)			
Bulbourethral gland, hemorrhage	1 (100%)			
Bulbourethral gland, necrosis	1 (100%)			
Urinary bladder	(65)	(50)	(46)	(45)
Infiltration cellular, lymphocyte	3 (5%)	8 (16%)	5 (11%)	5 (11%)
Inflammation, chronic active			1 (2%)	
Lumen, dilatation	6 (9%)	2 (4%)	2 (4%)	1 (2%)

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP[a]

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Disposition Summary				
Animals initially in study	65	48	48	50
Early deaths				
Moribund	4	8	10	18
Natural deaths	2	1	2	2
Survivors				
Moribund	10			5
Died last week of study	1	1	1	
Terminal sacrifice	46	37	35	25
Harvest	2	1		
Animals examined microscopically	65	48	48	50
Alimentary System				
Gallbladder	(59)	(45)	(44)	(47)
Vacuolization cytoplasmic	1 (2%)			
Intestine large, cecum	(63)	(47)	(45)	(48)
Hyperplasia, lymphoid	6 (10%)	6 (13%)	1 (2%)	
Intestine large, rectum	(63)	(47)	(45)	(47)
Intestine small, duodenum	(63)	(47)	(45)	(48)
Infiltration cellular, polymorphonuclear		1 (2%)		
Epithelium, hyperplasia		1 (2%)		
Intestine small, ileum	(63)	(47)	(45)	(48)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)		
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active			1 (2%)	
Intestine small, jejunum	(62)	(47)	(45)	(48)
Hyperplasia, lymphoid	2 (3%)	1 (2%)	1 (2%)	
Liver	(65)	(48)	(47)	(48)
Basophilic focus	7 (11%)	5 (10%)	5 (11%)	4 (8%)
Basophilic focus, multiple		1 (2%)		1 (2%)
Cholangiofibrosis		1 (2%)		
Clear cell focus	1 (2%)			
Cyst	1 (2%)			
Cyst multilocular				1 (2%)
Eosinophilic focus	1 (2%)	4 (8%)	2 (4%)	
Focal cellular change		1 (2%)		
Hepatodiaphragmatic nodule			1 (2%)	
Infiltration cellular, lymphocyte	3 (5%)	3 (6%)	4 (9%)	1 (2%)
Inflammation, chronic				1 (2%)
Inflammation, chronic active		1 (2%)		
Necrosis		1 (2%)		2 (4%)
Tension lipidosis	12 (18%)	10 (21%)	11 (23%)	8 (17%)
Vacuolization cytoplasmic	2 (3%)	2 (4%)	1 (2%)	2 (4%)
Oval cell, hyperplasia		1 (2%)		
Mesentery	(4)	(1)	(0)	(1)
Hemorrhage	1 (25%)			
Necrosis	1 (25%)			1 (100%)
Fat, necrosis	1 (25%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Alimentary System (continued)				
Pancreas	(64)	(47)	(45)	(48)
Cyst	2 (3%)	1 (2%)		
Cytoplasmic alteration		1 (2%)		
Edema		1 (2%)		
Infiltration cellular, lymphocyte	7 (11%)	3 (6%)	4 (9%)	7 (15%)
Inflammation, chronic active		1 (2%)		
Acinus, degeneration	6 (9%)	5 (11%)	3 (7%)	2 (4%)
Salivary glands	(64)	(47)	(45)	(48)
Infiltration cellular, lymphocyte	54 (84%)	39 (83%)	35 (78%)	38 (79%)
Mineralization				1 (2%)
Stomach, forestomach	(64)	(48)	(45)	(48)
Hyperkeratosis		1 (2%)		
Ulcer	2 (3%)	1 (2%)		
Epithelium, hyperplasia	2 (3%)	2 (4%)		3 (6%)
Stomach, glandular	(63)	(47)	(45)	(47)
Degeneration	1 (2%)			
Infiltration cellular, polymorphonuclear		2 (4%)		
Inflammation, chronic active	2 (3%)	1 (2%)		
Epithelium, hyperplasia	1 (2%)	1 (2%)		3 (6%)
Glands, hyperplasia		1 (2%)		
Cardiovascular System				
Blood vessel	(65)	(47)	(47)	(48)
Polyarteritis	1 (2%)			
Heart	(65)	(47)	(47)	(48)
Cardiomyopathy	1 (2%)	2 (4%)		
Inflammation	1 (2%)			
Inflammation, chronic active				1 (2%)
Polyarteritis	2 (3%)			
Endocrine System				
Adrenal cortex	(63)	(47)	(45)	(47)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)	4 (9%)	3 (6%)
Cyst	1 (2%)			
Depletion				1 (2%)
Hypertrophy	6 (10%)	3 (6%)	2 (4%)	1 (2%)
Inflammation, chronic active		1 (2%)		
Subcapsular, hyperplasia	47 (75%)	38 (81%)	37 (82%)	33 (70%)
Adrenal medulla	(63)	(46)	(44)	(46)
Hyperplasia				1 (2%)
Islets, pancreatic	(65)	(47)	(45)	(48)
Hyperplasia	7 (11%)	8 (17%)	9 (20%)	
Parathyroid gland	(52)	(38)	(33)	(34)
Cyst		1 (3%)		
Infiltration cellular, lymphocyte			2 (6%)	
Pituitary gland	(61)	(46)	(44)	(48)
Pars distalis, cyst		2 (4%)		
Pars distalis, hyperplasia	2 (3%)	1 (2%)	2 (5%)	1 (2%)
Thyroid gland	(64)	(48)	(45)	(48)
Ectopic thymus		1 (2%)		
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)		1 (2%)
Follicle, cyst	1 (2%)			1 (2%)
Follicle, degeneration	10 (16%)	5 (10%)	3 (7%)	2 (4%)

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
General Body System				
Tissue NOS	(1)	(2)	(0)	(0)
Abdominal, fibrosis		1 (50%)		
Abdominal, infiltration cellular, polymorphonuclear		1 (50%)		
Abdominal, inflammation, granulomatous		1 (50%)		
Abdominal, inflammation, chronic active		1 (50%)		
Genital System				
Coagulating gland	(2)	(1)	(1)	(0)
Lumen, dilatation	2 (100%)	1 (100%)	1 (100%)	
Epididymis	(63)	(47)	(45)	(48)
Atrophy		1 (2%)		
Fibrosis		1 (2%)		
Hypospermia	2 (3%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	3 (5%)	4 (9%)	3 (7%)	
Inflammation, chronic		1 (2%)		
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	
Mineralization			1 (2%)	
Spermatocele	1 (2%)	2 (4%)	1 (2%)	
Duct, degeneration	1 (2%)		1 (2%)	
Preputial gland	(64)	(48)	(44)	(48)
Cyst	4 (6%)	3 (6%)	8 (18%)	5 (10%)
Degeneration	32 (50%)	27 (56%)	22 (50%)	14 (29%)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)		1 (2%)
Inflammation, suppurative		3 (6%)	1 (2%)	
Inflammation, chronic active	6 (9%)	2 (4%)	3 (7%)	2 (4%)
Prostate	(64)	(47)	(43)	(48)
Infiltration cellular, lymphocyte	9 (14%)	4 (9%)	2 (5%)	3 (6%)
Inflammation, suppurative		1 (2%)		
Polyarteritis	1 (2%)			
Seminal vesicle	(63)	(48)	(45)	(49)
Amyloid deposition		1 (2%)		
Atrophy	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic active		1 (2%)		
Lumen, dilatation	8 (13%)	7 (15%)	1 (2%)	3 (6%)
Testes	(64)	(47)	(45)	(49)
Mineralization				1 (2%)
Seminiferous tubule, degeneration	7 (11%)	10 (21%)	7 (16%)	4 (8%)

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Hematopoietic System				
Bone marrow	(64)	(48)	(45)	(48)
Hyperplasia	6 (9%)		1 (2%)	3 (6%)
Pigmentation		1 (2%)		
Lymph node	(7)	(3)	(2)	(6)
Axillary, hyperplasia, lymphoid		1 (33%)	1 (50%)	
Axillary, infiltration cellular, plasma cell		1 (33%)		
Axillary, infiltration cellular, polymorphonuclear		1 (33%)		
Inguinal, hyperplasia, lymphoid		1 (33%)	1 (50%)	2 (33%)
Inguinal, infiltration cellular, plasma cell		1 (33%)		
Inguinal, infiltration cellular, polymorphonuclear		1 (33%)		
Inguinal, pigmentation				1 (17%)
Lumbar, hemorrhage	1 (14%)			
Lumbar, hyperplasia, lymphoid	3 (43%)	1 (33%)	1 (50%)	1 (17%)
Lumbar, infiltration cellular, plasma cell		1 (33%)		
Mediastinal, hyperplasia, lymphoid	2 (29%)			
Mediastinal, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, hyperplasia, lymphoid	1 (14%)			
Pancreatic, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, sinus, dilatation	1 (14%)			
Renal, hemorrhage	1 (14%)			
Renal, hyperplasia, lymphoid	2 (29%)	1 (33%)	1 (50%)	
Renal, infiltration cellular, histiocyte	1 (14%)			
Renal, infiltration cellular, plasma cell		1 (33%)		
Renal, infiltration cellular, polymorphonuclear		1 (33%)		
Lymph node, mandibular	(63)	(46)	(45)	(47)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	9 (14%)	10 (22%)	13 (29%)	4 (9%)
Hyperplasia, plasma cell	1 (2%)			
Infiltration cellular, plasma cell	1 (2%)	3 (7%)	2 (4%)	
Infiltration cellular, polymorphonuclear		1 (2%)		
Necrosis				1 (2%)
Pigmentation				1 (2%)
Lymph node, mesenteric	(63)	(46)	(45)	(48)
Angiectasis	10 (16%)	10 (22%)	11 (24%)	6 (13%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage	19 (30%)	13 (28%)	13 (29%)	15 (31%)
Hyperplasia, lymphoid	37 (59%)	29 (63%)	21 (47%)	19 (40%)
Infiltration cellular, histiocyte	4 (6%)	2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, mast cell	1 (2%)			1 (2%)
Infiltration cellular, plasma cell	2 (3%)	3 (7%)	1 (2%)	2 (4%)
Infiltration cellular, polymorphonuclear	1 (2%)	2 (4%)		1 (2%)
Inflammation, chronic active		1 (2%)		
Necrosis				1 (2%)
Thrombosis	1 (2%)		1 (2%)	
Sinus, dilatation	8 (13%)	8 (17%)	5 (11%)	5 (10%)

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Hematopoietic System (continued)				
Spleen	(63)	(47)	(45)	(48)
Angiectasis	1 (2%)			
Atrophy				1 (2%)
Depletion lymphoid		1 (2%)		
Hematopoietic cell proliferation	11 (17%)	7 (15%)	8 (18%)	12 (25%)
Hyperplasia, lymphoid	30 (48%)	20 (43%)	20 (44%)	20 (42%)
Thymus	(51)	(39)	(37)	(38)
Atrophy	23 (45%)	18 (46%)	19 (51%)	17 (45%)
Hyperplasia, lymphoid				1 (3%)
Infiltration cellular, plasma cell		1 (3%)		
Infiltration cellular, polymorphonuclear		1 (3%)		
Inflammation, chronic active		1 (3%)		
Necrosis				1 (3%)
Integumentary System				
Skin	(65)	(47)	(48)	(48)
Fibrosis		2 (4%)		2 (4%)
Hyperkeratosis	1 (2%)			
Inflammation, suppurative	1 (2%)			2 (4%)
Inflammation, chronic active	1 (2%)		1 (2%)	3 (6%)
Mineralization	1 (2%)			
Ulcer				5 (10%)
Epithelium, hyperplasia			1 (2%)	2 (4%)
Musculoskeletal System				
None				
Nervous System				
Brain, cerebrum	(64)	(47)	(47)	(48)
Mineralization	35 (55%)	20 (43%)	27 (57%)	15 (31%)
Respiratory System				
Lung	(64)	(47)	(45)	(48)
Congestion	1 (2%)			
Crystals	3 (5%)	1 (2%)	1 (2%)	
Infiltration cellular, histiocyte	3 (5%)		1 (2%)	
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)	2 (4%)	3 (6%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Alveolar epithelium, hyperplasia	4 (6%)	2 (4%)	2 (4%)	1 (2%)
Nose	(65)	(47)	(46)	(49)
Posterior to upper incisor, dysplasia	2 (3%)	2 (4%)	2 (4%)	

TABLE A4c
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Special Senses System				
Eye	(62)	(47)	(45)	(48)
Cataract	1 (2%)	2 (4%)		
Degeneration			1 (2%)	
Bilateral, cataract	1 (2%)	1 (2%)		
Cornea, inflammation, chronic active		1 (2%)		
Harderian gland	(64)	(47)	(45)	(48)
Cyst multilocular				1 (2%)
Infiltration cellular, lymphocyte	5 (8%)	1 (2%)	2 (4%)	9 (19%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Acinus, degeneration		1 (2%)		
Urinary System				
Kidney	(64)	(47)	(45)	(48)
Amyloid deposition		1 (2%)		
Cyst	3 (5%)	1 (2%)	3 (7%)	3 (6%)
Infiltration cellular, lymphocyte	6 (9%)	2 (4%)	9 (20%)	7 (15%)
Inflammation, chronic active		1 (2%)		
Nephropathy	54 (84%)	38 (81%)	31 (69%)	32 (67%)
Polyarteritis	1 (2%)			
Pelvis, dilatation		1 (2%)	1 (2%)	
Urethra	(1)	(0)	(0)	(0)
Bulbourethral gland, cyst	1 (100%)			
Bulbourethral gland, hemorrhage	1 (100%)			
Bulbourethral gland, necrosis	1 (100%)			
Urinary bladder	(65)	(47)	(46)	(48)
Infiltration cellular, lymphocyte	3 (5%)	7 (15%)	11 (24%)	7 (15%)
Inflammation, chronic active		1 (2%)		
Lumen, dilatation	6 (9%)	1 (2%)	1 (2%)	3 (6%)

TABLE A4d
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV[a]

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Disposition Summary				
Animals initially in study	65	48	51	15
Early deaths				
Moribund	4	6	5	6
Natural deaths	2	3	2	1
Survivors				
Moribund	10	1	5	
Died last week of study	1		1	
Terminal sacrifice	46	37	36	6
Harvest	2	1	2	2
Animals examined microscopically	65	48	51	15
Alimentary System				
Gallbladder	(59)	(45)	(47)	(13)
Vacuolization cytoplasmic	1 (2%)			
Intestine large, cecum	(63)	(45)	(48)	(14)
Hyperplasia, lymphoid	6 (10%)	1 (2%)	2 (4%)	2 (14%)
Intestine small, duodenum	(63)	(45)	(48)	(14)
Hyperplasia, lymphoid			1 (2%)	
Intestine small, ileum	(63)	(45)	(48)	(14)
Hyperplasia, lymphoid			1 (2%)	
Intestine small, jejunum	(62)	(45)	(48)	(14)
Hyperplasia, lymphoid	2 (3%)			1 (7%)
Liver	(65)	(48)	(50)	(15)
Basophilic focus	7 (11%)	2 (4%)	3 (6%)	
Basophilic focus, multiple		1 (2%)		
Clear cell focus	1 (2%)		2 (4%)	
Clear cell focus, multiple			1 (2%)	
Cyst	1 (2%)			
Eosinophilic focus	1 (2%)	2 (4%)	1 (2%)	1 (7%)
Fibrosis		1 (2%)		
Infiltration cellular, lymphocyte	3 (5%)	6 (13%)	3 (6%)	3 (20%)
Inflammation, chronic active		3 (6%)	2 (4%)	
Mixed cell focus			1 (2%)	
Necrosis		2 (4%)	2 (4%)	
Tension lipidosis	12 (18%)	13 (27%)	7 (14%)	
Vacuolization cytoplasmic	2 (3%)	2 (4%)	1 (2%)	
Mesentery	(4)	(0)	(2)	(0)
Hemorrhage	1 (25%)			
Necrosis	1 (25%)			
Fat, necrosis	1 (25%)		1 (50%)	
Pancreas	(64)	(45)	(49)	(15)
Cyst	2 (3%)			
Fibrosis		1 (2%)	1 (2%)	
Infiltration cellular, lymphocyte	7 (11%)	4 (9%)	6 (12%)	1 (7%)
Inflammation, chronic active		2 (4%)	1 (2%)	
Acinus, degeneration	6 (9%)	5 (11%)	4 (8%)	2 (13%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4d
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Alimentary System (continued)				
Salivary glands	(64)	(46)	(50)	(15)
Infiltration cellular, lymphocyte	54 (84%)	38 (83%)	40 (80%)	11 (73%)
Inflammation, chronic active		1 (2%)		
Stomach, forestomach	(64)	(45)	(50)	(15)
Cyst epithelial inclusion		1 (2%)		
Ulcer	2 (3%)			
Epithelium, hyperplasia	2 (3%)		1 (2%)	
Stomach, glandular	(63)	(45)	(48)	(14)
Degeneration	1 (2%)	1 (2%)		
Inflammation, chronic active	2 (3%)			1 (7%)
Necrosis		1 (2%)		
Ulcer				1 (7%)
Epithelium, hyperplasia	1 (2%)		1 (2%)	1 (7%)
Cardiovascular System				
Blood vessel	(65)	(48)	(50)	(15)
Polyarteritis	1 (2%)			1 (7%)
Heart	(65)	(48)	(50)	(15)
Cardiomyopathy	1 (2%)	1 (2%)		
Inflammation	1 (2%)			
Necrosis		1 (2%)		
Polyarteritis	2 (3%)			1 (7%)
Ventricle, dilatation		1 (2%)		
Endocrine System				
Adrenal cortex	(63)	(45)	(49)	(15)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)		
Cyst	1 (2%)			
Hypertrophy	6 (10%)	4 (9%)	2 (4%)	
Inflammation, chronic active		1 (2%)		
Subcapsular, hyperplasia	47 (75%)	36 (80%)	29 (59%)	12 (80%)
Adrenal medulla	(63)	(44)	(47)	(13)
Hyperplasia			1 (2%)	
Islets, pancreatic	(65)	(45)	(50)	(15)
Hyperplasia	7 (11%)	6 (13%)	9 (18%)	1 (7%)
Pituitary gland	(61)	(46)	(50)	(15)
Pars distalis, cyst		1 (2%)	2 (4%)	
Pars distalis, hyperplasia	2 (3%)	2 (4%)	1 (2%)	
Thyroid gland	(64)	(46)	(50)	(15)
Infiltration cellular, lymphocyte	3 (5%)	1 (2%)	1 (2%)	1 (7%)
Polyarteritis				1 (7%)
Follicle, cyst	1 (2%)	2 (4%)		1 (7%)
Follicle, degeneration	10 (16%)	1 (2%)	6 (12%)	2 (13%)
Follicular cell, hyperplasia			1 (2%)	
General Body System				
Tissue NOS	(1)	(2)	(1)	(2)

TABLE A4d
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Genital System				
Coagulating gland	(2)	(1)	(0)	(0)
Lumen, dilatation	2 (100%)	1 (100%)		
Epididymis	(63)	(45)	(50)	(15)
Hypospermia	2 (3%)	1 (2%)	1 (2%)	1 (7%)
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)	1 (2%)	
Inflammation, chronic active	1 (2%)	1 (2%)		
Polyarteritis				1 (7%)
Spermatocele	1 (2%)			
Duct, degeneration	1 (2%)			
Preputial gland	(64)	(47)	(50)	(15)
Cyst	4 (6%)	2 (4%)	4 (8%)	2 (13%)
Degeneration	32 (50%)	21 (45%)	20 (40%)	6 (40%)
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)		
Inflammation, chronic active	6 (9%)	2 (4%)	7 (14%)	1 (7%)
Bilateral, cyst			1 (2%)	
Prostate	(64)	(44)	(48)	(15)
Dilatation			2 (4%)	
Infiltration cellular, lymphocyte	9 (14%)	3 (7%)	9 (19%)	3 (20%)
Inflammation, chronic active		1 (2%)		
Polyarteritis	1 (2%)			1 (7%)
Seminal vesicle	(63)	(46)	(49)	(15)
Atrophy	1 (2%)	1 (2%)		
Inflammation, chronic active		1 (2%)		
Lumen, dilatation	8 (13%)	5 (11%)	3 (6%)	
Testes	(64)	(45)	(49)	(15)
Seminiferous tubule, degeneration	7 (11%)	3 (7%)	6 (12%)	2 (13%)
Hematopoietic System				
Bone marrow	(64)	(45)	(50)	(15)
Hyperplasia	6 (9%)		2 (4%)	1 (7%)
Lymph node	(7)	(4)	(3)	(3)
Hemorrhage			1 (33%)	
Inguinal, hyperplasia, lymphoid			1 (33%)	1 (33%)
Lumbar, hemorrhage	1 (14%)			
Lumbar, hyperplasia, lymphoid	3 (43%)			
Mediastinal, hyperplasia, lymphoid	2 (29%)			
Mediastinal, infiltration cellular, histiocyte	1 (14%)			
Mediastinal, inflammation, chronic active		1 (25%)		
Pancreatic, hyperplasia, lymphoid	1 (14%)			
Pancreatic, infiltration cellular, histiocyte	1 (14%)			
Pancreatic, sinus, dilatation	1 (14%)			
Renal, hemorrhage	1 (14%)			
Renal, hyperplasia, lymphoid	2 (29%)		1 (33%)	
Renal, infiltration cellular, histiocyte	1 (14%)			
Renal, inflammation, chronic active		1 (25%)		

TABLE A4d
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(63)	(46)	(49)	(14)
Hyperplasia, lymphoid	9 (14%)	4 (9%)	10 (20%)	2 (14%)
Hyperplasia, plasma cell	1 (2%)			
Infiltration cellular, plasma cell	1 (2%)	2 (4%)		
Inflammation, chronic active		1 (2%)		
Lymph node, mesenteric	(63)	(46)	(48)	(14)
Angiectasis	10 (16%)	6 (13%)	12 (25%)	4 (29%)
Fibrosis		1 (2%)		
Hemorrhage	19 (30%)	11 (24%)	16 (33%)	1 (7%)
Hyperplasia, lymphoid	37 (59%)	25 (54%)	31 (65%)	6 (43%)
Infiltration cellular, histiocyte	4 (6%)	1 (2%)	3 (6%)	
Infiltration cellular, mast cell	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, plasma cell	2 (3%)	1 (2%)	3 (6%)	2 (14%)
Infiltration cellular, polymorphonuclear	1 (2%)			
Inflammation, granulomatous		1 (2%)		
Inflammation, chronic active		1 (2%)		
Polyarteritis				1 (7%)
Thrombosis	1 (2%)		1 (2%)	
Sinus, dilatation	8 (13%)	8 (17%)	5 (10%)	
Spleen	(63)	(45)	(49)	(15)
Accessory spleen			1 (2%)	
Angiectasis	1 (2%)			
Hematopoietic cell proliferation	11 (17%)	5 (11%)	10 (20%)	8 (53%)
Hyperplasia, lymphoid	30 (48%)	21 (47%)	24 (49%)	4 (27%)
Inflammation, chronic active		1 (2%)		
Thymus	(51)	(35)	(44)	(12)
Atrophy	23 (45%)	15 (43%)	14 (32%)	8 (67%)
Integumentary System				
Skin	(65)	(48)	(51)	(15)
Fibrosis		1 (2%)	1 (2%)	
Hyperkeratosis	1 (2%)			
Inflammation, suppurative	1 (2%)	1 (2%)		
Inflammation, chronic active	1 (2%)		1 (2%)	1 (7%)
Mineralization	1 (2%)			
Necrosis				1 (7%)
Ulcer		1 (2%)		
Epithelium, hyperplasia			1 (2%)	
Musculoskeletal System				
Bone	(0)	(1)	(0)	(0)
Skeletal muscle	(0)	(0)	(1)	(0)
Nervous System				
Brain, cerebrum	(64)	(46)	(50)	(15)
Mineralization	35 (55%)	23 (50%)	17 (34%)	3 (20%)
Polyarteritis				1 (7%)

TABLE A4d
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Respiratory System				
Lung	(64)	(47)	(50)	(15)
Congestion	1 (2%)		1 (2%)	
Crystals	3 (5%)	2 (4%)	2 (4%)	1 (7%)
Infiltration cellular, histiocyte	3 (5%)	2 (4%)	2 (4%)	2 (13%)
Infiltration cellular, lymphocyte	3 (5%)	4 (9%)	3 (6%)	3 (20%)
Inflammation, chronic active	1 (2%)	1 (2%)		
Alveolar epithelium, hyperplasia	4 (6%)		1 (2%)	2 (13%)
Nose	(65)	(47)	(51)	(15)
Posterior to upper incisor, dysplasia	2 (3%)	4 (9%)	1 (2%)	
Special Senses System				
Eye	(62)	(45)	(49)	(14)
Cataract	1 (2%)			
Bilateral, cataract	1 (2%)			
Cornea, inflammation, chronic active		1 (2%)		
Cornea, ulcer		1 (2%)		
Harderian gland	(64)	(45)	(50)	(14)
Foreign body				1 (7%)
Infiltration cellular, lymphocyte	5 (8%)	3 (7%)	4 (8%)	3 (21%)
Inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	2 (14%)
Acinus, degeneration			1 (2%)	
Urinary System				
Kidney	(64)	(46)	(49)	(14)
Cyst	3 (5%)	3 (7%)		
Fibrosis		1 (2%)		
Infiltration cellular, lymphocyte	6 (9%)	5 (11%)	3 (6%)	1 (7%)
Inflammation, chronic active		2 (4%)		
Metaplasia, osseous		3 (7%)	1 (2%)	1 (7%)
Nephropathy	54 (84%)	35 (76%)	41 (84%)	12 (86%)
Polyarteritis	1 (2%)			1 (7%)
Pelvis, dilatation		1 (2%)		
Urethra	(1)	(2)	(0)	(0)
Bulbourethral gland, cyst	1 (100%)			
Bulbourethral gland, hemorrhage	1 (100%)	1 (50%)		
Bulbourethral gland, necrosis	1 (100%)			
Urinary bladder	(65)	(46)	(50)	(15)
Infiltration cellular, lymphocyte	3 (5%)	2 (4%)	4 (8%)	2 (13%)
Polyarteritis				1 (7%)
Lumen, dilatation	6 (9%)	2 (4%)	5 (10%)	2 (13%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE B6C3F1 MICE IN THE 2-YEAR TRANSPLACENTAL STUDY OF 3′-AZIDO-3′-DEOXYTHYMIDINE, LAMIVUDINE, NEVIRAPINE, AND NELFINAVIR MESYLATE

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TABLE B1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT[a]

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Disposition Summary				
Animals initially in study	64	48	47	48
Early deaths				
Moribund	7	3	7	8
Natural deaths	3	3	1	2
Survivors				
Moribund	6	2	3	
Died last week of study	1		1	
Terminal sacrifice	45	38	28	37
Harvest	2	2	7	1
Animals examined microscopically	64	47	47	48
Alimentary System				
Esophagus	(62)	(46)	(47)	(47)
Gallbladder	(60)	(44)	(45)	(46)
Intestine large, cecum	(60)	(46)	(46)	(46)
Intestine large, colon	(60)	(46)	(46)	(46)
Intestine large, rectum	(60)	(46)	(46)	(46)
Intestine small, duodenum	(60)	(45)	(47)	(46)
Adenoma		1 (2%)	1 (2%)	
Intestine small, ileum	(60)	(46)	(46)	(46)
Intestine small, jejunum	(60)	(46)	(45)	(46)
Liver	(61)	(46)	(46)	(47)
Hemangiosarcoma	2 (3%)	1 (2%)		
Hepatocellular adenoma	8 (13%)	6 (13%)	3 (7%)	4 (9%)
Hepatocellular adenoma, multiple	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Hepatocellular carcinoma	3 (5%)	3 (7%)	2 (4%)	1 (2%)
Mesentery	(8)	(11)	(4)	(8)
Pancreas	(62)	(46)	(46)	(46)
Fibrous histiocytoma	1 (2%)			
Salivary glands	(62)	(46)	(45)	(47)
Stomach, forestomach	(62)	(45)	(47)	(46)
Squamous cell papilloma		1 (2%)		
Stomach, glandular	(60)	(45)	(46)	(46)
Adenoma			1 (2%)	
Cardiovascular System				
Blood vessel	(62)	(46)	(46)	(48)
Heart	(63)	(46)	(47)	(47)
Endocrine System				
Adrenal cortex	(61)	(46)	(47)	(47)
Adenoma	1 (2%)			
Adrenal medulla	(60)	(43)	(45)	(46)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(62)	(46)	(46)	(47)
Adenoma	3 (5%)	2 (4%)	1 (2%)	1 (2%)
Parathyroid gland	(54)	(40)	(44)	(45)
Pituitary gland	(60)	(44)	(44)	(46)
Pars distalis, adenoma	6 (10%)	4 (9%)	4 (9%)	3 (7%)
Thyroid gland	(59)	(46)	(46)	(47)
Follicular cell, adenoma		1 (2%)		3 (6%)
Follicular cell, carcinoma				1 (2%)

TABLE B1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
General Body System				
Tissue NOS	(3)	(1)	(2)	(1)
Abdominal, fibrosarcoma, metastatic, skin			1 (50%)	
Abdominal, fibrous histiocytoma	1 (33%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (33%)			
Thoracic, sarcoma		1 (100%)		
Genital System				
Clitoral gland	(60)	(44)	(43)	(46)
Ovary	(60)	(45)	(45)	(47)
Cystadenoma	2 (3%)		1 (2%)	1 (2%)
Hemangioma			1 (2%)	1 (2%)
Uterus	(62)	(46)	(47)	(47)
Adenocarcinoma		1 (2%)		
Polyp stromal			1 (2%)	
Sarcoma stromal	1 (2%)		1 (2%)	
Hematopoietic System				
Bone marrow	(61)	(46)	(47)	(46)
Hemangiosarcoma				1 (2%)
Lymph node	(15)	(5)	(12)	(8)
Lymph node, mandibular	(61)	(46)	(44)	(47)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Lymph node, mesenteric	(60)	(45)	(46)	(45)
Sarcoma		1 (2%)		
Spleen	(62)	(46)	(47)	(47)
Hemangiosarcoma	2 (3%)			
Thymus	(55)	(44)	(42)	(46)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(63)	(45)	(46)	(47)
Adenocarcinoma	6 (10%)	1 (2%)	1 (2%)	3 (6%)
Skin	(63)	(46)	(47)	(48)
Subcutaneous tissue, fibrosarcoma	1 (2%)		2 (4%)	3 (6%)
Subcutaneous tissue, sarcoma	2 (3%)		2 (4%)	3 (6%)
Musculoskeletal System				
Bone, femur	(64)	(47)	(47)	(48)
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain, brain stem	(61)	(46)	(46)	(47)
Oligodendroglioma malignant			1 (2%)	
Brain, cerebellum	(62)	(46)	(46)	(47)
Brain, cerebrum	(62)	(46)	(46)	(47)
Oligodendroglioma malignant			1 (2%)	
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)

TABLE B1a
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Respiratory System				
Lung	(62)	(46)	(47)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Alveolar/bronchiolar adenoma	2 (3%)	5 (11%)	5 (11%)	4 (8%)
Alveolar/bronchiolar carcinoma	5 (8%)		3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Hepatocellular carcinoma, metastatic, Liver			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Nose	(62)	(46)	(47)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)		1 (2%)	
Trachea	(61)	(46)	(47)	(47)
Special Senses System				
Eye	(59)	(46)	(45)	(46)
Adenocarcinoma, metastatic, Harderian gland			1 (2%)	
Harderian gland	(60)	(46)	(47)	(46)
Adenocarcinoma	1 (2%)		1 (2%)	
Adenoma	5 (8%)	8 (17%)	4 (9%)	2 (4%)
Adenoma, multiple			1 (2%)	
Urinary System				
Kidney	(62)	(46)	(46)	(46)
Urinary bladder	(60)	(46)	(46)	(46)
Hemangioma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(64)	(47)	(47)	(48)
Histiocytic sarcoma	3 (5%)		2 (4%)	1 (2%)
Lymphoma malignant	24 (38%)	16 (34%)	18 (38%)	18 (38%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	51	31	37	35
Total primary neoplasms	82	55	59	52
Total animals with benign neoplasms	24	23	23	17
Total benign neoplasms	30	30	25	20
Total animals with malignant neoplasms	41	20	30	26
Total malignant neoplasms	52	25	34	32
Total animals with metastatic neoplasms	4		3	
Total metastatic neoplasms	6		4	
Total animals with malignant neoplasms of uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC[a]

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Disposition Summary				
Animals initially in study	64	48	51	48
Early deaths				
Moribund	7	9	8	4
Natural deaths	3	3	2	3
Survivors				
Moribund	6	2	2	1
Died last week of study	1		1	
Terminal sacrifice	45	32	35	35
Harvest	2	2	3	5
Animals examined microscopically	64	48	51	48
Alimentary System				
Esophagus	(62)	(47)	(50)	(48)
Gallbladder	(60)	(45)	(48)	(46)
Intestine large, cecum	(60)	(46)	(49)	(46)
Intestine large, colon	(60)	(46)	(50)	(46)
Intestine large, rectum	(60)	(46)	(50)	(46)
Intestine small, duodenum	(60)	(45)	(49)	(45)
Adenoma		2 (4%)		
Intestine small, ileum	(60)	(46)	(50)	(46)
Polyp adenomatous			1 (2%)	
Intestine small, jejunum	(60)	(46)	(48)	(46)
Liver	(61)	(47)	(50)	(48)
Hemangiosarcoma	2 (3%)			
Hemangiosarcoma, metastatic, spleen				1 (2%)
Hepatocellular adenoma	8 (13%)	3 (6%)	2 (4%)	4 (8%)
Hepatocellular adenoma, multiple	1 (2%)	1 (2%)		
Hepatocellular carcinoma	3 (5%)	2 (4%)	6 (12%)	2 (4%)
Mesentery	(8)	(11)	(7)	(6)
Pancreas	(62)	(46)	(49)	(48)
Fibrous histiocytoma	1 (2%)			
Salivary glands	(62)	(46)	(50)	(48)
Stomach, forestomach	(62)	(46)	(50)	(47)
Squamous cell papilloma			2 (4%)	
Squamous cell papilloma, multiple				1 (2%)
Stomach, glandular	(60)	(46)	(48)	(46)
Cardiovascular System				
Blood vessel	(62)	(45)	(49)	(48)
Heart	(63)	(48)	(50)	(48)
Carcinoma, metastatic, lung			1 (2%)	
Endocrine System				
Adrenal cortex	(61)	(47)	(50)	(47)
Adenoma	1 (2%)			
Subcapsular, adenoma			1 (2%)	
Adrenal medulla	(60)	(46)	(47)	(46)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(62)	(46)	(49)	(48)
Adenoma	3 (5%)			
Parathyroid gland	(54)	(36)	(40)	(38)

TABLE B1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Endocrine System (continued)				
Pituitary gland	(60)	(45)	(49)	(46)
Pars distalis, adenoma	6 (10%)	1 (2%)	7 (14%)	6 (13%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(59)	(46)	(50)	(47)
Follicular cell, adenoma		1 (2%)		
General Body System				
Tissue NOS	(3)	(0)	(2)	(1)
Abdominal, fibrous histiocytoma	1 (33%)			
Abdominal, sarcoma			1 (50%)	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (33%)			
Mediastinum, carcinoma, metastatic, lung			1 (50%)	
Genital System				
Clitoral gland	(60)	(44)	(49)	(44)
Ovary	(60)	(43)	(50)	(48)
Cystadenoma	2 (3%)	1 (2%)	2 (4%)	2 (4%)
Hemangioma			1 (2%)	
Hemangiosarcoma			1 (2%)	
Sertoli cell tumor benign				1 (2%)
Uterus	(62)	(46)	(50)	(48)
Hemangiosarcoma			1 (2%)	
Polyp stromal				1 (2%)
Sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(61)	(46)	(50)	(46)
Hemangiosarcoma			1 (2%)	
Hemangiosarcoma, metastatic, spleen				2 (4%)
Lymph node	(15)	(9)	(10)	(5)
Mediastinal, carcinoma, metastatic, lung			1 (10%)	
Lymph node, mandibular	(61)	(45)	(49)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Lymph node, mesenteric	(60)	(46)	(48)	(45)
Fibrous histiocytoma				1 (2%)
Spleen	(62)	(48)	(50)	(47)
Hemangiosarcoma	2 (3%)	1 (2%)	4 (8%)	3 (6%)
Thymus	(55)	(43)	(47)	(45)
Carcinoma, metastatic, lung			1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(63)	(45)	(50)	(45)
Adenocarcinoma	6 (10%)	3 (7%)	1 (2%)	2 (4%)
Adenoma			1 (2%)	
Skin	(63)	(46)	(50)	(48)
Hemangiosarcoma		1 (2%)		
Ear, hemangiosarcoma				1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Subcutaneous tissue, sarcoma	2 (3%)			

TABLE B1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Musculoskeletal System				
Bone	(0)	(0)	(0)	(1)
Bone, femur	(64)	(48)	(51)	(48)
Skeletal muscle	(1)	(1)	(1)	(1)
Hemangiosarcoma, metastatic, spleen				1 (100%)
Nervous System				
Brain, brain stem	(61)	(47)	(49)	(47)
Brain, cerebellum	(62)	(47)	(49)	(47)
Brain, cerebrum	(62)	(47)	(49)	(47)
Peripheral nerve	(1)	(1)	(1)	(0)
Spinal cord	(1)	(1)	(1)	(0)
Respiratory System				
Lung	(62)	(48)	(50)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Adenocarcinoma, metastatic, mammary gland			1 (2%)	
Alveolar/bronchiolar adenoma	2 (3%)	1 (2%)	3 (6%)	6 (13%)
Alveolar/bronchiolar carcinoma	5 (8%)	3 (6%)		4 (8%)
Alveolar/bronchiolar carcinoma, multiple			1 (2%)	
Fibrosarcoma, metastatic, skin		1 (2%)		
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Nose	(62)	(46)	(51)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Trachea	(61)	(46)	(50)	(47)
Special Senses System				
Eye	(59)	(45)	(49)	(46)
Harderian gland	(60)	(46)	(50)	(47)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	5 (11%)	3 (6%)	4 (9%)
Urinary System				
Kidney	(62)	(46)	(50)	(46)
Urinary bladder	(60)	(45)	(49)	(46)
Hemangioma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(64)	(48)	(51)	(48)
Histiocytic sarcoma	3 (5%)	1 (2%)	1 (2%)	1 (2%)
Leukemia		2 (4%)	1 (2%)	
Lymphoma malignant	24 (38%)	14 (29%)	10 (20%)	15 (31%)

TABLE B1b
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	51	31	34	34
Total primary neoplasms	82	45	55	56
Total animals with benign neoplasms	24	14	19	23
Total benign neoplasms	30	16	23	25
Total animals with malignant neoplasms	41	23	28	26
Total malignant neoplasms	52	29	32	31
Total animals with metastatic neoplasms	4	1	2	3
Total metastatic neoplasms	6	1	5	5
Total animals with malignant neoplasms of uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1c
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP[a]

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Disposition Summary				
Animals initially in study	64	48	48	49
Early deaths				
Moribund	7	12	7	5
Natural deaths	3	2	2	4
Survivors				
Moribund	6		2	
Died last week of study	1			
Terminal sacrifice	45	31	34	39
Harvest	2	3	3	1
Animals examined microscopically	64	48	48	49
Alimentary System				
Esophagus	(62)	(47)	(47)	(48)
Gallbladder	(60)	(45)	(45)	(45)
Intestine large, cecum	(60)	(45)	(46)	(45)
Sarcoma				1 (2%)
Intestine large, colon	(60)	(46)	(46)	(45)
Intestine large, rectum	(60)	(46)	(46)	(45)
Sarcoma, metastatic, skin				1 (2%)
Intestine small, duodenum	(60)	(46)	(46)	(45)
Adenoma				1 (2%)
Intestine small, ileum	(60)	(46)	(46)	(45)
Intestine small, jejunum	(60)	(46)	(46)	(46)
Adenoma			1 (2%)	
Liver	(61)	(46)	(47)	(47)
Hemangiosarcoma	2 (3%)		1 (2%)	
Hepatocellular adenoma	8 (13%)	4 (9%)	7 (15%)	2 (4%)
Hepatocellular adenoma, multiple	1 (2%)		2 (4%)	1 (2%)
Hepatocellular carcinoma	3 (5%)	1 (2%)	3 (6%)	2 (4%)
Hepatocellular carcinoma, multiple			1 (2%)	
Mesentery	(8)	(1)	(8)	(3)
Pancreas	(62)	(45)	(46)	(47)
Fibrous histiocytoma	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Salivary glands	(62)	(47)	(47)	(46)
Hemangiosarcoma			1 (2%)	
Stomach, forestomach	(62)	(46)	(46)	(47)
Squamous cell papilloma			1 (2%)	1 (2%)
Stomach, glandular	(60)	(46)	(46)	(46)
Cardiovascular System				
Blood vessel	(62)	(47)	(47)	(48)
Heart	(63)	(46)	(47)	(49)
Endocrine System				
Adrenal cortex	(61)	(45)	(46)	(48)
Adenoma	1 (2%)			
Subcapsular, adenoma				2 (4%)
Adrenal medulla	(60)	(43)	(45)	(44)
Pheochromocytoma benign	1 (2%)			

TABLE B1c
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(62)	(45)	(46)	(47)
Adenoma	3 (5%)			
Carcinoma			1 (2%)	
Parathyroid gland	(54)	(38)	(44)	(41)
Pituitary gland	(60)	(43)	(46)	(42)
Pars distalis, adenoma	6 (10%)	3 (7%)	2 (4%)	5 (12%)
Thyroid gland	(59)	(46)	(45)	(47)
C-cell, carcinoma		1 (2%)		
General Body System				
Tissue NOS	(3)	(1)	(0)	(0)
Abdominal, fibrous histiocytoma	1 (33%)			
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (33%)			
Genital System				
Clitoral gland	(60)	(46)	(46)	(46)
Ovary	(60)	(46)	(47)	(48)
Cystadenoma	2 (3%)	1 (2%)	2 (4%)	2 (4%)
Hemangiosarcoma		1 (2%)		
Luteoma				1 (2%)
Tubulostromal adenoma			1 (2%)	
Uterus	(62)	(46)	(46)	(48)
Hemangiosarcoma			1 (2%)	1 (2%)
Sarcoma stromal	1 (2%)	1 (2%)		
Hematopoietic System				
Bone marrow	(61)	(46)	(46)	(48)
Lymph node	(15)	(11)	(5)	(4)
Lymph node, mandibular	(61)	(47)	(47)	(47)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Lymph node, mesenteric	(60)	(46)	(46)	(47)
Hemangiosarcoma, metastatic, salivary glands			1 (2%)	
Spleen	(62)	(47)	(47)	(48)
Hemangiosarcoma	2 (3%)	1 (2%)	1 (2%)	
Hemangiosarcoma, metastatic, salivary glands			1 (2%)	
Thymus	(55)	(44)	(44)	(40)
Integumentary System				
Mammary gland	(63)	(47)	(47)	(47)
Adenocarcinoma	6 (10%)	3 (6%)		1 (2%)
Adenoma			1 (2%)	1 (2%)
Skin	(63)	(47)	(47)	(49)
Subcutaneous tissue, fibrosarcoma	1 (2%)		7 (15%)	
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma	2 (3%)		1 (2%)	1 (2%)

TABLE B1c
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Musculoskeletal System				
Bone, femur	(64)	(48)	(48)	(49)
Osteosarcoma			1 (2%)	
Skeletal muscle	(1)	(0)	(2)	(1)
Fibrosarcoma, metastatic, skin			1 (50%)	
Nervous System				
Brain, brain stem	(61)	(46)	(46)	(47)
Brain, cerebellum	(62)	(46)	(46)	(47)
Osteosarcoma		1 (2%)		
Brain, cerebrum	(62)	(46)	(46)	(47)
Peripheral nerve	(1)	(0)	(1)	(1)
Spinal cord	(1)	(0)	(1)	(1)
Respiratory System				
Lung	(62)	(46)	(47)	(48)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Alveolar/bronchiolar adenoma	2 (3%)	2 (4%)	4 (9%)	2 (4%)
Alveolar/bronchiolar carcinoma	5 (8%)	2 (4%)	3 (6%)	4 (8%)
Osteosarcoma				1 (2%)
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Nose	(62)	(48)	(47)	(49)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Rhabdomyosarcoma, metastatic, Harderian gland		1 (2%)		
Trachea	(61)	(46)	(45)	(47)
Special Senses System				
Eye	(59)	(45)	(45)	(45)
Harderian gland	(60)	(45)	(45)	(46)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	7 (16%)	4 (9%)	2 (4%)
Rhabdomyosarcoma		1 (2%)		
Special Senses System				
Kidney	(62)	(48)	(47)	(47)
Urinary bladder	(60)	(47)	(46)	(46)
Hemangioma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(64)	(48)	(48)	(49)
Histiocytic sarcoma	3 (5%)	1 (2%)		2 (4%)
Leukemia		1 (2%)		
Lymphoma malignant	24 (38%)	18 (38%)	13 (27%)	13 (27%)

TABLE B1c
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	51	34	34	33
Total primary neoplasms	82	49	60	46
Total animals with benign neoplasms	24	16	22	17
Total benign neoplasms	30	17	26	20
Total animals with malignant neoplasms	41	28	26	24
Total malignant neoplasms	52	32	34	26
Total animals with metastatic neoplasms	4	1	2	1
Total metastatic neoplasms	6	1	3	3
Total animals with malignant neoplasms of uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B1d
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV[a]

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Disposition Summary				
Animals initially in study	64	50	49	26
Early deaths				
Moribund	7	8	3	4
Natural deaths	3	5	1	1
Survivors				
Moribund	6	4	5	2
Died last week of study	1		1	
Terminal sacrifice	45	30	37	16
Harvest	2	3	2	3
Animals examined microscopically	64	50	49	26
Alimentary System				
Esophagus	(62)	(49)	(48)	(25)
Gallbladder	(60)	(46)	(47)	(25)
Intestine large, cecum	(60)	(45)	(47)	(25)
Intestine large, colon	(60)	(46)	(47)	(25)
Intestine large, rectum	(60)	(46)	(47)	(25)
Intestine small, duodenum	(60)	(45)	(47)	(25)
Intestine small, ileum	(60)	(45)	(47)	(25)
Intestine small, jejunum	(60)	(45)	(47)	(25)
Liver	(61)	(50)	(48)	(26)
Hemangiosarcoma	2 (3%)	1 (2%)		1 (4%)
Hepatocellular adenoma	8 (13%)	1 (2%)	7 (15%)	4 (15%)
Hepatocellular adenoma, multiple	1 (2%)		1 (2%)	
Hepatocellular carcinoma	3 (5%)	3 (6%)	5 (10%)	
Mesentery	(8)	(7)	(7)	(5)
Pancreas	(62)	(47)	(46)	(25)
Fibrous histiocytoma	1 (2%)			
Salivary glands	(62)	(47)	(47)	(25)
Stomach, forestomach	(62)	(47)	(47)	(25)
Squamous cell papilloma		1 (2%)		1 (4%)
Stomach, glandular	(60)	(46)	(47)	(25)
Cardiovascular System				
Blood vessel	(62)	(50)	(48)	(25)
Heart	(63)	(50)	(48)	(25)
Endocrine System				
Adrenal cortex	(61)	(48)	(49)	(25)
Adenoma	1 (2%)			
Subcapsular, adenoma		1 (2%)		
Adrenal medulla	(60)	(46)	(47)	(25)
Pheochromocytoma benign	1 (2%)			1 (4%)
Islets, pancreatic	(62)	(47)	(46)	(25)
Adenoma	3 (5%)	1 (2%)	1 (2%)	
Parathyroid gland	(54)	(42)	(42)	(21)
Pituitary gland	(60)	(47)	(45)	(23)
Pars distalis, adenoma	6 (10%)	3 (6%)	4 (9%)	1 (4%)
Thyroid gland	(59)	(47)	(48)	(25)
Follicular cell, carcinoma			1 (2%)	

TABLE B1d
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
General Body System				
Tissue NOS	(3)	(2)	(1)	(0)
Abdominal, fibrous histiocytoma	1 (33%)		1 (100%)	
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	1 (33%)			
Genital System				
Clitoral gland	(60)	(46)	(47)	(25)
Ovary	(60)	(47)	(48)	(25)
Cystadenoma	2 (3%)	1 (2%)	1 (2%)	2 (8%)
Granulosa cell tumor benign		1 (2%)	1 (2%)	
Yolk sac carcinoma			1 (2%)	
Uterus	(62)	(48)	(48)	(26)
Granular cell tumor benign		1 (2%)		
Hemangiosarcoma			1 (2%)	
Polyp stromal		1 (2%)		
Sarcoma		1 (2%)		
Sarcoma stromal	1 (2%)			
Hematopoietic System				
Bone marrow	(61)	(47)	(47)	(25)
Hemangiosarcoma, metastatic, spleen			1 (2%)	
Lymph node	(15)	(13)	(6)	(3)
Lumbar, fibrous histiocytoma			1 (17%)	
Lymph node, mandibular	(61)	(48)	(48)	(25)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Lymph node, mesenteric	(60)	(48)	(47)	(25)
Adenocarcinoma, metastatic, mammary gland			1 (2%)	
Spleen	(62)	(50)	(48)	(25)
Hemangiosarcoma	2 (3%)	3 (6%)	3 (6%)	
Hemangiosarcoma, metastatic, skin		1 (2%)		
Thymus	(55)	(44)	(48)	(25)
Integumentary System				
Mammary gland	(63)	(47)	(48)	(24)
Adenocarcinoma	6 (10%)	1 (2%)	3 (6%)	
Adenoma		1 (2%)		
Skin	(63)	(49)	(48)	(25)
Hemangiosarcoma		1 (2%)		
Trichoepithelioma				1 (4%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	3 (6%)		1 (4%)
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma	2 (3%)		1 (2%)	
Musculoskeletal System				
Bone, femur	(64)	(50)	(49)	(26)
Skeletal muscle	(1)	(0)	(0)	(0)

TABLE B1d
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Nervous System				
Brain, brain stem	(61)	(47)	(48)	(25)
Brain, cerebellum	(62)	(47)	(48)	(25)
Brain, cerebrum	(62)	(47)	(48)	(25)
Meninges, osteosarcoma		1 (2%)		
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Lung	(62)	(50)	(47)	(25)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Alveolar/bronchiolar adenoma	2 (3%)	3 (6%)	3 (6%)	1 (4%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)	
Alveolar/bronchiolar carcinoma	5 (8%)		3 (6%)	2 (8%)
Osteosarcoma, metastatic, brain, cerebrum		1 (2%)		
Osteosarcoma, metastatic, uncertain primary site	1 (2%)			
Nose	(62)	(48)	(47)	(26)
Adenocarcinoma, metastatic, Harderian gland	1 (2%)			
Trachea	(61)	(47)	(47)	(25)
Special Senses System				
Eye	(59)	(45)	(47)	(25)
Harderian gland	(60)	(46)	(46)	(25)
Adenocarcinoma	1 (2%)			
Adenoma	5 (8%)	5 (11%)	2 (4%)	2 (8%)
Urinary System				
Kidney	(62)	(47)	(47)	(25)
Urinary bladder	(60)	(47)	(47)	(25)
Hemangioma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(64)	(50)	(49)	(26)
Histiocytic sarcoma	3 (5%)	1 (2%)		1 (4%)
Leukemia		4 (8%)		
Lymphoma malignant	24 (38%)	9 (18%)	19 (39%)	9 (35%)

TABLE B1d
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	51	38	35	19
Total primary neoplasms	82	49	61	27
Total animals with benign neoplasms	24	18	20	10
Total benign neoplasms	30	21	22	13
Total animals with malignant neoplasms	41	26	25	12
Total malignant neoplasms	52	28	39	14
Total animals with metastatic neoplasms	4	2	2	
Total metastatic neoplasms	6	2	2	
Total animals with malignant neoplasms of uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	120 mg/kg	240 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/60 (8.3%)	8/46 (17.4%)	5/47 (10.6%)	2/46 (4.3%)
Adjusted rate[b]	9.0%	18.2%	11.9%	4.5%
Terminal rate[c]	5/44 (11.4%)	7/38 (18.4%)	3/28 (10.7%)	1/37 (2.7%)
First incidence (days)	737 (T)	718	610	574
Poly-3 test[d]	P=0.220N	P=0.146	P=0.453	P=0.318N
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	6/60 (10.0%)	8/46 (17.4%)	6/47 (12.8%)	2/46 (4.3%)
Adjusted rate	10.8%	18.2%	14.1%	4.5%
Terminal rate	6/44 (13.6%)	7/38 (18.4%)	3/28 (10.7%)	1/37 (2.7%)
First incidence (days)	737 (T)	718	601	574
Poly-3 test	P=0.182N	P=0.224	P=0.432	P=0.219N
Liver: Hepatocellular Adenoma				
Overall rate	9/61 (14.8%)	8/46 (17.4%)	4/46 (8.7%)	5/47 (10.6%)
Adjusted rate	15.9%	18.2%	9.9%	11.3%
Terminal rate	5/45 (11.1%)	7/38 (18.4%)	2/28 (7.1%)	4/37 (10.8%)
First incidence (days)	685	705	714	726
Poly-3 test	P=0.202N	P=0.482	P=0.291N	P=0.360N
Liver: Hepatocellular Carcinoma				
Overall rate	3/61 (4.9%)	3/46 (6.5%)	2/46 (4.3%)	1/47 (2.1%)
Adjusted rate	5.3%	6.8%	4.9%	2.3%
Terminal rate	2/45 (4.4%)	2/38 (5.3%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	669	586	714	737 (T)
Poly-3 test	P=0.278N	P=0.545	P=0.649N	P=0.399N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	12/61 (19.7%)	11/46 (23.9%)	5/46 (10.9%)	6/47 (12.8%)
Adjusted rate	21.0%	24.8%	12.3%	13.6%
Terminal rate	7/45 (15.6%)	9/38 (23.7%)	3/28 (10.7%)	5/37 (13.5%)
First incidence (days)	669	586	714	726
Poly-3 test	P=0.106N	P=0.419	P=0.199N	P=0.240N
Liver: Hemangiosarcoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	14/61 (23.0%)	11/46 (23.9%)	5/46 (10.9%)	6/47 (12.8%)
Adjusted rate	24.5%	24.8%	12.3%	13.6%
Terminal rate	9/45 (20.0%)	9/38 (23.7%)	3/28 (10.7%)	5/37 (13.5%)
First incidence (days)	669	586	714	726
Poly-3 test	P=0.047N	P=0.581	P=0.106N	P=0.131N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/62 (3.2%)	5/46 (10.9%)	5/47 (10.6%)	4/48 (8.3%)
Adjusted rate	3.5%	11.4%	11.9%	8.8%
Terminal rate	2/45 (4.4%)	3/38 (7.9%)	3/28 (10.7%)	3/37 (8.1%)
First incidence (days)	737 (T)	705	560	574
Poly-3 test	P=0.190	P=0.126	P=0.114	P=0.241
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/62 (8.1%)	1/46 (2.2%)	3/47 (6.4%)	1/48 (2.1%)
Adjusted rate	8.7%	2.3%	7.2%	2.2%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	2/28 (7.1%)	1/37 (2.7%)
First incidence (days)	579	727	610	741 (T)
Poly-3 test	P=0.166N	P=0.176N	P=0.541N	P=0.169N

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	120 mg/kg	240 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/62 (11.3%)	6/46 (13.0%)	8/47 (17.0%)	5/48 (10.4%)
Adjusted rate	12.1%	13.6%	18.8%	11.0%
Terminal rate	3/45 (6.7%)	3/38 (7.9%)	5/28 (17.9%)	4/37 (10.8%)
First incidence (days)	579	705	560	574
Poly-3 test	P=0.499	P=0.532	P=0.263	P=0.551N
Mammary Gland: Adenocarcinoma				
Overall rate	6/63 (9.5%)	1/45 (2.2%)	1/46 (2.2%)	3/47 (6.4%)
Adjusted rate	10.3%	2.3%	2.5%	6.8%
Terminal rate	2/45 (4.4%)	1/37 (2.7%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	567	737 (T)	743 (T)	686
Poly-3 test	P=0.244N	P=0.122N	P=0.139N	P=0.394N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	6/60 (10.0%)	4/44 (9.1%)	4/44 (9.1%)	3/46 (6.5%)
Adjusted rate	10.8%	9.5%	10.4%	7.0%
Terminal rate	2/44 (4.5%)	2/37 (5.4%)	3/27 (11.1%)	2/36 (5.6%)
First incidence (days)	579	718	729	711
Poly-3 test	P=0.326N	P=0.553N	P=0.609N	P=0.382N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/59 (0.0%)	1/46 (2.2%)	0/46 (0.0%)	3/47 (6.4%)
Adjusted rate	0.0%	2.3%	0.0%	6.8%
Terminal rate	0/45 (0.0%)	1/38 (2.6%)	0/27 (0.0%)	3/37 (8.1%)
First incidence (days)	—[e]	733 (T)	—	734 (T)
Poly-3 test	P=0.041	P=0.455	—[f]	P=0.083
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/59 (0.0%)	1/46 (2.2%)	0/46 (0.0%)	4/47 (8.5%)
Adjusted rate	0.0%	2.3%	0.0%	9.1%
Terminal rate	0/45 (0.0%)	1/38 (2.6%)	0/27 (0.0%)	4/37 (10.8%)
First incidence (days)	—	733 (T)	—	734 (T)
Poly-3 test	P=0.013	P=0.455	—	P=0.036
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/63 (1.6%)	0/46 (0.0%)	2/47 (4.3%)	3/48 (6.3%)
Adjusted rate	1.8%	0.0%	4.8%	6.6%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	0/28 (0.0%)	0/37 (0.0%)
First incidence (days)	739 (T)	—	633	633
Poly-3 test	P=0.070	P=0.533N	0.393	P=0.228
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	2/63 (3.2%)	0/46 (0.0%)	2/47 (4.3%)	3/48 (6.3%)
Adjusted rate	3.5%	0.0%	4.8%	6.6%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	735	—	707	598
Poly-3 test	P=0.184	P=0.298N	0.574	P=0.400
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	2/63 (3.2%)	0/46 (0.0%)	4/47 (8.5%)	5/48 (10.4%)
Adjusted rate	3.5%	0.0%	9.5%	10.9%
Terminal rate	1/45 (2.2%)	0/38 (0.0%)	1/28 (3.6%)	1/37 (2.7%)
First incidence (days)	735	—	633	598
Poly-3 test	P=0.028	P=0.298N	0.207	P=0.138

TABLE B2a
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	120 mg/kg	240 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	4/64 (6.3%)	1/47 (2.1%)	0/47 (0.0%)	1/48 (2.1%)
Adjusted rate	6.9%	2.3%	0.0%	2.2%
Terminal rate	4/45 (8.9%)	1/38 (2.6%)	0/28 (0.0%)	1/37 (2.7%)
First incidence (days)	732 (T)	739 (T)	—	744 (T)
Poly-3 test	P=0.088N	P=0.267N	P=0.112N	P=0.262N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/64 (7.8%)	1/47 (2.1%)	1/47 (2.1%)	2/48 (4.2%)
Adjusted rate	8.7%	2.3%	2.4%	4.5%
Terminal rate	5/45 (11.1%)	1/38 (2.6%)	0/28 (0.0%)	2/37 (5.4%)
First incidence (days)	732 (T)	739 (T)	707	742 (T)
Poly-3 test	P=0.194N	P=0.173N	P=0.195N	P=0.328N
All Organs: Malignant Lymphoma				
Overall rate	24/64 (37.5%)	16/47 (34.0%)	18/47 (38.3%)	18/48 (37.5%)
Adjusted rate	40.4%	36.0%	41.4%	39.3%
Terminal rate	18/45 (40.0%)	14/38 (36.8%)	10/28 (35.7%)	13/37 (35.1%)
First incidence (days)	583	705	560	616
Poly-3 test	P=0.517	P=0.397N	P=0.543	P=0.532N
All Organs: Benign Neoplasms				
Overall rate	24/64 (37.5%)	23/47 (48.9%)	23/47 (48.9%)	17/48 (35.4%)
Adjusted rate	40.7%	51.6%	52.6%	37.0%
Terminal rate	18/45 (40.0%)	20/38 (52.6%)	13/28 (46.4%)	13/37 (35.1%)
First incidence (days)	579	705	560	574
Poly-3 test	P=0.436N	P=0.182	P=0.157	P=0.424N
All Organs: Malignant Neoplasms				
Overall rate	41/64 (64.1%)	20/47 (42.6%)	30/47 (63.8%)	26/48 (54.2%)
Adjusted rate	66.0%	44.4%	64.9%	54.9%
Terminal rate	26/45 (57.8%)	17/38 (44.7%)	14/28 (50.0%)	17/37 (45.9%)
First incidence (days)	534	586	239	566
Poly-3 test	P=0.288N	P=0.019N	P=0.537N	P=0.162N
All Organs: Benign or Malignant Neoplasms				
Overall rate	51/64 (79.7%)	31/47 (66.0%)	37/47 (78.7%)	35/48 (72.9%)
Adjusted rate	81.3%	68.6%	79.5%	72.9%
Terminal rate	35/45 (77.8%)	26/38 (68.4%)	19/28 (67.9%)	24/37 (64.9%)
First incidence (days)	534	586	239	566
Poly-3 test	P=0.270N	P=0.092N	P=0.503N	P=0.203N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/60 (8.3%)	5/46 (10.9%)	3/50 (6.0%)	4/47 (8.5%)
Adjusted rate[b]	9.0%	11.8%	6.6%	9.4%
Terminal rate[c]	5/44 (11.4%)	4/32 (12.5%)	2/35 (5.7%)	3/35 (8.6%)
First incidence (days)	737 (T)	644	643	608
Poly-3 test[d]	P=0.463N	P=0.458	P=0.467N	P=0.617
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	6/60 (10.0%)	5/46 (10.9%)	3/50 (6.0%)	4/47 (8.5%)
Adjusted rate	10.8%	11.8%	6.6%	9.4%
Terminal rate	6/44 (13.6%)	4/32 (12.5%)	2/35 (5.7%)	3/35 (8.6%)
First incidence (days)	737 (T)	644	643	608
Poly-3 test	P=0.346N	P=0.570	P=0.346N	P=0.537N
Liver: Hepatocellular Adenoma				
Overall rate	9/61 (14.8%)	4/47 (8.5%)	2/50 (4.0%)	4/48 (8.3%)
Adjusted rate	15.9%	9.4%	4.4%	9.3%
Terminal rate	5/45 (11.1%)	4/32 (12.5%)	1/35 (2.9%)	4/35 (11.4%)
First incidence (days)	685	732 (T)	698	738 (T)
Poly-3 test	P=0.096	P=0.260N	P=0.061N	P=0.252N
Liver: Hepatocellular Carcinoma				
Overall rate	3/61 (4.9%)	2/47 (4.3%)	6/50 (12.0%)	2/48 (4.2%)
Adjusted rate	5.3%	4.7%	13.2%	4.6%
Terminal rate	2/45 (4.4%)	2/32 (6.3%)	5/35 (14.3%)	2/35 (5.7%)
First incidence (days)	669	736 (T)	662	745 (T)
Poly-3 test	P=0.370	P=0.627N	P=0.148	P=0.621N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	12/61 (19.7%)	6/47 (12.8%)	8/50 (16.0%)	6/48 (12.5%)
Adjusted rate	21.0%	14.1%	17.5%	13.9%
Terminal rate	7/45 (15.6%)	6/32 (18.8%)	6/35 (17.1%)	6/35 (17.1%)
First incidence (days)	669	732 (T)	662	738 (T)
Poly-3 test	P=0.235N	P=0.266N	P=0.422N	P=0.255N
Liver: Hemangiosarcoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	14/61 (23.0%)	6/47 (12.8%)	8/50 (16.0%)	6/48 (12.5%)
Adjusted rate	24.5%	14.1%	17.5%	13.9%
Terminal rate	9/45 (20.0%)	6/32 (18.8%)	6/35 (17.1%)	6/35 (17.1%)
First incidence (days)	669	732 (T)	662	738 (T)
Poly-3 test	P=0.119N	P=0.150N	P=0.267N	P=0.142N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/62 (3.2%)	1/48 (2.1%)	3/50 (6.0%)	6/48 (12.5%)
Adjusted rate	3.5%	2.3%	6.5%	13.7%
Terminal rate	2/45 (4.4%)	0/32 (0.0%)	1/35 (2.9%)	5/35 (14.3%)
First incidence (days)	737 (T)	608	587	585
Poly-3 test	P=0.022	P=0.592N	P=0.405	P=0.065
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/62 (8.1%)	3/48 (6.3%)	1/50 (2.0%)	4/48 (8.3%)
Adjusted rate	8.7%	7.0%	2.2%	9.1%
Terminal rate	1/45 (2.2%)	3/32 (9.4%)	0/35 (0.0%)	2/35 (5.7%)
First incidence (days)	579	732 (T)	587	588
Poly-3 test	P=0.432N	P=0.524N	P=0.164N	P=0.606

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/62 (11.3%)	4/48 (8.3%)	3/50 (6.0%)	9/48 (18.8%)
Adjusted rate	12.1%	9.2%	6.5%	20.3%
Terminal rate	3/45 (6.7%)	3/32 (9.4%)	1/35 (2.9%)	6/35 (17.1%)
First incidence (days)	579	608	587	585
Poly-3 test	P=0.203	P=0.442N	P=0.267N	P=0.198
Mammary Gland: Adenocarcinoma				
Overall rate	6/63 (9.5%)	3/45 (6.7%)	1/50 (2.0%)	2/45 (4.4%)
Adjusted rate	10.3%	7.1%	2.2%	4.8%
Terminal rate	2/45 (4.4%)	1/32 (3.1%)	0/35 (0.0%)	1/34 (2.9%)
First incidence (days)	567	608	698	715
Poly-3 test	P=0.097N	P=0.422N	P=0.108N	P=0.271N
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	6/63 (9.5%)	3/45 (6.7%)	2/50 (4.0%)	2/45 (4.4%)
Adjusted rate	10.3%	7.1%	4.4%	4.8%
Terminal rate	2/45 (4.4%)	1/32 (3.1%)	1/35 (2.9%)	1/34 (2.9%)
First incidence (days)	567	608	698	715
Poly-3 test	P=0.138N	P=0.422N	P=0.230N	P=0.271N
Pituitary Gland (Par Distalis): Adenoma				
Overall rate	6/60 (10.9%)	1/45 (2.2%)	7/49 (14.3%)	6/46 (13.0%)
Adjusted rate	10.8%	2.4%	15.7%	14.4%
Terminal rate	2/44 (4.5%)	1/31 (3.2%)	7/35 (20.0%)	6/35 (17.1%)
First incidence (days)	579	746 (T)	734 (T)	737 (T)
Poly-3 test	P=0.168	P=0.118N	P=0.333	P=0.409
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/63 (1.6%)	2/46 (4.3%)	4/50 (8.0%)	2/48 (4.2%)
Adjusted rate	1.8%	4.7%	8.8%	4.6%
Terminal rate	1/45 (2.2%)	0/32 (0.0%)	3/35 (8.6%)	1/35 (2.9%)
First incidence (days)	739 (T)	685	731	608
Poly-3 test	P=0.196	P=0.398	P=0.116	P=0.407
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	2/63 (3.2%)	2/46 (4.3%)	4/50 (8.0%)	2/48 (4.2%)
Adjusted rate	3.5%	4.7%	8.8%	4.6%
Terminal rate	1/45 (2.2%)	0/32 (0.0%)	3/35 (8.6%)	1/35 (2.9%)
First incidence (days)	735	685	731	608
Poly-3 test	P=0.338	P=0.584	P=0.237	P=0.594
Spleen: Hemangiosarcoma				
Overall rate	2/62 (3.2%)	1/48 (2.1%)	4/50 (8.0%)	3/47 (6.4%)
Adjusted rate	3.5%	2.3%	8.8%	7.1%
Terminal rate	2/45 (4.4%)	1/32 (3.1%)	3/35 (8.6%)	2/35 (5.7%)
First incidence (days)	733 (T)	733 (T)	595	705
Poly-3 test	P=0.155	P=0.596N	P=0.244	P=0.371
All Organs: Hemangiosarcoma				
Overall rate	4/64 (6.3%)	2/48 (4.2%)	6/51 (11.8%)	3/48 (6.3%)
Adjusted rate	6.9%	4.7%	12.7%	6.9%
Terminal rate	4/45 (8.9%)	2/32 (6.3%)	3/35 (8.6%)	2/35 (5.7%)
First incidence (days)	732 (T)	733 (T)	595	705
Poly-3 test	P=0.358	P=0.479N	P=0.251	P=0.652N

TABLE B2b
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/64 (7.8%)	2/48 (4.2%)	7/51 (13.7%)	3/48 (6.3%)
Adjusted rate	8.7%	4.7%	14.9%	6.9%
Terminal rate	5/45 (11.1%)	2/32 (6.3%)	4/35 (11.4%)	2/35 (5.7%)
First incidence (days)	732 (T)	733 (T)	595	705
Poly-3 test	P=0.433	P=0.350N	P=0.247	P=0. 520N
All Organs: Malignant Lymphoma				
Overall rate	24/64 (37.5%)	14/48 (29.2%)	10/51 (19.6%)	15/48 (31.3%)
Adjusted rate	40.4%	31.3%	21.1%	33.5%
Terminal rate	18/45 (40.0%)	8/32 (25.0%)	6/35 (17.1%)	10/35 (28.6%)
First incidence (days)	583	361	612	585
Poly-3 test	P=0.129N	P=0.224N	P=0.025N	P=0.303N
All Organs: Benign Neoplasms				
Overall rate	24/64 (37.5%)	14/48 (29.2%)	19/51 (37.3%)	23/48 (47.9%)
Adjusted rate	40.7%	32.0%	39.4%	50.7%
Terminal rate	18/45 (40.0%)	12/32 (37.5%)	12/35 (34.3%)	18/35 (51.4%)
First incidence (days)	579	608	587	585
Poly-3 test	P=0.153	P=0.239N	P=0.525N	P=0.205
All Organs: Malignant Neoplasms				
Overall rate	41/64 (64.1%)	23/48 (47.9%)	28/51 (54.9%)	26/48 (54.2%)
Adjusted rate	66.0%	48.7%	55.9%	56.6%
Terminal rate	26/45 (57.8%)	11/32 (34.4%)	16/35 (45.7%)	17/35 (48.6%)
First incidence (days)	534	361	538	585
Poly-3 test	P=0.214N	P=0.050N	P=0.184N	P=0.212N
All Organs: Benign or Malignant Neoplasms				
Overall rate	51/64 (79.7%)	31/48 (64.6%)	34/51 (66.7%)	34/48 (70.8%)
Adjusted rate	81.3%	65.1%	67.7%	73.2%
Terminal rate	33/45 (77.8%)	18/32 (56.3%)	21/35 (60.0%)	24/35 (68.6%)
First incidence (days)	534	361	538	585
Poly-3 test	P=0.174N	P=0.040N	P=0.069N	P=0.216N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/60 (8.3%)	7/45 (15.6%)	4/45 (8.9%)	2/46 (4.3%)
Adjusted rate[b]	9.0%	16.9%	9.4%	4.6%
Terminal rate[c]	5/44 (11.4%)	2/30 (6.7%)	2/33 (6.1%)	2/39 (5.1%)
First incidence (days)	737 (T)	526	592	731 (T)
Poly-3 test[d]	P=0.204N	P=0.197	P=0.614	P=0.324N
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	6/60 (10.0%)	7/45 (15.6%)	4/45 (8.9%)	2/46 (4.3%)
Adjusted rate	10.8%	16.9%	9.4%	4.6%
Terminal rate	6/44 (13.6%)	2/30 (6.7%)	2/33 (6.1%)	2/39 (5.1%)
First incidence (days)	737 (T)	526	592	731 (T)
Poly-3 test	P=0.135N	P=0.286	P=0.541N	P=0.225N
Liver: Hepatocellular Adenoma				
Overall rate	9/61 (14.8%)	4/46 (8.7%)	9/47 (19.1%)	3/47 (6.4%)
Adjusted rate	15.9%	9.9%	20.5%	6.8%
Terminal rate	5/45 (11.1%)	4/31 (12.9%)	6/34 (17.6%)	3/39 (7.7%)
First incidence (days)	685	731 (T)	598	733 (T)
Poly-3 test	P=0.238N	P=0.293N	P=0.367	P=0.137N
Liver: Hepatocellular Carcinoma				
Overall rate	3/61 (4.9%)	1/46 (2.2%)	4/47 (8.5%)	2/47 (4.3%)
Adjusted rate	5.3%	2.5%	9.2%	4.5%
Terminal rate	2/45 (4.4%)	1/31 (3.2%)	3/34 (8.8%)	2/39 (5.1%)
First incidence (days)	669	737 (T)	592	731 (T)
Poly-3 test	P=0.460	P=0.432N	P=0.361	P=0.610N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	12/61 (19.7%)	5/46 (10.9%)	12/47 (25.5%)	5/47 (10.6%)
Adjusted rate	21.0%	12.4%	27.0%	11.3%
Terminal rate	7/45 (15.6%)	5/31 (16.1%)	8/34 (23.5%)	5/39 (12.8%)
First incidence (days)	669	731 (T)	592	731 (T)
Poly-3 test	P=0.284N	P=0.201N	P=0.320	P=0.149N
Liver: Hemangiosarcoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	14/61 (23.0%)	5/46 (10.9%)	12/47 (25.5%)	5/47 (10.6%)
Adjusted rate	24.5%	12.4%	27.0%	11.3%
Terminal rate	9/45 (20.0%)	5/31 (16.1%)	8/34 (23.5%)	5/39 (12.8%)
First incidence (days)	669	731 (T)	592	731 (T)
Poly-3 test	P=0.153N	P=0.108N	P=0.478	P=0.073N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/62 (3.2%)	2/46 (4.3%)	4/47 (8.5%)	2/48 (4.2%)
Adjusted rate	3.5%	4.9%	9.3%	4.5%
Terminal rate	2/45 (4.4%)	1/31 (3.2%)	3/34 (8.8%)	2/39 (5.1%)
First incidence (days)	737 (T)	669	733 (T)	741 (T)
Poly-3 test	P=0.347	P=0.570	P=0.221	P=0.604
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/62 (8.1%)	2/46 (4.3%)	3/47 (6.4%)	4/48 (8.3%)
Adjusted rate	8.7%	4.9%	6.9%	9.0%
Terminal rate	1/45 (2.2%)	2/31 (6.5%)	1/34 (2.9%)	4/39 (10.3%)
First incidence (days)	579	739 (T)	592	733 (T)
Poly-3 test	P=0.517	P=0.380N	P=0.515N	P=0.616

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/62 (11.3%)	4/46 (8.7%)	7/47 (14.9%)	6/48 (12.5%)
Adjusted rate	12.1%	9.8%	16.0%	13.5%
Terminal rate	3/45 (6.7%)	3/31 (9.7%)	4/34 (11.8%)	6/39 (15.4%)
First incidence (days)	579	669	592	733 (T)
Poly-3 test	P=0.366	P=0.486N	P=0.394	P=0.539
Mammary Gland: Adenocarcinoma				
Overall rate	6/63 (9.5%)	3/47 (6.4%)	0/47 (0.0%)	1/47 (2.1%)
Adjusted rate	10.3%	7.2%	0.0%	2.2%
Terminal rate	2/45 (4.4%)	1/31 (3.2%)	0/34 (0.0%)	0/39 (0.0%)
First incidence (days)	567	547	—[e]	716
Poly-3 test	P=0.018N	P=0.429N	P=0.039N	P=0.113N
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	6/63 (9.5%)	3/47 (6.4%)	1/47 (2.1%)	2/47 (4.3%)
Adjusted rate	10.3%	7.2%	2.3%	4.5%
Terminal rate	2/45 (4.4%)	1/31 (3.2%)	1/34 (2.9%)	1/39 (2.6%)
First incidence (days)	567	547	734 (T)	716
Poly-3 test	P=0.090N	P=0.429N	P=0.121N	P=0.238N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	6/60 (10.0%)	3/43 (7.0%)	2/46 (4.3%)	5/42 (11.9%)
Adjusted rate	10.8%	8.0%	4.6%	12.8%
Terminal rate	2/44 (4.5%)	3/28 (10.7%)	1/34 (2.9%)	4/34 (11.8%)
First incidence (days)	579	733 (T)	706	644
Poly-3 test	P=0.546	P=0.467N	P=0.231N	P=0.507
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/63 (1.6%)	0/47 (0.0%)	7/47 (14.9%)	0/49 (0.0%)
Adjusted rate	1.8%	0.0%	15.8%	0.0%
Terminal rate	1/45 (2.2%)	0/31 (0.0%)	2/34 (5.9%)	0/39 (0.0%)
First incidence (days)	739 (T)	—	595	—
Poly-3 test	P=0.228	P=0.565N	0.011	P=0.549N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	2/63 (3.2%)	0/47 (0.0%)	8/47 (17.0%)	1/49 (2.0%)
Adjusted rate	3.5%	0.0%	18.1%	2.2%
Terminal rate	1/45 (2.2%)	0/31 (0.0%)	3/34 (8.8%)	0/39 (0.0%)
First incidence (days)	735	—	595	393
Poly-3 test	P=0.210	P=0.313N	0.016	P=0.578N
All Organs: Hemangiosarcoma				
Overall rate	4/64 (6.3%)	2/48 (4.2%)	4/48 (8.3%)	1/49 (2.0%)
Adjusted rate	6.9%	4.8%	9.1%	2.2%
Terminal rate	4/45 (8.9%)	1/31 (3.2%)	3/34 (8.8%)	1/39 (2.6%)
First incidence (days)	732 (T)	695	734 (T)	745 (T)
Poly-3 test	P=0.301N	P=0.497N	P=0.489	P=0.264N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/64 (7.8%)	2/48 (4.2%)	4/48 (8.3%)	1/49 (2.0%)
Adjusted rate	8.7%	4.8%	9.1%	2.2%
Terminal rate	5/45 (11.1%)	1/31 (3.2%)	3/34 (8.8%)	1/39 (2.6%)
First incidence (days)	732 (T)	695	734 (T)	745 (T)
Poly-3 test	P=0.193N	P=0.368N	P=0.609	P=0.171N

TABLE B2c
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	24/64 (37.5%)	18/48 (37.5%)	13/48 (27.1%)	13/49 (26.5%)
Adjusted rate	40.4%	42.0%	28.4%	28.4%
Terminal rate	18/45 (40.0%)	12/31 (38.7%)	8/34 (23.5%)	10/39 (25.6%)
First incidence (days)	583	638	383	412
Poly-3 test	P=0.059N	P=0.520	P=0.139N	P=0.138N
All Organs: Benign Neoplasms				
Overall rate	24/64 (37.5%)	16/48 (33.3%)	22/48 (45.8%)	17/49 (34.7%)
Adjusted rate	40.7%	36.8%	47.1%	37.7%
Terminal rate	18/45 (40.0%)	10/31 (32.3%)	13/34 (38.2%)	15/39 (38.5%)
First incidence (days)	579	526	383	644
Poly-3 test	P=0.508	P=0.420N	P=0.321	P=0.454N
All Organs: Malignant Neoplasms				
Overall rate	41/64 (64.1%)	28/48 (58.3%)	26/48 (54.2%)	24/49 (49.0%)
Adjusted rate	66.0%	61.2%	55.6%	51.1%
Terminal rate	26/45 (57.8%)	16/31 (51.6%)	17/34 (50.0%)	18/39 (46.2%)
First incidence (days)	534	526	383	393
Poly-3 test	P=0.053N	P=0.381N	P=0.181N	P=0.082N
All Organs: Benign or Malignant Neoplasms				
Overall rate	51/64 (79.7%)	34/48 (70.8%)	34/48 (70.8%)	33/49 (67.3%)
Adjusted rate	81.3%	72.8%	71.7%	69.7%
Terminal rate	35/45 (77.8%)	19/31 (61.3%)	23/34 (67.6%)	26/39 (66.7%)
First incidence (days)	534	526	383	393
Poly-3 test	P=0.087N	P=0.201N	P=0.163N	P=0.111N

(T) Terminal sacrifice

[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE B2d
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	5/60 (8.3%)	5/46 (10.9%)	2/46 (4.3%)	2/25 (8.0%)
Adjusted rate[b]	9.0%	12.2%	4.7%	8.8%
Terminal rate[c]	5/44 (11.4%)	4/30 (13.3%)	1/36 (2.8%)	1/16 (6.3%)
First incidence (days)	737 (T)	671	597	590
Poly-3 test[d]	P=0.361N	P=0.436	P=0.334N	P=0.652N
Harderian Gland: Adenoma or Adenocarcinoma				
Overall rate	6/60 (10.0%)	5/46 (10.9%)	2/46 (4.3%)	2/25 (8.0%)
Adjusted rate	10.8%	12.2%	4.7%	8.8%
Terminal rate	6/44 (13.6%)	4/30 (13.3%)	1/36 (2.8%)	1/16 (6.3%)
First incidence (days)	737 (T)	671	597	590
Poly-3 test	P=0.257N	P=0.548	P=0.233N	P=0.552N
Liver: Hepatocellular Adenoma				
Overall rate	9/61 (14.8%)	1/50 (2.0%)	8/48 (16.7%)	4/26 (15.4%)
Adjusted rate	15.9%	2.3%	18.2%	17.3%
Terminal rate	5/45 (11.1%)	1/30 (3.3%)	7/37 (18.9%)	2/16 (12.5%)
First incidence (days)	685	736 (T)	733	659
Poly-3 test	P=0.334	P=0.028N	P=0.480	P=0.568
Liver: Hepatocellular Carcinoma				
Overall rate	3/61 (4.9%)	3/50 (6.0%)	5/48 (10.4%)	0/26 (0.0%)
Adjusted rate	5.3%	7.0%	11.3%	0.0%
Terminal rate	2/45 (4.4%)	2/30 (6.7%)	4/37 (10.8%)	0/16 (0.0%)
First incidence (days)	669	664	610	—[e]
Poly-3 test	P=0.560N	P=0.531	P=0.232	P=0.324N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	12/61 (19.7%)	4/50 (8.0%)	12/48 (25.0%)	4/26 (15.4%)
Adjusted rate	21.0%	9.3%	27.1%	17.3%
Terminal rate	7/45 (15.6%)	3/30 (10.0%)	10/37 (27.0%)	2/16 (12.5%)
First incidence (days)	669	664	610	659
Poly-3 test	P=0.421	P=0.094N	P=0.317	P=0.472N
Liver: Hemangiosarcoma, Hepatocellular Adenoma, or Hepatocellular Carcinoma				
Overall rate	14/61 (23.0%)	5/50 (10.0%)	12/48 (25.0%)	5/26 (19.2%)
Adjusted rate	24.5%	11.6%	27.1%	21.2%
Terminal rate	9/45 (20.0%)	4/30 (13.3%)	10/37 (27.0%)	2/16 (12.5%)
First incidence (days)	669	664	610	593
Poly-3 test	P=0.499	P=0.084N	P=0.475	P=0.486N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/62 (3.2%)	3/50 (6.0%)	4/47 (8.5%)	1/25 (4.0%)
Adjusted rate	3.5%	7.0%	9.3%	4.4%
Terminal rate	2/45 (4.4%)	3/30 (10.0%)	4/37 (10.8%)	0/16 (0.0%)
First incidence (days)	737 (T)	738 (T)	740 (T)	600
Poly-3 test	P=0.314	P=0.374	P=0.220	P=0.679
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/62 (8.1%)	0/50 (0.0%)	3/47 (6.4%)	2/25 (8.0%)
Adjusted rate	8.7%	0.0%	7.0%	8.8%
Terminal rate	1/45 (2.2%)	0/30 (0.0%)	3/37 (8.1%)	1/16 (6.3%)
First incidence (days)	579	—	741 (T)	671
Poly-3 test	P=0.554	P=0.064N	P=0.523N	P=0.659

TABLE B2d
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	7/62 (11.3%)	3/50 (6.0%)	7/47 (14.9%)	3/25 (12.0%)
Adjusted rate	12.1%	7.0%	16.2%	13.0%
Terminal rate	3/45 (6.7%)	3/30 (10.0%)	7/37 (18.9%)	1/16 (6.3%)
First incidence (days)	579	738 (T)	740 (T)	600
Poly-3 test	P=0.354	P=0.306N	P=0.384	P=0.605
Mammary Gland: Adenocarcinoma				
Overall rate	6/63 (9.5%)	1/47 (2.1%)	3/48 (6.3%)	0/24 (0.0%)
Adjusted rate	10.3%	2.4%	6.8%	0.0%
Terminal rate	2/45 (4.4%)	0/30 (0.0%)	2/37 (5.4%)	0/16 (0.0%)
First incidence (days)	567	555	645	—
Poly-3 test	P=0.092N	P=0.127N	P=0.397N	P=0.140N
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	6/63 (9.5%)	2/47 (4.3%)	3/48 (2.1%)	0/24 (0.0%)
Adjusted rate	10.3%	4.7%	6.8%	0.0%
Terminal rate	2/45 (4.4%)	0/30 (0.0%)	2/37 (5.4%)	0/16 (0.0%)
First incidence (days)	567	555	645	—
Poly-3 test	P=0.094N	P=0.260N	P=0.397N	P=0.140N
Ovary: Cystadenoma				
Overall rate	2/60 (3.3%)	1/47 (2.1%)	1/48 (2.1%)	2/25 (8.0%)
Adjusted rate	3.6%	2.4%	2.3%	8.9%
Terminal rate	2/44 (4.5%)	1/30 (3.3%)	0/37 (0.0%)	0/16 (0.0%)
First incidence (days)	733 (T)	736 (T)	708	694
Poly-3 test	P=0.315	P=0.599N	P=0.580N	P=352N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	6/60 (10.0%)	3/47 (6.4%)	4/45 (8.9%)	1/23 (4.3%)
Adjusted rate	10.8%	7.2%	9.5%	4.9%
Terminal rate	2/44 (4.5%)	3/30 (10.0%)	2/36 (5.6%)	1/14 (7.1%)
First incidence (days)	579	739 (T)	597	743 (T)
Poly-3 test	P=0.313N	P=0.404N	P=0.552N	P=0.372N
Spleen: Hemangiosarcoma				
Overall rate	2/62 (3.2%)	3/50 (6.0%)	3/48 (6.3%)	0/25 (0.0%)
Adjusted rate	3.5%	6.9%	6.8%	0.0%
Terminal rate	2/45 (4.4%)	1/30 (3.3%)	2/37 (5.4%)	0/16 (0.0%)
First incidence (days)	733 (T)	601	610	—
Poly-3 test	P=0.503N	P=0.383	P=0.388	P=0.459N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/63 (1.6%)	3/49 (6.1%)	0/48 (0.0%)	1/25 (4.0%)
Adjusted rate	1.8%	7.0%	0.0%	4.5%
Terminal rate	1/45 (2.2%)	2/30 (6.7%)	0/37 (0.0%)	1/16 (6.3%)
First incidence (days)	739 (T)	700	—	743 (T)
Poly-3 test	P=0.578	P=0.209	0.553N	P=0.539
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	2/63 (3.2%)	3/49 (6.1%)	1/48 (2.1%)	1/25 (4.0%)
Adjusted rate	3.5%	7.0%	2.3%	4.5%
Terminal rate	1/45 (2.2%)	2/30 (6.7%)	0/37 (0.0%)	1/16 (6.3%)
First incidence (days)	735	700	733	743 (T)
Poly-3 test	P=0.526N	P=0.370	0.591N	P=0.672

TABLE B2d
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	4/64 (6.3%)	4/50 (8.0%)	3/49 (6.1%)	1/26 (3.8%)
Adjusted rate	6.9%	9.2%	6.7%	4.3%
Terminal rate	4/45 (8.9%)	2/30 (6.7%)	2/37 (5.4%)	0/16 (0.0%)
First incidence (days)	732 (T)	601	610	593
Poly-3 test	P=0.405N	P=0.484	P=0.633N	P=0.528N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	5/64 (7.8%)	5/50 (10.0%)	3/49 (6.1%)	1/26 (3.8%)
Adjusted rate	8.7%	11.5%	6.7%	4.3%
Terminal rate	5/45 (11.1%)	3/30 (10.0%)	2/37 (5.4%)	0/16 (0.0%)
First incidence (days)	732 (T)	601	610	593
Poly-3 test	P=0.277N	P=0.449	P=0.499N	P=0.420N
All Organs: Leukemia				
Overall rate	0/64 (0.0%)	4/50 (8.0%)	0/49 (0.0%)	0/28 (0.0%)
Adjusted rate	0.0%	9.0%	0.0%	0.0%
Terminal rate	0/45 (0.0%)	0/30 (0.0%)	0/37 (0.0%)	0/16 (0.0%)
First incidence (days)	—	508	—	—
Poly-3 test	P=0.457N	P=0.033	—[f]	—
All Organs: Malignant Lymphoma				
Overall rate	24/64 (37.5%)	9/50 (18.0%)	19/49 (38.8%)	9/26 (34.6%)
Adjusted rate	40.4%	20.2%	41.3%	39.1%
Terminal rate	18/45 (40.0%)	3/30 (10.0%)	13/37 (35.1%)	7/16 (43.8%)
First incidence (days)	583	489	610	659
Poly-3 test	P=0.445	P=0.021N	P=0.543	P=0.555N
All Organs: Benign Neoplasms				
Overall rate	24/64 (37.5%)	18/50 (36.0%)	20/49 (40.8%)	10/26 (38.5%)
Adjusted rate	40.7%	40.9%	44.0%	41.0%
Terminal rate	18/45 (40.0%)	14/30 (46.7%)	16/37 (43.2%)	4/16 (25.0%)
First incidence (days)	579	601	597	590
Poly-3 test	P=0.457	P=0.576	P=0.448	P=0.590
All Organs: Malignant Neoplasms				
Overall rate	41/64 (64.1%)	26/50 (52.0%)	25/49 (51.0%)	12/26 (46.2%)
Adjusted rate	66.0%	53.2%	53.0%	49.0%
Terminal rate	26/45 (57.8%)	10/30 (33.3%)	17/37 (45.9%)	7/16 (43.8%)
First incidence (days)	534	489	362	492
Poly-3 test	P=0.059N	P=0.119N	P=0.116N	P=0.109N
All Organs: Benign or Malignant Neoplasms				
Overall rate	51/64 (79.7%)	38/50 (76.0%)	35/49 (71.4%)	19/26 (73.1%)
Adjusted rate	81.3%	77.4%	72.9%	73.1%
Terminal rate	35/45 (77.8%)	21/30 (70.0%)	25/37 (67.6%)	9/16 (56.3%)
First incidence (days)	534	489	362	492
Poly-3 test	P=0.151N	P=0.387N	P=0.200N	P=0.278N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B3
Historical Incidence of Neoplasms in Control Female B6C3F1/Nctr BR Mice[a]

Study	Thyroid Gland (Follicular Cell) Adenoma	Thyroid Gland (Follicular Cell) Adenoma or Carcinoma	Skin Fibrous Histiocytoma, Fibrosarcoma, Sarcoma, or Myxosarcoma	Lung Alveolar/bronchiolar Adenoma
Sulfamethazine	5/180 (2.8%)	5/180 (2.8%)	0/181 (0.0%)	5/182 (2.7%)
Doxylamine	0/44 (0.0%)	0/44 (0.0%)	1/48 (2.1%)	3/48 (6.3%)
Pyrilamine	0/47 (0.0%)	0/47 (0.0%)	1/48 (2.1%)	1/48 (2.1%)
Triprolidine	1/45 (2.2%)	1/45 (2.2%)	0/46 (0.0%)	3/47 (6.4%)
Fumonisin B_1	0/46 (0.0%)	0/46 (0.0%)	1/47 (2.1%)	2/47 (4.3%)
Chloral Hydrate	1/141 (0.7%)	1/141 (0.7%)	1/139 (0.7%)	8/143 (5.6%)
Urethane and Ethanol	1/47 (2.1%)	1/47 (2.1%)	4/48 (8.3%)	4/48 (8.3%)
Malachite Green	1/47 (2.1%)	1/47 (2.1%)	0/48 (0.0%)	4/48 (8.3%)
Leucomalachite Green	1/46 (2.2%)	1/46 (2.2%)	0/46 (0.0%)	3/47 (6.4%)
Total	10/643 (1.6%)	10/643 (1.6%)	8/651 (1.6%)	33/658 (5.0%)
Range	0.0%-2.8%	0.0%-2.8%	0.0%-8.3%	2.1%-8.3%

[a] Data as of June 9, 2009. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT[a]

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Disposition Summary				
Animals initially in study	64	48	47	48
Early deaths				
Moribund	7	3	7	8
Natural deaths	3	3	1	2
Survivors				
Moribund	6	2	3	
Died last week of study	1		1	
Terminal sacrifice	45	38	28	37
Harvest	2	2	7	1
Animals examined microscopically	64	47	47	48
Alimentary System				
Esophagus	(62)	(46)	(47)	(47)
Gallbladder	(60)	(44)	(45)	(46)
Cyst		2 (5%)	1 (2%)	
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, chronic			1 (2%)	
Intestine large, cecum	(60)	(46)	(46)	(46)
Hyperplasia, lymphoid		2 (4%)		2 (4%)
Serosa, hyperplasia	1 (2%)			
Intestine large, colon	(60)	(46)	(46)	(46)
Intestine large, rectum	(60)	(46)	(46)	(46)
Intestine small, duodenum	(60)	(45)	(47)	(46)
Diverticulum		1 (2%)		
Intestine small, ileum	(60)	(46)	(46)	(46)
Hyperplasia, lymphoid		2 (4%)		1 (2%)
Inflammation, chronic active	1 (2%)			
Intestine small, jejunum	(60)	(46)	(45)	(46)
Hyperplasia, lymphoid	1 (2%)	1 (2%)		1 (2%)
Liver	(61)	(46)	(46)	(47)
Angiectasis			1 (2%)	
Basophilic focus	2 (3%)	3 (7%)	2 (4%)	2 (4%)
Congestion				1 (2%)
Cyst	1 (2%)			
Cyst multilocular			1 (2%)	
Eosinophilic focus	3 (5%)		1 (2%)	2 (4%)
Eosinophilic focus, multiple		1 (2%)		
Hematopoietic cell proliferation	2 (3%)		2 (4%)	1 (2%)
Hemorrhage				1 (2%)
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, lymphocyte	18 (30%)	12 (26%)	13 (28%)	13 (28%)
Inflammation, chronic active	5 (8%)	3 (7%)	2 (4%)	2 (4%)
Mineralization	1 (2%)			
Mixed cell focus	1 (2%)	1 (2%)	2 (4%)	
Necrosis	3 (5%)		5 (11%)	2 (4%)
Tension lipidosis	7 (11%)	5 (11%)	2 (4%)	11 (23%)
Vacuolization cytoplasmic	32 (52%)	19 (41%)	23 (50%)	18 (38%)
Mesentery	(8)	(11)	(4)	(8)
Cyst		1 (9%)		
Hemorrhage		2 (18%)		
Fat, necrosis	8 (100%)	11 (100%)	3 (75%)	8 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Alimentary System (continued)				
Pancreas	(62)	(46)	(46)	(46)
Cyst	1 (2%)	1 (2%)		
Cytoplasmic alteration				1 (2%)
Edema		1 (2%)		
Infiltration cellular, lymphocyte	23 (37%)	13 (28%)	15 (33%)	18 (39%)
Inflammation, chronic active		1 (2%)		
Necrosis		1 (2%)		
Acinus, degeneration	2 (3%)	2 (4%)	1 (2%)	1 (2%)
Fat, necrosis		1 (2%)		
Salivary glands	(62)	(46)	(45)	(47)
Infiltration cellular, lymphocyte	47 (76%)	39 (85%)	40 (89%)	38 (81%)
Acinus, degeneration			1 (2%)	
Stomach, forestomach	(62)	(45)	(47)	(46)
Ulcer	1 (2%)			
Epithelium, hyperplasia	2 (3%)		1 (2%)	
Stomach, glandular	(60)	(45)	(46)	(46)
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, chronic active		1 (2%)		
Mineralization			1 (2%)	
Epithelium, hyperplasia		1 (2%)		
Cardiovascular System				
Blood vessel	(62)	(46)	(46)	(48)
Heart	(63)	(46)	(47)	(47)
Inflammation, suppurative	1 (2%)			
Polyarteritis				1 (2%)
Endocrine System				
Adrenal cortex	(61)	(46)	(47)	(47)
Accessory adrenal cortical nodule	6 (10%)	3 (7%)	4 (9%)	4 (9%)
Angiectasis			1 (2%)	
Cyst		1 (2%)		
Hypertrophy	1 (2%)			
Vacuolization cytoplasmic	3 (5%)	6 (13%)	4 (9%)	6 (13%)
Subcapsular, hyperplasia	60 (98%)	45 (98%)	46 (98%)	46 (98%)
Adrenal medulla	(60)	(43)	(45)	(46)
Islets, pancreatic	(62)	(46)	(46)	(47)
Hyperplasia	1 (2%)	4 (9%)	3 (7%)	2 (4%)
Infiltration cellular, lymphocyte	1 (2%)			1 (2%)
Parathyroid gland	(54)	(40)	(44)	(45)
Cyst	1 (2%)	1 (3%)		
Hypertrophy	1 (2%)			
Pituitary gland	(60)	(44)	(44)	(46)
Hemorrhage		1 (2%)		
Pars distalis, angiectasis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pars distalis, cyst	2 (3%)	6 (14%)	2 (5%)	1 (2%)
Pars distalis, hyperplasia	8 (13%)	7 (16%)	10 (23%)	10 (22%)
Thyroid gland	(59)	(46)	(46)	(47)
Cyst				1 (2%)
Ectopic thymus	1 (2%)			
Infiltration cellular, lymphocyte	2 (3%)	1 (2%)		4 (9%)
Follicle, degeneration	9 (15%)	7 (15%)	4 (9%)	9 (19%)
Follicular cell, hyperplasia		1 (2%)		
Follicular cell, hypertrophy	2 (3%)			

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
General Body System				
Tissue NOS	(3)	(1)	(2)	(1)
Abdominal, inflammation, chronic active	1 (33%)			
Fat, necrosis				1 (100%)
Genital System				
Clitoral gland	(60)	(44)	(43)	(46)
Atrophy				2 (4%)
Degeneration	55 (92%)	41 (93%)	41 (95%)	39 (85%)
Ovary	(60)	(45)	(45)	(47)
Angiectasis			1 (2%)	
Atrophy	57 (95%)	44 (98%)	39 (87%)	44 (94%)
Cyst	22 (37%)	14 (31%)	11 (24%)	13 (28%)
Hematocyst			2 (4%)	
Infiltration cellular, lymphocyte			2 (4%)	
Bilateral, cyst	3 (5%)	3 (7%)	4 (9%)	4 (9%)
Germinal epithelium, hyperplasia			2 (4%)	
Uterus	(62)	(46)	(47)	(47)
Angiectasis		1 (2%)		
Hydrometra	9 (15%)	5 (11%)	4 (9%)	6 (13%)
Thrombosis		1 (2%)		
Endometrium, hyperplasia, cystic	52 (84%)	38 (83%)	42 (89%)	41 (87%)
Hematopoietic System				
Bone marrow	(61)	(46)	(47)	(46)
Hyperplasia	2 (3%)	2 (4%)	3 (6%)	
Lymph node	(15)	(5)	(12)	(8)
Hemorrhage	1 (7%)		1 (8%)	
Hyperplasia, lymphoid			1 (8%)	
Axillary, autolysis	1 (7%)			
Axillary, hyperplasia, lymphoid		1 (20%)		2 (25%)
Bronchial, autolysis	1 (7%)			
Iliac, autolysis	1 (7%)			
Lumbar, autolysis	1 (7%)			
Lumbar, hyperplasia, lymphoid	5 (33%)		1 (8%)	2 (25%)
Lumbar, infiltration cellular, plasma cell	1 (7%)			
Lumbar, infiltration cellular, polymorphonuclear	2 (13%)			
Mediastinal, autolysis	1 (7%)			
Mediastinal, hyperplasia, lymphoid	1 (7%)			
Pancreatic, hyperplasia, lymphoid		1 (20%)		
Renal, autolysis	1 (7%)			
Renal, hyperplasia, lymphoid	1 (7%)		1 (8%)	2 (25%)
Renal, infiltration cellular, polymorphonuclear	1 (7%)			
Sinus, dilatation	1 (7%)		2 (17%)	
Lymph node, mandibular	(61)	(46)	(44)	(47)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)		1 (2%)	
Hyperplasia, lymphoid	16 (26%)	12 (26%)	9 (20%)	9 (19%)
Infiltration cellular, plasma cell	1 (2%)			
Infiltration cellular, polymorphonuclear	1 (2%)			

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(60)	(45)	(46)	(45)
Angiectasis				1 (2%)
Autolysis	1 (2%)			1 (2%)
Cyst		1 (2%)		
Hemorrhage		2 (4%)		
Hyperplasia			1 (2%)	
Hyperplasia, lymphoid	20 (33%)	16 (36%)	17 (37%)	16 (36%)
Infiltration cellular, histiocyte	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, polymorphonuclear	1 (2%)	1 (2%)		2 (4%)
Thrombosis	1 (2%)			
Sinus, dilatation		1 (2%)		1 (2%)
Spleen	(62)	(46)	(47)	(47)
Autolysis	1 (2%)			1 (2%)
Erythrophagocytosis	1 (2%)			
Hematopoietic cell proliferation	14 (23%)	8 (17%)	13 (28%)	11 (23%)
Hyperplasia, lymphoid	32 (52%)	25 (54%)	25 (53%)	28 (60%)
Necrosis			1 (2%)	
Pigmentation		1 (2%)		
Thymus	(55)	(44)	(42)	(46)
Angiectasis		1 (2%)		
Atrophy	13 (24%)	13 (30%)	12 (29%)	16 (35%)
Autolysis				1 (2%)
Hyperplasia, lymphoid	12 (22%)	12 (27%)	6 (14%)	5 (11%)
Mineralization				1 (2%)
Fat, necrosis				1 (2%)
Integumentary System				
Mammary gland	(63)	(45)	(46)	(47)
Lactation	2 (3%)	2 (4%)	1 (2%)	
Alveolus, hyperplasia	2 (3%)	1 (2%)	2 (4%)	1 (2%)
Skin	(63)	(46)	(47)	(48)
Inflammation, chronic active			1 (2%)	
Ulcer			1 (2%)	
Musculoskeletal System				
Bone, femur	(64)	(47)	(47)	(48)
Fibro-osseous lesion		1 (2%)	1 (2%)	
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain, brain stem	(61)	(46)	(46)	(47)
Compression	1 (2%)	1 (2%)	3 (7%)	1 (2%)
Hemorrhage	1 (2%)	1 (2%)		
Brain, cerebellum	(62)	(46)	(46)	(47)
Meninges, infiltration cellular, lymphocyte				1 (2%)
Brain, cerebrum	(62)	(46)	(46)	(47)
Mineralization	41 (66%)	26 (57%)	22 (48%)	22 (47%)
Meninges, infiltration cellular, lymphocyte				1 (2%)
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)

TABLE B4a
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Respiratory System				
Lung	(62)	(46)	(47)	(48)
Autolysis				1 (2%)
Congestion			1 (2%)	
Crystals	5 (8%)	1 (2%)	3 (6%)	3 (6%)
Hemorrhage	1 (2%)			1 (2%)
Infiltration cellular, histiocyte	8 (13%)	1 (2%)	4 (9%)	4 (8%)
Infiltration cellular, lymphocyte	19 (31%)	14 (30%)	13 (28%)	14 (29%)
Inflammation, chronic active		2 (4%)		1 (2%)
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (5%)	1 (2%)	2 (4%)	1 (2%)
Nose	(62)	(46)	(47)	(48)
Inflammation, suppurative	1 (2%)			
Posterior to upper incisor, dysplasia			1 (2%)	
Trachea	(61)	(46)	(47)	(47)
Special Senses System				
Eye	(59)	(46)	(45)	(46)
Cataract		1 (2%)	1 (2%)	1 (2%)
Bilateral, cataract			1 (2%)	
Cornea, inflammation, chronic active	1 (2%)		1 (2%)	1 (2%)
Cornea, necrosis				1 (2%)
Harderian gland	(60)	(46)	(47)	(46)
Infiltration cellular, lymphocyte	7 (12%)	6 (13%)	8 (17%)	6 (13%)
Inflammation, chronic active				1 (2%)
Urinary System				
Kidney	(62)	(46)	(46)	(46)
Amyloid deposition	1 (2%)	1 (2%)		
Hemorrhage		1 (2%)		
Hyaline droplet			2 (4%)	1 (2%)
Hydronephrosis		1 (2%)		
Infiltration cellular, lymphocyte	17 (27%)	15 (33%)	16 (35%)	12 (26%)
Metaplasia, osseous	2 (3%)	3 (7%)		3 (7%)
Nephropathy	31 (50%)	23 (50%)	27 (59%)	25 (54%)
Adventitia, inflammation, chronic active		1 (2%)		
Adventitia, fat, necrosis		1 (2%)		
Urinary bladder	(60)	(46)	(46)	(46)
Edema			1 (2%)	
Infiltration cellular, lymphocyte	20 (33%)	21 (46%)	19 (41%)	20 (43%)
Inflammation, chronic active			1 (2%)	
Lumen, dilatation	1 (2%)			

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT and 3TC[a]

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Disposition Summary				
Animals initially in study	64	48	51	48
Early deaths				
Moribund	7	9	8	4
Natural deaths	3	3	2	3
Survivors				
Moribund	6	2	2	1
Died last week of study	1		1	
Terminal sacrifice	45	32	35	35
Harvest	2	2	3	5
Animals examined microscopically	64	48	51	48
Alimentary System				
Esophagus	(62)	(47)	(50)	(48)
Gallbladder	(60)	(45)	(48)	(46)
Infiltration cellular, lymphocyte		1 (2%)		
Intestine large, cecum	(60)	(46)	(49)	(46)
Hyperplasia, lymphoid		1 (2%)	2 (4%)	1 (2%)
Serosa, hyperplasia	1 (2%)			
Intestine large, colon	(60)	(46)	(50)	(46)
Intestine large, rectum	(60)	(46)	(50)	(46)
Hyperplasia, lymphoid		1 (2%)		1 (2%)
Intestine small, duodenum	(60)	(45)	(49)	(45)
Intestine small, ileum	(60)	(46)	(50)	(46)
Hyperplasia, lymphoid		1 (2%)		
Inflammation, chronic active	1 (2%)			
Intestine small, jejunum	(60)	(46)	(48)	(46)
Hyperplasia, lymphoid	1 (2%)			
Liver	(61)	(47)	(50)	(48)
Angiectasis			1 (2%)	
Basophilic focus	2 (3%)	2 (4%)	3 (6%)	7 (15%)
Basophilic focus, multiple			1 (2%)	
Clear cell focus			2 (4%)	1 (2%)
Congestion			1 (2%)	
Cyst	1 (2%)			
Eosinophilic focus	3 (5%)	1 (2%)	1 (2%)	2 (4%)
Hematocyst			1 (2%)	
Hematopoietic cell proliferation	2 (3%)		1 (2%)	
Hepatodiaphragmatic nodule				1 (2%)
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, lymphocyte	18 (30%)	10 (21%)	17 (34%)	13 (27%)
Inflammation, chronic active	5 (8%)	2 (4%)	2 (4%)	2 (4%)
Mineralization	1 (2%)			
Mixed cell focus	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Necrosis	3 (5%)	1 (2%)	1 (2%)	
Tension lipidosis	7 (11%)	8 (17%)	6 (12%)	6 (13%)
Vacuolization cytoplasmic	32 (52%)	21 (45%)	28 (56%)	16 (33%)
Mesentery	(8)	(11)	(7)	(6)
Hematocyst			1 (14%)	
Hemorrhage		1 (9%)	1 (14%)	2 (33%)
Infiltration cellular, lymphocyte			1 (14%)	1 (17%)
Fat, necrosis	8 (100%)	9 (82%)	6 (86%)	5 (83%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Alimentary System (continued)				
Pancreas	(62)	(46)	(49)	(48)
Cyst	1 (2%)			
Infiltration cellular, lymphocyte	23 (37%)	16 (35%)	19 (39%)	18 (38%)
Acinus, degeneration	2 (3%)	1 (2%)	1 (2%)	
Salivary glands	(62)	(46)	(50)	(48)
Infiltration cellular, lymphocyte	47 (76%)	38 (83%)	46 (92%)	39 (81%)
Acinus, degeneration				1 (2%)
Stomach, forestomach	(62)	(46)	(50)	(47)
Hyperkeratosis				2 (4%)
Keratin cyst			1 (2%)	
Ulcer	1 (2%)			
Epithelium, hyperplasia	2 (3%)	2 (4%)		1 (2%)
Stomach, glandular	(60)	(46)	(48)	(46)
Degeneration, hyaline				2 (4%)
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, chronic active		2 (4%)		2 (4%)
Epithelium, hyperplasia		2 (4%)		2 (4%)
Cardiovascular System				
Blood vessel	(62)	(45)	(49)	(48)
Heart	(63)	(48)	(50)	(48)
Cardiomyopathy			1 (2%)	
Inflammation, suppurative	1 (2%)			
Mineralization			1 (2%)	
Necrosis		1 (2%)		
Endocrine System				
Adrenal cortex	(61)	(47)	(50)	(47)
Accessory adrenal cortical nodule	6 (10%)	2 (4%)	1 (2%)	1 (2%)
Cyst		2 (4%)		
Hypertrophy	1 (2%)	1 (2%)		
Vacuolization cytoplasmic	3 (5%)	1 (2%)	3 (6%)	1 (2%)
Subcapsular, hyperplasia	60 (98%)	47 (100%)	49 (98%)	44 (94%)
Adrenal medulla	(60)	(46)	(47)	(46)
Islets, pancreatic	(62)	(46)	(49)	(48)
Hyperplasia	1 (2%)	4 (9%)	3 (6%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)			
Parathyroid gland	(54)	(36)	(40)	(38)
Cyst	1 (2%)			
Hyperplasia		1 (3%)		
Hypertrophy	1 (2%)			
Pituitary gland	(60)	(45)	(49)	(46)
Pars distalis, angiectasis	1 (2%)		1 (2%)	2 (4%)
Pars distalis, cyst	2 (3%)	2 (4%)	2 (4%)	3 (7%)
Pars distalis, hyperplasia	8 (13%)	6 (13%)	11 (22%)	10 (22%)
Thyroid gland	(59)	(46)	(50)	(47)
Cyst			1 (2%)	
Ectopic thymus	1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte	2 (3%)			2 (4%)
C-cell, hyperplasia				1 (2%)
Follicle, degeneration	9 (15%)	6 (13%)	4 (8%)	8 (17%)
Follicular cell, hyperplasia			2 (4%)	1 (2%)
Follicular cell, hypertrophy	2 (3%)		1 (2%)	

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
General Body System				
Tissue NOS	(3)	(0)	(2)	(1)
Hemorrhage				1 (100%)
Infiltration cellular, lymphocyte				1 (100%)
Abdominal, inflammation, chronic active	1 (33%)			
Genital System				
Clitoral gland	(60)	(44)	(49)	(44)
Atrophy		1 (2%)		1 (2%)
Degeneration	55 (92%)	41 (93%)	45 (92%)	42 (95%)
Ovary	(60)	(43)	(50)	(48)
Atrophy	57 (95%)	36 (84%)	42 (84%)	39 (81%)
Cyst	22 (37%)	13 (30%)	17 (34%)	15 (31%)
Hematocyst		3 (7%)	1 (2%)	4 (8%)
Bilateral, cyst	3 (5%)	3 (7%)	2 (4%)	1 (2%)
Fat, necrosis		1 (2%)		
Uterus	(62)	(46)	(50)	(48)
Angiectasis		1 (2%)	1 (2%)	
Hydrometra	9 (15%)	1 (2%)	1 (2%)	6 (13%)
Endometrium, hyperplasia, cystic	52 (84%)	41 (89%)	49 (98%)	41 (85%)
Hematopoietic System				
Bone marrow	(61)	(46)	(50)	(46)
Hyperplasia	2 (3%)	1 (2%)	3 (6%)	
Pigmentation				1 (2%)
Lymph node	(15)	(9)	(10)	(5)
Cyst			1 (10%)	
Hemorrhage	1 (7%)		1 (10%)	
Axillary, autolysis	1 (7%)			
Bronchial, autolysis	1 (7%)			
Iliac, autolysis	1 (7%)			
Lumbar, autolysis	1 (7%)			
Lumbar, fibrosis			1 (10%)	
Lumbar, hyperplasia, lymphoid	5 (33%)	2 (22%)	1 (10%)	1 (20%)
Lumbar, infiltration cellular, plasma cell	1 (7%)			1 (20%)
Lumbar, infiltration cellular, polymorphonuclear	2 (13%)			
Mediastinal, autolysis	1 (7%)			
Mediastinal, hyperplasia, lymphoid	1 (7%)	2 (22%)		1 (20%)
Pancreatic, hyperplasia, lymphoid			1 (10%)	
Renal, autolysis	1 (7%)			
Renal, hyperplasia, lymphoid	1 (7%)	2 (22%)		1 (20%)
Renal, infiltration cellular, plasma cell				1 (20%)
Renal, infiltration cellular, polymorphonuclear	1 (7%)			
Sinus, dilatation	1 (7%)			
Lymph node, mandibular	(61)	(45)	(49)	(48)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)			
Hyperplasia, lymphoid	16 (26%)	15 (33%)	16 (33%)	12 (25%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)		
Infiltration cellular, polymorphonuclear	1 (2%)		1 (2%)	

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(60)	(46)	(48)	(45)
Angiectasis		1 (2%)		
Autolysis	1 (2%)			
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	20 (33%)	20 (43%)	23 (48%)	21 (47%)
Infiltration cellular, histiocyte	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, plasma cell	1 (2%)	3 (7%)	2 (4%)	3 (7%)
Infiltration cellular, polymorphonuclear	1 (2%)		2 (4%)	
Inflammation, chronic active				2 (4%)
Thrombosis	1 (2%)			
Spleen	(62)	(48)	(50)	(47)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)			
Hematopoietic cell proliferation	14 (23%)	10 (21%)	17 (34%)	13 (28%)
Hyperplasia, lymphoid	32 (52%)	31 (65%)	32 (64%)	27 (57%)
Thymus	(55)	(43)	(47)	(45)
Atrophy	13 (24%)	15 (35%)	13 (28%)	18 (40%)
Hyperplasia, lymphoid	12 (22%)	7 (16%)	9 (19%)	3 (7%)
Integumentary System				
Mammary gland	(63)	(45)	(50)	(45)
Galactocele			1 (2%)	
Lactation	2 (3%)			
Alveolus, hyperplasia	2 (3%)		1 (2%)	1 (2%)
Skin	(63)	(46)	(50)	(48)
Fibrosis				1 (2%)
Musculoskeletal System				
Bone	(0)	(0)	(0)	(1)
Bone, femur	(64)	(48)	(51)	(48)
Fibro-osseous lesion		1 (2%)	1 (2%)	1 (2%)
Skeletal muscle	(1)	(1)	(1)	(1)
Nervous System				
Brain, brain stem	(61)	(47)	(49)	(47)
Compression	1 (2%)		2 (4%)	1 (2%)
Hemorrhage	1 (2%)			
Brain, cerebellum	(62)	(47)	(49)	(47)
Brain, cerebrum	(62)	(47)	(49)	(47)
Mineralization	41 (66%)	28 (60%)	25 (51%)	25 (53%)
Peripheral nerve	(1)	(1)	(1)	(0)
Spinal cord	(1)	(1)	(1)	(0)

TABLE B4b
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Respiratory System				
Lung	(62)	(48)	(50)	(48)
Congestion			1 (2%)	
Crystals	5 (8%)	2 (4%)		2 (4%)
Hemorrhage	1 (2%)		1 (2%)	
Infiltration cellular, histiocyte	8 (13%)	3 (6%)		2 (4%)
Infiltration cellular, lymphocyte	19 (31%)	14 (29%)	16 (32%)	14 (29%)
Inflammation, chronic				1 (2%)
Inflammation, chronic active		1 (2%)	1 (2%)	
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (5%)	1 (2%)	2 (4%)	1 (2%)
Nose	(62)	(46)	(51)	(48)
Inflammation, suppurative	1 (2%)			
Trachea	(61)	(46)	(50)	(47)
Special Senses System				
Eye	(59)	(45)	(49)	(46)
Cataract		1 (2%)	1 (2%)	1 (2%)
Bilateral, cataract		1 (2%)		
Cornea, inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Harderian gland	(60)	(46)	(50)	(47)
Cyst			1 (2%)	
Infiltration cellular, lymphocyte	7 (12%)	2 (4%)	4 (8%)	4 (9%)
Inflammation, chronic active		1 (2%)		
Acinus, degeneration			1 (2%)	1 (2%)
Urinary System				
Kidney	(62)	(46)	(50)	(46)
Amyloid deposition	1 (2%)			
Hyaline droplet		2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	17 (27%)	13 (28%)	17 (34%)	16 (35%)
Metaplasia, osseous	2 (3%)	1 (2%)	2 (4%)	1 (2%)
Nephropathy	31 (50%)	26 (57%)	23 (46%)	18 (39%)
Urinary bladder	(60)	(45)	(49)	(46)
Infiltration cellular, lymphocyte	20 (33%)	24 (53%)	26 (53%)	22 (48%)
Lumen, dilatation	1 (2%)	1 (2%)		

TABLE B4c
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP[a]

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Disposition Summary				
Animals initially in study	64	48	48	49
Early deaths				
Moribund	7	12	7	5
Natural deaths	3	2	2	4
Survivors				
Moribund	6		2	
Died last week of study	1			
Terminal sacrifice	45	31	34	39
Harvest	2	3	3	1
Animals examined microscopically	64	48	48	49
Alimentary System				
Esophagus	(62)	(47)	(47)	(48)
Gallbladder	(60)	(45)	(45)	(45)
Cyst		1 (2%)		
Intestine large, cecum	(60)	(45)	(46)	(45)
Hyperplasia, lymphoid			3 (7%)	1 (2%)
Serosa, hyperplasia	1 (2%)			
Intestine large, colon	(60)	(46)	(46)	(45)
Intestine large, rectum	(60)	(46)	(46)	(45)
Intestine small, duodenum	(60)	(46)	(46)	(45)
Intestine small, ileum	(60)	(46)	(46)	(45)
Inflammation, chronic active	1 (2%)			
Intestine small, jejunum	(60)	(46)	(46)	(46)
Hyperplasia, lymphoid	1 (2%)			
Liver	(61)	(46)	(47)	(47)
Angiectasis			1 (2%)	
Basophilic focus	2 (3%)	3 (7%)	4 (9%)	7 (15%)
Cyst	1 (2%)	1 (2%)		
Cyst multilocular		1 (2%)		
Deformity				1 (2%)
Eosinophilic focus	3 (5%)		1 (2%)	
Fatty change			1 (2%)	
Hematopoietic cell proliferation	2 (3%)	2 (4%)		
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, lymphocyte	18 (30%)	13 (28%)	12 (26%)	16 (34%)
Inflammation, chronic active	5 (8%)	4 (9%)		3 (6%)
Mineralization	1 (2%)			
Mixed cell focus	1 (2%)		1 (2%)	1 (2%)
Necrosis	3 (5%)	1 (2%)	1 (2%)	1 (2%)
Tension lipidosis	7 (11%)	7 (15%)	10 (21%)	9 (19%)
Vacuolization cytoplasmic	32 (52%)	26 (57%)	24 (51%)	10 (21%)
Mesentery	(8)	(1)	(8)	(3)
Fat, necrosis	8 (100%)	1 (100%)	8 (100%)	3 (100%)
Pancreas	(62)	(45)	(46)	(47)
Cyst	1 (2%)	1 (2%)		
Infiltration cellular, lymphocyte	23 (37%)	20 (44%)	14 (30%)	14 (30%)
Mineralization				1 (2%)
Acinus, degeneration	2 (3%)	1 (2%)		3 (6%)
Salivary glands	(62)	(47)	(47)	(46)
Infiltration cellular, lymphocyte	47 (76%)	33 (70%)	36 (77%)	38 (83%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4c
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(62)	(46)	(46)	(47)
Hyperplasia			1 (2%)	
Inflammation, chronic active		1 (2%)		
Keratin cyst			1 (2%)	
Ulcer	1 (2%)		1 (2%)	
Epithelium, hyperplasia	2 (3%)	3 (7%)	1 (2%)	4 (9%)
Stomach, glandular	(60)	(46)	(46)	(46)
Infiltration cellular, lymphocyte	1 (2%)			
Epithelium, hyperplasia		1 (2%)		
Cardiovascular System				
Blood vessel	(62)	(47)	(47)	(48)
Heart	(63)	(46)	(47)	(49)
Cardiomyopathy				1 (2%)
Inflammation, suppurative	1 (2%)			
Inflammation, chronic active			1 (2%)	
Endocrine System				
Adrenal cortex	(61)	(45)	(46)	(48)
Accessory adrenal cortical nodule	6 (10%)	4 (9%)	1 (2%)	1 (2%)
Hypertrophy	1 (2%)		1 (2%)	1 (2%)
Vacuolization cytoplasmic	3 (5%)	2 (4%)	1 (2%)	
Subcapsular, hyperplasia	60 (98%)	45 (100%)	46 (100%)	46 (96%)
Adrenal medulla	(60)	(43)	(45)	(44)
Hyperplasia				1 (2%)
Islets, pancreatic	(62)	(45)	(46)	(47)
Hyperplasia	1 (2%)	3 (7%)	4 (9%)	4 (9%)
Infiltration cellular, lymphocyte	1 (2%)			
Parathyroid gland	(54)	(38)	(44)	(41)
Cyst	1 (2%)			
Hyperplasia				1 (2%)
Hypertrophy	1 (2%)			
Infiltration cellular, lymphocyte		1 (3%)	1 (2%)	1 (2%)
Pituitary gland	(60)	(43)	(46)	(42)
Pars distalis, angiectasis	1 (2%)		1 (2%)	4 (10%)
Pars distalis, cyst	2 (3%)	1 (2%)		4 (10%)
Pars distalis, hyperplasia	8 (13%)	8 (19%)	7 (15%)	13 (31%)
Rathke's cleft, dilatation			1 (2%)	
Thyroid gland	(59)	(46)	(45)	(47)
Cyst		1 (2%)	1 (2%)	1 (2%)
Ectopic thymus	1 (2%)	1 (2%)	1 (2%)	
Infiltration cellular, lymphocyte	2 (3%)	1 (2%)	3 (7%)	1 (2%)
Follicle, degeneration	9 (15%)	9 (20%)	7 (16%)	9 (19%)
Follicular cell, hyperplasia				1 (2%)
Follicular cell, hypertrophy	2 (3%)			
General Body System				
Tissue NOS	(3)	(1)	(0)	(0)
Abdominal, inflammation, chronic active	1 (33%)			

TABLE B4c
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Genital System				
Clitoral gland	(60)	(46)	(46)	(46)
Atrophy		1 (2%)	1 (2%)	
Degeneration	55 (92%)	42 (91%)	43 (93%)	45 (98%)
Inflammation, suppurative			1 (2%)	
Ovary	(60)	(46)	(47)	(48)
Atrophy	57 (95%)	38 (83%)	42 (89%)	40 (83%)
Cyst	22 (37%)	21 (46%)	20 (43%)	21 (44%)
Hematocyst		1 (2%)		
Infiltration cellular, lymphocyte			2 (4%)	1 (2%)
Bilateral, cyst	3 (5%)	5 (11%)	2 (4%)	8 (17%)
Parenchymal cell, degeneration				1 (2%)
Uterus	(62)	(46)	(46)	(48)
Hydrometra	9 (15%)	1 (2%)	4 (9%)	1 (2%)
Endometrium, hyperplasia, cystic	52 (84%)	44 (96%)	42 (91%)	44 (92%)
Hematopoietic System				
Bone marrow	(61)	(46)	(46)	(48)
Hyperplasia	2 (3%)	1 (2%)	1 (2%)	1 (2%)
Lymph node	(15)	(11)	(5)	(4)
Hemorrhage	1 (7%)			1 (25%)
Hyperplasia, lymphoid				1 (25%)
Axillary, autolysis	1 (7%)			
Bronchial, autolysis	1 (7%)			
Iliac, autolysis	1 (7%)			
Lumbar, autolysis	1 (7%)			
Lumbar, hyperplasia, lymphoid	5 (33%)			
Lumbar, infiltration cellular, plasma cell	1 (7%)			
Lumbar, infiltration cellular, polymorphonuclear	2 (13%)			
Mediastinal, autolysis	1 (7%)			
Mediastinal, hyperplasia, lymphoid	1 (7%)		1 (20%)	
Mediastinal, infiltration cellular, plasma cell			1 (20%)	
Renal, autolysis	1 (7%)			
Renal, hyperplasia, lymphoid	1 (7%)			
Renal, infiltration cellular, polymorphonuclear	1 (7%)			
Sinus, dilatation	1 (7%)			1 (25%)
Lymph node, mandibular	(61)	(47)	(47)	(47)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)			
Hyperplasia, lymphoid	16 (26%)	11 (23%)	18 (38%)	18 (38%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, polymorphonuclear	1 (2%)			
Pigmentation				1 (2%)
Lymph node, mesenteric	(60)	(46)	(46)	(47)
Angiectasis			1 (2%)	
Autolysis	1 (2%)			
Hematopoietic cell proliferation			1 (2%)	
Hyperplasia, lymphoid	20 (33%)	10 (22%)	21 (46%)	18 (38%)
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, plasma cell	1 (2%)		1 (2%)	2 (4%)
Infiltration cellular, polymorphonuclear	1 (2%)			
Thrombosis	1 (2%)			
Sinus, dilatation			2 (4%)	

TABLE B4c
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Hematopoietic System (continued)				
Spleen	(62)	(47)	(47)	(48)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)			
Hematopoietic cell proliferation	14 (23%)	10 (21%)	15 (32%)	9 (19%)
Hyperplasia, lymphoid	32 (52%)	25 (53%)	30 (64%)	30 (63%)
Thrombosis			1 (2%)	
Thymus	(55)	(44)	(44)	(40)
Angiectasis				1 (3%)
Atrophy	13 (24%)	11 (25%)	11 (25%)	6 (15%)
Hyperplasia, lymphoid	12 (22%)	4 (9%)	10 (23%)	10 (25%)
Integumentary System				
Mammary gland	(63)	(47)	(47)	(47)
Lactation	2 (3%)			
Metaplasia, squamous				1 (2%)
Alveolus, hyperplasia	2 (3%)		1 (2%)	2 (4%)
Skin	(63)	(47)	(47)	(49)
Fibrosis				1 (2%)
Inflammation, chronic active				1 (2%)
Ulcer				1 (2%)
Musculoskeletal System				
Bone, femur	(64)	(48)	(48)	(49)
Fibro-osseous lesion		2 (4%)	1 (2%)	
Skeletal muscle	(1)	(0)	(2)	(1)
Nervous System				
Brain, brain stem	(61)	(46)	(46)	(47)
Compression	1 (2%)		1 (2%)	2 (4%)
Degeneration			1 (2%)	
Hemorrhage	1 (2%)			
Brain, cerebellum	(62)	(46)	(46)	(47)
Brain, cerebrum	(62)	(46)	(46)	(47)
Mineralization	41 (66%)	25 (54%)	16 (35%)	19 (40%)
Ventricle, dilatation			1 (2%)	
Peripheral nerve	(1)	(0)	(1)	(1)
Axon, degeneration				1 (100%)
Spinal cord	(1)	(0)	(1)	(1)
Degeneration			1 (100%)	
Inflammation, chronic active			1 (100%)	
Axon, degeneration				1 (100%)

TABLE B4c
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Respiratory System				
Lung	(62)	(46)	(47)	(48)
Autolysis				1 (2%)
Crystals	5 (8%)		2 (4%)	1 (2%)
Hemorrhage	1 (2%)		1 (2%)	
Infiltration cellular, histiocyte	8 (13%)	1 (2%)	3 (6%)	1 (2%)
Infiltration cellular, lymphocyte	19 (31%)	8 (17%)	15 (32%)	13 (27%)
Inflammation, chronic active		1 (2%)	1 (2%)	
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (5%)	2 (4%)	2 (4%)	1 (2%)
Bronchiole, hyperplasia		1 (2%)		
Nose	(62)	(48)	(47)	(49)
Inflammation, suppurative	1 (2%)	1 (2%)		
Posterior to upper incisor, dysplasia			1 (2%)	
Trachea	(61)	(46)	(45)	(47)
Special Senses System				
Eye	(59)	(45)	(45)	(45)
Cataract		1 (2%)	2 (4%)	1 (2%)
Bilateral, cataract		1 (2%)	1 (2%)	
Cornea, inflammation, chronic active	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Cornea, ulcer		1 (2%)		1 (2%)
Retina, degeneration			1 (2%)	
Harderian gland	(60)	(45)	(45)	(46)
Infiltration cellular, lymphocyte	7 (12%)	5 (11%)	5 (11%)	5 (11%)
Inflammation, chronic active			1 (2%)	
Epithelium, hyperplasia				1 (2%)
Urinary System				
Kidney	(62)	(48)	(47)	(47)
Amyloid deposition	1 (2%)	1 (2%)		
Hyaline droplet				1 (2%)
Infiltration cellular, lymphocyte	17 (27%)	15 (31%)	15 (32%)	11 (23%)
Metaplasia, osseous	2 (3%)	1 (2%)		
Nephropathy	31 (50%)	19 (40%)	22 (47%)	30 (64%)
Urinary bladder	(60)	(47)	(46)	(46)
Infiltration cellular, lymphocyte	20 (33%)	24 (51%)	25 (54%)	21 (46%)
Lumen, dilatation	1 (2%)			

TABLE B4d
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV[a]

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Disposition Summary				
Animals initially in study	64	50	49	26
Early deaths				
Moribund	7	8	3	4
Natural deaths	3	5	1	1
Survivors				
Moribund	6	4	5	2
Died last week of study	1		1	
Terminal sacrifice	45	30	37	16
Harvest	2	3	2	3
Animals examined microscopically	64	50	49	26
Alimentary System				
Esophagus	(62)	(49)	(48)	(25)
Gallbladder	(60)	(46)	(47)	(25)
Degeneration, hyaline				1 (4%)
Infiltration cellular, lymphocyte		1 (2%)		1 (4%)
Intestine large, cecum	(60)	(45)	(47)	(25)
Hyperplasia, lymphoid		3 (7%)	2 (4%)	
Serosa, hyperplasia	1 (2%)			
Intestine large, colon	(60)	(46)	(47)	(25)
Intestine large, rectum	(60)	(46)	(47)	(25)
Intestine small, duodenum	(60)	(45)	(47)	(25)
Intestine small, ileum	(60)	(45)	(47)	(25)
Inflammation, chronic active	1 (2%)			
Intestine small, jejunum	(60)	(45)	(47)	(25)
Hyperplasia, lymphoid	1 (2%)	2 (4%)		
Epithelium, hyperplasia		1 (2%)		
Liver	(61)	(50)	(48)	(26)
Angiectasis		2 (4%)		
Autolysis			1 (2%)	1 (4%)
Basophilic focus	2 (3%)	1 (2%)	2 (4%)	1 (4%)
Cyst	1 (2%)		1 (2%)	
Eosinophilic focus	3 (5%)		3 (6%)	1 (4%)
Hematopoietic cell proliferation	2 (3%)	2 (4%)	2 (4%)	
Infiltration cellular, histiocyte	1 (2%)			
Infiltration cellular, lymphocyte	18 (30%)	13 (26%)	15 (31%)	6 (23%)
Inflammation, chronic active	5 (8%)	1 (2%)	7 (15%)	4 (15%)
Mineralization	1 (2%)			
Mixed cell focus	1 (2%)	1 (2%)	1 (2%)	1 (4%)
Necrosis	3 (5%)	3 (6%)	3 (6%)	
Tension lipidosis	7 (11%)	6 (12%)	5 (10%)	3 (12%)
Vacuolization cytoplasmic	32 (52%)	19 (38%)	25 (52%)	11 (42%)
Mesentery	(8)	(7)	(7)	(5)
Hemorrhage		1 (14%)		
Infiltration cellular, lymphocyte			1 (14%)	
Fat, necrosis	8 (100%)	5 (71%)	5 (71%)	5 (100%)
Pancreas	(62)	(47)	(46)	(25)
Cyst	1 (2%)		2 (4%)	
Cytoplasmic alteration		1 (2%)		
Infiltration cellular, lymphocyte	23 (37%)	19 (40%)	14 (30%)	10 (40%)
Acinus, degeneration	2 (3%)	3 (6%)	2 (4%)	2 (8%)
Duct, dilatation		1 (2%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4d
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Alimentary System (continued)				
Salivary glands	(62)	(47)	(47)	(25)
Infiltration cellular, lymphocyte	47 (76%)	36 (77%)	40 (85%)	21 (84%)
Polyarteritis		1 (2%)		
Stomach, forestomach	(62)	(47)	(47)	(25)
Ulcer	1 (2%)	1 (2%)		
Epithelium, hyperplasia	2 (3%)	1 (2%)	1 (2%)	
Stomach, glandular	(60)	(46)	(47)	(25)
Infiltration cellular, lymphocyte	(2%)			
Cardiovascular System				
Blood vessel	(62)	(50)	(48)	(25)
Heart	(63)	(50)	(48)	(25)
Cardiomyopathy		1 (2%)		1 (4%)
Inflammation, suppurative	1 (2%)			
Polyarteritis		1 (2%)		
Endocrine System				
Adrenal cortex	(61)	(48)	(49)	(25)
Accessory adrenal cortical nodule	6 (10%)	2 (4%)	1 (2%)	
Cyst			1 (2%)	1 (4%)
Hypertrophy	1 (2%)			
Vacuolization cytoplasmic	3 (5%)	1 (2%)	2 (4%)	
Subcapsular, hyperplasia	60 (98%)	47 (98%)	47 (96%)	25 (100%)
Adrenal medulla	(60)	(46)	(47)	(25)
Hyperplasia			1 (2%)	
Islets, pancreatic	(62)	(47)	(46)	(25)
Hyperplasia	1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)			
Parathyroid gland	(54)	(42)	(42)	(21)
Cyst	1 (2%)			1 (5%)
Hypertrophy	1 (2%)			
Infiltration cellular, lymphocyte				1 (5%)
Pituitary gland	(60)	(47)	(45)	(23)
Pars distalis, angiectasis	1 (2%)	1 (2%)	4 (9%)	
Pars distalis, cyst	2 (3%)			
Pars distalis, hyperplasia	8 (13%)	11 (23%)	11 (24%)	2 (9%)
Thyroid gland	(59)	(47)	(48)	(25)
Cyst		1 (2%)	2 (4%)	
Ectopic thymus	1 (2%)		1 (2%)	
Infiltration cellular, lymphocyte	2 (3%)	5 (11%)	1 (2%)	1 (4%)
Inflammation, chronic active			1 (2%)	
Polyarteritis		1 (2%)		1 (4%)
Follicle, degeneration	9 (15%)	7 (15%)	4 (8%)	7 (28%)
Follicular cell, hypertrophy	2 (3%)			
General Body System				
Tissue NOS	(3)	(2)	(1)	(0)
Abdominal, fibrosis			1 (100%)	
Abdominal, inflammation, chronic active	1 (33%)	1 (50%)	1 (100%)	
Abdominal, keratin cyst		1 (50%)		
Fat, necrosis		1 (50%)		

TABLE B4d
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Genital System				
Clitoral gland	(60)	(46)	(47)	(25)
Atrophy			1 (2%)	
Degeneration	55 (92%)	42 (91%)	43 (91%)	24 (96%)
Inflammation, suppurative				1 (4%)
Ovary	(60)	(47)	(48)	(25)
Atrophy	57 (95%)	40 (85%)	43 (90%)	23 (92%)
Cyst	22 (37%)	13 (28%)	19 (40%)	8 (32%)
Cyst dermoid		1 (2%)		
Infiltration cellular, lymphocyte		2 (4%)	1 (2%)	
Inflammation, chronic active			1 (2%)	
Bilateral, cyst	3 (5%)	3 (6%)	2 (4%)	4 (16%)
Uterus	(62)	(48)	(48)	(26)
Angiectasis		2 (4%)		
Autolysis				1 (4%)
Hydrometra	9 (15%)	6 (13%)	7 (15%)	2 (8%)
Endometrium, hyperplasia, cystic	52 (84%)	42 (88%)	40 (83%)	22 (85%)
Hematopoietic System				
Bone marrow	(61)	(47)	(47)	(25)
Hyperplasia	2 (3%)	3 (6%)	2 (4%)	1 (4%)
Lymph node	(15)	(13)	(6)	(3)
Hemorrhage	1 (7%)			
Axillary, autolysis	1 (7%)			
Axillary, hyperplasia, lymphoid		1 (8%)		
Bronchial, autolysis	1 (7%)			
Iliac, autolysis	1 (7%)			
Lumbar, autolysis	1 (7%)			
Lumbar, cyst			1 (17%)	
Lumbar, hyperplasia, lymphoid	5 (33%)	2 (15%)		
Lumbar, infiltration cellular, plasma cell	1 (7%)	1 (8%)		
Lumbar, infiltration cellular, polymorphonuclear	2 (13%)			
Lumbar, inflammation, chronic active			1 (17%)	
Lumbar, polyarteritis		1 (8%)		
Mediastinal, autolysis	1 (7%)			
Mediastinal, hyperplasia, lymphoid	1 (7%)			
Pancreatic, hyperplasia, lymphoid		1 (8%)		
Renal, autolysis	1 (7%)			
Renal, hyperplasia, lymphoid	1 (7%)	2 (15%)		
Renal, infiltration cellular, polymorphonuclear	1 (7%)			
Sinus, dilatation	1 (7%)			
Lymph node, mandibular	(61)	(48)	(48)	(25)
Autolysis	1 (2%)			
Erythrophagocytosis	1 (2%)			
Hyperplasia, lymphoid	16 (26%)	13 (27%)	14 (29%)	6 (24%)
Infiltration cellular, plasma cell	1 (2%)	1 (2%)		
Infiltration cellular, polymorphonuclear	1 (2%)			
Lymph node, mesenteric	(60)	(48)	(47)	(25)
Autolysis	1 (2%)			
Hyperplasia, lymphoid	20 (33%)	27 (56%)	21 (45%)	11 (44%)
Infiltration cellular, histiocyte	1 (2%)		2 (4%)	
Infiltration cellular, plasma cell	1 (2%)	4 (8%)	1 (2%)	
Infiltration cellular, polymorphonuclear	1 (2%)			
Thrombosis	1 (2%)			

TABLE B4d
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Hematopoietic System (continued)				
Spleen	(62)	(50)	(48)	(25)
Autolysis	1 (2%)		1 (2%)	
Erythrophagocytosis	1 (2%)			
Hematopoietic cell proliferation	14 (23%)	12 (24%)	9 (19%)	8 (32%)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	32 (52%)	32 (64%)	27 (56%)	14 (56%)
Necrosis			1 (2%)	
Thymus	(55)	(44)	(48)	(25)
Atrophy	13 (24%)	18 (41%)	6 (13%)	7 (28%)
Hyperplasia, lymphoid	12 (22%)	8 (18%)	13 (27%)	4 (16%)
Integumentary System				
Mammary gland	(63)	(47)	(48)	(24)
Lactation	2 (3%)	1 (2%)		
Alveolus, hyperplasia	2 (3%)	1 (2%)	3 (6%)	
Skin	(63)	(49)	(48)	(25)
Infiltration cellular, lymphocyte		1 (2%)		
Musculoskeletal System				
Bone, femur	(64)	(50)	(49)	(26)
Fibro-osseous lesion				1 (4%)
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain, brain stem	(61)	(47)	(48)	(25)
Compression	1 (2%)	2 (4%)	3 (6%)	
Hemorrhage	1 (2%)			
Brain, cerebellum	(62)	(47)	(48)	(25)
Hemorrhage		1 (2%)		
Infiltration cellular, lymphocyte				1 (4%)
Vacuolization cytoplasmic				1 (4%)
Ventricle, dilatation			1 (2%)	
Brain, cerebrum	(62)	(47)	(48)	(25)
Cyst epithelial inclusion		1 (2%)		
Hemorrhage		1 (2%)		
Mineralization	41 (66%)	25 (53%)	18 (38%)	5 (20%)
Ventricle, dilatation			1 (2%)	
Peripheral nerve	(1)	(0)	(0)	(0)
Spinal cord	(1)	(0)	(0)	(0)
Respiratory System				
Lung	(62)	(50)	(47)	(25)
Congestion			1 (2%)	
Crystals	5 (8%)	1 (2%)	1 (2%)	2 (8%)
Hemorrhage	1 (2%)			
Infiltration cellular, histiocyte	8 (13%)	1 (2%)	4 (9%)	2 (8%)
Infiltration cellular, lymphocyte	19 (31%)	14 (28%)	9 (19%)	4 (16%)
Inflammation, chronic active		1 (2%)		
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	3 (5%)	1 (2%)	1 (2%)	1 (4%)
Nose	(62)	(48)	(47)	(26)
Inflammation, suppurative	1 (2%)			
Trachea	(61)	(47)	(47)	(25)

TABLE B4d
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Transplacental Study of AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Special Senses System				
Eye	(59)	(45)	(47)	(25)
Cataract		1 (2%)	1 (2%)	1 (4%)
Degeneration			1 (2%)	
Inflammation, chronic active			1 (2%)	
Metaplasia, squamous				1 (4%)
Bilateral, cataract			1 (2%)	
Cornea, inflammation, chronic active	1 (2%)			1 (4%)
Harderian gland	(60)	(46)	(46)	(25)
Cyst		1 (2%)		
Infiltration cellular, lymphocyte	7 (12%)	4 (9%)	4 (9%)	2 (8%)
Inflammation, chronic		1 (2%)		
Inflammation, chronic active		1 (2%)		
Acinus, degeneration		2 (4%)		
Acinus, hyperplasia				1 (4%)
Urinary System				
Kidney	(62)	(47)	(47)	(25)
Amyloid deposition	1 (2%)			
Hyaline droplet		2 (4%)		
Hydronephrosis			1 (2%)	
Hyperplasia, lymphoid				1 (4%)
Infiltration cellular, lymphocyte	17 (27%)	7 (15%)	15 (32%)	5 (20%)
Inflammation, chronic active			1 (2%)	
Metaplasia, osseous	2 (3%)	1 (2%)		
Nephropathy	31 (50%)	30 (64%)	23 (49%)	12 (48%)
Polyarteritis		1 (2%)		
Urinary bladder	(60)	(47)	(47)	(25)
Infiltration cellular, lymphocyte	20 (33%)	19 (40%)	23 (49%)	10 (40%)
Polyarteritis		1 (2%)		
Lumen, dilatation	1 (2%)			

APPENDIX C
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Bacterial mutagenicity testing procedures followed the protocols reported by Zeiger *et al*. (1992), with slight modifications. AZT, 3TC, NVP, and NFV were all sent by NCTR to the testing laboratory, ILS, Inc., and were coded prior to screening. Test samples were incubated with *Salmonella typhimurium* tester strains TA98 and TA100 and *Escherichia coli* strain WP2 *uvrA*/pKM101 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver) for 20 minutes at 37º C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days incubation at 37º C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of test compound. The highest concentrations tested with AZT and NFV were limited by toxicity. 3TC and NVP gave no evidence of toxicity and were tested up to the limit concentration of 6,000 µg/plate.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold-increase required for a chemical to be judged positive or weakly positive.

RESULTS

The same lots of AZT, 3TC, NVP, and NFV that were used in the 2-year animal studies were tested for bacterial mutagenicity in *S. typhimurium* and *E. coli* (Tables C1 through C4). The highest concentrations tested with AZT and NFV were limited by toxicity. 3TC and NVP showed no evidence of toxicity and were therefore tested up to 6,000 µg/plate, the limit concentration established by the assay protocol.

Of the four compounds tested, only AZT (0.03 to 6.0 µg/plate) was found to be mutagenic; significant increases in revertant colonies were seen in the *E. coli* strain WP2 *uvrA*/pKM101, with and without induced rat liver metabolic activation enzymes (S9), suggesting that the observed mutagenic activity did not require metabolic transformation of the parent compound. The highest number of mutant colonies was seen at AZT concentrations of 0.25 to 0.5 µg/plate, with and without S9. AZT was not mutagenic in *S. typhimurium* strains TA98 or TA100.

TABLE C1
Mutagenicity of AZT in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	92 ± 3	100 ± 6	117 ± 9	94 ± 3
	0.03	122 ± 9			111 ± 10
	0.10	115 ± 8	125 ± 6	127 ± 8	101 ± 8
	0.25	132 ± 10	119 ± 5	114 ± 3	101 ± 6
	0.5	123 ± 7	111 ± 9	90 ± 7	100 ± 6
	1	114 ± 5	116 ± 12	92 ± 14	102 ± 9
	3	114 ± 5	102 ± 2	75 ± 3	97 ± 4
	6		68 ± 6[b]	51 ± 6[b]	
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		958 ± 15	843 ± 13	2,633 ± 260	2,716 ± 80
TA98	0	28 ± 4	27 ± 2	27 ± 2	21 ± 3
	0.03	31 ± 5	22 ± 3	28 ± 2	21 ± 2
	0.10	31 ± 5	29 ± 5	25 ± 4	23 ± 3
	0.25	37 ± 1	27 ± 4	27 ± 5	32 ± 2
	0.5	34 ± 5	29 ± 3	30 ± 5	23 ± 4
	1	18 ± 1	23 ± 3	21 ± 2	16 ± 3
	3	9 ± 3[b]	14 ± 3[b]	9 ± 2[b]	6 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		439 ± 12	424 ± 102	1,739 ± 160	1,884 ± 104
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	176 ± 9	166 ± 5	214 ± 16	221 ± 10
	0.03	233 ± 4	193 ± 9	258 ± 8	269 ± 11
	0.10	247 ± 7	209 ± 10	257 ± 7	266 ± 10
	0.25	284 ± 5	250 ± 17	238 ± 9	311 ± 14
	0.5	290 ± 26	184 ± 26	181 ± 27	307 ± 6
	1	139 ± 24	55 ± 4[b]	52 ± 2[b]	149 ± 10
	3	29 ± 4[b]	4 ± 2[b]	4 ± 2[b]	1 ± 0[b]
Trial summary		Weakly positive	Equivocal	Negative	Weakly positive
Positive control		1,075 ± 176	1,165 ± 46	1,269 ± 39	1,345 ± 66

[a] Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b] Slight toxicity
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Mutagenicity of 3TC in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	89 ± 3	120 ± 6	118 ± 8	90 ± 2
	12.5	85 ± 5	113 ± 15	135 ± 9	84 ± 5
	50	87 ± 3	130 ± 8	136 ± 1	97 ± 11
	125	89 ± 2	128 ± 11	119 ± 4	82 ± 7
	500	100 ± 7	112 ± 1	132 ± 10	88 ± 4
	1,500	78 ± 3	114 ± 7	127 ± 5	84 ± 2
	6,000	87 ± 7	120 ± 5	128 ± 6	88 ± 9
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		494 ± 12	843 ± 35	2,573 ± 80	1,060 ± 115
TA98	0	25 ± 3	22 ± 2	40 ± 5	29 ± 3
	12.5	21 ± 2	21 ± 2	37 ± 1	29 ± 4
	50	22 ± 4	23 ± 4	31 ± 2	23 ± 8
	125	22 ± 1	20 ± 4	32 ± 5	32 ± 6
	500	27 ± 2	25 ± 4	34 ± 2	31 ± 2
	1,500	23 ± 4	28 ± 5	39 ± 3	33 ± 6
	6,000	25 ± 1	25 ± 4	44 ± 3	36 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control		470 ± 26	510 ± 28	822 ± 3	826 ± 40
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	224 ± 18	210 ± 8	246 ± 21	262 ± 22
	12.5	184 ± 6	192 ± 5	240 ± 5	222 ± 2
	50	204 ± 8	216 ± 2	246 ± 20	234 ± 13
	125	214 ± 9	217 ± 9	263 ± 14	255 ± 10
	500	195 ± 10	190 ± 5	251 ± 10	239 ± 17
	1,500	205 ± 5	203 ± 6	260 ± 12	254 ± 5
	6,000	215 ± 8	221 ± 6	259 ± 5	269 ± 10
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,020 ± 68	1,316 ± 40	1,246 ± 42	1,181 ± 72

[a] Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C3
Mutagenicity of NVP in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	105 ± 10	127 ± 10	130 ± 11	114 ± 8
	12.5	123 ± 4			104 ± 3
	50	118 ± 1	129 ± 10	121 ± 5	110 ± 3
	125	106 ± 4	115 ± 6	138 ± 8	104 ± 12
	500	119 ± 13	122 ± 6	134 ± 5	114 ± 1
	1,500	107 ± 9	121 ± 11	131 ± 6	108 ± 2
	3,000		126 ± 10[b]	134 ± 8[b]	
	6,000	108 ± 8[b]	138 ± 3[b]	112 ± 1[b]	83 ± 5[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		883 ± 51	864 ± 10	2,478 ± 129	2,625 ± 226
TA98	0	22 ± 1	27 ± 3	36 ± 5	34 ± 3
	12.5	23 ± 4			28 ± 4
	50	24 ± 0	25 ± 1	29 ± 1	38 ± 5
	125	23 ± 5	24 ± 5	30 ± 3	31 ± 4
	500	22 ± 2	28 ± 2	38 ± 4	29 ± 1
	1,500	27 ± 8	37 ± 5	36 ± 3	49 ± 5
	3,000		22 ± 4[b]	23 ± 2[b]	
	6,000	15 ± 2[b]	19 ± 3[b]	18 ± 2[b]	40 ± 5[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		446 ± 44	624 ± 45	1,119 ± 45	1,086 ± 13
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	199 ± 4	153 ± 13	189 ± 18	226 ± 8
	12.5	177 ± 7			218 ± 6
	50	197 ± 7	150 ± 10	183 ± 7	214 ± 12
	125	193 ± 8	185 ± 12	204 ± 6	210 ± 10
	500	187 ± 5	153 ± 2	214 ± 6	225 ± 6
	1,500	197 ± 20	158 ± 8	199 ± 6	222 ± 15
	3,000		151 ± 22[b]	204 ± 10[b]	
	6,000	258 ± 13[b]	209 ± 16[b]	207 ± 9[b]	292 ± 9[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,162 ± 58	1,090 ± 54	1,502 ± 17	1,289 ± 109

[a] Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] Precipitate on plate

[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C4
Mutagenicity of NFV in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100	0	108 ± 3	102 ± 3	97 ± 8	105 ± 4
	10	114 ± 12	101 ± 5		
	20	112 ± 7	99 ± 2	82 ± 5	89 ± 4
	50	108 ± 6	93 ± 7	84 ± 4	91 ± 5
	125	101 ± 9	130 ± 2	104 ± 12	108 ± 5
	250	120 ± 5	96 ± 8[b]	95 ± 5	96 ± 8
	500	88 ± 11[b]	92 ± 3[b]	59 ± 5[b]	70 ± 10
	1,500			69 ± 1[b]	68 ± 5[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control[c]		857 ± 15	862 ± 41	2,181 ± 126	1,847 ± 139
TA98	0	34 ± 4	19 ± 5	36 ± 1	40 ± 5
	10	18 ± 1	19 ± 1		
	20	31 ± 4	20 ± 5	29 ± 4	44 ± 1
	50	16 ± 4[b]	18 ± 3[b]	34 ± 6	42 ± 4
	125	18 ± 3[b]	21 ± 1[b]	33 ± 4	43 ± 2
	250	13 ± 1[b]	15 ± 3[b]	39 ± 8	31 ± 1
	500	19 ± 1[b]	20 ± 3[b]	29 ± 2[b]	33 ± 4
	1,500			23 ± 3[b]	25 ± 2[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		473 ± 25	719 ± 13	1,082 ± 58	1,721 ± 58
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	181 ± 10	183 ± 14	199 ± 9	206 ± 8
	10	165 ± 2	172 ± 7		
	20	159 ± 9	165 ± 3	222 ± 19	198 ± 3
	50	170 ± 5[b]	129 ± 12[b]	213 ± 10	205 ± 1
	125	184 ± 3[b]	134 ± 7[b]	207 ± 4	195 ± 10
	250	153 ± 1[b]	156 ± 6[b]	182 ± 3	193 ± 5
	500	135 ± 16[b]	147 ± 3[b]	194 ± 13	187 ± 3
	1,500			225 ± 13	220 ± 11
Trial summary		Negative	Negative	Negative	Negative
Positive control		909 ± 20	1,033 ± 48	1,287 ± 51	1,431 ± 150

[a] Study was performed at ILS, Inc. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] Precipitate on plate

[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

AZT, 3TC, NVP, and NFV were obtained from Cipla Ltd., Mumbai Central (Mumbai, India) in single lots F00573, B10250, FX1009, and HX1292, respectively. Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR) and Galbraith Laboratories, Inc. (Knoxville, TN). To ensure stability, the bulk chemicals were stored in the original cardboard containers at room temperature protected from light inside multiple, high-density polyethylene bags. Reports on analyses performed in support of the AZT, 3TC, NVP, and NFV transplacental study are on file at the NCTR.

AZT

Lot F00573 of the chemical, a white-to-beige crystalline solid, was identified as AZT by the study laboratory using proton nuclear magnetic resonance (NMR) spectroscopy, direct exposure probe/electron ionization (DEP/EI) mass spectrometry (MS), and liquid chromatography combined with mass spectrometry (LC-MS). All spectra were consistent with the structure of AZT, literature spectra, and/or the spectra of an AZT sample obtained from Sigma-Aldrich® Corporation (St. Louis, MO). A representative proton NMR spectrum is presented in Figure D1. The melting point range of lot F00573 was determined to be 122.0° to 123.1° C by Galbraith Laboratories, Inc.

Karl Fischer titration and elemental analyses of lot F00573 were performed by Galbraith Laboratories, Inc., and the study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy and high-performance liquid chromatography (HPLC). HPLC was conducted with a Waters Millennium^{32} system using photodiode array (PDA) detection at 254 nm (Waters Corporation, Milford, MA). The analytical column was a Nova-Pak® (3.9 mm × 150 mm, 4 µm particle size, and 60 Å pore size) C18 column (Waters Corporation). The mobile phase (1 mL/minute) was held at 5% acetonitrile:95% water for 5 minutes and then linearly changed to 95% acetonitrile:5% water over 20 minutes, followed by a final 5 minute hold.

For lot F00573, Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for AZT. Total impurity was estimated at 0.3% to 0.4% by proton NMR. HPLC-PDA detected no impurities with peak areas exceeding 0.1% of the total peak area and estimated a purity of approximately 99.9%. The overall purity of lot F00573 was determined to be 99% or greater.

3TC

Lot B10250 of the chemical, a white-to-off-white crystalline solid, was identified as 3TC by the study laboratory using proton NMR spectroscopy, DEP/EI-MS, and LC-MS. All spectra were consistent with the structure of 3TC and/or the spectra of a 3TC sample obtained from GlaxoWellcome (Research Triangle Park, NC). A representative proton NMR spectrum is presented in Figure D2.

Karl Fischer titration and elemental analyses of lot B10250 were performed by Galbraith Laboratories, Inc., and the study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy and the same HPLC-PDA system used to estimate the purity of lot F00573 of AZT.

For lot B10250, Karl Fischer titration indicated less than 0.097% water. Elemental analyses for carbon, hydrogen, nitrogen, and sulfur were in agreement with the theoretical values for 3TC. Total impurity was estimated at 0.5% by proton NMR spectroscopy. HPLC-PDA detected one impurity with a peak area of 1.1% of the total peak area and estimated a purity of approximately 98.9%. The overall purity of lot B10250 was estimated to be approximately 99%.

NVP

Lot FX1009 of the chemical, a white-to-off-white crystalline powder, was identified as NVP by the study laboratory using proton NMR spectroscopy, DEP/EI-MS, gas chromatography/electron ionization (GC/EI) MS, and LC-MS. All spectra were consistent with the structure of NVP, literature spectra, and/or the spectra of an NVP sample

obtained from Boehringer/Ingelheim (Ridgefield, CT). A representative proton NMR spectrum is presented in Figure D3.

Karl Fischer titration and elemental analyses of lot FX1009 were performed by Galbraith Laboratories, Inc., and the study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy and the same HPLC-PDA system used to estimate the purity of lot F00573 of AZT.

For lot FX1009, Karl Fischer titration indicated less than 0.14% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for NVP. Total impurity was estimated at 0.2% by proton NMR. HPLC-PDA detected a single peak, indicating that the test article was 100% pure. The overall purity of lot FX1009 was estimated to be at least 99.5%.

NFV

Lot HX1292 of the chemical, a white-to-off-white amorphous powder, was identified as NFV by the study laboratory using proton and carbon-13 NMR spectroscopy, DEP/EI-MS, and LC-MS. All spectra were consistent with the structure of NFV. Representative proton and carbon-13 NMR spectra are presented in Figures D4 and D5, respectively. The melting point range of lot HX1292 was determined to be 135.1° to 146.8° C by Galbraith Laboratories, Inc.

Karl Fischer titration and elemental analyses of lot HX1292 were performed by Galbraith Laboratories, Inc., and the study laboratory assessed the purity of the bulk chemical by proton NMR spectroscopy, GC with flame ionization detection (GC-FID), and the same HPLC-PDA system used to estimate the purity of lot F00573 of AZT.

For lot HX1292, Karl Fischer titration indicated 2.92% water. Elemental analyses for carbon, hydrogen, nitrogen, and sulfur were in agreement with the theoretical values for NFV. Proton NMR data suggested that the lot was contaminated with approximately 2.1% tetrahydrofuran, 0.7% diethyl ether, and 0.1% to 0.2% impurities structurally related to NFV, indicating a total of approximately 3% organic impurities. The presence of tetrahydrofuran in lot HX1292 was corroborated by GC-FID, and the organic purity of this lot was estimated to be approximately 97%. HPLC-PDA detected one impurity peak with an area of 0.20% of the total peak area and estimated a purity of approximately 99.8%. Based on these preliminary results, the overall purity of lot HX1292 was estimated to be 97%.

Subsequent experiments were conducted to determine a method for vacuum removal of tetrahydrofuran and diethyl ether from lot HX1292. A procedure was developed for drying the test article for 24 hours at 60° C under 30 inches of mercury vacuum. Characterization of the dried test article by proton NMR spectroscopy, HPLC-MS, and HPLC-PDA indicated that it was not significantly altered by the purification steps and that the concentrations of tetrahydrofuran and diethyl ether were reduced to 0.64% and 0.16%, respectively. Because the total impurities were reduced to approximately 1% by weight, the organic purity of the dried test article was estimated to be approximately 99%. HPLC-PDA of the dried test article detected one impurity with a peak area of 0.7% of the total peak area and estimated a purity of 99.3%. The overall purity of the dried sample of lot HX1292 was determined to be approximately 99%. Only dried samples of lot HX1292 were used in the dose formulations for the animal studies.

Methylcellulose/Tween®80 Vehicle

The vehicle used for dose formulations in this study was a 0.2% methylcellulose/0.1% Tween® 80 aqueous solution. This vehicle was selected based upon preliminary experiments to find a vehicle that gave suitable suspensions with the drug combinations. Methylcellulose was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in one batch (062K0144-1) and Tween® 80 was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) in one lot (13127CA-1). Proton and carbon-13 NMR analyses of both chemicals were performed by the study laboratory. For methylcellulose, proton and carbon-13 NMR spectra of batch 062K0144-1 were similar to those of a methylcellulose sample obtained from Fischer Scientific (Fair Lawn, NJ), and no resonances from small molecule impurities were detected. For Tween® 80, the proton NMR spectrum of lot 13127CA-1 was consistent with the structure of the chemical, and the carbon-13 NMR spectrum of this lot was consistent with a literature spectrum (Bugay and Findlay, 1999); both spectra of lot 13127CA-1 showed smaller resonances indicative of minor impurities.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing the test chemicals with an aqueous solution of 0.2% methylcellulose/0.1% Tween® 80 to give the required concentrations (Table D1). The dose formulations were stored at room temperature in capped glass vials for up to 21 days.

Homogeneity and stability studies of two high-dose and two low-dose suspensions of the test chemicals in the methylcellulose/Tween® 80 vehicle were conducted by the study laboratory using HPLC. For these analyses, the same Waters HPLC-PDA system was used as for the bulk chemical purity determinations except that the solvent program was a 3 minute linear gradient from 100% mobile phase A (methanol:water, 5:95; 0.005 M sodium phosphate monobasic, 0.003 M sodium pentanesulfonic acid; pH 2.5) to 100% mobile phase B (methanol:water, 90:10; 0.005 M sodium phosphate monobasic, 0.003 M sodium pentanesulfonic acid; pH 2.5) followed by a 10.5 minute hold. The two high-dose mixtures were composed of AZT (20 mg/mL), 3TC (10 mg/mL), and NVP (13.3 mg/mL) or AZT (20 mg/mL), 3TC (10 mg/mL), and NFV (83 mg/mL). The two low-dose mixtures were composed of AZT (6.7 mg/mL), 3TC (3.3 mg/mL), and NVP (4.4 mg/mL) or AZT (6.7 mg/mL), 3TC (3.3 mg/mL), and NFV (27.7 mg/mL). Homogeneity was confirmed, and stability was confirmed for 21 days for dose formulations stored in capped glass vials at room temperature.

At four time points during the transplacental dosing period, analyses of the dose formulations of the antiretroviral drugs were conducted by the study laboratory using HPLC-PDA by the system described above for the homogeneity and stability studies. Of the 43 samples measured for concentration of a test chemical, 38 were within 10% of the target concentration (Table D2).

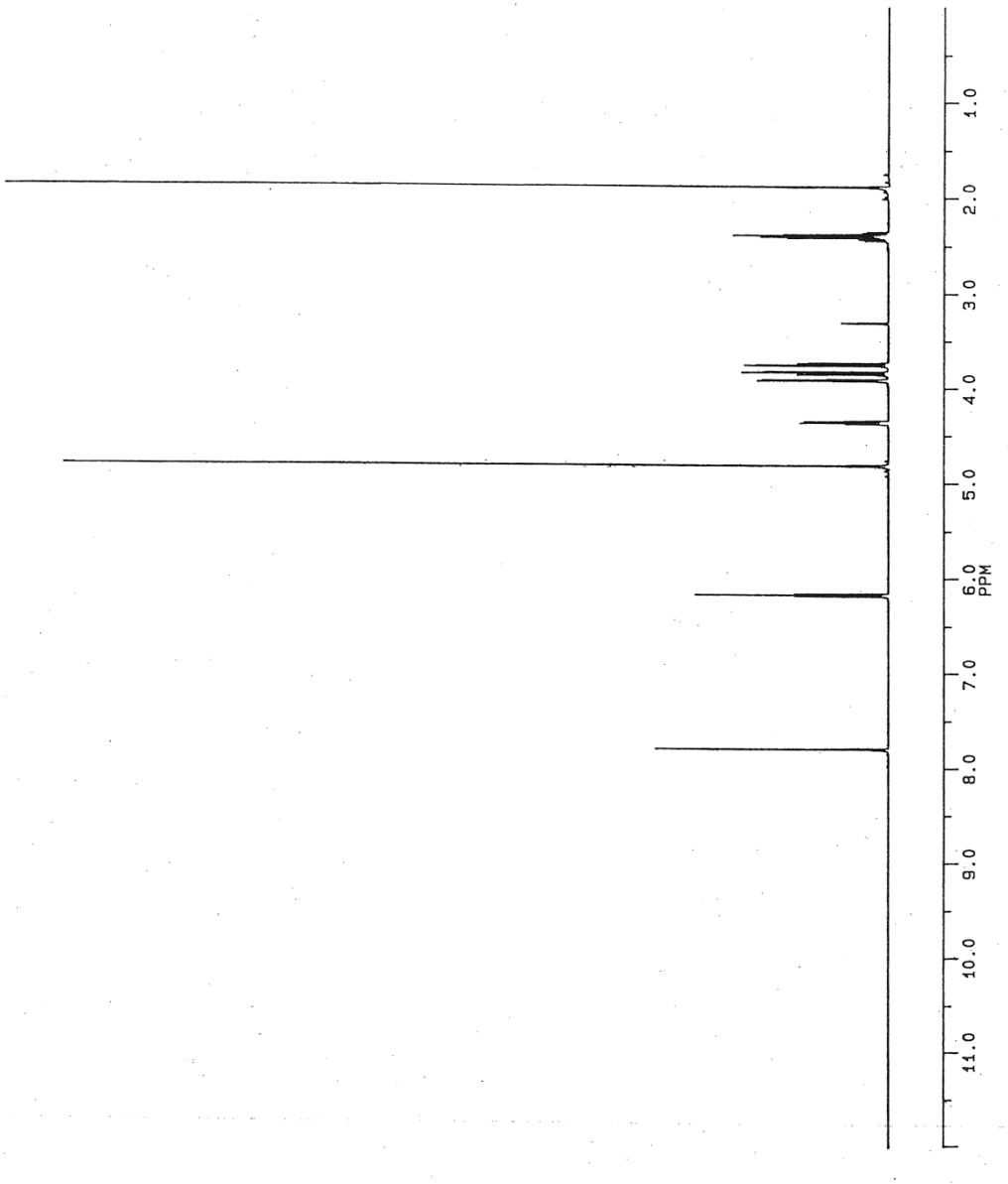

FIGURE D1
Proton Nuclear Magnetic Resonance Spectrum of AZT

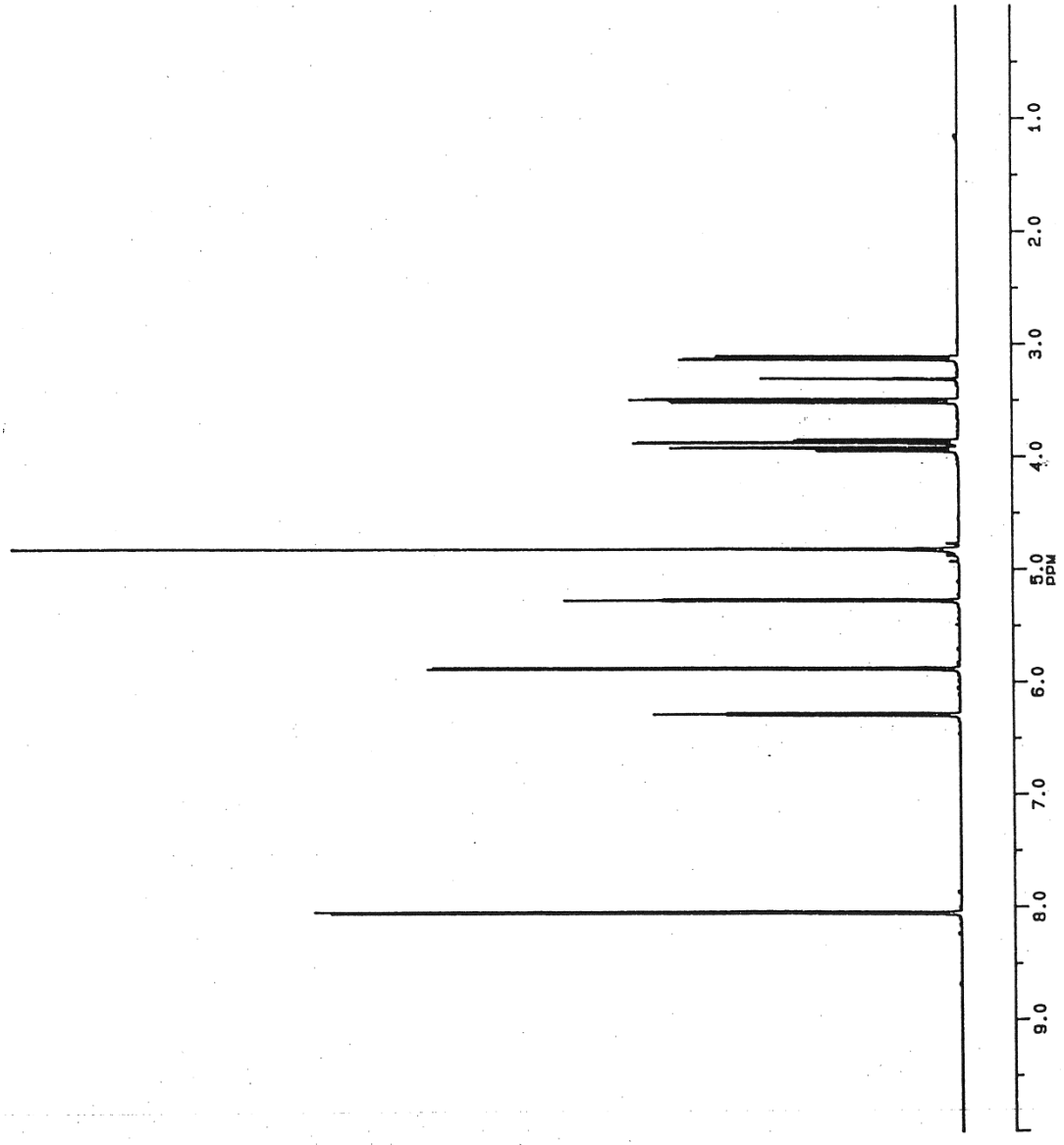

FIGURE D2
Proton Nuclear Magnetic Resonance Spectrum of 3TC

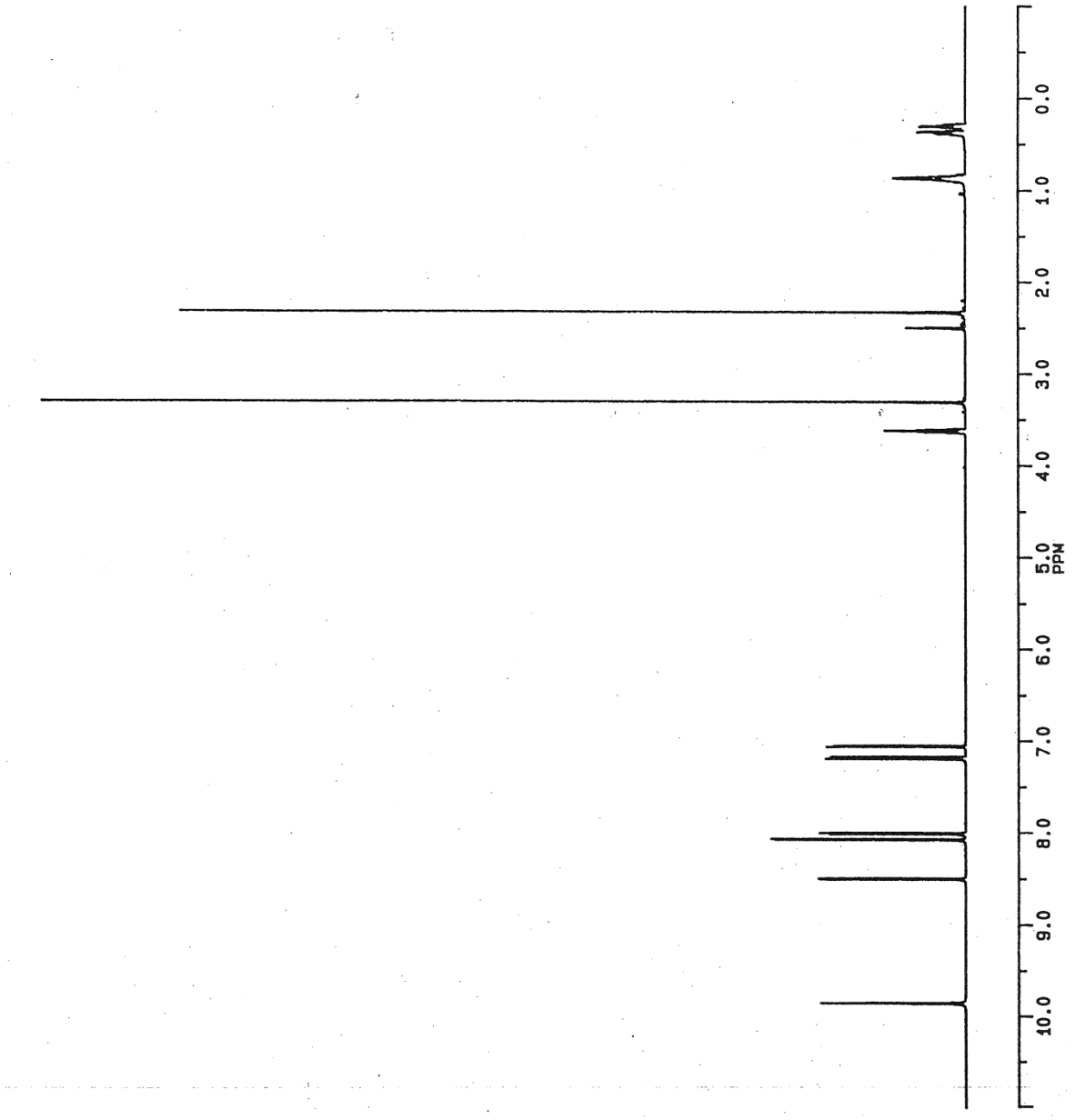

FIGURE D3
Proton Nuclear Magnetic Resonance Spectrum of NVP

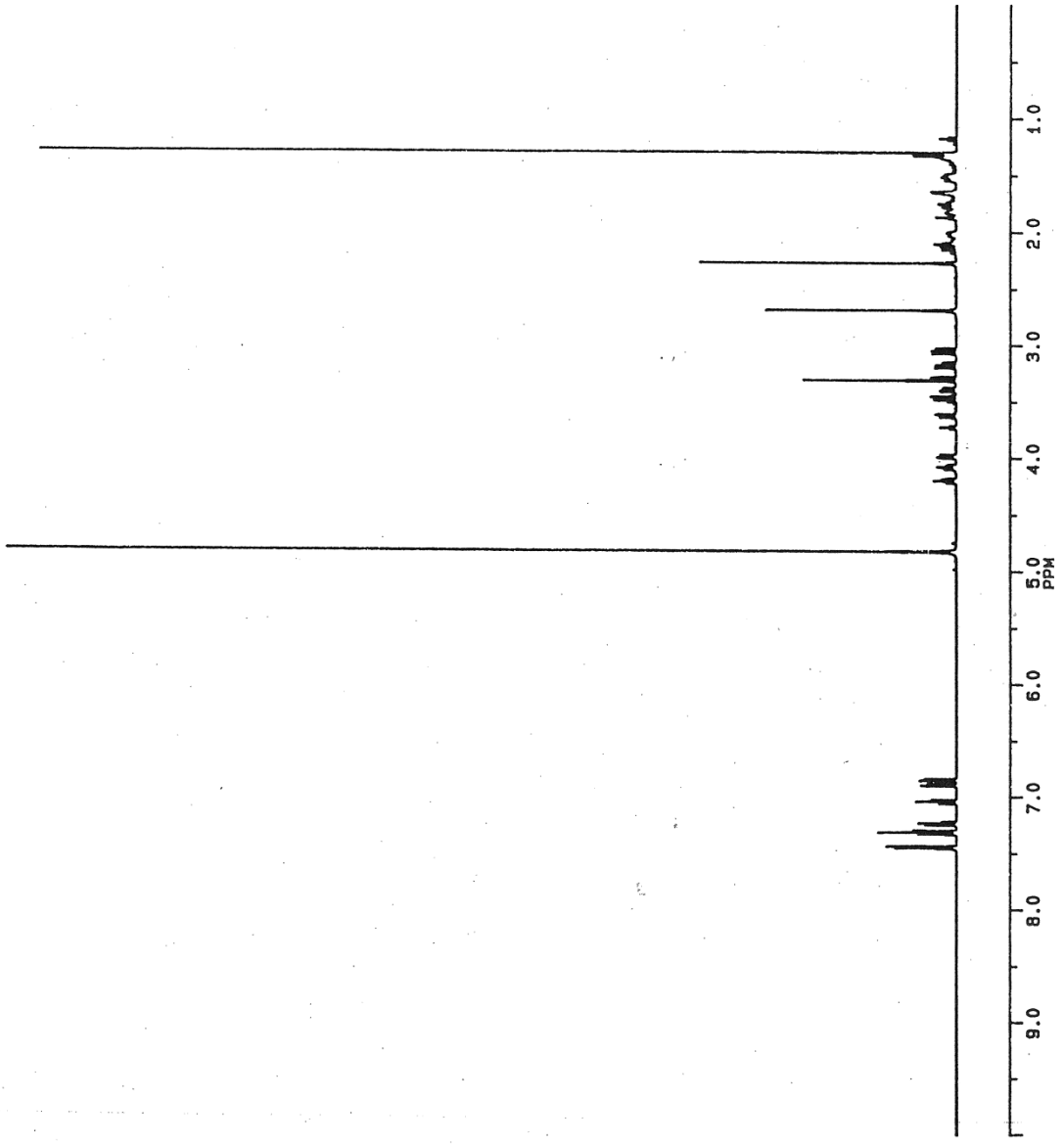

FIGURE D4
Proton Nuclear Magnetic Resonance Spectrum of NFV

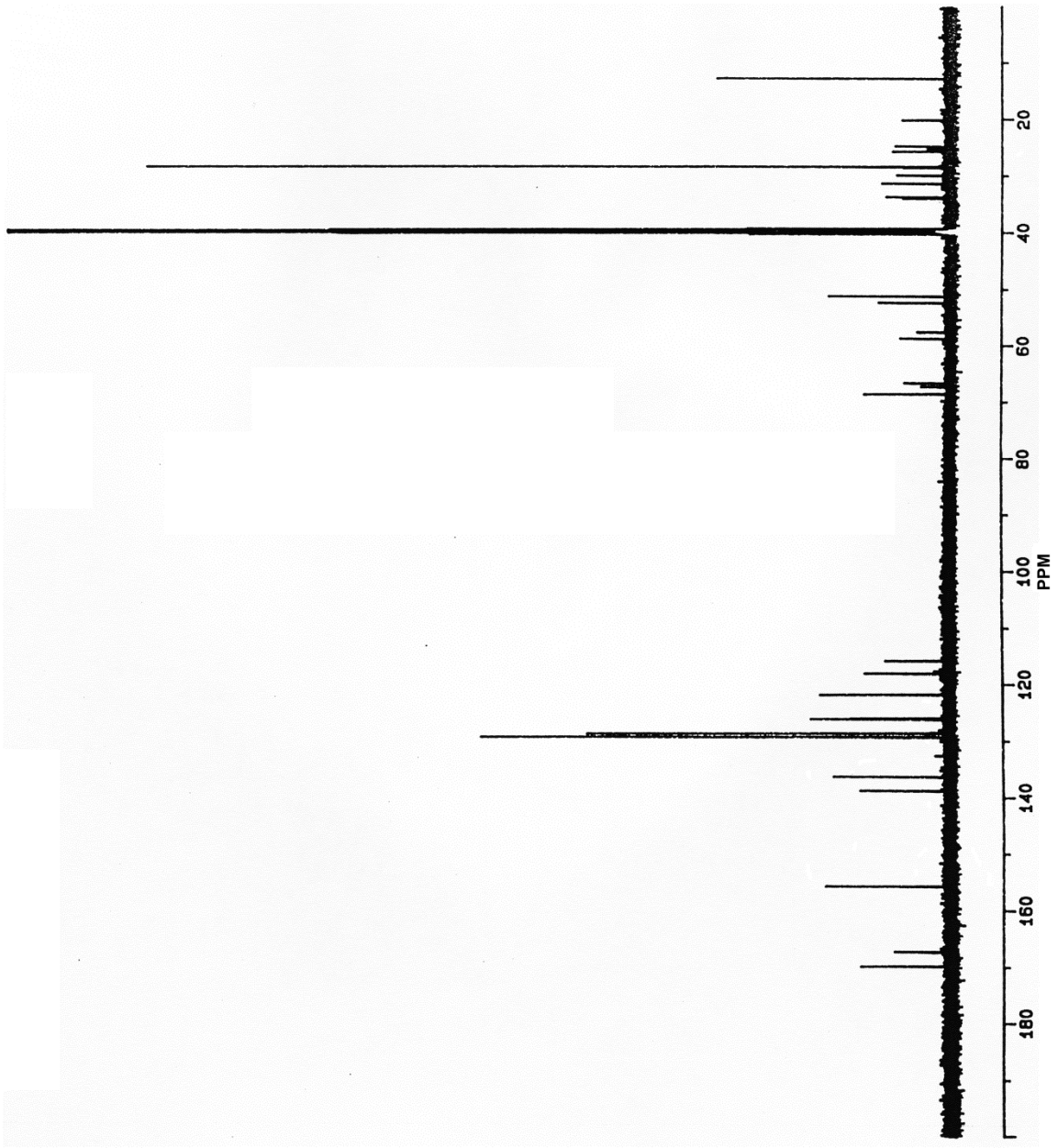

FIGURE D5
Carbon-13 Nuclear Magnetic Resonance Spectrum of NFV

TABLE D1
Preparation and Storage of Dose Formulations in the Transplacental Study of AZT, 3TC, NVP, and NFV

Preparation
An aqueous solution of 0.2% methylcellulose and 0.1% Tween®80 was added to weighed amounts of the test chemicals, and the mixtures were stirred with a magnetic stirrer to form a solution or suspension depending upon the specific formulation.

Chemical Lot Numbers
AZT, F00573
3TC, B10250
NVP, FX1009
NFV, HX1292

Maximum Storage Time
21 days

Storage Conditions
Stored in capped glass vials at room temperature

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

TABLE D2
Results of Analyses of Dose Formulations Administered to Mouse Dams in the Transplacental Study of AZT, 3TC, NVP, and NFV

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
AZT					
	July 22, 2003		12	10.2 ± 1.1	–15
	July 29, 2003		4	3.68 ± 0.18	–8
	July 29, 2003		12	11.4 ± 0.3	–5
	May 11, 2004		8	8.4 ± 0.03	+5
	May 11, 2004		4	4.19 ± 0.03	+5
AZT and 3TC					
	July 22, 2003				
		AZT	12	11.4 ± 0.6	–5
		3TC	6	5.78 ± 0.32	–4
	July 29, 2003				
		AZT	4	3.83 ± 0.07	–4
		3TC	2	1.95 ± 0.06	–3
	May 11, 2004				
		AZT	8	8.30 ± 0.03	+4
		3TC	4	3.98 ± 0.01	–1
	May 11, 2004				
		AZT	4	4.09 ± 0.01	+2
		3TC	2	2.02 ± 0.01	+1
AZT, 3TC, and NVP					
	July 22, 2003				
		AZT	12	11.3 ± 0.4	–6
		3TC	6	5.68 ± 0.27	–5
		NVP	8.4	8.11 ± 0.23	–3
	July 29, 2003				
		AZT	4	3.79 ± 0.06	–5
		3TC	2	1.88 ± 0.03	–6
		NVP	2.8	2.69 ± 0.03	–4
	May 11, 2004				
		AZT	12	12.2 ± 0.3	+2
		3TC	6	6.01 ± 0.21	0
		NVP	8.4	7.78 ± 0.24	–7

TABLE D2
Results of Analyses of Dose Formulations Administered to Mouse Dams in the Transplacental Study of AZT, 3TC, NVP, and NFV

Dose Formulation	Date Prepared		Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
AZT, 3TC, and NVP (continued)					
	May 11, 2004				
		AZT	8	8.26 ± 0.07	+3
		3TC	4	3.96 ± 0.03	–1
		NVP	5.6	5.52 ± 0.05	–1
	May 11, 2004				
		AZT	4	4.14 ± 0.06	+4
		3TC	2	2.05 ± 0.03	+3
		NVP	2.8	2.76 ± 0.03	–1
	June 14, 2004				
		AZT	12	12.6 ± 0.1	+5
		3TC	6	5.95 ± 0.03	–1
		NVP	8.4	7.33 ± 0.06	–13
AZT, 3TC, and NFV					
	July 22, 2003				
		AZT	12	10.9 ± 0.3	–9
		3TC	6	5.44 ± 0.06	–9
		NFV	50.4	47.8 ± 1.1	–5
	July 29, 2003				
		AZT	4	3.55 ± 0.31	–11
		3TC	2	1.78 ± 0.15	–11
		NFV	16.8	15.2 ± 1.5	–10
	May 11, 2004				
		AZT	8	7.84 ± 0.05	–2
		3TC	4	3.78 ± 0.04	–6
		NFV	33.6	29.9 ± 0.1	–11
	May 11, 2004				
		AZT	4	3.86 ± 0.10	–4
		3TC	2	1.94 ± 0.06	–3
		NFV	16.8	15.9 ± 0.5	–5

[a] Results of triplicate analyses (mean ± standard deviation). Dosing volume=20 mL/kg; 2 mg/mL=40 mg/kg, 2.8 mg/mL=56 mg/kg, 4 mg/mL=80 mg/kg, 5.6 mg/mL=112 mg/kg, 6 mg/mL=120 mg/kg, 8 mg/mL=160 mg/kg, 8.4 mg/mL=168 mg/kg, 12 mg/mL=240 mg/kg, 16.8 mg/mL=336 mg/kg, 33.6 mg/mL=672 mg/kg, 50.4 mg/mL=1,008 mg/kg.

APPENDIX E
LITTER SUCCESS AND SURVIVAL

TABLE E1 Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT 200

TABLE E2 Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT and 3TC 201

TABLE E3 Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT, 3TC, and NVP 202

TABLE E4 Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT, 3TC, and NFV 203

TABLE E1
Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT

	0 mg/kg	80 mg/kg	160 mg/kg	240 mg/kg
Number of Litters/Plugged Dams				
Load 1	5/8	9/9	8/9	8/9
Load 2	7/9	5/8	9/9	6/8
Load 3	8/9	5/5	3/4	–/–
Total	20/26	19/22	20/22	14/17
Males/Females Born Alive				
Load 1	25/22	40/33	30/39	38/38
Load 2	27/33	24/21	34/40	31/26
Load 3	29/36	24/14	13/11	–/–
Total	81/91	88/68	77/90	69/64
Pups Born Dead				
Load 1	1	1	0	0
Load 2	0	0	2	0
Load 3	0	1	0	–
Total	1	2	2	0
Pups Dead or Missing on Postnatal Day 1				
Load 1	1	0	1	1
Load 2	1	0	0	1
Load 3	0	0	1	–
Total	2	0	2	2
Males/Females on Postnatal Day 1[a]				
Load 1	20/20	36(2)/29(4)	25/37	30(1)/32
Load 2	24(1)/30	21/19	34/36(2)	26/22
Load 3	23/25	20/8	10/7	–/–
Total	67(1)/75	77(2)/46(4)	69/80(2)	56(1)/54
Litters with Eight Pups on Postnatal Day 1				
Load 1	5	8	7	7
Load 2	6	5	9	6
Load 3	8	4	3	–
Total	19	17	19	13
Males/Females Alive on Postnatal Day 14				
Load 1	20/20	33/27	18/27	29/32
Load 2	24/30	21/19	34/36	26/22
Load 3	23/25	20/8	10/7	–/–
Total	67/75	74/54	62/70	55/54
Males/Females Alive at Weaning on Postnatal Day 21[a]				
Load 1	20/20	32/27(3)[c]	17/27	29/32
Load 2	24(1)[b]/30	21/19	34/36	26/21
Load 3	23/25	20/8	10/7	–/–
Total	67(1)[b]/75	73/54(3)[c]	61/70	55/53
Males/Females Loaded to the Study on Postnatal Day 28[d]				
Load 1	20(5)/20(5)	31(9)/30(8)	16(6)/20(6)	26(8)28(8)
Load 2	25(7)/28(7)	17(5)/18(5)	32(9)/27(9)	22(6)/20(6)
Load 3	20(8)/16(8)	–/–	–/–	–/–
Total	65(20)/64(20)	48(14)/48(13)	48(15)/47(15)	48(14)/48(14)

[a] Parenthetical value is the number of additional fosters.
[b] The foster was loaded to the in-life phase.
[c] The three fosters were loaded to the in-life phase.
[d] Parenthetical value is the number of litters.

TABLE E2
Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT and 3TC

	0 mg/kg	80/40 mg/kg	160/80 mg/kg	240/120 mg/kg
Number of Litters/Plugged Dams				
Load 1	5/8	9/9	7/9	8/9
Load 2	7/9	5/8	8/8	7/8
Load 3	8/9	4/4	4/4	–/–
Total	20/26	18/21	19/21	15/17
Males/Females Born Alive				
Load 1	25/22	34/40	28/30	33/43
Load 2	27/33	25/17	31/37	28/27
Load 3	29/36	18/15	14/19	–/–
Total	81/91	77/72	73/86	61/70
Pups Born Dead				
Load 1	1	0	0	1
Load 2	0	1	0	0
Load 3	0	0	0	–
Total	1	1	0	1
Pups Dead or Missing on Postnatal Day 1				
Load 1	1	1	0	0
Load 2	1	0	0	0
Load 3	0	0	0	–
Total	2	1	0	0
Males/Females on Postnatal Day 1[a]				
Load 1	20/20	30(2)/37(1)	27(1)[b]/27(1)	28/35(1)
Load 2	24(1)/30	23/16	29/32(3)	26/23(2)
Load 3	23/25	15/9	10/14	–/–
Total	67(1)/75	68(2)/62(1)	66(1)[b]/73(4)	54/58(3)
Litters with Eight Pups on Postnatal Day 1				
Load 1	5	8	7	8
Load 2	6	4	8	6
Load 3	8	4	4	–
Total	19	16	19	14
Males/Females Alive on Postnatal Day 14				
Load 1	20/20	30/36	27/27	28/33
Load 2	24/30	17/13	29/32	26/23
Load 3	23/25	12/6	10/14	–/–
Total	67/75	59/55	66/73	54/56
Males/Females Alive at Weaning on Postnatal Day 21[a]				
Load 1	20/20	30/36	25/27	28/33
Load 2	24(1)[c]/30	17/13	29/32	26/23
Load 3	23/25	12/6	10/14	–/–
Total	67(1)[c]/75	59/55	64/73	54/56
Males/Females Loaded to the Study on Postnatal Day 28[d]				
Load 1	20(5)/20(5)	30(9)/35(9)	23(7)/21(7)	26(8)/28(8)
Load 2	25(7)/28(7)	17(4)/13(4)	25(8)/26(8)	22(7)/20(7)
Load 3	20(8)/16(8)	4(3)/ –	–/4(4)	–/–
Total	65(20)/64(20)	51(16)/48(13)	48(15)/51(19)	48(15)/48(15)

[a] Parenthetical value is the number of additional fosters.
[b] The foster was loaded as a sentinel.
[c] The foster was loaded to the in-life phase.
[d] Parenthetical value is the number of litters.

TABLE E3
Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT, 3TC, and NVP

	0 mg/kg	80/40/56 mg/kg	160/80/112 mg/kg	240/120/168 mg/kg
Number of Litters/Plugged Dams				
Load 1	5/8	9/9	9/9	8/9
Load 2	7/9	8/8	7/8	7/8
Load 3	8/9	3/3	1/3	3/4
Total	20/26	20/20	17/20	18/21
Males/Females Born Alive				
Load 1	25/22	40/36	40/34	39/35
Load 2	27/33	32/37	29/32	24/37
Load 3	29/36	12/10	3/4	11/14
Total	81/91	84/83	72/70	74/86
Pups Born Dead				
Load 1	1	1	1	1
Load 2	0	2	1	1
Load 3	0	0	0	0
Total	1	3	2	2
Pups Dead or Missing on Postnatal Day 1				
Load 1	1	0	2	0
Load 2	1	1	2	4
Load 3	0	0	0	0
Total	2	1	4	4
Males/Females on Postnatal Day 1[a]				
Load 1	20/20	39/33	32(1)/30	35/29
Load 2	24(1)/30	26(2)/33	26/29(1)	23(1)/31(1)
Load 3	23/25	9/8	3/3	8/10
Total	67(1)/75	74(2)/74	58(1)/62(1)	66(1)/70(1)
Litters with Eight Pups on Postnatal Day 1				
Load 1	5	9	5	8
Load 2	6	7	7	7
Load 3	8	2	1	3
Total	19	18	13	18
Males/Females Alive on Postnatal Day 14				
Load 1	20/20	37/31	32/30	20/16
Load 2	24/30	26/33	24/28	23/29
Load 3	23/25	9/8	3/3	8/10
Total	67/75	72/72	59/61	51/55
Males/Females Alive at Weaning on Postnatal Day 21[a]				
Load 1	20/20	37/31	32/30	19/16
Load 2	24(1)[b]/30	25/33	23/28	23/29
Load 3	23/25	9/8	3/3	8/10
Total	67(1)[b]/75	71/72	58/61	50/55
Males/Females Loaded to the Study Postnatal Day 28[c]				
Load 1	20(5)/20(5)	27(9)/25(8)	39(9)/27(9)	19(5)/16(5)
Load 2	25(7)/28(7)	22(8)/23(8)	18(7)/21(7)	23(7)/29(7)
Load 3	20(8)/16(8)	–/–	–/–	8(3)/4(3)
Total	65(20)/64(20)	49(17)/48(16)	48(16)/48(16)	50(15)/49(15)

[a] Parenthetical value is the number of additional fosters.
[b] The foster was loaded to the in-life phase.
[c] Parenthetical value is the number of litters.

TABLE E4
Litter Parameters and Pup Survival for B6C3F1 Mice Exposed to AZT, 3TC, and NFV

	0 mg/kg	80/40/336 mg/kg	160/80/672 mg/kg	240/120/1,008 mg/kg
Number of Litters/Plugged Dams				
Load 1	5/8	6/8	5/8	8/9
Load 2	7/9	9/9	6/9	13/14
Load 3	8/9	5/5	9/9	–/–
Total	20/26	20/22	20/26	21/23
Males/Females Born Alive				
Load 1	25/22	27/26	17/17	23/24
Load 2	27/33	43/27	24/31	31/42
Load 3	29/36	18/19	33/31	–/–
Total	81/91	88/72	74/79	54/66
Pups Born Dead				
Load 1	1	2	3	17
Load 2	0	0	0	3
Load 3	0	0	1	–
Total	1	2	4	20
Pups Dead or Missing on Postnatal Day 1				
Load 1	1	9	0	16
Load 2	1	1	0	36
Load 3	0	0	0	–
Total	2	10	0	52
Males/Females on Postnatal Day 1[a]				
Load 1	20/20	20/20	15/17	15/12
Load 2	24(1)/30	38(1)/26	21/27	30/39
Load 3	23/25	14/16	25/22	–/–
Total	67(1)/75	72(1)/62	61/66	45/51
Litters with Eight Pups on Postnatal Day 1				
Load 1	5	5	4	2
Load 2	6	7	6	6
Load 3	8	5	7	–
Total	19	17	17	8
Males/Females Alive on Postnatal Day 14				
Load 1	20/20	15/16	14/10	5/8
Load 2	24/30	34/22	17/23	10/18
Load 3	23/25	14/16	25/22	–/–
Total	67/75	63/54	56/55	15/26
Males/Females Alive at Weaning on Postnatal Day 21[a]				
Load 1	20/20	15/16	14/10	5/8
Load 2	24(1)[b]/30	34/22	17/23	10/18
Load 3	23/25	14/16	25/22	–/–
Total	67(1)[b]/75	63/54	56/55	15/26
Males/Females Loaded to the Study on Postnatal Day 28[c]				
Load 1	20(5)/20(5)	15(4)/16(4)	14(3)/10(3)	5(2)/8(2)
Load 2	25(7)/28(7)	33(9)/22(8)	17(5)/23(5)	10(4)/18(4)
Load 3	20(8)/16(8)	–/12(5)	20(8)/16(8)	–/–
Total	65(20)/64(20)	48(13)/50(17)	51(16)/49(16)	15(6)/26(6)

[a] Parenthetical value is the number of additional fosters.
[b] The foster was loaded to the in-life phase.
[c] Parenthetical value is the number of litters.

APPENDIX F
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-31 RAT AND MOUSE RATION

TABLE F1 Ingredients of NIH-31 Rat and Mouse Ration 206
TABLE F2 Vitamins and Minerals in NIH-31 Rat and Mouse Ration 206
TABLE F3 Nutrient Composition of NIH-31 Rat and Mouse Ration 207
TABLE F4 Contaminant Levels in NIH-31 Rat and Mouse Ration 207

TABLE F1
Ingredients of NIH-31 Rat and Mouse Ration

Ingredients[a]	Percent by Weight
Ground whole hard wheat	35.5
Ground #2 yellow shelled corn	21.0
Ground whole oats	10.0
Wheat middlings	10.0
Fish meal (60% protein)	9.0
Soybean meal (48.5% protein)	5.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60% protein)	2.0
Dicalcium phosphate[b]	1.5
Soy oil	1.5
Brewer's dried yeast	1.0
Ground limestone[b]	0.5
Premixes (vitamin and mineral)	0.5
Salt	0.5

[a] Ingredients are ground to pass through a U.S. Standard Screen No. 16 before mixing.
[b] Specific ingredient requirement is for cadmium content not to exceed 1 mg/kg.

TABLE F2
Vitamins and Minerals in NIH-3l Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
Choline	700 g	Choline chloride
dl-α-tocopheryl acetate	15 g	
Folic acid	1 g	
Niacin	20 g	
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.12 g	*d*-Biotin
Minerals		
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganese oxide
Iron	60 g	Iron sulfate
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 pounds) of finished product

TABLE F3
Nutrient Composition of NIH-3l Rat and Mouse Ration[a]

Nutrient	Mean ± Standard Deviation	Number of Samples
Crude protein (% by weight)	20.7 ± 0.9	19
Crude fat (% by weight)	5.61 ± 0.86	19
Volatiles (% by weight)	7.29 ± 1.39	19
Vitamins		
A (µg/g)	10.9 ± 1.9	19
E (µg/g)	57.3 ± 5.1	19
B_1 (mg/g)	0.092 ± 0.005	19
Minerals		
Selenium (µg/g)	0.40 ± 0.12	19

[a] Analyses for nutrient content of NIH-31 diet were performed by standard operating procedures developed and/or validated by the NCTR Division of Chemistry.

TABLE F4
Contaminant Levels in NIH-3l Rat and Mouse Ration[a]

	Mean ± Standard Deviation	Number of Samples (Number Positive)
Contaminants		
Arsenic (µg/g)	0.11 ± 0.07	19 (15)
Cadmium (µg/g)	< MDL	19 (0)
Lead (µg/g)	0.41 ± 0.24	19 (15)
Aflatoxin B_1 (ppb)	< MDL	19 (0)
Aflatoxin B_2 (ppb)	< MDL	19 (0)
Aflatoxin G_1 (ppb)	< MDL	19 (0)
Aflatoxin G_2 (ppb)	< MDL	19 (0)
Total fumonisin (ppb)	367 ± 227	19 (19)
Pesticides (ppb)		
Heptachlor	< MDL	5 (0)
Total DDT[b]	< MDL	5 (0)
Dieldrin	< MDL	5 (0)
PCB	< MDL	5 (0)
Malathion	< MDL	5 (0)
Lindane	< MDL	5 (0)

[a] Analyses for nutrient and contamination content of NIH-31 diet were performed by standard operating procedures developed and/or validated by the NCTR Division of Chemistry. MDL = minimum detectable level.
[b] DDE+DDT+DDD

APPENDIX G
SENTINEL ANIMAL PROGRAM

METHODS 210
RESULTS 211

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from randomly selected sentinel mice during the transplacental carcinogenicity study. Blood from each animal was collected and allowed to clot, and the serum was separated. Prior to February 15, 2005, the samples were processed by enzyme-linked immunosorbent assay (ELISA) and, thereafter, by the multiplex fluorescent immunoassay (MFI) by the Research Animal Diagnostic Laboratory at the University of Missouri (Columbia, MO) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Collection
ELISA	
Ectromelia virus	7, 28, 49, 54, and 79 weeks
EDIM (epizootic diarrhea of infant mice)	7, 28, 49, 54, and 79 weeks
LCM (lymphocytic choriomeningitis virus)	7, 28, 49, 54, and 79 weeks
MMV (minute virus of mice)	7, 28, 49, 54, and 79 weeks
MHV (mouse hepatitis virus)	7, 28, 49, 54, and 79 weeks
MPV (mouse parvovirus)	7, 28, 49, 54, and 79 weeks
Mycoplasma pulmonis	7, 28, 49, 54, and 79 weeks
Parvovirus NS-1	7, 28, 49, 54, and 79 weeks
PVM (pneumonia virus of mice)	7, 28, 49, 54, and 79 weeks
Polyoma virus	7, 28, 49, 54, and 79 weeks
Reovirus 3	7, 28, 49, 54, and 79 weeks
Sendai	7, 28, 49, 54, and 79 weeks
TMEV GDVII (Theiler's murine encephalomyelitis virus)	7, 28, 49, 54, and 79 weeks
MFI	
Ectromelia virus	101, 102, 103, 105, 131, and 148 weeks
EDIM	101, 102, 103, 105, 131, and 148 weeks
LCM	101, 102, 103, 105, 131, and 148 weeks
MMV	101, 102, 103, 105, 131, and 148 weeks
MHV	101, 102, 103, 105, 131, and 148 weeks
MPV	101, 102, 103, 105, 131, and 148 weeks
M. pulmonis	101, 102, 103, 105, 131, and 148 weeks
Parvo NS-1	101, 102, 103, 105, 131, and 148 weeks
PVM	101, 102, 103, 105, 131, and 148 weeks
Polyoma virus	101, 102, 103, 105, 131, and 148 weeks
Reovirus 3	101, 102, 103, 105, 131, and 148 weeks
Sendai	101, 102, 103, 105, 131, and 148 weeks
TMEV GDVII	101, 102, 103, 105, 131, and 148 weeks

RESULTS

All serology test results were negative. Thirty sentinel animals were positive by polymerase chain reaction testing for *Helicobacter hepaticus.*