

NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 235



**CARCINOGENESIS BIOASSAY
OF
ZEARALENONE
(CAS NO. 17924-92-4)
IN F344/N RATS AND B6C3F₁ MICE
(FEED STUDY)**

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

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

**NTP TECHNICAL REPORT
ON THE
CARCINOGENESIS BIOASSAY
OF
ZEARALENONE
(CAS NO. 17924-92-4)
IN F344/N RATS AND B6C3F₁ MICE
(FEED STUDY)**



**NATIONAL TOXICOLOGY PROGRAM
Box 12233
Research Triangle Park
North Carolina 27709
and
Bethesda, Maryland 20205**

October 1982

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

NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, North Carolina 27709 (919-541-3991).

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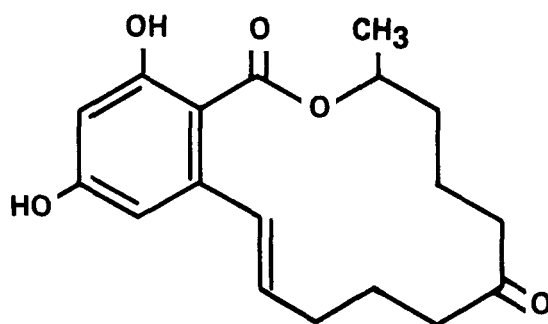
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CARCINOGENESIS BIOASSAY OF ZEARALENONE



TRANS-ZEARALENONE

CAS NO. 17924-92-4
C₁₈H₂₂O₅ Mol. Wt. 318.36

ABSTRACT

A carcinogenesis bioassay of zearalenone, an estrogenic mycotoxin, was conducted by feeding diets containing 25 or 50 ppm zearalenone to groups of 50 F344/N rats of each sex and 50 or 100 ppm to groups of 50 B6C3F1 mice of each sex for 103 weeks. Groups of 50 rats and 50 mice of each sex served as controls. Estimates based on food consumption data indicate that the low- and high-dose rats received daily doses of about 1 and 2 mg, respectively, of zearalenone per kg body weight. Low-dose and high-dose mice received estimated daily doses of about 7-10 and 14-20 mg, respectively, of zearalenone per kg body weight.

Survival of dosed and control rats of each sex was comparable. Mean body weight gains of dosed rats of each sex were lower than those of the corresponding controls; depression in mean body weight gain was dose related. Final body weights of dosed rats were <9% lower than those of control rats. The average daily feed consumption by dosed rats of each sex was 91%-102% that of the controls.

Inflammation of the prostate, testicular atrophy, and hepatocellular cytoplasmic vacuolization (male rats), and nephrosis (male and female rats) were compound-related. Retinopathy and cataracts occurred in low- and high-dose male rats and in low-dose female rats, and were associated with closeness to fluorescent light. No compound-related, increased tumor incidences were observed in rats in the chronic study.

Survival of dosed and control mice of each sex was comparable. Mean body weight gains of high-dose male and low-dose female mice were lower than those of the controls. Terminal body weights of dosed mice were <8% below those of control mice. The average daily feed consumption by dosed mice of each sex was 97%-99% that of the controls.

Myelofibrosis in the bone marrow, uterine fibrosis, and cystic ducts in the mammary gland were related to administration of zearalenone in female mice. The incidence of hepatocellular adenomas in female mice was dose related ($P \leq 0.003$), and the incidence of these tumors in high-dose female mice was significantly higher ($P \leq 0.006$) than those in the controls (control, 0/50; low-dose, 2/49; high-dose, 7/49). Pituitary adenomas occurred with statistically significant positive trends ($P \leq 0.022$ for males and $P \leq 0.001$ for females). The incidences of these tumors in high-dose mice were significantly increased relative to controls ($P \leq 0.032$ for males: 0/40, 4/45, 6/44; and $P \leq 0.003$ for females: 3/46, 2/43, 13/42).

Under the conditions of this bioassay, zearalenone was not carcinogenic for F344/N rats of either sex. Zearalenone should be considered carcinogenic in B6C3F1 mice, as evidenced by the increased proportion of male and female mice with pituitary adenomas and by the increased proportion of female mice with hepatocellular adenomas.

CONTRIBUTORS

The bioassay of zearalenone was conducted at Southern Research Institute under a subcontract to Tracor Jitco, Inc., the prime contractor for the Carcinogenesis Testing Program. The 13-week study was begun in June 1977 and completed in September 1977. The chronic study was begun in February 1978 and completed in February 1980.

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The chemicals used in this bioassay of zearalenone were analyzed by the Midwest Research Institute, 425 Volker Blvd., Kansas City, Missouri 64110; reanalysis of the bulk chemical and analyses of dosed feed mixtures were performed by Southern Research Institute.

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SUMMARY OF PEER REVIEW COMMENTS ON THE BIOASSAY OF ZEARALENONE

On December 16, 1981, this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9:00 a.m. in Conference Room A, Landow Building, 7910 Woodmont Avenue, Bethesda, Maryland.

Dr. Schwetz, a primary reviewer for the report on the bioassay of zearalenone, agreed with the conclusion that zearalenone was not carcinogenic for rats and suggested that the increase in male and female B6C3F1 mice with pituitary adenomas and in females with hepatocellular adenomas should be considered as indirect evidence of carcinogenicity since there were no observed carcinomas in those organs. He suggested that some mention should be made in the discussion about the relationship between exposure to fluorescent lights and the occurrence of retinopathies and cataracts in rats.

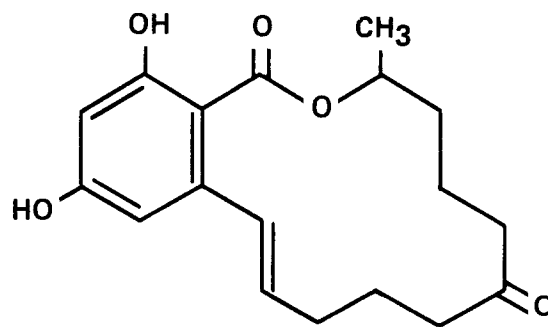
As a second principal reviewer, Dr. Holland pointed out that testicular interstitial tumors in the rat were decreased by zearalenone administration and that potentially serious ophthalmic lesions associated with the compound were induced. He indicated that this latter finding may be one of the more important results to come from the study and should be given more attention. He opined that there was no documentation that this effect was due to fluorescent lights in the animal room.

A discussion followed pertaining to the mechanism(s) by which tumors may be induced in animals exposed to hormonally active chemicals such as zearalenone, which has estrogenic activity. There was some disagreement on the wording of the conclusions based on increased incidences of pituitary and hepatocellular adenomas in mice.

Dr. Schwetz moved that the bioassay report on zearalenone be accepted with revisions as indicated. Dr. Vore seconded the motion and the report was approved by the Peer Review Panel (nine affirmative votes with two abstentions: Dr. Highland and Dr. Mirer).

I. INTRODUCTION

I. INTRODUCTION



TRANS-ZEARALENONE

CAS NO. 17924-92-4

$C_{18}H_{22}O_5$ Mol. Wt. 318.36

Trans-zearalenone — (3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1H-2-benzoxacyclo-tetradecin-1,7 (8H)-dione)—is a non-steroid estrogenic mycotoxin produced by numerous species of *Fusarium*, but especially *F. roseum* (also known as *F. graminearum*). Under conditions of high moisture *F. roseum* colonizes in maize, barley, oats, and wheat and also invades stored corn (Mirocha et al., 1974). (See the reviews by Christensen, 1979, and by Ciegler, 1975, for a full discussion of the history, production of fungal species, chemistry, and physiological effects of zearalenone and its derivatives).

Mycotoxicosis and swine estrogenism have been associated for over 50 years. Stob et al., (1962) isolated an anabolic uterotrophic compound from corn infected with *F. graminearum* (the imperfect stage of *Gibberella zeae*); this material was later identified as zearalenone by Urry et al., (1977). Zearalenone is also known as F-2 toxin and as a resorcylic acid lactone (Christensen et al., 1965; Mirocha et al., 1968).

Outbreaks of this *Fusarium* syndrome have been reported in the United States as well as in Europe (Marasas et al., 1979a); outbreaks are associated with above-average rainfall and infestation of stored corn with *Fusarium* (Mirocha and Christensen, 1974).

The estrogenic syndrome in field and experimental animals is characterized by a number of reproductive organ changes that are probably mediated through the endocrine system. Ingestion of moldy feed by pigs resulted in fetal death, infertility, reduced litter size, abortion, and other

related problems (Nelson et al., 1966; Radnai, 1974). Chang et al. (1979) reported that the feeding of pure zearalenone to healthy, multiparous sows during the pre-estrus or gestation period resulted in these reproductive deficiencies.

Mirocha et al., (1968) reported that cattle fed hay with a high (14 mg/kg) content of zearalenone showed reduced fertility. In experimental animals (young female pigs), as little as 1 mg of zearalenone per day for 8 days induced vulvar tumefaction (Mirocha and Christensen, 1974). At higher doses of zearalenone, young female pigs developed enlarged vulvae and mammary glands; prolapse of the vagina was commonly seen (Mirocha and Christensen, 1974). These effects were reversible upon cessation of zearalenone administration. Histological effects reported included uterine wall edema and epithelial metaplasia in the cervix and vagina (Kurtz et al., 1969).

The histological changes found in immature female pigs are the same when the gilt is administered pure zearalenone, *Fusarium* infested feed, or estradiol (Kurtz et al., 1969). Zearalenone inhibited the production of follicle stimulating hormone (FSH), depressed the maturation of ovarian follicles, and showed a luteotrophic activity which extended the lifespan of corpora lutea and resulted in pseudopregnancy (Chang et al., 1979). Young male pigs administered zearalenone underwent a feminizing effect with testicular atrophy, mammary gland enlargement, and preputial gland swelling (Mirocha and Christensen, 1974).

I. INTRODUCTION

Zearalenone may be teratogenic in pigs, but in most reports of stillbirth, fetal resorption, or undersize litters, moldy feed rather than zearalenone specifically was considered to be the causative factor (reviewed by Mirocha and Christensen, 1974). Studies using zearalenone as a teratogenic agent in swine were carried out by Chang et al. (1979). Infertility, pseudopregnancy, reduced litter size, fetal malformation, and probable fetal resorption were noted in this study, but there was no clear evidence of teratogenicity. In multigeneration studies of the teratogenicity of zearalenone in rats, Bailey et al. (1976) were unable to find teratogenic effects at daily intakes of 0.1-10 mg zearalenone per kilogram of body weight. Ruddick et al. (1976) administered zearalenone at levels of 0.075-10 mg/kg to pregnant rats; fetal skeletal anomalies were found in the offspring of rats given oral doses of zearalenone above 1 mg/kg body weight.

The known uterotrophic action of zearalenone is, on a cellular level, translated into cellular proliferation and increased rate of synthesis of target cell protein, DNA, and RNA (Ueno and Yagasaki, 1975; Ueno et al., 1974). Zearalenone and its reduced derivative, zeranol, compete with estradiol for unfilled nuclear estrogen receptor sites in a human breast cancer cell line (Martin et al., 1978), in rat mammary glands (Boyd and Wittliff, 1978), in the immature rat uterus (Katzellenbogen et al., 1979), in bovine liver (Ingerski and Stan, 1979), and in the calf uterus (Kiang et al., 1978). The filled cytoplasmic estrogen receptors are translocated to the nucleus, where subsequent biochemical events are influenced. In the immature mouse uterus, zeranol was the strongest competitor for cytoplasmic estradiol receptor sites, stronger than either zearalenone or diethylstilbestrol (DES) (Greenman et al., 1979). In their physiological, cellular, and subcellular actions, zearalenone and zeranol mimic both estradiol, the natural female mammalian circulating hormone, and DES, the synthetic anabolic non-steroid estrogen. The physiological and carcinogenic properties of DES were reviewed in IARC publications in 1974 and 1979.

Zearalenone is produced by large-scale deep fermentation. (See Hidy et al., 1977, for a review of the commercial processes.) The recovered zearalenone is converted via a 4H reduction to its reduced (3*S*,7*R*) derivative zeranol, commercially known as Ralgro®*. Zeranol was approved by the FDA in 1969 for use as a growth promoter in cattle and sheep (Federal Register, 1969). Feedlot cattle and sheep receive ear implants of zeranol;

growth, carcass grade, and feed conversion are improved (Brown, 1970; Sharp and Dyer, 1971; Borger et al., 1973). Implantation is discontinued 40-65 days prior to slaughter for sheep and 65 days prior to slaughter for cattle to reach the FDA's zero-residue (less than 20 ppb) requirement for tissue content of zeranol (US CFR, 1973; US CFR, 1974). Zeranol and zearalenone show similar physiological activities in all systems tested.

Zearalenone, when administered to whole animals, is excreted either unchanged or as zearalenol (2H reduction) (Ueno and Tashiro, 1981). The excretory products can be either the intact molecule or the glucuronides (Mirocha et al., 1981). Rat liver homogenates also catalyze both the conjugation and the reduction of zearalenone (Kiessling and Pettersson, 1978). While the reversible reduction of zearalenone to zearalenol can be catalyzed by a bacterial beta-hydroxy steroid dehydrogenase (Kiessling and Pettersson, 1978), there is no evidence for or against this route in mammalian tissue.

There has been no direct evidence that zearalenone, or its metabolic products, are carcinogens. Schoental (1978; 1979) has pointed out that mycotoxins may be responsible for unexplained variations in background tumor rates in laboratory animals. Marasas et al. (1979b) reported that in the African Republic of Transkei there is a correlation between geographical distribution of esophageal cancer and levels of *Fusarium* infestation of corn, the main dietary staple.

Livestock feeds contaminated by mycotoxin-producing microorganisms may present a health hazard to man, because the toxins can pass into milk or edible tissues (Schoental, 1975). When zearalenone was administered to lactating sows, it was metabolized and appeared in the milk as zearalenol (Palyusik et al., 1980). Suckling female piglets fed exclusively on this milk developed red and swollen vulvae, the typical sign of zearalenone mycotoxicosis.

Although zearalenone and zearalenol were positive in the *rec⁻* assay in *Bacillus subtilis* M45 (Ueno and Kubota, 1976), zearalenone is reported to be not mutagenic for *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98, and TA

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I. INTRODUCTION

100, with or without activation by rat liver microsomes (Kuczuk et al., 1978; Wehner et al., 1978; NTP Technical Bulletin, 1980).

Zearalenone was tested by the Bioassay Program because of the potential for chance exposure of the general population to *Fusarium*-infested foods or grains, the use of a chemically

reduced (but metabolically unrelated) form, zeranol, as a cattle or lamb growth promoter, and because it is a non-steroidal anabolic estrogenic compound showing physiological and sub-cellular activities similar to those of DES, a non-steroidal anabolic estrogen for which there is sufficient evidence of carcinogenicity in animals and in humans (IARC, 1979).

II. MATERIALS AND METHODS

CHEMICAL ANALYSIS

PRECHRONIC STUDIES

Single-Dose Study

Fourteen-Day Study

Thirteen-Week Study

CHRONIC STUDY

Clinical Examinations and Pathology

Data Recording and Statistical Methods

II. MATERIALS AND METHODS: CHEMICAL ANALYSIS

CHEMICAL ANALYSIS

The trans-zearalenone (CAS No. 17924-92-4) used in this bioassay was obtained from Commercial Solvents Corp., IMC Chemical Group, Inc. (Terre Haute, IN), in three batches. Lot No. 3-75 (Batch 01) was used for the single-dose and 14-day studies; Lot No. 3-75 (Batch 02) was used for the 13-week studies and for the first 19 months of the chronic studies; and Lot No. 51079 (Batch 03) was used for the remaining 5 months of the chronic studies. The third batch was a homogeneous mixture of two lots obtained from the manufacturers (Lot Nos. 626973 and 626990).

Results of analyses of the three batches of zearalenone at Midwest Research Institute (MRI) were consistent with the literature values (Appendix E). A trace impurity in each of three batches was detected by thin-layer chromatography; and a minor impurity with an area 0.04% that of the major peak was detected before the major peak by high-pressure liquid chromatography only in Lot No. 51079 (Appendix E).

Southern Research Institute (SoRI) reanalyzed the bulk chemical, which was stored in the dark at 5°C, versus a reference sample, which was stored at -20°C, periodically throughout the entire study. Reanalysis included infrared spectroscopy

and gas-liquid chromatography (3% OV-1 column after derivatization with a Tri-Sil/BSA reagent). The results of these analyses indicated no change in the chemical throughout the study.

MRI determined that chemical/feed mixtures were stable at temperatures up to 45°C for 2 weeks (Appendix F) and thus required no special storage. (See Table I for storage conditions of formulated diets at SoRI.) The GLC procedure (Appendix G, Table G1) utilized by SoRI during the subchronic and the first year of the chronic studies for the analyses of formulated diets was based upon extraction of the dosed feed, cleanup of the extraction on a Florasil column, conversion of zearalenone to the trimethylsilyl derivative, and analysis of the derivatized zearalenone by gas chromatography. This procedure proved troublesome in that it produced equivocal results due to reaction of the silylating reagent with feed components. Subsequently, a more reliable procedure was developed based on the HPLC method used for chemical reanalysis. The new method was used during the second year of the chronic study.

Bulk zearalenone was stored in the dark at 5°C.

PRECHRONIC STUDIES

Single-Dose Study

Groups of five male and five female F344/N rats were administered single doses of 250 to 4,000 mg/kg zearalenone in corn oil by gavage. Groups of five male and five female B6C3F1 mice received doses of 125 to 2,000 mg/kg by the same route. Animals were killed on day 16. Details of animal maintenance and examination are provided in Table I.

Fourteen-Day Study

Groups of five male and five female F344/N rats and groups of five male and five female B6C3F1 mice were fed diets containing 6,000, 12,500, 25,000, 50,000, or 100,000 ppm zearalenone for 14 days (Table I). Animals were observed twice daily for mortality and were weighed on days 1 and 15. Necropsies were performed on all animals on days 16-21.

Thirteen-Week Study

Subchronic studies were conducted to evaluate the cumulative toxicity of zearalenone and to determine the concentrations to be used in the chronic study.

Groups of 9 or 10 F344/N rats of each sex and groups of 10 B6C3F1 mice of each sex were fed diets containing 0, 30, 100, 300, 1,000, or 3,000 ppm zearalenone for 13 weeks (Table I). Dosed feed, control diets, and water were available *ad libitum*.

Animals were checked for mortality and signs of morbidity twice daily. Those animals that were judged moribund were killed and necropsied. Each animal was given a clinical examination weekly, including palpation for tissue masses or swelling. Body weight and feed consumption data were collected weekly.

II. MATERIALS AND METHODS: CHEMICAL ANALYSIS

At the end of the 13-week study, survivors were killed with carbon dioxide. Necropsies were performed on all animals found dead during the study, unless precluded in whole or in part by autolysis or cannibalization, and on animals that survived to the end of the study. Thus the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group. The following specimens were examined for control and high-dose groups: gross lesions, tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, bone marrow, bone (rib), thymus,

trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/-prostate/testes or ovaries/uterus, brain, and pituitary.

Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Histopathologic examination for all other dosed groups was limited to the pituitary, adrenals, urinary bladder, bone, bone marrow, testes or ovaries, prostate or uterus, and seminal vesicles.

CHRONIC STUDY

Groups of 50 rats of each sex were fed diets containing 0, 25, or 50 ppm zearalenone for 103 weeks, and groups of 50 mice of each sex were fed diets containing 0, 50, or 100 ppm zearalenone for the same period of time. Control and dosed groups were of the same strain, sex, and age range and from the same source and shipment. All animals shared the same room, and all aspects of animal care and maintenance were similar (Table 1). No other chemicals were on test in that room.

The mixing schedule and storage conditions were based on the results of a stability study. Sample diets formulated with 1,400 or 100,000 ppm zearalenone and stored for 2 weeks at -20° to 45°C were found to be stable (Appendix F). The concentrations of zearalenone were determined in blindly selected batches of formulated diets (Appendix G).

Clinical Examinations and Pathology

Morbidity and mortality checks were made twice daily. Body weights, feed consumption by cage, and clinical signs were recorded monthly. The mean body weight of each group was calculated by dividing the total weight of all surviving animals in the group by the number of surviving animals in the group. The average feed consumption per animal was calculated by dividing the total feed consumption measured for all cages by the number of surviving animals in the group. Moribund animals and animals that survived to the end of the bioassay were killed using carbon dioxide and necropsied.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following were examined microscopically: tissue masses, abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, thigh muscle, sciatic nerve, bone marrow, femur, thymus, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/-prostate/testes or ovaries/uterus, pituitary, brain, and any grossly abnormal tissues or organs. Special staining techniques were used as necessary.

Necropsies were performed on all animals found dead and on those killed at the end of the study, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

The histopathologic examination for the rat study was performed by Dr. Farnell and for the mouse study by Dr. Giles. The slides, individual animal lifetime records, gross and microscopic necropsy results, animal tumor pathology description, and tumor pathology summary tables were sent to a second pathologist for quality review. This review included reconciliation of the tumor

II. MATERIALS AND METHODS: CHRONIC STUDY

pathology summary tables with the individual animal records, verification of tissue and slide count, as well as a review of the quality of a percentage of the slides. The second pathologist, in addition, reviewed all tumor diagnoses and all tissues in which there was an increased incidence of tumors. Any discrepancies between the reports of the original pathologist and the reviewing pathologist as well as all tumor target tissue (liver, pituitary) slides were submitted to and evaluated by the NTP Pathology Working Group as described by Ward et al. (1978). The classification of neoplastic nodules was done according to the recommendations of Squire and Levitt (1975) and the National Academy of Sciences (1980). The diagnoses represent a consensus of contracting pathologists and the NTP Pathology Working Group.

Data Recording and Statistical Methods

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

For the statistical analysis of tumor incidence data, two different methods of adjusting for intercurrent mortality were employed. Each used the classical methods for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high- and low-dosed groups with controls and tests for overall dose-response trends.

The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel methods to obtain an overall P-value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975).

The second method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "incidental"; i.e., they were merely observed at autopsy in animals dying of an unrelated cause. According to this approach, the proportions of animals found to have tumors in dosed and control groups were compared in each of the five time intervals: 0-52 weeks, 53-78 weeks, 79-92 weeks, week 93 to the week before terminal kill, and the terminal kill period. The denominators of these proportions were the number of animals actually autopsied during the time interval. The individual time interval comparisons were then combined by the previously described methods to obtain a single overall result. (See Peto et al., 1980, for the computational details of both methods.)

In addition to these tests, one other set of statistical analyses was carried out and reported in the tables analyzing primary tumors: the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test for dose-response trends (Armitage, 1971; Gart et al., 1979). These tests were based on the overall proportion of tumor-bearing animals. All reported P values are one-sided.

II. MATERIALS AND METHODS: CHRONIC STUDY

For studies in which there is little effect of compound administration on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by

the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death.

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS

| | Single-Dose | 14-Day Study | 13-Week Study | 2-Year Study |
|---------------------------------------|--|--|---|--|
| Experimental Design | | | | |
| Size of Test Groups | 5 males and 5 females of each species | 5 males and 5 females of each species | 9 or 10 male rats; 10 female rats; 10 male and 10 female mice | 50 males and 50 females of each species |
| Doses | Rats: 250, 500, 1,000, 2,000, or 4,000 mg/kg zearalenone in corn oil by gavage Mice: 125, 250, 500, 1,000, or 2,000 mg/kg zearalenone in corn oil by gavage | Rats and Mice: 6,000, 12,500, 25,000, 50,000, or 100,000 ppm zearalenone in feed | Rats and Mice: 0, 30, 100, 300, 1,000, or 3,000 ppm zearalenone in feed | Rats: 0, 25, or 50 ppm zearalenone in feed Mice: 0, 50, or 100 ppm zearalenone in feed |
| Duration of Dosing | Single dose; killed on day 16 | 14 days; rats killed on days 16-17; mice killed on days 16-21 | 13 weeks; killed on days 92-96 | Start dates were Feb. 7, 1978, for rats and Feb 14, 1978, for mice. Rats killed on days 729-744; mice on days 735-756. |
| Type and Frequency of Observation | Observed twice daily for mortality | Same as Single-Dose Study | Observed twice daily; weighed once per week | Observed twice daily; weighed every 4-5 weeks |
| Necropsy and Histologic Examination | No necropsy or histologic examination | All animals necropsied; no histologic examination | All animals necropsied. Complete histologic exam on controls and high-dose; pituitary, adrenal, urinary bladder, bone, bone marrow, testes or ovaries, prostate or uterus and seminal vesicles examined on all other animals. | All animals necropsied; complete histologic exam on all animals |
| Animals and Animal Maintenance | | | | |
| Species | F344/N Rats; B6C3F ₁ Mice | Same as Single-Dose Study | Same as Single-Dose Study | Same as Single-Dose Study |
| Animal Source | Frederick Cancer Research Center (Frederick, MD) | Same as Single-Dose Study | Same as Single-Dose Study | Harlan Industries, Inc. (Indianapolis, IN) |
| Time Held Before Start of Test | Rats, 8 days; mice, 7 days | 8 days | 7 days | 12 days |
| Age When Placed on Study | 6 weeks | 5 weeks | 5 weeks | Rats: 5 weeks Mice: 7 weeks |
| Method of Animal Distribution | Animals assigned to cages according to table of random numbers; cages assigned to test group according to new table of random numbers | Same as Single-Dose Study | Same as Single-Dose Study | Same as Single-Dose Study |
| Feed | Wayne Lab Blox® meal, Allied Mills, Inc. (Chicago, IL) Available <i>ad libitum</i> | Same as Single-Dose Study | Same as Single-Dose Study | Same as Single-Dose Study |
| Bedding | Beta Chips®, Northeastern Products Corp. (Warrensburg, NY); replaced biweekly | Same as Single-Dose Study | Same as Single-Dose Study | Beta Chips® and Sawdust, PWI, Inc. (Lowville, NY) |
| Water | Water bottles | Tap water via automatic system, Edstrom Industries (Waterford, WI) | Same as 14-Day Study | Same as 14-Day Study |

TABLE I. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS (Continued)

| | Single-Dose | 14-Day Study | 13-Week Study | 2-Year Study |
|--|--|--|--|---|
| Cages | Rats and Mice: Stainless steel, Hahn Roofing and Sheet Metal Co. (Birmingham, AL) | Rats: Stainless steel, Hahn Roofing and Sheet Metal Co. (Birmingham, AL) Mice: Polycarbonate, Lab Products, Inc. (Garfield, NJ) | Polycarbonate, Lab Products, Inc. (Garfield, NJ); changed twice per week | Same as 13-Week Study |
| Animals per Cage | 5 | 5 | 5 | 5 |
| Cage Filters | Remay polyester, Dupont #2024 Snow Filtration Co. (Cincinnati, OH); replaced every 2 weeks | Same as Single-Dose Study | Same as Single-Dose Study | Same as Single-Dose Study |
| Animal Room Environment | Rats and Mice: temperature $23^{\circ} \pm 3^{\circ}\text{C}$; uncontrolled humidity | Rats: temperature $23^{\circ} \pm 3^{\circ}\text{C}$; uncontrolled humidity; 9 hours fluorescent light; air changed 15 times per hour Mice: temperature $23^{\circ} \pm 3^{\circ}\text{C}$; uncontrolled humidity; 12 hours fluorescent light; room air changed 15 times per hour | Temperature $23^{\circ} \pm 3^{\circ}\text{C}$; uncontrolled humidity; 12 hours fluorescent light; room air changed 15 times per hour | Same as 13-Week Study |
| Other Chemicals on Test in Same Room | D-Mannitol, stannous chloride, ziram, propyl gallate | Rats: D-mannitol, ziram, propyl gallate Mice: propyl gallate | None | None |
| Chemical/Vehicle and Chemical/Feed Mixture Preparation | Weighed amount of zearalenone mixed with corn oil | Weighed amount of zearalenone mixed with small amount of ground feed; premix and additional meal mixed in Patterson-Kelly® Twin-Shell V-Blender (equipped with an intensifier bar) for 5 minutes | Same as 14-Day Study | Same as 14-Day Study |
| Maximum Storage Time | — | 14 days | 14 days first 4 weeks; 7 days thereafter | 14 days |
| Storage Conditions | — | Stored in sealed plastic containers at room temperature | Same as 14-Day Study | Stored in plastic bags at 5°C the first week, and at $21^{\circ} - 23^{\circ}\text{C}$ the second week |

III. RESULTS

RATS

PRECHRONIC STUDIES

Single-Dose Study

Fourteen-Day Study

Thirteen-Week Study

CHRONIC STUDY

Body Weights and Clinical Signs

Zearalenone Intake

Survival

Pathology and Statistical Analyses of Results

MICE

PRECHRONIC STUDIES

Single-Dose Study

Fourteen-Day Study

Thirteen-Week Study

CHRONIC STUDY

Body Weights and Clinical Signs

Zearalenone Intake

Survival

Pathology and Statistical Analyses of Results

III. RESULTS: RATS—PRECHRONIC STUDIES

PRECHRONIC STUDIES

Single-Dose Study

All rats lived to the end of the 15-day observation period. Rats of each sex receiving 2,000 or 4,000 mg/kg zearalenone were slightly inactive after dosing, but they returned to normal by day 2.

Fourteen-Day Study

All animals survived to the end of the dosing period. A generalized, dose-related decrease in mean body weight gain was observed (Table 2). Male rats fed 100,000 ppm zearalenone lost weight. Ruffled, wet fur and diarrhea were seen in 3/5 or 4/5 rats in each group receiving 12,500 to 100,000 ppm. Distention of the uterine horns was observed in 4/5 or 5/5 females in each dosed group.

Thirteen-Week Study

No compound-related deaths occurred. Weight gain was depressed by more than 17% in

rats of either sex receiving 100 ppm or more zearalenone, but feed consumption by dosed and control groups was comparable (Table 3).

Atrophy of the seminal vesicles and testes and fibromuscular hyperplasia of the prostate occurred in 90%-100% of the male rats fed 1,000 or 3,000 ppm zearalenone (Table 4). Ductular hyperplasia of the mammary gland occurred in 6/10 males and 10/10 females fed 3,000 ppm. Chromophobe hyperplasia of the pituitary occurred in 6/10 males and 7/10 females fed 3,000 ppm, 2/9 males and 2/10 females fed 1,000 ppm, and 1/10 females fed 100 ppm; this lesion was not seen in controls. Osteopetrosis was seen in most dosed groups; it was observed in 90%-100% of the females fed 100 ppm or more and in 90%-100% of the males fed 1,000 or 3,000 ppm.

Because of the depression of weight gain, endocrine system involvement, and osteopetrosis at higher dose levels, doses of 25 and 50 ppm zearalenone in feed were selected for both male and female rats in the chronic study.

TABLE 2. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF RATS FED DIETS CONTAINING ZEARELENONE FOR 14 DAYS

| Dose (ppm) | Survival (a) | Mean Body Weight (grams) | | |
|----------------|--------------|--------------------------|--------------|--------------|
| | | Initial | Final | Change |
| Males | | | | |
| 6,000 | 5/5 | 80.4 ± 2.11 | 95.2 ± 1.07 | +14.8 ± 1.59 |
| 12,500 | 5/5 | 82.8 ± 3.87 | 101.2 ± 2.35 | +18.4 ± 4.26 |
| 25,000 | 5/5 | 85.6 ± 4.31 | 95.6 ± 2.66 | +10.0 ± 5.32 |
| 50,000 | 5/5 | 80.8 ± 1.56 | 80.6 ± 1.81 | - 0.2 ± 1.66 |
| 100,000 | 5/5 | 82.4 ± 5.48 | 76.4 ± 2.77 | - 6.0 ± 3.36 |
| Females | | | | |
| 6,000 | 5/5 | 67.6 ± 2.86 | 91.6 ± 2.06 | +24.0 ± 1.79 |
| 12,500 | 5/5 | 67.8 ± 2.56 | 86.4 ± 2.01 | +18.6 ± 1.29 |
| 25,000 | 5/5 | 73.0 ± 3.11 | 86.8 ± 1.93 | +13.8 ± 1.69 |
| 50,000 | 5/5 | 61.6 ± 1.63 | 77.6 ± 1.96 | +16.0 ± 1.67 |
| 100,000 | 5/5 | 60.8 ± 2.06 | 65.4 ± 4.01 | + 4.6 ± 2.09 |

(a) Number surviving/number per group.

TABLE 3. DOSAGE, SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS FED DIETS CONTAINING ZEARALENONE FOR 13 WEEKS

| Dose (ppm) | Survival (a) | Mean Body Weight (grams) | | | Weight Change Relative to Control (c) (percent) | Average Daily Feed Consumption (grams) |
|----------------|--------------|--------------------------|--------------|---------------|---|--|
| | | Initial | Final (b) | Change | | |
| Males | | | | | | |
| 0 | 10/10 | 88.7 ± 2.88 | 325.5 ± 7.14 | +236.8 ± 8.00 | | 19.9 |
| 30 | 9/9 (d) | 87.0 ± 4.38 | 304.4 ± 2.87 | +214.4 ± 4.51 | - 9.5 | 18.8 |
| 100 | 10/10 | 81.6 ± 5.25 | 254.0 ± 2.65 | +172.4 ± 4.54 | -27.2 | 18.8 |
| 300 | 10/10 | 80.5 ± 3.44 | 201.6 ± 3.69 | +121.1 ± 2.57 | -48.9 | 18.6 |
| 1,000 | 9/9 (d) | 77.1 ± 2.39 | 179.3 ± 1.78 | +102.2 ± 2.59 | -56.8 | 19.5 |
| 3,000 | 10/10 | 86.6 ± 5.46 | 173.6 ± 1.90 | + 87.0 ± 5.42 | -63.3 | 19.1 |
| Females | | | | | | |
| 0 | 10/10 | 79.2 ± 2.70 | 191.7 ± 1.47 | +112.5 ± 2.36 | | 13.2 |
| 30 | 10/10 | 78.5 ± 3.43 | 187.7 ± 2.09 | +109.2 ± 3.92 | - 2.9 | 12.4 |
| 100 | 10/10 | 77.8 ± 2.38 | 170.2 ± 2.60 | + 92.4 ± 3.80 | -17.9 | 12.7 |
| 300 | 10/10 | 80.1 ± 3.40 | 158.1 ± 3.34 | + 78.0 ± 3.41 | -30.7 | 14.2 |
| 1,000 | 10/10 | 82.6 ± 2.79 | 149.1 ± 1.91 | + 66.5 ± 3.34 | -40.9 | 13.8 |
| 3,000 | 10/10 | 77.2 ± 2.51 | 145.6 ± 3.00 | + 68.4 ± 2.62 | -39.2 | 14.4 |

(a) Number surviving/ number initially in the group.

(b) Mean body weight of survivors of the group ± standard error of the mean.

(c) Weight change of the dosed group relative to that of the controls □

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(d) One female rat was found in this group of males and was killed.

TABLE 4. INCIDENCE OF COMPOUND-RELATED HISTOPATHOLOGIC EFFECTS IN THE 13-WEEK STUDY OF ZEARALENONE IN RATS

| Dose (ppm) | Pituitary | | Mammary Gland | Uterus | Prostate | Seminal Vesicles | Testes | | Bone |
|----------------|-------------|---------|----------------------|-------------------------|---------------------------|------------------|---------|------------------|---------------|
| | Hyperplasia | Adenoma | Ductular Hyperplasia | Endometrial Hyperplasia | Fibromuscular Hyperplasia | Atrophy | Atrophy | Aspermatogenesis | Osteopetrosis |
| Males | | | | | | | | | |
| 0 | 0/10 | 0/10 | 0/10 | — | 0/10 | 0/9 | 0/10 | 0/10 | 0/10 |
| 30 | 0/9 | 0/9 | 0/9 | — | 0/9 | 1/9 | 0/9 | 0/9 | 0/9 |
| 100 | 0/10 | 0/10 | 0/10 | — | 0/10 | 7/10 | 0/10 | 0/10 | 0/10 |
| 300 | 0/10 | 0/10 | 0/10 | — | 3/10 | 10/10 | 7/10 | 6/10 | 0/10 |
| 1,000 | 2/9 | 0/10 | 0/10 | — | 9/9 | 9/9 | 9/9 | 9/9 | 9/9 |
| 3,000 | 6/10 | 0/10 | 6/10 | — | 9/10 | 9/10 | 10/10 | 10/10 | 10/10 |
| Females | | | | | | | | | |
| 0 | 0/10 | 0/10 | 0/10 | 0/10 | — | — | — | — | 0/10 |
| 30 | 0/10 | 0/10 | 0/10 | 0/10 | — | — | — | — | 5/10 |
| 100 | 1/10 | 0/10 | 0/10 | 1/10 | — | — | — | — | 10/10 |
| 300 | 0/10 | 0/10 | 0/10 | 2/10 | — | — | — | — | 9/10 |
| 1,000 | 2/10 | 0/10 | 0/10 | 8/10 | — | — | — | — | 10/10 |
| 3,000 | 7/10 | 0/10 | 10/10 | 10/10 | — | — | — | — | 10/10 |

III. RESULTS: RATS—CHRONIC STUDY

CHRONIC STUDY

Body Weights and Clinical Signs

Mean body weight gains of dosed males (throughout the study) and dosed females (during the second year of the study) were lower than those of the controls (Figure 1 and Appendix H, Table H1). The decrement in weight gain was dose related. The average daily feed consumption per rat by low- and high-dose rats was 102% (16.6/16.3) and 91% (14.8/16.3) that of the controls for males and 96% (10.8/11.3) and 98% (11.1/11.3) for females (Appendix I, Table I1). No compound-related clinical signs were observed.

Zearalenone Intake

The average daily intake of zearalenone by male and female rats at various intervals during the 104-week study is shown in Table J1 (Appendix J). The low-dose animals (male and female) received about 1 mg of zearalenone per kg body weight per day during the major portion of the study; high-dose animals received about twice this daily amount. These daily intake amounts should be considered as useful approximations that are dependent on the accuracy of the measurement of feed consumption.

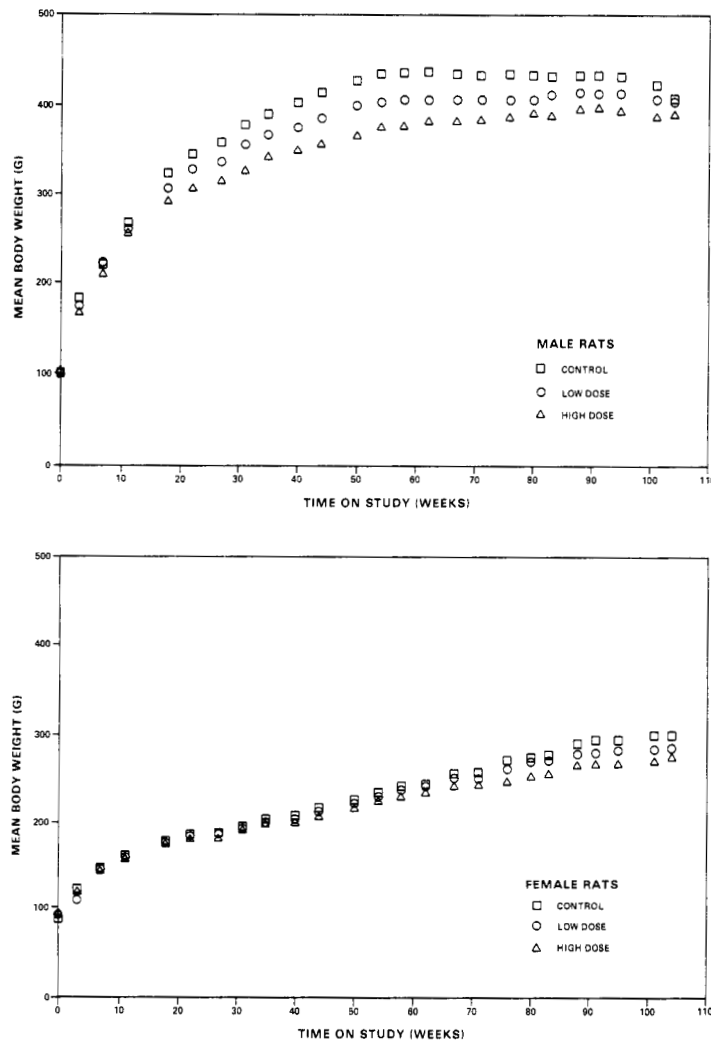


Figure 1. Growth Curves for Rats Fed Diets Containing Zearalenone

III. RESULTS: RATS—CHRONIC STUDY

Survival

Estimates of the probabilities of survival of male and female rats fed diets containing zearalenone at the concentrations of this bioassay, together with those of the control groups, are shown by the Kaplan and Meier curves in Figure 2. No significant differences in survival were observed between any groups of male or any groups of females.

In male rats, 39/50 (78%) of the controls, 38/50 (76%) of the low-dose, and 37/50 (74%) of

the high-dose group lived to the termination period of the study at 104-106 weeks. In female rats, 40/50 (80%) of the controls, 38/50 (76%) of the low-dose, and 41/50 (82%) of the high-dose group lived to the termination period of the study at 104-106 weeks. The survival incidence of control male rats includes one animal that died a natural death during the termination period; this animal is listed in Appendix Table A1 as dying a natural death.

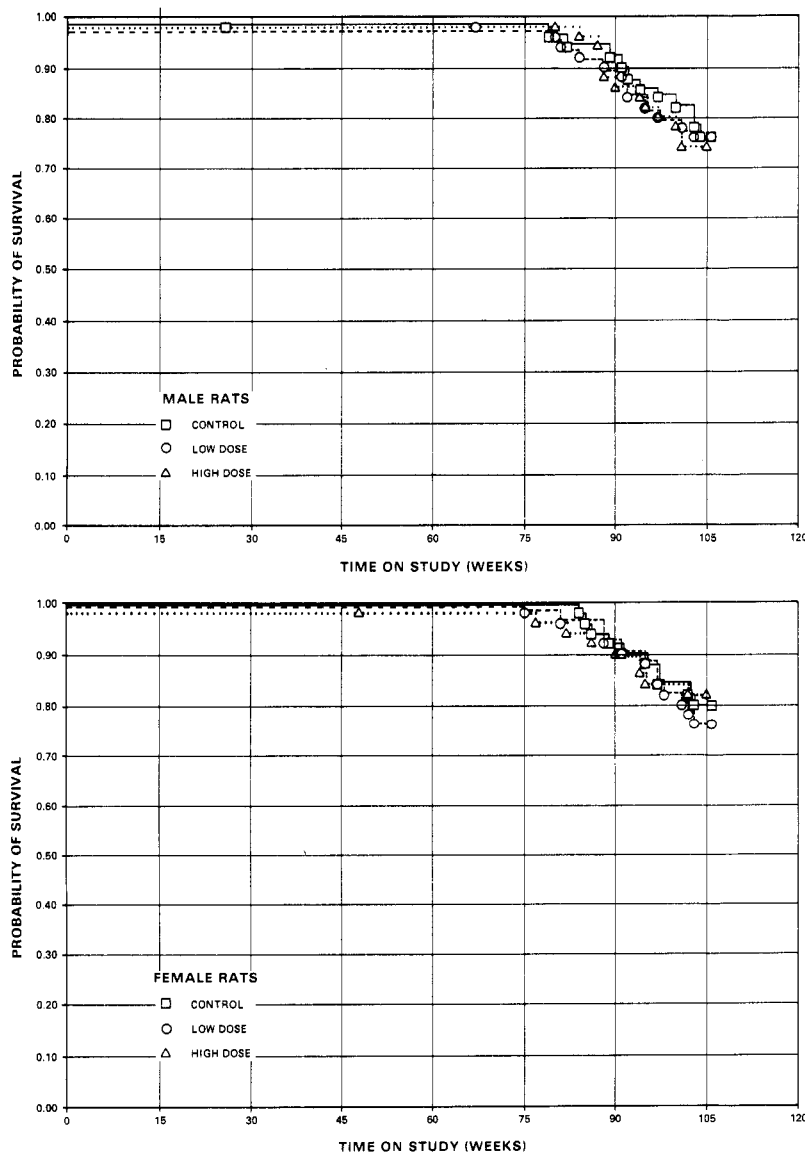


Figure 2. Survival Curves for Rats Fed Diets Containing Zearalenone

III. RESULTS: RATS—CHRONIC STUDY

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for each individual animal in the male rat and female rat studies, respectively. Tables 6 and 7 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups.

Findings on nonneoplastic lesions are summarized in Table 5 and Appendix C, Tables C1 and C2.

Kidney: Nephrosis, typical of nephropathy seen in aging F344/N rats, was observed in increased incidence in dosed rats of either sex.

Eye: Retinopathy and cataracts were observed in increased incidence in low- and high-dose male rats (Table C1) and in low-dose female rats (Table C2).

Liver: Cytoplasmic vacuolization was found in increased incidence in dosed male rats.

Prostate: Inflammation was observed in increased incidence in dosed male rats.

Testis: Compound-related atrophy was seen in male rats; incidence: control, 1/50 (2%); low-dose, 26/50 (52%); high-dose, 17/50 (34%). Interstitial-cell tumors occurred with a statistically significant negative trend ($P=0.042$, incidental tumor test; incidence: control, 45/50, 90%; low-dose, 43/50, 86%; high-dose, 39/50, 78%).

Pituitary: Low-dose male rats showed a significant ($P<0.05$) increase in pituitary adenomas; however, there was no significant dose-related trend (Table 5). When pituitary adenomas and carcinomas were combined for analysis, none of the tests indicated a significant change in tumor rate (Table 5).

TABLE 5. INCIDENCES OF SELECTED NONNEOPLASTIC LESIONS IN RATS ADMINISTERED ZEARALENONE IN THE CHRONIC STUDY

| | Controls | Low Dose | High Dose |
|----------------------------------|-------------|-------------|-------------|
| Kidney: Nephrosis | | | |
| Males | 30/50 (60%) | 43/50 (86%) | 41/50 (82%) |
| Females | 10/50 (20%) | 24/50 (48%) | 26/50 (52%) |
| Eye: Retinopathy | | | |
| Males | 0/50 (0%) | 14/50 (28%) | 27/50 (54%) |
| Females | 3/50 (6%) | 28/50 (56%) | 8/50 (16%) |
| Eye: Cataracts | | | |
| Males | 0/50 (0%) | 11/50 (22%) | 25/50 (50%) |
| Females | 4/50 (8%) | 26/50 (52%) | 6/50 (12%) |
| Liver: Cytoplasmic Vacuolization | | | |
| Males | 0/50 (0%) | 6/50 (12%) | 8/50 (16%) |
| Prostate: Inflammation | 15/49 (31%) | 31/50 (62%) | 22/50 (44%) |
| Testis: Atrophy | 1/50 (2%) | 26/50 (52%) | 17/50 (34%) |

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS (a)

| | Control | Low Dose | High Dose |
|--|------------|-------------|-------------|
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/49 (6%) | 2/50 (4%) | 1/47 (2%) |
| Adjusted (c) | 7.0% | 4.9% | 2.8% |
| Terminal (d) | 1/39 (3%) | 1/38 (3%) | 1/36 (3%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.246N | P=0.513N | P=0.335N |
| Incidental Tumor Test | P=0.202N | P=0.474N | P=0.286N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.234N | P=0.490N | P=0.324N |
| Hematopoietic System: Undifferentiated Leukemia | | | |
| Tumor Rates | | | |
| Overall (b) | 9/50 (18%) | 4/50 (8%) | 7/50 (14%) |
| Adjusted (c) | 21.7% | 9.6% | 15.4% |
| Terminal (d) | 7/39 (18%) | 2/38 (5%) | 2/37 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.362N | P=0.135N | P=0.429N |
| Incidental Tumor Test | P=0.262N | P=0.120N | P=0.310N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.330N | P=0.117N | P=0.393N |
| Hematopoietic System: Lymphoma or Leukemia | | | |
| Tumor Rates | | | |
| Overall (b) | 9/50 (18%) | 5/50 (10%) | 8/50 (16%) |
| Adjusted (c) | 21.7% | 12.1% | 17.8% |
| Terminal (d) | 7/39 (18%) | 3/38 (8%) | 3/37 (8%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.477N | P=0.215N | P=0.534N |
| Incidental Tumor Test | P=0.376N | P=0.198N | P=0.420N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.444N | P=0.194N | P=0.500N |
| Pituitary: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 5/46 (11%) | 13/49 (27%) | 9/50 (18%) |
| Adjusted (c) | 13.3% | 30.3% | 22.7% |
| Terminal (d) | 4/36 (11%) | 8/37 (22%) | 7/37 (19%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.192 | P=0.042 | P=0.204 |
| Incidental Tumor Test | P=0.266 | P=0.048 | P=0.246 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.236 | P=0.045 | P=0.243 |
| Pituitary: Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 6/46 (13%) | 13/49 (27%) | 10/50 (20%) |
| Adjusted (c) | 15.1% | 30.3% | 24.6% |
| Terminal (d) | 4/36 (11%) | 8/37 (22%) | 7/37 (19%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.199 | P=0.075 | P=0.216 |
| Incidental Tumor Test | P=0.290 | P=0.095 | P=0.290 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.246 | P=0.082 | P=0.262 |

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS (a) (Continued)

| | Control | Low Dose | High Dose |
|---|------------|------------|------------|
| Adrenal: Pheochromocytoma | | | |
| Tumor Rates | | | |
| Overall (b) | 5/50 (10%) | 5/50 (10%) | 5/50 (10%) |
| Adjusted (c) | 12.3% | 11.9% | 12.7% |
| Terminal (d) | 4/39 (10%) | 2/38 (5%) | 4/37 (11%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.535 | P=0.610 | P=0.601 |
| Incidental Tumor Test | P=0.530N | P=0.626N | P=0.615N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.566 | P=0.630 | P=0.630 |
| Adrenal: All Pheochromocytomas | | | |
| Tumor Rates | | | |
| Overall (b) | 7/50 (14%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted (c) | 16.8% | 11.9% | 17.1% |
| Terminal (d) | 5/39 (13%) | 2/38 (5%) | 5/37 (14%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.523 | P=0.404N | P=0.575 |
| Incidental Tumor Test | P=0.511N | P=0.389N | P=0.576N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.559 | P=0.380N | P=0.613 |
| Thyroid: C-Cell Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/49 (6%) | 7/50 (14%) | 6/50 (12%) |
| Adjusted (c) | 7.2% | 17.9% | 15.4% |
| Terminal (d) | 1/39 (3%) | 6/38 (16%) | 5/37 (14%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.189 | P=0.153 | P=0.224 |
| Incidental Tumor Test | P=0.241 | P=0.146 | P=0.290 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.219 | P=0.167 | P=0.254 |
| Thyroid: C-Cell Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/49 (6%) | 8/50 (16%) | 8/50 (16%) |
| Adjusted (c) | 7.2% | 19.6% | 20.1% |
| Terminal (d) | 1/39 (3%) | 6/38 (16%) | 6/37 (16%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.081 | P=0.100 | P=0.093 |
| Incidental Tumor Test | P=0.118 | P=0.102 | P=0.136 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.095 | P=0.106 | P=0.106 |
| Pancreatic Islets: Islet-Cell Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/49 (6%) | 3/50 (6%) | 0/50 (0%) |
| Adjusted (c) | 7.4% | 7.9% | 0.0% |
| Terminal (d) | 2/39 (5%) | 3/38 (8%) | 0/37 (0%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.114N | P=0.648 | P=0.136N |
| Incidental Tumor Test | P=0.098N | P=0.652 | P=0.105N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.098N | P=0.651N | P=0.117N |

TABLE 6. ANALYSIS OF PRIMARY TUMORS IN MALE RATS (a) (Continued)

| | Control | Low Dose | High Dose |
|---|-------------|-------------|-------------|
| Mammary Gland: Fibroadenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted (c) | 2.6% | 6.5% | 5.4% |
| Terminal (d) | 1/39 (3%) | 0/38 (0%) | 2/37 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.383 | P=0.300 | P=0.482 |
| Incidental Tumor Test | P=0.451 | P=0.390 | P=0.482 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.400 | P=0.309 | P=0.500 |
| Testis: All Interstitial-Cell Tumors | | | |
| Tumor Rates | | | |
| Overall (b) | 45/50 (90%) | 43/50 (86%) | 39/50 (78%) |
| Adjusted (c) | 97.8% | 91.7% | 86.4% |
| Terminal (d) | 38/39 (97%) | 34/38 (89%) | 31/37 (84%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.231N | P=0.514N | P=0.257N |
| Incidental Tumor Test | P=0.042N | P=0.396N | P=0.056N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.064N | P=0.380N | P=0.086N |

(a) Dosed groups received doses of 25 or 50 ppm of zearalenone in the diet.

(b) Number of tumor bearing animals/number of animals examined at the site.

(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

(d) Observed tumor incidence at terminal kill.

(e) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS (a)

| | Control | Low Dose | High Dose |
|--|-------------|-------------|-------------|
| Hematopoietic System: Undifferentiated Leukemia | | | |
| Tumor Rates | | | |
| Overall (b) | 7/50 (14%) | 7/50 (14%) | 2/50 (4%) |
| Adjusted (c) | 16.7% | 16.3% | 4.9% |
| Terminal (d) | 6/40 (15%) | 4/38 (11%) | 2/41 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.075N | P=0.583 | P=0.078N |
| Incidental Tumor Test | P=0.096N | P=0.566 | P=0.090N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.073N | P=0.613 | P=0.080N |
| Hematopoietic System: Lymphoma or Leukemia | | | |
| Tumor Rates | | | |
| Overall (b) | 8/50 (16%) | 7/50 (14%) | 3/50 (6%) |
| Adjusted (c) | 19.2% | 16.3% | 6.9% |
| Terminal (d) | 7/40 (18%) | 4/38 (11%) | 2/41 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.086N | P=0.529N | P=0.099N |
| Incidental Tumor Test | P=0.114N | P=0.550N | P=0.123N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.084N | P=0.500N | P=0.100N |
| Pituitary: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 13/49 (27%) | 15/50 (30%) | 14/49 (29%) |
| Adjusted (c) | 28.5% | 33.2% | 29.9% |
| Terminal (d) | 8/40 (20%) | 9/38 (24%) | 8/40 (20%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.446 | P=0.382 | P=0.488 |
| Incidental Tumor Test | P=0.388 | P=0.460 | P=0.479 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.456 | P=0.437 | P=0.500 |
| Pituitary: Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 14/49 (29%) | 16/50 (32%) | 14/49 (29%) |
| Adjusted (c) | 30.0% | 34.8% | 29.9% |
| Terminal (d) | 8/40 (20%) | 9/38 (24%) | 8/40 (20%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.525 | P=0.385 | P=0.567 |
| Incidental Tumor Test | P=0.467 | P=0.475 | P=0.560 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.544 | P=0.440 | P=0.588 |
| Thyroid: C-Cell Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 5/49 (10%) | 6/50 (12%) | 4/49 (8%) |
| Adjusted (c) | 12.5% | 14.7% | 9.5% |
| Terminal (d) | 5/40 (13%) | 4/38 (11%) | 3/40 (7%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.437N | P=0.470 | P=0.500N |
| Incidental Tumor Test | P=0.463N | P=0.513 | P=0.520N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.434N | P=0.514 | P=0.500N |

TABLE 7. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS (a) (Continued)

| | Control | Low Dose | High Dose |
|---|------------|-------------|------------|
| Thyroid: C-Cell Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 5/49 (10%) | 7/50 (14%) | 6/49 (12%) |
| Adjusted (c) | 12.5% | 17.2% | 14.1% |
| Terminal (d) | 5/40 (13%) | 5/38 (13%) | 4/40 (10%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.439 | P=0.350 | P=0.500 |
| Incidental Tumor Test | P=0.405 | P=0.388 | P=0.468 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.439 | P=0.394 | P=0.500 |
| Mammary Gland: Fibroadenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 9/50 (18%) | 14/50 (28%) | 9/50 (18%) |
| Adjusted (c) | 20.9% | 34.7% | 20.9% |
| Terminal (d) | 7/40 (18%) | 12/38 (32%) | 7/41 (17%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.530N | P=0.148 | P=0.587N |
| Incidental Tumor Test | P=0.513 | P=0.152 | P=0.546 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.548 | P=0.171 | P=0.602 |
| Uterus: Endometrial Stromal Polyp | | | |
| Tumor Rates | | | |
| Overall (b) | 4/50 (8%) | 8/49 (16%) | 5/49 (10%) |
| Adjusted (c) | 10.0% | 20.6% | 12.1% |
| Terminal (d) | 4/40 (10%) | 7/37 (19%) | 4/40 (10%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.437 | P=0.146 | P=0.500 |
| Incidental Tumor Test | P=0.410 | P=0.138 | P=0.485 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.424 | P=0.168 | P=0.487 |

(a) Dosed groups received doses of 25 or 50 ppm of zearalenone in the diet.

(b) Number of tumor bearing animals/number of animals examined at the site.

(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

(d) Observed tumor incidence at terminal kill.

(e) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

III. RESULTS: MICE—PRECHRONIC STUDIES

PRECHRONIC STUDIES

Single-Dose Study

One male mouse administered 1,000 mg/kg zearalenone died due to gavage error. All other mice lived to the end of the observation period.

Fourteen-Day Study

All animals survived to the end of the dosing period. A generalized, dose-related decrease in mean body weight gain was observed (Table 8).

Two of five male mice fed 100,000 ppm and 4/5 and 3/5 female mice fed 50,000 or 100,000 ppm, respectively, lost weight. (No controls were used in this study.)

Distention of the uterine horns was observed in 2/5 to 4/5 female mice in each dosed group. All dosed mice had small thymuses. Distention of the urinary bladder was seen in 2/5 and 3/5 male mice receiving 12,500 or 25,000 ppm, respectively.

TABLE 8. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF MICE FED DIETS CONTAINING ZEARALENONE FOR 14 DAYS

| Dose (ppm) | Survival (a) | Mean Body Weight (grams) | | |
|----------------|--------------|--------------------------|-------------|-------------|
| | | Initial | Final | Change (b) |
| Males | | | | |
| 6,000 | 5/5 | 20.4 ± 1.21 | 21.0 ± 1.14 | +0.6 ± 0.24 |
| 12,500 | 5/5 | 19.8 ± 0.66 | 20.8 ± 0.66 | +1.0 ± 0.00 |
| 25,000 | 5/5 | 20.4 ± 0.75 | 21.2 ± 0.58 | +0.8 ± 0.49 |
| 50,000 | 5/5 | 18.4 ± 0.75 | 19.2 ± 0.86 | +0.8 ± 0.20 |
| 100,000 | 5/5 | 20.0 ± 0.63 | 19.6 ± 1.03 | -0.4 ± 0.51 |
| Females | | | | |
| 6,000 | 5/5 | 17.8 ± 0.58 | 18.6 ± 0.60 | +0.8 ± 0.20 |
| 12,500 | 5/5 | 16.8 ± 0.37 | 18.8 ± 0.37 | +2.0 ± 0.32 |
| 25,000 | 5/5 | 16.8 ± 0.37 | 17.2 ± 0.37 | +0.4 ± 0.40 |
| 50,000 | 5/5 | 18.0 ± 0.63 | 17.4 ± 0.40 | -0.6 ± 0.81 |
| 100,000 | 5/5 | 17.6 ± 0.40 | 16.8 ± 0.37 | -0.8 ± 0.20 |

(a) Number surviving/number per group.

(b) Weight change of the group ± standard error of the mean.

III. RESULTS: MICE—PRECHRONIC STUDIES

Thirteen-Week Study

Two of the 10 female mice fed 3,000 ppm died. Weight gain was depressed by 14% or more in male mice fed 300 ppm or more (Table 9). Feed consumption by dosed and control mice was comparable.

Atrophy of the seminal vesicles and cytoplasmic vacuolization of the adrenal were found in 10/10 or 9/10 male mice fed 1,000 or 3,000 ppm zearalenone, and squamous metaplasia of the prostate was seen in 8/10 males fed 3,000 ppm (Table 10). Osteopetrosis was observed in 10/10 males and 6/8 females fed 3,000 ppm, 10/10 males and 5/10 females fed 1,000 ppm, 7/10

males and 5/10 females fed 300 ppm, 4/10 males and 2/10 females fed 100 ppm, and 0/10 male and 0/10 female controls. Myelofibrosis of the bone marrow was seen in 10/10 males fed 3,000 ppm or 1,000 ppm, 6/8 females fed 3,000 ppm, and 3/10 females fed 1,000 ppm. Endometrial hyperplasia of the uterus was seen in 1/10 to 10/10 female mice in all dosed groups, but the incidence was not dose related.

Because of the severity of the lesions observed in mice at doses of 300 ppm or more, doses of 50 and 100 ppm zearalenone in feed were selected for mice in the chronic study.

TABLE 9. DOSAGE, SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE FED DIETS CONTAINING ZEARELENONE FOR 13 WEEKS

| Dose (ppm) | Survival (a) | Mean Body Weight (grams) | | | Weight Change Relative to Control (c) (percent) | Average Daily Feed Consumption (grams) |
|----------------|--------------|--------------------------|------------|-------------|---|--|
| | | Initial | Final (b) | Change | | |
| Males | | | | | | |
| 0 | 10/10 | 19.0 ±0.39 | 31.2 ±0.49 | +12.2 ±0.20 | | 7.7 |
| 30 | 10/10 | 19.6 ±0.34 | 31.4 ±0.54 | +11.8 ±0.36 | - 3.3 | 7.4 |
| 100 | 10/10 | 19.6 ±0.37 | 32.2 ±0.65 | +12.6 ±0.67 | + 3.3 | 7.9 |
| 300 | 10/10 | 19.2 ±0.51 | 29.6 ±0.64 | +10.4 ±0.70 | -14.8 | 7.9 |
| 1,000 | 10/10 | 18.9 ±0.55 | 28.3 ±0.82 | + 9.4 ±0.40 | -23.0 | 8.1 |
| 3,000 | 10/10 | 19.6 ±0.43 | 26.5 ±0.78 | + 6.9 ±0.55 | -43.4 | 8.1 |
| Females | | | | | | |
| 0 | 10/10 | 16.6 ±0.56 | 24.8 ±0.20 | + 8.2 ±0.63 | | 8.0 |
| 30 | 10/10 | 16.2 ±0.47 | 24.8 ±0.36 | + 8.6 ±0.54 | + 4.9 | 7.7 |
| 100 | 10/10 | 16.6 ±0.31 | 24.8 ±0.33 | + 8.2 ±0.36 | 0.0 | 7.8 |
| 300 | 10/10 | 16.0 ±0.37 | 24.3 ±0.50 | + 8.3 ±0.30 | + 1.2 | 7.3 |
| 1,000 | 10/10 | 15.5 ±0.60 | 23.6 ±0.65 | + 8.1 ±0.60 | - 1.2 | 6.7 |
| 3,000 | 8/10 | 15.9 ±0.50 | 23.8 ±0.53 | + 7.9 ±0.68 | - 3.7 | 7.0 |

(a) Number surviving/number initially in the group.

(b) Mean weight change of the survivors of the group ± standard error of the mean.

(c) Weight change of the dosed group relative to that of the controls □

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

TABLE 10. INCIDENCE OF COMPOUND-RELATED HISTOPATHOLOGIC EFFECTS IN THE 13-WEEK STUDY OF ZEARALENONE IN MICE

| Dose (ppm) | Adrenal (Zona Reticularis) | Uterus | Vagina | Prostate | Seminal Vesicles | Testes | | Urinary Bladder | Bone | Bone Marrow | |
|----------------|----------------------------|-------------------------|----------------|---------------------|------------------|---------|------------------|---------------------|---------------|---------------|--------------------------|
| | Cytoplasmic Vacuolization | Endometrial Hyperplasia | Hyperkeratosis | Squamous Metaplasia | Atrophy | Atrophy | Aspermatogenesis | Fibrous hyperplasia | Osteopetrosis | Myelofibrosis | Neutrophilic hyperplasia |
| Males | | | | | | | | | | | |
| 0 | 0/10 | — | — | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 30 | 0/10 | — | — | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| 100 | 0/10 | — | — | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 | 4/10 | 0/10 | 0/10 |
| 300 | 0/10 | — | — | 0/10 | 1/10 | 0/10 | 0/10 | 0/10 | 7/10 | 1/10 | 0/10 |
| 1,000 | 9/10 | — | — | 3/10 | 10/10 | 4/10 | 1/10 | 0/10 | 10/10 | 10/10 | 0/10 |
| 3,000 | 9/10 | — | — | 8/10 | 10/10 | 4/10 | 1/10 | 2/10 | 10/10 | 10/10 | 0/10 |
| Females | | | | | | | | | | | |
| 0 | 0/10 | 0/10 | 0/10 | — | — | — | — | 0/10 | 0/10 | 0/10 | 0/10 |
| 30 | 0/10 | 10/10 | 0/10 | — | — | — | — | 0/10 | 0/10 | 0/10 | 0/10 |
| 100 | 0/10 | 7/10 | 0/10 | — | — | — | — | 0/10 | 2/10 | 0/10 | 0/10 |
| 300 | 6/10 | 1/10 | 0/10 | — | — | — | — | 0/10 | 5/10 | 0/10 | 0/10 |
| 1,000 | 4/10 | 6/10 | 0/10 | — | — | — | — | 0/10 | 5/10 | 3/10 | 2/10 |
| 3,000 | 6/8 | 7/8 | 4/8 | — | — | — | — | 1/8 | 6/8 | 6/8 | 1/8 |

III. RESULTS: MICE—CHRONIC STUDY

CHRONIC STUDY

Body Weights and Clinical Signs

No significant reduction in body weight gain was apparent in the male mice during the second half of the chronic study. Low-dose female mice showed a reduced rate of weight gain, but this was not evident in the high-dose female mice (Figure 3 and Appendix H, Table H2). The average daily feed consumption per mouse in the low- and high-dose groups was 99% (6.8/6.9) and 97% (6.7/6.9) that of the controls for males and 97% (6.7/6.9) for both dosed groups of females (Appendix I, Table I2). No other compound-related clinical signs were observed.

Zearalenone Intake

The average daily intake of zearalenone by male and female mice at various intervals during the 104-week study is shown in Table J2 (Appendix J). The low-dose animals (male and female) received about 7-10 mg of zearalenone per kg body weight per day during the major portion of the study; high-dose animals received about twice this daily amount. These daily intake amounts should be considered as useful approximations that are dependent on the accuracy of the measurement of feed consumption.

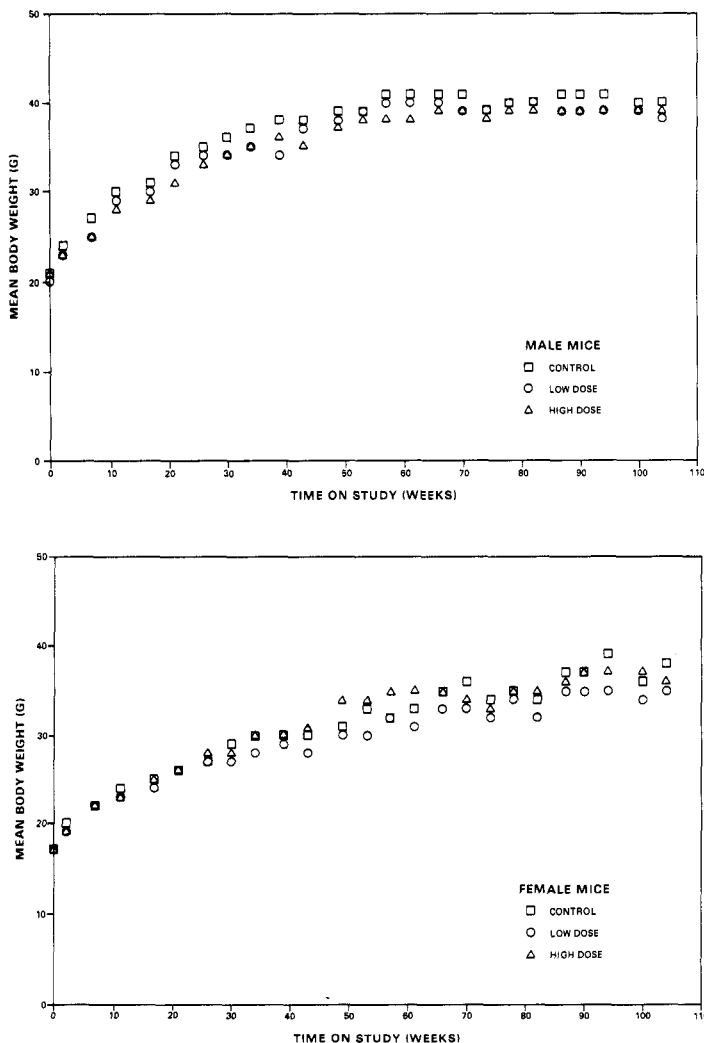


Figure 3. Growth Curves for Mice Fed Diets Containing Zearalenone

III. RESULTS: MICE—CHRONIC STUDY

Survival

Estimates of the probabilities of survival of male and female mice fed diets containing zearalenone at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 4. No significant differences in survival were observed between any groups of either sex.

In male mice, 42/50 (84%) of the controls, 40/50 (80%) of the low-dose, and 44/50 (88%) of the high-dose group lived to the termination period of the study at 105-108 weeks. In female mice, 37/50 (74%) of the controls, 37/50 (74%) of the low-dose, and 32/50 (64%) of the high-dose

group lived to the termination period of the study at 105-108 weeks. The 10 male control mice listed in Appendix B, Table B1, animal disposition summary as "scheduled sacrifice" have been included in the termination period. These animals were killed at week 105. The eight female control mice listed as "scheduled sacrifice" in Appendix B, Table B2, animal disposition summary have also been included in the termination period; these mice were killed at weeks 105 (six animals) and 106 (two animals). In addition, one male and one female mouse in the low-dose groups died natural deaths during the termination period; for statistical purposes, these animals were considered to be killed at termination.

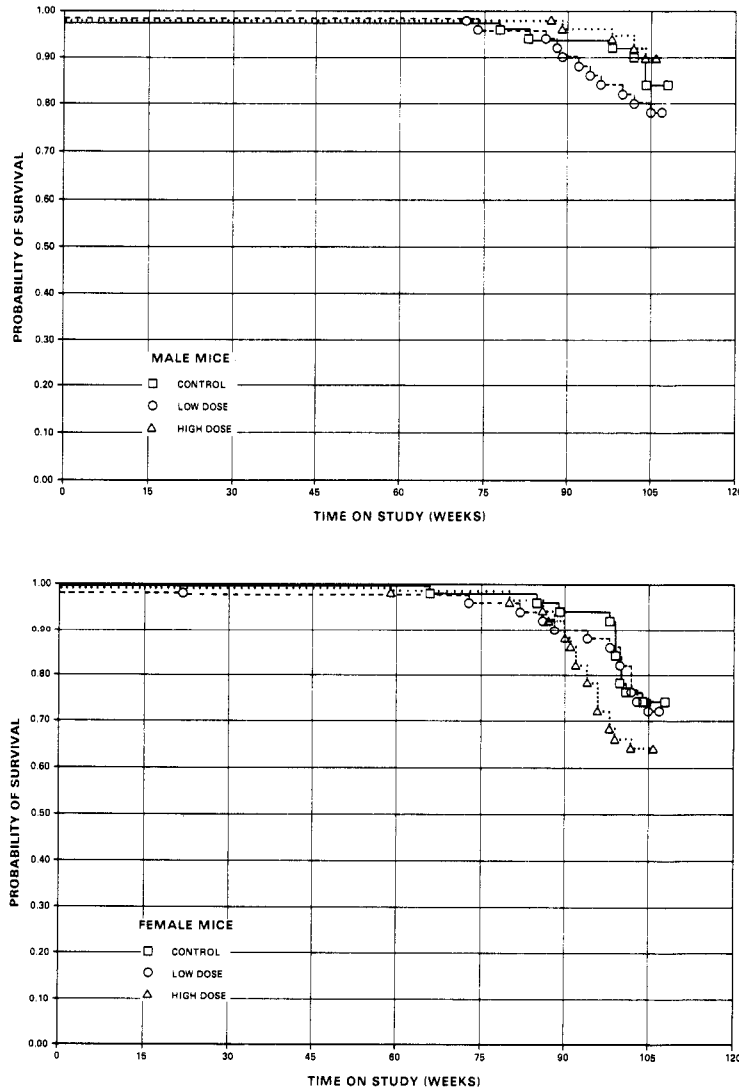


Figure 4. Survival Curves for Mice Fed Diets Containing Zearalenone

III. RESULTS: MICE—CHRONIC STUDY

Pathology and Statistical Analyses of Results

Histopathologic findings on neoplasms occurring in mice are summarized in Appendix B, Tables B1 and B2; Tables B3 and B4 give the survival and tumor status for each individual animal in the male and female mouse studies, respectively. Findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2. Tables 11 and 12 contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups.

Bone Marrow: Myelofibrosis was observed at increased incidences and with increased severity in dosed female mice (control, 8/49, 16%; low-dose, 32/48, 67%; high-dose 34/49, 69%).

Uterus: Fibrosis in the uterus/myometrium was observed at increased incidences in dosed female mice (control, 1/50, 2%; low-dose, 5/49, 10%; high-dose, 17/48, 35%).

Mammary Gland: Dosed females had increased incidences of cystic ducts (control, 0/50, 0%; low-dose, 3/49, 6%; high-dose, 15/49, 31%). Adenocarcinomas were observed at increased incidences in dosed female mice (control, 1/50, 2%; low-dose, 3/49, 6%; high-dose, 3/49, 6%); these increases were not statistically different from controls. The carcinoma in the control animal was type B; in the dosed females, four tumors were type C, one was adenosquamous, and one was type A.

Liver: Hepatocellular adenomas occurred in female mice with a statistically significant

($P \leq 0.003$) positive trend. The incidences in the high-dose group were statistically significant ($P \leq 0.006$) in individual comparisons with the control group (0/50, 2/49, 7/49). The occurrence of liver tumors in dosed male mice was not statistically significant.

Compared with hepatocytes elsewhere in the liver, those in lesions diagnosed as hepatocellular adenomas were often larger, had cytoplasm that was more vacuolated, and often had cytoplasm with a different tinctorial quality. Hepatocellular carcinomas generally had a more disorderly arrangement of hepatocytes than did hepatocellular adenomas, and there was usually evidence of invasive growth into adjoining hepatic tissue. The arrangement of hepatocytes into trabeculae or islands of neoplastic hepatocytes was a criterion for diagnosing lesions as hepatocellular carcinomas. No toxic hepatic lesions were found.

Pituitary: Adenomas occurred in both male and female mice with statistically significant positive trends ($P \leq 0.022$ for males and $P \leq 0.001$ for females). The increased incidences of adenomas were statistically significant in high-dose males ($P \leq 0.032$) and high-dose females ($P \leq 0.003$); see Tables 11 and 12 for incidence rates.

Pituitary adenomas were usually characterized by a nodular, solid proliferation of pleomorphic epithelial cells which was frequently associated with blood-filled vascular spaces and hemosiderin deposition. The diagnosis of pituitary carcinoma was made when one or more of the following were observed: unusually large growths with bizarrely shaped epithelial cells, an increased mitotic index, or invasion into the brain.

TABLE 11. ANALYSIS OF PRIMARY TUMORS IN MALE MICE (a)

| | Control | Low Dose | High Dose |
|---|-------------|------------|-------------|
| Lung: Alveolar/Bronchiolar Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 7/50 (14%) | 5/49 (10%) | 8/50 (16%) |
| Adjusted (c) | 16.7% | 12.8% | 18.2% |
| Terminal (d) | 7/42 (17%) | 5/39 (13%) | 8/44 (18%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.479 | P=0.431N | P=0.539 |
| Incidental Tumor Test | P=0.479 | P=0.431N | P=0.539 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.442 | P=0.394N | P=0.500 |
| Lung: Alveolar/Bronchiolar Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 4/50 (8%) | 3/49 (6%) | 3/50 (6%) |
| Adjusted (c) | 9.2% | 7.3% | 6.8% |
| Terminal (d) | 4/42 (7%) | 2/39 (5%) | 3/44 (7%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.403N | P=0.543N | P=0.478N |
| Incidental Tumor Test | P=0.454N | P=0.564N | P=0.517N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.421N | P=0.511N | P=0.500N |
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 11/50 (22%) | 8/49 (16%) | 11/50 (22%) |
| Adjusted (c) | 25.5% | 19.8% | 25.0% |
| Terminal (d) | 10/42 (24%) | 7/39 (18%) | 11/44 (25%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.505N | P=0.373N | P=0.549N |
| Incidental Tumor Test | P=0.538 | P=0.385N | P=0.573N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.549 | P=0.323N | P=0.595 |
| Hematopoietic System: Malignant Lymphoma, Lymphocytic Type | | | |
| Tumor Rates | | | |
| Overall (b) | 1/50 (2%) | 4/50 (8%) | 4/50 (8%) |
| Adjusted (c) | 2.4% | 10.0% | 8.4% |
| Terminal (d) | 1/42 (2%) | 4/40 (10%) | 2/44 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.167 | P=0.165 | P=0.199 |
| Incidental Tumor Test | P=0.206 | P=0.165 | P=0.319 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.147 | P=0.181 | P=0.181 |
| Hematopoietic System: Malignant Lymphoma, Mixed Type | | | |
| Tumor Rates | | | |
| Overall (b) | 5/50 (10%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted (c) | 11.1% | 4.9% | 4.5% |
| Terminal (d) | 2/42 (5%) | 1/40 (3%) | 2/44 (5%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.147N | P=0.259N | P=0.211N |
| Incidental Tumor Test | P=0.200N | P=0.262N | P=0.290N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.147N | P=0.218N | P=0.218N |

TABLE 11. ANALYSIS OF PRIMARY TUMORS IN MALE MICE (a) (Continued)

| | Control | Low Dose | High Dose |
|--|-------------|-------------|-------------|
| Hematopoietic System: Malignant Lymphoma, All Types | | | |
| Tumor Rates | | | |
| Overall (b) | 6/50 (12%) | 7/50 (14%) | 6/50 (12%) |
| Adjusted (c) | 13.3% | 16.3% | 12.7% |
| Terminal (d) | 3/42 (7%) | 5/40 (13%) | 4/44 (9%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.531N | P=0.451 | P=0.591N |
| Incidental Tumor Test | P=0.503 | P=0.423 | P=0.576N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.559 | P=0.500 | P=0.620 |
| Liver: Hepatocellular Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 4/50 (8%) | 3/50 (6%) | 7/49 (14%) |
| Adjusted (c) | 9.5% | 7.3% | 15.9% |
| Terminal (d) | 4/42 (10%) | 2/40 (5%) | 7/44 (16%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.218 | P=0.532N | P=0.288 |
| Incidental Tumor Test | P=0.191 | P=0.540N | P=0.288 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.187 | P=0.500N | P=0.251 |
| Liver: Hepatocellular Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 15/50 (30%) | 19/50 (38%) | 7/49 (14%) |
| Adjusted (c) | 33.0% | 44.0% | 15.9% |
| Terminal (d) | 12/42 (29%) | 16/40 (40%) | 7/44 (16%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.041N | P=0.217 | P=0.040N |
| Incidental Tumor Test | P=0.071N | P=0.237 | P=0.104N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.052N | P=0.263 | P=0.050N |
| Liver: Hepatocellular Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 19/50 (38%) | 22/50 (44%) | 14/49 (29%) |
| Adjusted (c) | 41.9% | 49.9% | 31.8% |
| Terminal (d) | 16/42 (38%) | 18/40 (45%) | 14/44 (32%) |
| Statistical Tests 2(e) | | | |
| Life Table | P=0.147N | P=0.277 | P=0.162N |
| Incidental Tumor Test | P=0.237N | P=0.295 | P=0.308N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.196N | P=0.342 | P=0.217N |
| Pituitary: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 0/40 (0%) | 4/45 (9%) | 6/44 (14%) |
| Adjusted (c) | 0.0% | 9.9% | 13.8% |
| Terminal (d) | 0/34 (0%) | 2/37 (5%) | 4/40 (10%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.022 | P=0.065 | P=0.026 |
| Incidental Tumor Test | P=0.010 | P=0.046 | P=0.032 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.017 | P=0.074 | P=0.017 |

TABLE 11. ANALYSIS OF PRIMARY TUMORS IN MALE MICE (a) (Continued)

| | Control | Low Dose | High Dose |
|---|------------|-------------|-------------|
| Pituitary: Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 0/40 (0%) | 5/45 (11%) | 6/44 (14%) |
| Adjusted (c) | 0.0% | 11.7% | 13.8% |
| Terminal (d) | 0/34 (0%) | 2/37 (5%) | 4/40 (10%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.027 | P=0.036 | P=0.026 |
| Incidental Tumor Test | P=0.006 | P=0.025 | P=0.032 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.022 | P=0.037 | P=0.017 |
| Adrenal: Cortical Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 2/50 (4%) | 1/50 (2%) | 4/49 (8%) |
| Adjusted (c) | 4.8% | 2.1% | 9.0% |
| Terminal (d) | 2/42 (5%) | 0/40 (0%) | 3/43 (7%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.250 | P=0.511N | P=0.348 |
| Incidental Tumor Test | P=0.255 | P=0.367N | P=0.309 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.232 | P=0.500N | P=0.329 |
| Harderian Gland: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 6/50 (12%) | 10/50 (20%) | 10/50 (20%) |
| Adjusted (c) | 14.3% | 23.6% | 22.1% |
| Terminal (d) | 6/42 (14%) | 8/40 (20%) | 9/44 (20%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.209 | P=0.179 | P=0.236 |
| Incidental Tumor Test | P=0.199 | P=0.218 | P=0.215 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.178 | P=0.207 | P=0.207 |

(a) Dosed groups received doses of 50 or 100 ppm of zearalenone in the diet.

(b) Number of tumor bearing animals/number of animals examined at the site.

(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

(d) Observed tumor incidence at terminal kill.

(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE (a)

| | Control | Low Dose | High Dose |
|---|-------------|------------|-------------|
| Lung: Alveolar/Bronchiolar Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 2/48 (4%) | 4/48 (8%) | 1/49 (2%) |
| Adjusted (c) | 5.6% | 10.0% | 3.1% |
| Terminal (d) | 2/36 (6%) | 3/37 (8%) | 1/32 (3%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.450N | P=0.347 | P=0.541N |
| Incidental Tumor Test | P=0.343N | P=0.427 | P=0.541N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.398N | P=0.339 | P=0.492N |
| Lung: Alveolar/Bronchiolar Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/48 (6%) | 4/48 (8%) | 1/49 (2%) |
| Adjusted (c) | 7.6% | 10.0% | 3.1% |
| Terminal (d) | 2/36 (6%) | 3/37 (8%) | 1/32 (3%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.299N | P=0.504 | P=0.363N |
| Incidental Tumor Test | P=0.209N | P=0.571 | P=0.348N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.245N | P=0.500 | P=0.301N |
| Hematopoietic System: Malignant Lymphoma, Lymphocytic Type | | | |
| Tumor Rates | | | |
| Overall (b) | 3/50 (6%) | 2/49 (4%) | 3/49 (6%) |
| Adjusted (c) | 8.1% | 5.4% | 9.0% |
| Terminal (d) | 3/37 (8%) | 2/37 (5%) | 2/32 (6%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.513 | P=0.500N | P=0.581 |
| Incidental Tumor Test | P=0.524 | P=0.500N | P=0.597 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.578 | P=0.510N | P=0.651 |
| Hematopoietic System: Malignant Lymphoma, Histiocytic Type | | | |
| Tumor Rates | | | |
| Overall (b) | 2/50 (4%) | 0/49 (0%) | 3/49 (6%) |
| Adjusted (c) | 5.3% | 0.0% | 7.2% |
| Terminal (d) | 1/37 (3%) | 0/37 (0%) | 0/32 (0%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.339 | P=0.243N | P=0.432 |
| Incidental Tumor Test | P=0.358 | P=0.264N | P=0.458 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.384 | P=0.253N | P=0.490 |
| Hematopoietic System: Malignant Lymphoma, Mixed Type | | | |
| Tumor Rates | | | |
| Overall (b) | 10/50 (20%) | 6/49 (12%) | 10/49 (20%) |
| Adjusted (c) | 24.4% | 15.6% | 25.7% |
| Terminal (d) | 7/37 (19%) | 5/37 (14%) | 5/32 (16%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.429 | P=0.210N | P=0.462 |
| Incidental Tumor Test | P=0.529N | P=0.220N | P=0.547N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.534 | P=0.220N | P=0.579 |

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE (a) (Continued)

| | Control | Low Dose | High Dose |
|--|-------------|------------|-------------|
| Hematopoietic System: Malignant Lymphoma, All Types | | | |
| Tumor Rates | | | |
| Overall (b) | 15/50 (30%) | 9/49 (18%) | 16/49 (33%) |
| Adjusted (c) | 36.2% | 22.5% | 38.0% |
| Terminal (d) | 11/37 (30%) | 7/37 (19%) | 7/32 (22%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.307 | P=0.129N | P=0.327 |
| Incidental Tumor Test | P=0.428 | P=0.138N | P=0.490 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.431 | P=0.132N | P=0.473 |
| Liver: Hepatocellular Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 0/50 (0%) | 2/49 (4%) | 7/49 (14%) |
| Adjusted (c) | 0.0% | 4.9% | 21.0% |
| Terminal (d) | 0/37 (0%) | 1/37 (3%) | 6/32 (19%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.002 | P=0.235 | P=0.005 |
| Incidental Tumor Test | P=0.002 | P=0.215 | P=0.006 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.003 | P=0.242 | P=0.006 |
| Liver: Hepatocellular Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/50 (6%) | 6/49 (12%) | 3/49 (6%) |
| Adjusted (c) | 8.1% | 14.0% | 8.3% |
| Terminal (d) | 3/37 (8%) | 2/37 (5%) | 2/32 (6%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.487 | P=0.253 | P=0.602 |
| Incidental Tumor Test | P=0.564 | P=0.194 | P=0.636N |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.561 | P=0.233 | P=0.651 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/50 (6%) | 7/49 (14%) | 10/49 (20%) |
| Adjusted (c) | 8.1% | 16.4% | 28.7% |
| Terminal (d) | 3/37 (8%) | 3/37 (8%) | 8/32 (25%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.014 | P=0.169 | P=0.019 |
| Incidental Tumor Test | P=0.020 | P=0.124 | P=0.029 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.026 | P=0.151 | P=0.033 |
| Pituitary: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/46 (7%) | 2/43 (5%) | 13/42 (31%) |
| Adjusted (c) | 8.6% | 5.5% | 40.9% |
| Terminal (d) | 3/35 (9%) | 1/33 (3%) | 10/28 (36%) |
| Statistical Tests (e) | | | |
| Life Table | P<0.001 | P=0.517N | P=0.002 |
| Incidental Tumor Test | P<0.001 | P=0.558N | P=0.002 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.001 | P=0.532N | P=0.003 |

TABLE 12. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE (a) (Continued)

| | Control | Low Dose | High Dose |
|---|-----------|-----------|-------------|
| Pituitary: Adenoma or Carcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 3/46 (7%) | 2/43 (5%) | 15/42 (36%) |
| Adjusted (c) | 8.6% | 5.5% | 44.4% |
| Terminal (d) | 3/35 (9%) | 1/33 (3%) | 10/28 (3%) |
| Statistical Tests (e) | | | |
| Life Table | P<0.001 | P=0.517N | P<0.001 |
| Incidental Tumor Test | P<0.001 | P=0.558N | P<0.001 |
| Cochran-Armitage Trend, Fisher Exact Tests | P<0.001 | P=0.532N | P=0.001 |
| Mammary Gland: Adenocarcinoma | | | |
| Tumor Rates | | | |
| Overall (b) | 1/50 (2%) | 3/49 (6%) | 3/49 (6%) |
| Adjusted (c) | 2.7% | 7.1% | 7.4% |
| Terminal (d) | 1/37 (3%) | 1/37 (3%) | 1/32 (3%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.209 | P=0.311 | P=0.275 |
| Incidental Tumor Test | P=0.416 | P=0.322 | P=0.493 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.233 | P=0.301 | P=0.301 |
| Harderian Gland: Adenoma | | | |
| Tumor Rates | | | |
| Overall (b) | 1/50 (2%) | 0/49 (0%) | 3/49 (6%) |
| Adjusted (c) | 2.7% | 0.0% | 9.4% |
| Terminal (d) | 1/37 (3%) | 0/37 (0%) | 3/32 (9%) |
| Statistical Tests (e) | | | |
| Life Table | P=0.144 | P=0.500N | P=0.254 |
| Incidental Tumor Test | P=0.144 | P=0.500N | P=0.254 |
| Cochran-Armitage Trend, Fisher Exact Tests | P=0.173 | P=0.505N | P=0.301 |

(a) Dosed groups received doses of 50 or 100 ppm of zearalenone in the diet.

(b) Number of tumor bearing animals/number of animals examined at the site.

(c) Kaplan-Meier estimated lifetime tumor incidence after adjusting for intercurrent mortality.

(d) Observed tumor incidence at terminal kill.

(e) Beneath the control incidence are the P-values associated with the trend test. Beneath the dosed group incidence are the P-values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as non-fatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend is indicated by (N).

IV. DISCUSSION AND CONCLUSIONS

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Rat Study

Zearalenone and zearalenol have been shown to bind to the uterine cytoplasmic estrogen receptors of Holtzman and Sprague-Dawley rats and to elicit the translocation of the cytosol-receptor complex to the nucleus (Katzenellenbogen et al., 1979; Kiang et al., 1978). Zearalenone has also been found to bind to the mammary gland estrogen receptors of Sprague-Dawley rats (Boyd and Wittliff, 1978). The physiological consequences of these subcellular events were shown clearly in the subchronic tests. In the 14-day study, distention of the uterine horns occurred in 4/5 or 5/5 F344/N rats fed diets containing 6,000-100,000 ppm zearalenone. Endometrial hyperplasia of the uterus was observed in 2/10, 8/10, and 10/10 female rats administered 300, 1,000, or 3,000 ppm for 13 weeks; at the 3,000-ppm dose, ductular gland hyperplasia of the mammary gland was found in 6/10 males and 10/10 females.

Effects on the pituitary, prostate, testes, and bone in rats may also be linked to the binding of zearalenone or its metabolite to steroid receptors. Chromophobe hyperplasia of the pituitary occurred in 1/10 females fed 100 ppm, 2/10 females and 2/9 males fed 1,000 ppm, and 6/10 males and 7/10 females fed 3,000 ppm zearalenone for 13 weeks. Other hyperplastic effects on the pituitary have also been observed in rats administered other estrogenic compounds. Increased incidences of pituitary tumors have been found in rats and mice in other studies after they received implantations of cholesterol pellets containing diethylstilbesterol (Andervont et al., 1960; DeNicola et al., 1978) and after subcutaneous injections of estradiol benzoate (Gardner, 1941) or diethylstilbesterol dipropionate (Jacobi et al., 1975). In this NCI/NTP chronic study an increased incidence of pituitary tumors occurred in B6C3F1 mice but not in F344/N rats.

Implants of zearalenone induce squamous metaplasia and hyperplasia of calf prostate glands (Rothenbacher et al., 1975) as well as testicular atrophy (Ralston, 1978). In the present 13-week study, fibromuscular hyperplasia of the prostate was seen in 9/9 and 9/10 male rats fed diets containing 1,000 or 3,000 ppm. In the chronic study, inflammation of the prostate occurred in 31/50 and 22/50 male rats administered 25 or 50 ppm (control incidence, 15/49).

Atrophy of the seminal vesicles occurred in 90%-100% of the male rats fed 1,000 or 3,000 ppm zearalenone for 13 weeks; atrophy of the testes was found at increased incidences relative

to controls in males administered 25 or 50 ppm for 103 weeks. Aspermatogenesis was found in 60%-100% of the animals receiving 300-3,000 ppm in the 13-week study. Interstitial-cell tumors of the testes occurred with a statistically significant negative trend in rats fed 25 or 50 ppm for 103 weeks ($P=0.042$ in the incidental tumor test; control 45/50, 90%; low-dose, 43/50, 86%; high-dose 39/50, 78%). The decreased incidence of testicular interstitial-cell tumors is probably related to testicular atrophy.

Osteopetrosis occurred with a dose-related incidence in female rats administered diets containing 30-3,000 ppm zearalenone for 13 weeks. Incidences of 90%-100% were found in groups of females administered 100 ppm or more and in males administered 1,000 or 3,000 ppm. Female rats were considerably more sensitive to this chemically-induced effect (5/10 at 30 ppm) than were males. Yet, osteopetrosis was not observed in either male or female rats in the chronic study. Bailey et al. (1976) reported that ingestion of more than 0.1 mg zearalenone per kg per day increased the number and extent of distribution of medullary trabeculae in the femurs of male and female rats in a multigeneration study of the toxic effects of zearalenone. The absence of this response in the current study is unexplainable.

Studies on the metabolic fate of ^3H -zearalenone in Wistar rats have shown that radioactivity accumulates in both the liver and kidney (Ueno et al., 1977). Cytoplasmic vacuolization of the liver was observed at increased incidences in male rats fed diets containing 25 or 50 ppm zearalenone in the chronic study. The vacuolization, however, was minimal and was focal in most instances. Nephrosis, typical of that seen in aging F344/N rats, was observed at increased incidences in males and females fed 25 or 50 ppm zearalenone (control males, 30/50, 60%; low-dose males, 43/50, 86%; high-dose males, 41/50, 82%; control females, 10/50, 20%; low-dose females, 24/50, 48%; high-dose females, 26/50, 52%).

Clinically, opacity or cloudiness of the lens was noted at increased incidences in male rats fed diets containing 25 or 50 ppm zearalenone and in female rats fed 25 ppm. The increased incidences were also associated with animals in the uppermost rows of cages and with proximity to fluorescent light. Inasmuch as the eye was not one of the organs sampled routinely, only those eyes with clinical or gross abnormalities were examined microscopically. Almost all rats in which opacity or cloudiness of the lens was

IV. DISCUSSION AND CONCLUSIONS

observed were also found to have cataractous or shrunken lenses and retinal degeneration or atrophy. The relationship between cataracts, the test chemical, light intensity, and retinal degeneration (which was usually severe in the present study) is not known.

Lenticular problems associated with exposure of test or control animals to fluorescent light have been noted in several Bioassay Program studies at this same laboratory (NTP 1982a; NTP 1982b; NTP 1982c).

For male and female rats there were no compound-related increases in any tumor type.

Mouse Study

Effects similar to those found in rats were also observed in mice. Distention of the uterine horns occurred in 2/5 to 4/5 females receiving 6,000-100,000 ppm zearalenone for 14 days. Fibrosis of the uterus/myometrium was seen at increased incidences in mice administered 50 or 100 ppm for 103 weeks. The number of females with cystic ducts in the mammary glands was increased in the dosed groups in the chronic study.

Atrophy of the seminal vesicles was seen in all male mice fed diets containing 1,000 or 3,000 ppm zearalenone in the 13-week subchronic study and vacuolization of the adrenal glands was observed in 90% of the male mice fed diets containing the same doses of the test chemical; squamous metaplasia of the prostate occurred in 8/10 males administered the 3,000-ppm dose.

Osteopetrosis was observed in 10/10 males and 6/8 females fed diets containing 3,000 ppm zearalenone for 13 weeks, in 10/10 males and 5/10 females administered 1,000 ppm, in 7/10 males and 5/10 females receiving 300 ppm, and in 4/10 males and 2/10 females administered 100 ppm. Osteopetrosis, noted at most dose levels in the 13-week study, was not seen in the chronic study. Certain minimal bony changes were noted in the femurs of the chronic study high-dose female mice; these changes were characterized by increased fibrous osteodystrophy. Sass and Montali (1980) recently reported that the spontaneous myelofibrosis found in B6C3F1 mice resembles the estrogen-induced myelofibrosis described by other investigators (Seaman et al., 1979; Sokoloff et al., 1967; Silberberg and Silberberg, 1970). In the current experiment, myelofibrosis of the bone marrow occurred in 10/10 males fed diets containing 3,000 or 1,000 ppm for 13 weeks and in 6/8 females fed 3,000 ppm and 3/10 females fed 1,000 ppm; this effect occurred with increased incidence and severity (compared with

the controls) for females receiving 50 or 100 ppm zearalenone for 103 weeks.

Administration of zearalenone to mice in the present study was associated with increased incidences of neoplastic lesions of the liver and of the pituitary.

The incidence of hepatocellular adenomas was dose-related in female mice administered diets containing 0, 50, or 100 ppm zearalenone for 103 weeks ($P \leq 0.003$); the incidences in females receiving the highest dose were significantly ($P \leq 0.006$) higher than those in the controls. The incidence of hepatocellular adenomas for females administered 50 or 100 ppm zearalenone was 2/49 (4%) and 7/49 (14%), respectively. The incidence in the control group was 0/50 (0%), compared with an average rate of 14/498 (2.8%) for control female B6C3F1 mice at this laboratory. The historical incidence of liver tumors in untreated control female B6C3F1 mice in all Bioassay Program laboratories is summarized in Appendix K, Table K1. The incidence of hepatocellular carcinomas (3/50, 6/49, 3/49) was similar for the control and dosed groups.

Pituitary adenomas occurred with statistically significant positive trends in mice administered 50 or 100 ppm zearalenone for 103 weeks ($P \leq 0.022$ for males and $P \leq 0.001$ for females). The increased incidences of pituitary adenomas in mice receiving the higher dose were each statistically significant ($P \leq 0.032$ in males, $P \leq 0.003$ in females). These increases are considered to be due to the dietary administration of zearalenone. In addition to the pituitary adenomas, two other high-dose female mice had pituitary carcinoma. The incidence of pituitary adenomas and carcinomas (of all types) in untreated control B6C3F1 mice at this laboratory is 0/399 for males and 21/428 (4.9%) for females (individual group incidences ranged from 0% to 6/48, 12.5%) (Appendix K, Tables K2 and K3). Pituitary tumors have also been found at increased incidences in various strains of rats and mice, including F344 males and C57BL males, administered other estrogenic compounds such as diethylstilbesterol (Andervont et al., 1960), estradiol benzoate (Gardner, 1941), or diethylstilbesterol dipropionate (Jacobi et al., 1975).

The body weight gain reduction anticipated from the 13-week study was not duplicated throughout the chronic study. Based on weight gain data only, it appears that the mice could have tolerated higher doses of zearalenone. However, based on histological findings in both male and female mice, it appears likely that the

IV. DISCUSSION AND CONCLUSIONS

levels used in the chronic study approached a maximum tolerated dose.

As can be observed from the analysis of the data given in Tables 11 and 12, the significance of the dose-related increase in hepatocellular adenomas in female mice is not markedly affected when adenomas or carcinomas are considered together. Hepatocellular adenomas are considered to be part of the process of hepatic carcinogenesis (Stewart et al., 1980) which is induced by hepatocarcinogens but not by non-carcinogens. National and international agencies (IARC, 1980; IRLG, 1979) have stated that few, if any, chemicals produce only benign tumors in any species; agents that significantly increase the incidence of benign tumors are today viewed with as much suspicion as they would have been had the induced tumors been diagnosed as malignant. In general, existing evidence supports the progression, in many tissues, of adenomas to carcinomas and, accordingly, a chemical that induces adenomas represents a potential carcinogenic risk to humans (IARC, 1980).

Despite the similarities between zearalenone and DES in their physiological responses and subcellular actions, there appear to be significant differences in tumor target tissues. Following oral doses of DES, Sprague-Dawley rats developed mammary fibroadenomas, pituitary tumors (not specified as adenomas or carcinomas),

hepatomas, and a hemangio-endothelioma (Gibson et al., 1967). Such tumors were not seen in the F344/N rats in this study. Various strains of mice receiving oral doses of DES (gavage or feed) developed mammary tumors, uterine horn adenocarcinomas, cervical adenocarcinomas, and osteosarcomas (reviewed in IARC, 1979). The B6C3F1 mice used in this study did not develop tumors in these locations. It is not presently clear if these target tissue differences are due to strain, to the chemical tested (despite physiologically similar mechanisms), or to both.

Administration of zearalenone was related to the occurrence of inflammation of the prostate, testicular atrophy, and cytoplasmic vacuolization of the liver in male F344/N rats and to nephrosis in rats of either sex. In female B6C3F1 mice, zearalenone increased the incidence of bone marrow myelofibrosis, uterine fibrosis, and mammary cystic ducts.

Conclusions: Under the conditions of this bioassay, zearalenone was not carcinogenic for F344/N rats of either sex. Zearalenone should be considered carcinogenic in B6C3F1 mice, as evidenced by the increased proportion of male and female mice with pituitary adenomas and by the increased proportion of female mice with hepatocellular adenomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED DIETS CONTAINING ZEARALENONE

TABLE A1.

**SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS
CONTAINING ZEARELENONE**

| | CONTROL | LOW DOSE | HIGH DOSE |
|--------------------------------------|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *MEDIASTINUM | (50) | (50) | (50) |
| FIBROUS HISTIOCYTOMA, MALIGNANT | 1 (2%) | | |
| *SKIN | (50) | (50) | (50) |
| PAPILLOMA, NOS | | | 1 (2%) |
| SQUAMOUS CELL PAPILLOMA | 2 (4%) | 1 (2%) | |
| BASAL-CELL TUMOR | 1 (2%) | | |
| SEBACEOUS ADENOMA | | | 1 (2%) |
| ADENOSQUAMOUS CARCINOMA | | | 1 (2%) |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| FIBROMA | 2 (4%) | | |
| FIBROSARCOMA | 1 (2%) | | 1 (2%) |
| LIPOMA | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| *NOSE | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILLOMA | | 1 (2%) | |
| #TRACHEAL GLAND | (49) | (43) | (46) |
| ADENOCARCINOMA, NOS | 1 (2%) | | |
| #LUNG | (49) | (50) | (47) |
| CARCINOMA, NOS, METASTATIC | | 1 (2%) | |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 3 (6%) | 1 (2%) | |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 2 (4%) | 1 (2%) | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIG. LYMPHOMA, LYMPHOCYTIC TYPE | | 1 (2%) | |
| MALIGNANT LYMPHOMA, MIXED TYPE | | | 1 (2%) |
| UNDIFFERENTIATED LEUKEMIA | 9 (18%) | 4 (8%) | 7 (14%) |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| DIGESTIVE SYSTEM | | | |
| #SALIVARY GLAND | (49) | (49) | (50) |
| SARCOMA, NOS | 1 (2%) | | |
| #LIVER | (50) | (50) | (50) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------|----------------|------------------|
| NEOPLASTIC NODULE HEPATOCELLULAR CARCINOMA | 2 (4%) | | 1 (2%) 1 (2%) |
| #COLONIC MUCOUS MEMBR ADENOMATOUS POLYP, NOS | (49) | (49) 1 (2%) | (48) |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| SARCOMA, NOS | 1 (2%) | | |
| NEPHROBLASTOMA | 1 (2%) | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (46) | (49) | (50) |
| NEOPLASM, NOS | 1 (2%) | | |
| CARCINOMA, NOS | 1 (2%) | | 1 (2%) |
| ADENOMA, NOS | 5 (11%) | 13 (27%) | 9 (18%) |
| #ADRENAL | (50) | (50) | (50) |
| PHEOCHROMOCYTOMA | 5 (10%) | 5 (10%) | 5 (10%) |
| PHEOCHROMOCYTOMA, MALIGNANT | 2 (4%) | | 2 (4%) |
| #THYROID | (49) | (50) | (50) |
| FOLLICULAR-CELL ADENOMA | | | 1 (2%) |
| C-CELL ADENOMA | 3 (6%) | 7 (14%) | 6 (12%) |
| C-CELL CARCINOMA | | 1 (2%) | 2 (4%) |
| #PANCREATIC ISLETS | (49) | (50) | (50) |
| ISLET-CELL ADENOMA | 2 (4%) | 2 (4%) | |
| ISLET-CELL CARCINOMA | 1 (2%) | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| CARCINOMA, NOS | 1 (2%) | | |
| ADENOMA, NOS | | | 1 (2%) |
| FIBROMA | 1 (2%) | | |
| FIBROADENOMA | 1 (2%) | 3 (6%) | 2 (4%) |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| CARCINOMA, NOS | 1 (2%) | | |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| ADENOMA, NOS | 1 (2%) | 2 (4%) | 1 (2%) |
| #PROSTATE | (49) | (50) | (49) |
| ADENOCARCINOMA, NOS | | | 1 (2%) |
| #TESTIS | (50) | (50) | (50) |
| INTERSTITIAL-CELL TUMOR | 45 (90%) | 42 (84%) | 39 (78%) |
| INTERSTITIAL-CELL TUMOR, MALIGNANT | | 1 (2%) | |
| *SCROTUM | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILOMA | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|--------------------------|--------------------------|
| NERVOUS SYSTEM | | | |
| #BRAIN ASTROCYTOMA | (50) 1 (2%) | (50) | (50) |
| #PALLIUM GLIOMA, NOS | (50) | (50) 1 (2%) | (50) |
| SPECIAL SENSE ORGANS | | | |
| *ZYMBAL'S GLAND SQUAMOUS CELL CARCINOMA | (50) | (50) 1 (2%) | (50) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *MEDIASTINUM SQUAMOUS CELL CARCINOMA, METASTA | (50) 1 (2%) | (50) | (50) |
| *ABDOMINAL CAVITY MESOTHELIOMA BENIGN | (50) 1 (2%) | (50) | (50) |
| *MESENTERY MESOTHELIOMA BENIGN | (50) | (50) 1 (2%) | (50) |
| *TUNICA VAGINALIS MESOTHELIOMA BENIGN | (50) 1 (2%) | (50) | (50) |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS ALVEOLAR/BRONCHIOLAR CA, METASTA PHEOCHROMOCYTOMA, METASTATIC MESOTHELIOMA, NOS | (50) 1 (2%) | (50) 1 (2%) 1 (2%) | (50) 1 (2%) 1 (2%) |
| THIGH CARCINOMA, NOS | | 1 | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|---------|----------|-----------|
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH ^a | 6 | 4 | 3 |
| MORIBUND SACRIFICE | 6 | 8 | 10 |
| SCHEDULED SACRIFICE | 6 | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 32 | 38 | 37 |
| ANIMAL MISSING | | | |
| ^a INCLUDES AUTOLYZED ANIMALS | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 50 | 50 | 48 |
| TOTAL PRIMARY TUMORS | 102 | 93 | 87 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 47 | 47 | 46 |
| TOTAL BENIGN TUMORS | 73 | 80 | 67 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 21 | 10 | 15 |
| TOTAL MALIGNANT TUMORS | 26 | 12 | 18 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 2 | 2 | 1 |
| TOTAL SECONDARY TUMORS | 2 | 2 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT | 3 | 1 | 2 |
| TOTAL UNCERTAIN TUMORS | 3 | 1 | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| * PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS | | | |
| # SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN | | | |

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS
CONTAINING ZEARALENONE

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILLOMA | | 1 (2%) | |
| KERATOACANTHOMA | 1 (2%) | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| CARCINOMA, NOS | | 1 (2%) | |
| SARCOMA, NOS | | 2 (4%) | |
| FIBROMA | | 2 (4%) | |
| RESPIRATORY SYSTEM | | | |
| #TRACHEA | (42) | (46) | (47) |
| C-CELL CARCINOMA, INVASIVE | | | 1 (2%) |
| #LUNG | (48) | (50) | (50) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 1 (2%) | 2 (4%) | 1 (2%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | | | 1 (2%) |
| SARCOMA, NOS, METASTATIC | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIG. LYMPHOMA, HISTIOCYTIC TYPE | | | 1 (2%) |
| UNDIFFERENTIATED LEUKEMIA | 7 (14%) | 7 (14%) | 2 (4%) |
| #BONE MARROW | (49) | (48) | (48) |
| MALIG. LYMPHOMA, HISTIOCYTIC TYPE | 1 (2%) | | |
| CIRCULATORY SYSTEM | | | |
| NONE | | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|----------------------------|----------|----------|-----------|
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (50) |
| NEOPLASTIC NODULE | | 1 (2%) | 1 (2%) |
| HEPATOCELLULAR CARCINOMA | | | 1 (2%) |
| #STOMACH | (50) | (50) | (50) |
| SARCOMA, NOS | 1 (2%) | | |
| URINARY SYSTEM | | | |
| NONE | | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (49) | (50) | (49) |
| CARCINOMA, NOS | 1 (2%) | 1 (2%) | |
| ADENOMA, NOS | 13 (27%) | 15 (30%) | 14 (29%) |
| #ADRENAL | (50) | (50) | (50) |
| CORTICAL ADENOMA | | | 1 (2%) |
| PHEOCHROMOCYTOMA | | 1 (2%) | |
| #THYROID | (49) | (50) | (49) |
| ADENOMA, NOS | 1 (2%) | | |
| FOLLICULAR-CELL ADENOMA | 1 (2%) | | |
| C-CELL ADENOMA | 5 (10%) | 6 (12%) | 4 (8%) |
| C-CELL CARCINOMA | | 1 (2%) | 2 (4%) |
| #PARATHYROID | (43) | (45) | (44) |
| ADENOMA, NOS | | | 1 (2%) |
| #PANCREATIC ISLETS | (50) | (50) | (50) |
| ISLET-CELL ADENOMA | 1 (2%) | | |
| ISLET-CELL CARCINOMA | | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| ADENOCARCINOMA, NOS | 1 (2%) | 2 (4%) | 1 (2%) |
| CYSTADENOMA, NOS | | | 1 (2%) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------------|-----------------|-----------------|
| FIBROADENOMA | 9 (18%) | 14 (28%) | 9 (18%) |
| *PREPUTIAL GLAND CARCINOMA, NOS | (50) 1 (2%) | (50) | (50) 2 (4%) |
| *VAGINA GRANULAR-CELL TUMOR, BENIGN | (50) 1 (2%) | (50) | (50) |
| #UTERUS ENDOMETRIAL STROMAL POLYP | (50) 4 (8%) | (49) 8 (16%) | (49) 5 (10%) |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *EYELID FIBROSARCOMA | (50) | (50) 1 (2%) | (50) |
| *ZYMBAI'S GLAND SQUAMOUS CELL CARCINOMA | (50) 1 (2%) | (50) | (50) |
| MUSCULOSKELETAL SYSTEM | | | |
| *MUSCLE OF BACK RHABDOMYOSARCOMA | (50) | (50) | (50) 1 (2%) |
| BODY CAVITIES | | | |
| *THORAX FIBROMA | (50) | (50) | (50) 1 (2%) |
| *MEDIASTINUM ADENOCARCINOMA, NOS | (50) | (50) 1 (2%) | (50) |
| *ABDOMINAL WALL FIBROMA | (50) 1 (2%) | (50) | (50) |
| *MESENTERY LIPOMA | (50) 1 (2%) | (50) | (50) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|---------|----------|----------------|
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS C-CELL CARCINOMA, METASTATIC | (50) | (50) | (50) 1 (2%) |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH ^a | 2 | | |
| MORIBUND SACRIFICE | 8 | 12 | 9 |
| SCHEDULED SACRIFICE | 6 | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 34 | 38 | 41 |
| ANIMAL MISSING | | | |
| ^a INCLUDES AUTOLYZED ANIMALS | | | |
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 32 | 40 | 33 |
| TOTAL PRIMARY TUMORS | 52 | 67 | 49 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 29 | 35 | 28 |
| TOTAL BENIGN TUMORS | 39 | 49 | 39 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 13 | 15 | 9 |
| TOTAL MALIGNANT TUMORS | 13 | 17 | 9 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | 1 | 1 |
| TOTAL SECONDARY TUMORS | | 1 | 2 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT | | 1 | 1 |
| TOTAL UNCERTAIN TUMORS | | 1 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| * PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS | | | |
| # SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN | | | |

TABLE A4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE 2-YEAR STUDY OF ZEARALENONE

LOW DOSE

| ANIMAL NUMBER | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WEEKS ON STUDY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SKIN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| SQUAMOUS CELL PAPILLOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| SUBCUTANEOUS TISSUE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| CARCINOMA, NOS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| SARCOMA, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FIBROMA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LUNGS AND BRONCHI | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| SARCOMA, NOS, METASTATIC | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TRACHEA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BONE MARROW | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| SPLEEN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LYMPH NODES | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| THYMUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HEART | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SALIVARY GLAND | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LIVER | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NEOPLASTIC NODULE | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| BILE DUCT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| GALLBLADDER & COMMON BILE DUCT | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | | |
| PANCREAS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ESOPHAGUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| STOMACH | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SMALL INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LARGE INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| KIDNEY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY BLADDER | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PITUITARY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| CARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ADENOMA, NOS | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| ADRENAL | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PHEOCHROMOCYTOMA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| THYROID | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-CELL ADENOMA | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C-CELL CARCINOMA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| PARATHYROID | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PANCREATIC ISLETS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ISLET-CELL CARCINOMA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MAMMARY GLAND | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ADENOCARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| FIBROADENOMA | X | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| UTERUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ENDOMETRIAL STROMAL POLYP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| OVARY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BRAIN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| EYE APPENDAGES | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| FIBROSARCOMA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MEDIASTINUM | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| ADENOCARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MULTIPLE ORGANS NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | |
| UNDIFFERENTIATED LEUKEMIA | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | X | |

+: TISSUE EXAMINED MICROSCOPICALLY
 -: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
 X: TUMOR INCIDENCE
 N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
 : NO TISSUE INFORMATION SUBMITTED
 C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
 A: AUTOLYSIS
 M: ANIMAL MISSING
 B: NO NECROPSY PERFORMED

TABLE A4. FEMALE RATS: TUMOR PATHOLOGY (CONTINUED) LOW DOSE

| ANIMAL NUMBER | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | TOTAL TISSUES/TUMORS |
|--------------------------------|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----------------------|
| WEEKS ON STUDY | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
| INTEGUMENTARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| SKIN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50x |
| SQUAMOUS CELL PAPILLOMA | | | | | | | | | | | | | | | | | | | | | | 1 |
| SUBCUTANEOUS TISSUE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50x |
| CARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 1 |
| SARCOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 2 |
| FIBROMA | | | | | | | | | | | | | | | | | | | | | | 2 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| LUNGS AND BRONCHI | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ALVEOLAR/BRONCHIOLAR ADENOMA | | | | | | | | | | | | | | | | | | | | | | 2 |
| SARCOMA, NOS, METASTATIC | | | | | | | | | | | | | | | | | | | | | | 1 |
| TRACHEA | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 46 |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| BONE MARROW | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| SPLEEN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| LYMPH NODES | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| THYMUS | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| HEART | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| SALIVARY GLAND | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| LIVER | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| NEOPLASTIC NODULE | | | | | | | | | | | | | | | | | | | | | | 1 |
| BILE DUCT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| GALLBLADDER & COMMON BILE DUCT | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 50x |
| PANCREAS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ESOPHAGUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| STOMACH | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| SMALL INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| LARGE INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| KIDNEY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| URINARY BLADDER | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 48 |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| PITUITARY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| CARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 1 |
| ADENOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 15 |
| ADRENAL | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| PHEOCHROMOCYTOMA | | | | | | | | | | | | | | | | | | | | | | 1 |
| THYROID | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| C-CELL ADENOMA | | | | | | | | | | | | | | | | | | | | | | 6 |
| C-CELL CARCINOMA | | | | | | | | | | | | | | | | | | | | | | 1 |
| PARATHYROID | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 |
| PANCREATIC ISLETS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| ISLET-CELL CARCINOMA | | | | | | | | | | | | | | | | | | | | | | 1 |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| MAMMARY GLAND | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50x |
| ADENOCARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 2 |
| FIBROADENOMA | | | | | | | | | | | | | | | | | | | | | | 14 |
| UTERUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| ENDOMETRIAL STROMAL POLYP | | | | | | | | | | | | | | | | | | | | | | 8 |
| OVARY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | |
| BRAIN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 |
| SPECIAL SENSE ORGANS | | | | | | | | | | | | | | | | | | | | | | |
| EYE APPENDAGES | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 50x |
| FIBROSARCOMA | | | | | | | | | | | | | | | | | | | | | | 1 |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | |
| MEDIASTINUM | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 50x |
| ADENOCARCINOMA, NOS | | | | | | | | | | | | | | | | | | | | | | 1 |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | |
| MULTIPLE ORGANS NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | 50x |
| UNDIFFERENTIATED LEUKEMIA | | | | | | | | | | | | | | | | | | | | | | 7 |

* ANIMALS NECROPSIED
 +: TISSUE EXAMINED MICROSCOPICALLY
 -: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
 X: TUMOR INCIDENCE
 N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
 : NO TISSUE INFORMATION SUBMITTED
 C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
 A: AUTOLYSIS
 M: ANIMAL MISSING
 B: NO NECROPSY PERFORMED

TABLE A4.

INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE 2-YEAR STUDY OF ZEARALENONE

HIGH DOSE

| ANIMAL NUMBER | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | |
|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|---|
| WEEKS ON STUDY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| RESPIRATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LUNGS AND BRONCHI ALVEOLAR/BRONCHIOLAR ADENOMA ALVEOLAR/BRONCHIOLAR CARCINOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| TRACHEA C-CELL CARCINOMA, INVASIVE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| HEMATOPOIETIC SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BONE MARROW | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SPLEEN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LYMPH NODES | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| THYMUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| CIRCULATORY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| HEART | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| DIGESTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SALIVARY GLAND | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LIVER NEOPLASTIC NODULE HEPATOCELLULAR CARCINOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BILE DUCT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| GALLBLADDER & COMMON BILE DUCT | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| PANCREAS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ESOPHAGUS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| STOMACH | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| SMALL INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| LARGE INTESTINE | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| KIDNEY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| URINARY BLADDER | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ENDOCRINE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PITUITARY ADENOMA, NOS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ADRENAL CORTICAL ADENOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| THYROID C-CELL ADENOMA C-CELL CARCINOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PARATHYROID ADENOMA, NOS | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| REPRODUCTIVE SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MAMMARY GLAND ADENOCARCINOMA, NOS CYSTADENOMA, NOS FIBROADENOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| PREPUTIAL/CLITORAL GLAND ADENOMA, NOS | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| UTERUS ENDOMETRIAL STROMAL POLYP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| OVARY | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| NERVOUS SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| BRAIN | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MUSCULOSKELETAL SYSTEM | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MUSCLE RHABDOMYOSARCOMA | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BODY CAVITIES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PLEURA FIBROMA | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| ALL OTHER SYSTEMS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MULTIPLE ORGANS NOS C-CELL CARCINOMA, METASTATIC MALIG. LYMPHOMA, HISTIOCYTIC TYPE UNDIFFERENTIATED LEUKEMIA | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

+: TISSUE EXAMINED MICROSCOPICALLY
 -: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
 X: TUMOR INCIDENCE
 N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
 : NO TISSUE INFORMATION SUBMITTED
 C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
 A: AUTOLYSIS
 M: ANIMAL MISSING
 B: NO NECROPSY PERFORMED

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED DIETS CONTAINING ZEARALENONE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS
CONTAINING ZEARALENONE

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| SQUAMOUS CELL PAPILOMA | 1 (2%) | | |
| FIBROSARCOMA | | 1 (2%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| SARCOMA, NOS | 1 (2%) | 1 (2%) | |
| FIBROSARCOMA | | 1 (2%) | |
| NEURILEMOMA, MALIGNANT | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (49) | (50) |
| HEPATOCELLULAR CARCINOMA, METAST | 1 (2%) | 3 (6%) | |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 7 (14%) | 5 (10%) | 8 (16%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 4 (8%) | 3 (6%) | 3 (6%) |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | 1 (2%) | 1 (2%) | 4 (8%) |
| MALIG.LYMPHOMA, HISTIOCYTIC TYPE | | 1 (2%) | |
| MALIGNANT LYMPHOMA, MIXED TYPE | 5 (10%) | 2 (4%) | 1 (2%) |
| #SPLEEN | (50) | (50) | (49) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | 1 (2%) | |
| #PEYER'S PATCH | (49) | (49) | (49) |
| MALIGNANT LYMPHOMA, MIXED TYPE | | | 1 (2%) |
| #DUODENUM | (49) | (49) | (49) |
| MALIG.LYMPHOMA, LYMPHOCYTIC TYPE | | 2 (4%) | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------------------------|----------------------------|----------------------------|
| CIRCULATORY SYSTEM | | | |
| *MULTIPLE ORGANS HEMANGIOSARCOMA | (50) 1 (2%) | (50) | (50) 1 (2%) |
| *SUBCUT TISSUE HEMANGIOSARCOMA | (50) 1 (2%) | (50) | (50) |
| #SPLEEN HEMANGIOMA HEMANGIOSARCOMA | (50) 1 (2%) | (50) 1 (2%) 1 (2%) | (49) 1 (2%) |
| #PANCREAS HEMANGIOMA | (50) 1 (2%) | (49) | (49) |
| DIGESTIVE SYSTEM | | | |
| #LIVER HEPATOCELLULAR ADENOMA HEPATOCELLULAR CARCINOMA | (50) 4 (8%) 15 (30%) | (50) 3 (6%) 19 (38%) | (49) 7 (14%) 7 (14%) |
| URINARY SYSTEM | | | |
| #KIDNEY HEPATOCELLULAR CARCINOMA, METAST | (50) 1 (2%) | (50) | (50) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY CARCINOMA, NOS ADENOMA, NOS | (40) | (45) 1 (2%) 4 (9%) | (44) 6 (14%) |
| #ADRENAL CORTICAL ADENOMA PHEOCHROMOCYTOMA | (50) 2 (4%) | (50) 1 (2%) | (49) 4 (8%) 1 (2%) |
| #THYROID FOLLICULAR-CELL ADENOMA | (50) 2 (4%) | (49) 1 (2%) | (49) |
| #PANCREATIC ISLETS ISLET-CELL ADENOMA | (50) | (49) 1 (2%) | (49) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| REPRODUCTIVE SYSTEM | | | |
| #TESTIS | (50) | (50) | (50) |
| INTERSTITIAL-CELL TUMOR | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| #HARDERIAN GLAND | (50) | (50) | (50) |
| ADENOMA, NOS | 6 (12%) | 10 (20%) | 10 (20%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| SARCOMA, NOS | 1 (2%) | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH ^a | 6 | 4 | 1 |
| MORIBUND SACRIFICE | 2 | 7 | 4 |
| SCHEDULED SACRIFICE | 10 | | |
| TERMINAL SACRIFICE | 32 | 39 | 44 |
| ACCIDENTALLY KILLED | | | 1 |
| ANIMAL MISSING | | | |
| ^a INCLUDES AUTOLYZED ANIMALS | | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|---------|----------|-----------|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 37 | 41 | 38 |
| TOTAL PRIMARY TUMORS | 54 | 60 | 55 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 20 | 21 | 29 |
| TOTAL BENIGN TUMORS | 24 | 26 | 37 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 24 | 29 | 17 |
| TOTAL MALIGNANT TUMORS | 30 | 34 | 18 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | 1 | 3 | |
| TOTAL SECONDARY TUMORS | 2 | 3 | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| * PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS | | | |
| # SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN | | | |

TABLE B2.

**SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS
CONTAINING ZEARALENONE**

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 49 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 49 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (49) | (49) |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| *SUBCUT TISSUE | (50) | (49) | (49) |
| FIBROSARCOMA | | 1 (2%) | |
| OSTEOSARCOMA | | | 1 (2%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (48) | (48) | (49) |
| HEPATOCELLULAR CARCINOMA, METAST | | 1 (2%) | 1 (2%) |
| ALVEOLAR/BRONCHIOLAR ADENOMA | 2 (4%) | 4 (8%) | 1 (2%) |
| ALVEOLAR/BRONCHIOLAR CARCINOMA | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| *MULTIPLE ORGANS | (50) | (49) | (49) |
| MALIGNANT LYMPHOMA, NOS | | 1 (2%) | |
| MALIG. LYMPHOMA, LYMPHOCYTIC TYPE | 2 (4%) | 2 (4%) | 3 (6%) |
| MALIG. LYMPHOMA, HISTIOCYTIC TYPE | 2 (4%) | | 3 (6%) |
| MALIGNANT LYMPHOMA, MIXED TYPE | 9 (18%) | 5 (10%) | 9 (18%) |
| #SPLEEN | (50) | (49) | (49) |
| MALIG. LYMPHOMA, LYMPHOCYTIC TYPE | 1 (2%) | | |
| MALIGNANT LYMPHOMA, MIXED TYPE | 1 (2%) | | |
| #MESENTERIC L. NODE | (50) | (48) | (49) |
| MALIGNANT LYMPHOMA, MIXED TYPE | | 1 (2%) | |
| #PEYER'S PATCH | (48) | (45) | (49) |
| MALIGNANT LYMPHOMA, MIXED TYPE | | | 1 (2%) |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------------|---------------------------|----------------------------|
| CIRCULATORY SYSTEM | | | |
| *MULTIPLE ORGANS HEMANGIOSARCOMA | (50) | (49) | (49) 1 (2%) |
| #SPLEEN HEMANGIOSARCOMA | (50) 1 (2%) | (49) | (49) 1 (2%) |
| #UTERUS HEMANGIOMA | (50) 1 (2%) | (49) | (48) |
| #OVARY HEMANGIOMA | (44) | (48) 1 (2%) | (48) |
| DIGESTIVE SYSTEM | | | |
| #LIVER HEPATOCELLULAR ADENOMA HEPATOCELLULAR CARCINOMA | (50) 3 (6%) | (49) 2 (4%) 6 (12%) | (49) 7 (14%) 3 (6%) |
| #STOMACH SQUAMOUS CELL PAPILLOMA | (48) | (48) 1 (2%) | (49) |
| URINARY SYSTEM | | | |
| NONE | | | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY CARCINOMA, NOS ADENOMA, NOS | (46) 3 (7%) | (43) 2 (5%) | (42) 2 (5%) 13 (31%) |
| #ADRENAL CORTICAL ADENOMA PHEOCHROMOCYTOMA | (50) | (49) 1 (2%) 1 (2%) | (47) 1 (2%) |
| #THYROID FOLLICULAR-CELL ADENOMA | (47) 1 (2%) | (48) 1 (2%) | (47) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND ADENOCARCINOMA, NOS | (50) 1 (2%) | (49) 3 (6%) | (49) 3 (6%) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ADENOSQUAMOUS CARCINOMA | | 1 (2%) | |
| #UTERUS | (50) | (49) | (48) |
| ENDOMETRIAL STROMAL POLYP | 1 (2%) | | |
| #CERVIX UTERI | (50) | (49) | (48) |
| SQUAMOUS CELL CARCINOMA | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| *HARDERIAN GLAND | (50) | (49) | (49) |
| ADENOMA, NOS | 1 (2%) | | 3 (6%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *BONE | (50) | (49) | (49) |
| OSTEOSARCOMA | | | 1 (2%) |
| BODY CAVITIES | | | |
| NONE | | | |
| ALL OTHER SYSTEMS | | | |
| LEG | | | |
| OSTEOSARCOMA | 1 | | |
| NEURILEMOMA, MALIGNANT | 1 | | |
| ANIMAL DISPOSITION SUMMARY | | | |
| - ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| NATURAL DEATH ^a | 7 | 10 | 4 |
| MORIBUND SACRIFICE | 6 | 4 | 14 |
| SCHEDULED SACRIFICE | 8 | | |
| ACCIDENTALLY KILLED | | | |
| TERMINAL SACRIFICE | 29 | 36 | 32 |
| ANIMAL MISSING | | | |
| ^a INCLUDES AUTOLYZED ANIMALS | | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|---------|----------|-----------|
| TUMOR SUMMARY | | | |
| TOTAL ANIMALS WITH PRIMARY TUMORS* | 28 | 25 | 40 |
| TOTAL PRIMARY TUMORS | 34 | 33 | 53 |
| TOTAL ANIMALS WITH BENIGN TUMORS | 8 | 13 | 22 |
| TOTAL BENIGN TUMORS | 9 | 13 | 25 |
| TOTAL ANIMALS WITH MALIGNANT TUMORS | 23 | 18 | 27 |
| TOTAL MALIGNANT TUMORS | 25 | 20 | 28 |
| TOTAL ANIMALS WITH SECONDARY TUMORS# | | 1 | 1 |
| TOTAL SECONDARY TUMORS | | 1 | 1 |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC | | | |
| TOTAL UNCERTAIN TUMORS | | | |
| * PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS | | | |
| # SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN | | | |

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED DIETS CONTAINING ZEARALENONE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
FED DIETS CONTAINING ZEARALENONE

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| EPIDERMAL INCLUSION CYST | | 1 (2%) | |
| ATROPHY, NOS | | | 1 (2%) |
| HYPERPLASIA, FOCAL | | | 1 (2%) |
| SKIN TAG | 2 (4%) | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| EPIDERMAL INCLUSION CYST | 2 (4%) | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG/BRONCHIOLE | (49) | (50) | (47) |
| HYPERPLASIA, NOS | 3 (6%) | 1 (2%) | 1 (2%) |
| HYPERPLASIA, ADENOMATOUS | | 1 (2%) | |
| #LUNG | (49) | (50) | (47) |
| INFLAMMATION, INTERSTITIAL | 1 (2%) | | |
| PNEUMONIA, CHRONIC MURINE | 1 (2%) | | |
| HYPERPLASIA, ALVEOLAR EPITHELIUM | 3 (6%) | 1 (2%) | 1 (2%) |
| #ALVEOLAR EPITHELIUM | (49) | (50) | (47) |
| HYPERPLASIA, ADENOMATOUS | | 1 (2%) | |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (49) | (49) | (50) |
| HISTIOCYTOSIS | | 1 (2%) | |
| HYPOPLASIA, HEMATOPOIETIC | 1 (2%) | | |
| #SPLEEN | (50) | (50) | (50) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|-----------------------------|----------|----------|-----------|
| FIBROSIS, FOCAL | | 1 (2%) | |
| HEMATOPOIESIS | 1 (2%) | 2 (4%) | |
| #MANDIBULAR L. NODE | (50) | (50) | (50) |
| DILATATION, NOS | | | 1 (2%) |
| INFLAMMATION, NOS | | | 2 (4%) |
| HYPERPLASIA, NOS | | 5 (10%) | 1 (2%) |
| HYPERPLASIA, CYSTIC | 2 (4%) | | |
| HYPERPLASIA, LYMPHOID | | 1 (2%) | |
| #MESENTERIC L. NODE | (50) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| HYPERPLASIA, RETICULUM CELL | | | 1 (2%) |
| #LIVER | (50) | (50) | (50) |
| LEUKOCYTOSIS, NOS | | 5 (10%) | |
| HEMATOPOIESIS | | 1 (2%) | |
| #KIDNEY | (50) | (50) | (50) |
| HYPERPLASIA, LYMPHOID | 1 (2%) | 1 (2%) | |
| CIRCULATORY SYSTEM | | | |
| #LUNG | (49) | (50) | (47) |
| THROMBOSIS, NOS | 1 (2%) | | |
| #HEART | (49) | (50) | (50) |
| INFLAMMATION, CHRONIC | 1 (2%) | | 4 (8%) |
| #MYOCARDIUM | (49) | (50) | (50) |
| INFLAMMATION, NOS | | 2 (4%) | |
| INFLAMMATION, INTERSTITIAL | | | 1 (2%) |
| INFLAMMATION, CHRONIC | 18 (37%) | | 3 (6%) |
| FIBROSIS | 1 (2%) | 6 (12%) | |
| FIBROSIS, FOCAL | | 1 (2%) | |
| FIBROSIS, DIFFUSE | 2 (4%) | 2 (4%) | 6 (12%) |
| #CARDIAC VALVE | (49) | (50) | (50) |
| INFLAMMATION, FIBRINOUS | 1 (2%) | | |
| *ARTERY | (50) | (50) | (50) |
| INFLAMMATION, FOCAL | 1 (2%) | | |
| DIGESTIVE SYSTEM | | | |
| #LIVER | (50) | (50) | (50) |
| FIBROSIS | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|-----------------------------|---------|----------|-----------|
| CHOLANGIOFIBROSIS | 1 (2%) | | |
| NECROSIS, NOS | 1 (2%) | | |
| CYTOPLASMIC VACUOLIZATION | | 6 (12%) | 8 (16%) |
| BASOPHILIC CYTO CHANGE | | | 1 (2%) |
| ATROPHY, FOCAL | 1 (2%) | | |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| ANGIECTASIS | | | 1 (2%) |
| #HEPATIC LOBULE | (50) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #LIVER/CENTRIOLOBULAR | (50) | (50) | (50) |
| ATROPHY, NOS | 1 (2%) | | 3 (6%) |
| #BILE DUCT | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 7 (14%) | 4 (8%) | 1 (2%) |
| HYPERPLASIA, FOCAL | | | 1 (2%) |
| #PANCREAS | (49) | (50) | (50) |
| INFLAMMATION, CHRONIC | 9 (18%) | 1 (2%) | |
| INFLAMMATION, CHRONIC FOCAL | 1 (2%) | | |
| ATROPHY, NOS | | 1 (2%) | |
| ATROPHY, FOCAL | | | 2 (4%) |
| #PANCREATIC ACINUS | (49) | (50) | (50) |
| ATROPHY, NOS | | 1 (2%) | |
| ATROPHY, FOCAL | | 4 (8%) | 3 (6%) |
| #GASTRIC MUCOSA | (50) | (50) | (50) |
| INFLAMMATION, NOS | | | 1 (2%) |
| HYPERPLASIA, NOS | | | 1 (2%) |
| #GASTRIC SUBMUCOSA | (50) | (50) | (50) |
| INFLAMMATION, NOS | | | 1 (2%) |
| #CARDIAC STOMACH | (50) | (50) | (50) |
| ULCER, NOS | | 1 (2%) | |
| INFLAMMATION ACTIVE CHRONIC | | 1 (2%) | |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | |
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| HYDRONEPHROSIS | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|-----------------------------|----------|----------|-----------|
| CYST, NOS | | | 1 (2%) |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| INFLAMMATION, GRANULOMATOUS | 1 (2%) | | |
| NEPHROPATHY | | | 1 (2%) |
| NEPHROSIS, NOS | 30 (60%) | 43 (86%) | 41 (82%) |
| INFARCT, NOS | 1 (2%) | | |
| HEMOSIDEROSIS | | 1 (2%) | |
| #URINARY BLADDER | (48) | (50) | (49) |
| INFLAMMATION, HEMORRHAGIC | | 1 (2%) | |
| *URETHRA | (50) | (50) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| INFLAMMATION, NOS | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (46) | (49) | (50) |
| HYPERPLASIA, NOS | | 2 (4%) | 3 (6%) |
| HYPERPLASIA, FOCAL | 1 (2%) | | 1 (2%) |
| #ADRENAL | (50) | (50) | (50) |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| #ADRENAL CORTEX | (50) | (50) | (50) |
| CYTOPLASMIC VACUOLIZATION | | 2 (4%) | 1 (2%) |
| CYTOLOGIC ALTERATION, NOS | | 1 (2%) | |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| #ADRENAL MEDULLA | (50) | (50) | (50) |
| FOCAL CELLULAR CHANGE | | | 1 (2%) |
| #THYROID | (49) | (50) | (50) |
| CYSTIC FOLLICLES | | | 1 (2%) |
| DEGENERATION, CYSTIC | | 7 (14%) | 5 (10%) |
| HYPERPLASIA, CYSTIC | | | 2 (4%) |
| HYPERPLASIA, C-CELL | 1 (2%) | 1 (2%) | 3 (6%) |
| #PARATHYROID | (41) | (43) | (43) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #PANCREATIC ISLETS | (49) | (50) | (50) |
| HYPERPLASIA, NOS | 1 (2%) | | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|-----------------------------------|----------|----------|-----------|
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| CYST, NOS | 3 (6%) | 13 (26%) | 5 (10%) |
| CYSTIC DUCTS | 1 (2%) | | 14 (28%) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| HYPERPLASIA, NOS | 1 (2%) | | |
| HYPERPLASIA, CYSTIC | 1 (2%) | | 2 (4%) |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| CYST, NOS | | 1 (2%) | 1 (2%) |
| CYSTIC DUCTS | | | 1 (2%) |
| INFLAMMATION, NOS | | 1 (2%) | |
| INFLAMMATION, SUPPURATIVE | 1 (2%) | 1 (2%) | |
| INFLAMMATION ACTIVE CHRONIC | | 1 (2%) | |
| INFLAMMATION, GRANULOMATOUS | 1 (2%) | | |
| HYPERPLASIA, NOS | 3 (6%) | 1 (2%) | 2 (4%) |
| HYPERPLASIA, CYSTIC | | 1 (2%) | 1 (2%) |
| *PROSTATE | (49) | (50) | (49) |
| CYST, NOS | | 1 (2%) | |
| HEMORRHAGE | | 1 (2%) | |
| INFLAMMATION, NOS | | | 1 (2%) |
| INFLAMMATION, SUPPURATIVE | 10 (20%) | 29 (58%) | 20 (41%) |
| INFLAMMATION ACUTE AND CHRONIC | | | 1 (2%) |
| INFLAMMATION, CHRONIC | 3 (6%) | 2 (4%) | |
| INFLAMMATION, CHRONIC SUPPURATIVE | 2 (4%) | | |
| FIBROSIS, DIFFUSE | 2 (4%) | | |
| HYPERPLASIA, FOCAL | 1 (2%) | 2 (4%) | 1 (2%) |
| *SEMINAL VESICLE | (50) | (50) | (50) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| *COAGULATING GLAND | (50) | (50) | (50) |
| HEMORRHAGE | | 1 (2%) | |
| *TESTIS | (50) | (50) | (50) |
| INFARCT, NOS | | 1 (2%) | |
| CALCIFICATION, FOCAL | | 1 (2%) | |
| ATROPHY, NOS | 1 (2%) | 26 (52%) | 17 (34%) |
| HYPERPLASIA, INTERSTITIAL CELL | | | 2 (4%) |
| *EPIDIDYMIS | (50) | (50) | (50) |
| GRANULOMA, SPERMATIC | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|----------------|----------------|
| NERVOUS SYSTEM | | | |
| #BRAIN HEMORRHAGE | (50) 3 (6%) | (50) | (50) |
| #HYPOTHALAMUS COMPRESSION | (50) 1 (2%) | (50) 2 (4%) | (50) 1 (2%) |
| SPECIAL SENSE ORGANS | | | |
| *EYE HEMORRHAGE | (50) | (50) 1 (2%) | (50) |
| RETINOPATHY | | 14 (28%) | 27 (54%) |
| CATARACT | | 11 (22%) | 25 (50%) |
| *EYE/RETINA DEFORMITY, NOS | (50) | (50) | (50) 1 (2%) |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| ATROPHY, NOS | 7 (14%) | | 2 (4%) |
| *EYE/CRYSTALLINE LENS MINERALIZATION | (50) | (50) | (50) 1 (2%) |
| FIBROSIS | 1 (2%) | | |
| *ZYMBAL'S GLAND HYPERPLASIA, CYSTIC | (50) | (50) | (50) 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| NONE | | | |
| BODY CAVITIES | | | |
| *MESENTERY STEATITIS | (50) 3 (6%) | (50) 3 (6%) | (50) 2 (4%) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| LIPOGRANULOMA | | 1 (2%) | |
| ADHESION, NOS | | | 1 (2%) |
| NECROSIS, FAT | | 5 (10%) | 3 (6%) |
| ALL OTHER SYSTEMS | | | |
| OMENTUM NECROSIS, FAT | 1 | 2 | 1 |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NONE | | | |
| * NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
FED DIETS CONTAINING ZEARALENONE

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| ULCER, NOS | | 1 (2%) | |
| HYPERPLASIA, NOS | 1 (2%) | | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | | 2 (4%) |
| RESPIRATORY SYSTEM | | | |
| #LUNG/BRONCHIOLE | (48) | (50) | (50) |
| HYPERPLASIA, ADENOMATOUS | | 1 (2%) | |
| #LUNG | (48) | (50) | (50) |
| CONGESTION, NOS | | 1 (2%) | |
| EDEMA, NOS | | 1 (2%) | |
| HYPERPLASIA, ALVEOLAR EPITHELIUM | 1 (2%) | | 1 (2%) |
| #ALVEOLAR EPITHELIUM | (48) | (50) | (50) |
| HYPERPLASIA, ADENOMATOUS | | 2 (4%) | |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (49) | (48) | (48) |
| HYPERPLASIA, NOS | 1 (2%) | | |
| #SPLEEN | (50) | (50) | (50) |
| HEMOSIDEROSIS | 2 (4%) | | 3 (6%) |
| ATROPHY, FOCAL | 1 (2%) | | |
| HYPERPLASIA, HEMATOPOIETIC | 1 (2%) | | |
| HEMATOPOIESIS | | 2 (4%) | |
| #MANDIBULAR L. NODE | (50) | (50) | (49) |
| HEMOSIDEROSIS | | | 1 (2%) |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|-----------------|----------------|
| HYPERPLASIA, NOS | | 3 (6%) | 3 (6%) |
| #MEDIASTINAL L.NODE HYPERPLASIA, NOS | (50) | (50) 1 (2%) | (49) |
| *FEMUR MYELOFIBROSIS | (50) | (50) | (50) 1 (2%) |
| #LUNG HYPERPLASIA, LYMPHOID | (48) | (50) 2 (4%) | (50) |
| #LIVER LEUKOCYTOSIS, NOS | (50) 1 (2%) | (50) 5 (10%) | (50) |
| #THYMUS HYPERPLASIA, EPITHELIAL | (43) | (47) 1 (2%) | (46) |
| CIRCULATORY SYSTEM | | | |
| *MULTIPLE ORGANS PERIARTERITIS | (50) 1 (2%) | (50) | (50) |
| *MEDIASTINUM THROMBUS, MURAL | (50) | (50) | (50) 1 (2%) |
| #HEART INFLAMMATION, CHRONIC | (49) | (50) 1 (2%) | (50) |
| #BASE OF HEART INFLAMMATION, FOCAL | (49) | (50) 1 (2%) | (50) |
| #MYOCARDIUM INFLAMMATION, NOS | (49) 1 (2%) | (50) | (50) |
| INFLAMMATION, MULTIFOCAL | | 1 (2%) | |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| FIBROSIS | | 1 (2%) | |
| FIBROSIS, DIFFUSE | 2 (4%) | | 1 (2%) |
| *MESENTERIC ARTERY INFLAMMATION, CHRONIC | (50) 1 (2%) | (50) | (50) |
| NECROSIS, FIBRINOID | 1 (2%) | | |
| #LIVER THROMBOSIS, NOS | (50) | (50) | (50) 1 (2%) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|---------|----------------|-----------|
| DIGESTIVE SYSTEM | | | |
| #PAROTID GLAND INFLAMMATION, CHRONIC FOCAL | (50) | (49) 1 (2%) | (50) |
| #LIVER | (50) | (50) | (50) |
| INFLAMMATION, FOCAL GRANULOMATOUS | | 1 (2%) | |
| NECROSIS, FOCAL | 1 (2%) | | |
| METAMORPHOSIS FATTY | 1 (2%) | 2 (4%) | |
| CYTOPLASMIC CHANGE, NOS | | | 1 (2%) |
| CYTOPLASMIC VACUOLIZATION | 2 (4%) | 2 (4%) | 2 (4%) |
| BASOPHILIC CYTO CHANGE | 1 (2%) | 1 (2%) | 3 (6%) |
| FOCAL CELLULAR CHANGE | | 1 (2%) | |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| #LIVER/CENTRILOBULAR | (50) | (50) | (50) |
| DEGENERATION, NOS | 1 (2%) | | |
| METAMORPHOSIS FATTY | | 2 (4%) | 1 (2%) |
| CYTOPLASMIC VACUOLIZATION | | 1 (2%) | 1 (2%) |
| ATROPHY, NOS | 3 (6%) | 1 (2%) | 1 (2%) |
| #LIVER/MIDLOBULAR | (50) | (50) | (50) |
| METAMORPHOSIS FATTY | | 1 (2%) | |
| #LIVER/PERIportal | (50) | (50) | (50) |
| METAMORPHOSIS FATTY | | 1 (2%) | |
| #LIVER/HEPATOcytes | (50) | (50) | (50) |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| #BILE DUCT | (50) | (50) | (50) |
| CYST, NOS | | 1 (2%) | |
| HYPERPLASIA, NOS | 5 (10%) | | |
| #PANCREAS | (50) | (50) | (50) |
| CYSTIC DUCTS | | 1 (2%) | |
| #PANCREATIC ACINUS | (50) | (50) | (50) |
| ATROPHY, NOS | 2 (4%) | 1 (2%) | |
| ATROPHY, FOCAL | 1 (2%) | 5 (10%) | 1 (2%) |
| #ESOPHAGUS | (48) | (50) | (50) |
| INFLAMMATION, NOS | | 1 (2%) | |
| #CARDIAC STOMACH | (50) | (50) | (50) |
| INFLAMMATION, VESICULAR | 1 (2%) | | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|-----------------------------|----------|----------|-----------|
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (50) | (50) |
| HYDRONEPHROSIS | 1 (2%) | | |
| NEPHROSIS, NOS | 10 (20%) | 24 (48%) | 26 (52%) |
| NECROSIS, NOS | | 1 (2%) | |
| #KIDNEY/PELVIS | (50) | (50) | (50) |
| INFLAMMATION, ACUTE/CHRONIC | 1 (2%) | | |
| CALCIFICATION, NOS | | 1 (2%) | |
| #URINARY BLADDER | (49) | (48) | (48) |
| HYPERPLASIA, EPITHELIAL | | 1 (2%) | |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (49) | (50) | (49) |
| HYPERPLASIA, NOS | 6 (12%) | 4 (8%) | 7 (14%) |
| HYPERPLASIA, FOCAL | 1 (2%) | | 1 (2%) |
| #ANTERIOR PITUITARY | (49) | (50) | (49) |
| HEMORRHAGIC CYST | | | 1 (2%) |
| ANGIECTASIS | | | 1 (2%) |
| #ADRENAL | (50) | (50) | (50) |
| CALCIFICATION, FOCAL | | 1 (2%) | |
| CYTOPLASMIC VACUOLIZATION | 2 (4%) | | |
| #ADRENAL CORTEX | (50) | (50) | (50) |
| HEMORRHAGIC CYST | | 1 (2%) | |
| CYTOPLASMIC VACUOLIZATION | 1 (2%) | 1 (2%) | |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| #ADRENAL MEDULLA | (50) | (50) | (50) |
| HEMORRHAGIC CYST | | | 1 (2%) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| #THYROID | (49) | (50) | (49) |
| ULTIMOBRANCHIAL CYST | 2 (4%) | | |
| CYSTIC FOLLICLES | | | 1 (2%) |
| HYPERPLASIA, CYSTIC | 1 (2%) | | |
| HYPERPLASIA, C-CELL | 9 (18%) | 6 (12%) | 7 (14%) |
| #THYROID PARAFOLLICUL | (49) | (50) | (49) |
| HYPERTROPHY, FOCAL | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|----------------------------|----------|----------|-----------|
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (50) | (50) |
| GALACTOCELE | | | 1 (2%) |
| CYST, NOS | 10 (20%) | 34 (68%) | 10 (20%) |
| CYSTIC DUCTS | 18 (36%) | | 18 (36%) |
| ABSCESS, NOS | | 1 (2%) | |
| INFLAMMATION, CHRONIC | | | 1 (2%) |
| HYPERPLASIA, CYSTIC | 1 (2%) | | 1 (2%) |
| ADENOSIS | | 2 (4%) | 2 (4%) |
| *MAMMARY LOBULE | (50) | (50) | (50) |
| HYPERPLASIA, NOS | | 1 (2%) | 1 (2%) |
| *PREPUTIAL GLAND | (50) | (50) | (50) |
| CYSTIC DUCTS | | | 1 (2%) |
| INFLAMMATION, NOS | | 1 (2%) | |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | 2 (4%) |
| HYPERPLASIA, CYSTIC | 2 (4%) | 2 (4%) | 1 (2%) |
| *CLITORAL GLAND | (50) | (50) | (50) |
| HYPERPLASIA, FOCAL | 1 (2%) | | |
| #UTERUS | (50) | (49) | (49) |
| HYDROMETRA | 1 (2%) | 3 (6%) | |
| HEMATOMETRA | | | 1 (2%) |
| #CERVIX UTERI | (50) | (49) | (49) |
| HYPERPLASIA, EPITHELIAL | | | 1 (2%) |
| #UTERUS/ENDOMETRIUM | (50) | (49) | (49) |
| HYPERPLASIA, FOCAL | 1 (2%) | 1 (2%) | |
| HYPERPLASIA, CYSTIC | 2 (4%) | 5 (10%) | |
| #OVARY | (50) | (49) | (49) |
| CYST, NOS | 2 (4%) | 1 (2%) | |
| FOLLICULAR CYST, NOS | | | 4 (8%) |
| CORPUS LUTEUM | 1 (2%) | | |
| NERVOUS SYSTEM | | | |
| *CHOROID PLEXUS LATER | (50) | (50) | (50) |
| ANGIECTASIS | 1 (2%) | | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------------|----------------|----------------|
| #HYPOTHALAMUS COMPRESSION | (49) 1 (2%) | (50) 2 (4%) | (50) 3 (6%) |
| SPECIAL SENSE ORGANS | | | |
| *EYE | (50) | (50) | (50) |
| HEMORRHAGE | | 6 (12%) | 1 (2%) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| RETINOPATHY | 3 (6%) | 28 (56%) | 8 (16%) |
| CATARACT | 4 (8%) | 26 (52%) | 6 (12%) |
| *EYE/CORNEA | (50) | (50) | (50) |
| INFLAMMATION, FOCAL | | | 1 (2%) |
| *EYE/RETINA | (50) | (50) | (50) |
| ATROPHY, NOS | | 3 (6%) | 1 (2%) |
| *EYE/CRYSTALLINE LENS | (50) | (50) | (50) |
| MINERALIZATION | | 1 (2%) | |
| *ZYMBAL'S GLAND | (50) | (50) | (50) |
| HYPERPLASIA, CYSTIC | | | 1 (2%) |
| *MIDDLE EAR | (50) | (50) | (50) |
| INFLAMMATION, SUPPURATIVE | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *MUSCLE OF HEAD | (50) | (50) | (50) |
| INFLAMMATION, NOS | | 1 (2%) | |
| BODY CAVITIES | | | |
| *MESENTERY | (50) | (50) | (50) |
| STEATITIS | 3 (6%) | 2 (4%) | |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| NECROSIS, FAT | 3 (6%) | 2 (4%) | 4 (8%) |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS | (50) | (50) | (50) |
| HEMORRHAGE | 1 (2%) | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------------|-----------------|------------------|
| TAIL HYPERKERATOSIS | | | 1 |
| FOOT HYPERKERATOSIS | | | 1 |
| OMENTUM NECROSIS, FAT | 2 | | 1 |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 4 | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED DIETS CONTAINING ZEARALENONE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
FED DIETS CONTAINING ZEARELENONE

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|----------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 50 | 50 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 50 | 50 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (50) | (50) |
| INFLAMMATION, ACUTE FOCAL | 3 (6%) | | |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| INFLAMMATION, CHRONIC FOCAL | 3 (6%) | 2 (4%) | |
| *SUBCUT TISSUE | (50) | (50) | (50) |
| INFLAMMATION ACUTE AND CHRONIC | | | 1 (2%) |
| INFLAMMATION, CHRONIC FOCAL | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (50) | (49) | (50) |
| CONGESTION, NOS | 1 (2%) | | |
| BRONCHOPNEUMONIA, FOCAL | 14 (28%) | 6 (12%) | 12 (24%) |
| PNEUMONIA, CHRONIC MURINE | | | 1 (2%) |
| HYPERPLASIA, ADENOMATOUS | 1 (2%) | | |
| HYPERPLASIA, ALVEOLAR EPITHELIUM | 1 (2%) | | |
| HEMATOPOIETIC SYSTEM | | | |
| #HARDERIAN GLAND | (50) | (50) | (50) |
| HYPERPLASIA, LYMPHOID | 7 (14%) | 7 (14%) | 8 (16%) |
| #SPLEEN | (50) | (50) | (49) |
| HYPERPLASIA, NOS | | 1 (2%) | |
| HYPERPLASIA, LYMPHOID | | 1 (2%) | |
| #PANCREATIC L. NODE | (50) | (49) | (50) |
| INFLAMMATION, GRANULOMATOUS | | 1 (2%) | |
| #MESENTERIC L. NODE | (50) | (49) | (50) |
| ANGIECTASIS | 1 (2%) | | 1 (2%) |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|----------------|-----------|
| #SACRAL LYMPH NODE HYPERPLASIA, LYMPHOID | (50) 1 (2%) | (49) | (50) |
| #INGUINAL LYMPH NODE HYPERPLASIA, LYMPHOID | (50) 3 (6%) | (49) | (50) |
| #PEYER'S PATCH HYPERPLASIA, LYMPHOID | (49) | (49) 1 (2%) | (49) |
| #THYMUS CYST, NOS | (48) | (39) 1 (3%) | (44) |
| CIRCULATORY SYSTEM | | | |
| #HEART INFLAMMATION, CHRONIC FOCAL | (50) 1 (2%) | (50) | (50) |
| DIGESTIVE SYSTEM | | | |
| #LIVER CYST, NOS | (50) 1 (2%) | (50) 1 (2%) | (49) |
| INFLAMMATION, CHRONIC | | 1 (2%) | |
| FIBROSIS | | 1 (2%) | |
| NECROSIS, NOS | 1 (2%) | 2 (4%) | |
| NECROSIS, CASEOUS | | | 1 (2%) |
| CYTOLOGIC ALTERATION, NOS | 4 (8%) | 1 (2%) | 1 (2%) |
| #PANCREAS DILATATION/DUCTS | (50) 1 (2%) | (49) | (49) |
| ATROPHY, NOS | 1 (2%) | | |
| URINARY SYSTEM | | | |
| #KIDNEY HYDRONEPHROSIS | (50) | (50) 1 (2%) | (50) |
| CYST, NOS | 1 (2%) | | |
| INFLAMMATION, SUPPURATIVE | | 1 (2%) | |
| INFLAMMATION, ACUTE SUPPURATIVE | 1 (2%) | | |
| #URINARY BLADDER LYMPHOCYITIC INFLAMMATORY INFILTR | (49) | (50) 1 (2%) | (50) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|--------------------------|------------------------------------|--|
| ENDOCRINE SYSTEM | | | |
| #PITUITARY HYPERPLASIA, FOCAL | (40) | (45) | (44) 1 (2%) |
| #ADRENAL CORTEX HYPERPLASIA, FOCAL | (50) | (50) | (49) 1 (2%) |
| #ADRENAL MEDULLA HYPERPLASIA, FOCAL | (50) | (50) 1 (2%) | (49) |
| #THYROID CYST, NOS CYSTIC FOLLICLES | (50) | (49) 1 (2%) | (49) 1 (2%) 2 (4%) |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND CYSTIC DUCTS | (50) | (50) | (50) 2 (4%) |
| *PREPUTIAL GLAND CYST, NOS CYSTIC DUCTS INFLAMMATION, ACUTE SUPPURATIVE INFLAMMATION ACUTE AND CHRONIC | (50) 3 (6%) 2 (4%) | (50) 2 (4%) 1 (2%) 2 (4%) | (50) 3 (6%) 1 (2%) 1 (2%) 1 (2%) |
| #PROSTATE INFLAMMATION, ACUTE SUPPURATIVE | (50) 1 (2%) | (50) | (50) |
| *SEMINAL VESICLE DISTENTION INFLAMMATION, CHRONIC | (50) 1 (2%) | (50) 1 (2%) | (50) |
| #TESTIS FIBROSIS NECROSIS, NOS | (50) | (50) 1 (2%) 1 (2%) | (50) |
| NERVOUS SYSTEM | | | |
| NONE | | | |
| SPECIAL SENSE ORGANS | | | |
| #HARDERIAN GLAND INFLAMMATION, ACUTE SUPPURATIVE | (50) 1 (2%) | (50) | (50) |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|----------------|----------------|
| INFLAMMATION, ACUTE/CHRONIC | 1 (2%) | | |
| MUSCULOSKELETAL SYSTEM | | | |
| *ABDOMINAL MUSCLE INFLAMMATION, ACUTE SUPPURATIVE | (50) 1 (2%) | (50) | (50) |
| BODY CAVITIES | | | |
| *PERITONEUM INFLAMMATION, SUPPURATIVE INFLAMMATION, ACUTE SUPPURATIVE | (50) | (50) 1 (2%) | (50) 1 (2%) |
| *MESENTERY NECROSIS, FAT | (50) 2 (4%) | (50) 1 (2%) | (50) |
| ALL OTHER SYSTEMS | | | |
| NONE | | | |
| SPECIAL MORPHOLOGY SUMMARY | | | |
| NO LESION REPORTED | 1 | 4 | 6 |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE D2.

**SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
FED DIETS CONTAINING ZEARALENONE**

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|---------|----------|-----------|
| ANIMALS INITIALLY IN STUDY | 50 | 50 | 50 |
| ANIMALS NECROPSIED | 50 | 49 | 49 |
| ANIMALS EXAMINED HISTOPATHOLOGICALLY | 50 | 49 | 49 |
| INTEGUMENTARY SYSTEM | | | |
| *SKIN | (50) | (49) | (49) |
| EDEMA, NOS | | 1 (2%) | |
| INFLAMMATION ACUTE AND CHRONIC | | | 1 (2%) |
| INFLAMMATION, FOCAL GRANULOMATOUS | 1 (2%) | | |
| *SUBCUT TISSUE | (50) | (49) | (49) |
| INFLAMMATION ACUTE AND CHRONIC | | 1 (2%) | |
| RESPIRATORY SYSTEM | | | |
| #LUNG | (48) | (48) | (49) |
| BRONCHOPNEUMONIA, FOCAL | 3 (6%) | 4 (8%) | 3 (6%) |
| BRONCHOPNEUMONIA, CHRONIC | | | 1 (2%) |
| CHOLESTEROL DEPOSIT | | | 1 (2%) |
| HYPERPLASIA, ALVEOLAR EPITHELIUM | | | 1 (2%) |
| HEMATOPOIETIC SYSTEM | | | |
| #BONE MARROW | (49) | (48) | (49) |
| MYELOFIBROSIS | 8 (16%) | 32 (67%) | 34 (69%) |
| *SPLEEN | (50) | (49) | (49) |
| ABSCESS, NOS | 1 (2%) | | |
| #MEDIASTINAL L. NODE | (50) | (48) | (49) |
| ABSCESS, NOS | 1 (2%) | | |
| HYPERPLASIA, NOS | | | 1 (2%) |
| #CELIAC LYMPH NODE | (50) | (48) | (49) |
| HYPERPLASIA, LYMPHOID | 1 (2%) | | |
| #RENAL LYMPH NODE | (50) | (48) | (49) |
| HYPERPLASIA, LYMPHOID | 1 (2%) | | |
| # NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY | | | |
| * NUMBER OF ANIMALS NECROPSIED | | | |

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|---|----------------|----------------|----------------|
| #ILIAC LYMPH NODE HYPERPLASIA, NOS | (50) | (48) | (49) 1 (2%) |
| #LIVER MYELOPOIESIS | (50) 1 (2%) | (49) | (49) |
| #PEYER'S PATCH HYPERPLASIA, LYMPHOID | (48) 1 (2%) | (45) | (49) |
| CIRCULATORY SYSTEM | | | |
| *MUSCLE HIP/THIGH PERIVASCULITIS | (50) | (49) | (49) 1 (2%) |
| #THYROID PERIVASCULITIS | (47) | (48) | (47) 1 (2%) |
| DIGESTIVE SYSTEM | | | |
| #LIVER CYST, NOS | (50) | (49) 1 (2%) | (49) 3 (6%) |
| INFLAMMATION, CHRONIC FOCAL | | 1 (2%) | |
| NECROSIS, NOS | | 3 (6%) | |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| CYTOLOGIC ALTERATION, NOS | 2 (4%) | | |
| HEPATOCTOMEALY | | 1 (2%) | 2 (4%) |
| ATROPHY, NOS | | 1 (2%) | |
| ANGIECTASIS | | | 3 (6%) |
| #PANCREAS | (49) | (48) | (49) |
| INFLAMMATION ACUTE AND CHRONIC | 1 (2%) | | 1 (2%) |
| INFLAMMATION, CHRONIC | 1 (2%) | | |
| INFLAMMATION, CHRONIC FOCAL | | 1 (2%) | |
| FIBROSIS | | | 1 (2%) |
| FIBROSIS, FOCAL | 1 (2%) | | |
| ATROPHY, NOS | 3 (6%) | 1 (2%) | 1 (2%) |
| #STOMACH | (48) | (48) | (49) |
| FOREIGN BODY, NOS | | 1 (2%) | |
| INFLAMMATION, ACUTE FOCAL | | 1 (2%) | |
| INFLAMMATION, ACUTE SUPPURATIVE | | | 1 (2%) |
| INFLAMMATION, FOCAL GRANULOMATOUS | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|----------------------------------|----------|----------|-----------|
| URINARY SYSTEM | | | |
| #KIDNEY | (50) | (48) | (49) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | 1 (2%) | | |
| #URINARY BLADDER | (49) | (48) | (49) |
| LYMPHOCYTIC INFLAMMATORY INFILTR | 1 (2%) | | |
| INFLAMMATION, ACUTE FOCAL | | | 1 (2%) |
| ENDOCRINE SYSTEM | | | |
| #PITUITARY | (46) | (43) | (42) |
| HYPERPLASIA, FOCAL | | 3 (7%) | |
| ANGIECTASIS | | | 1 (2%) |
| #ADRENAL CORTEX | (50) | (49) | (47) |
| CYST, NOS | | 1 (2%) | |
| CYTOPLASMIC VACUOLIZATION | | | 1 (2%) |
| ATROPHY, NOS | | | 1 (2%) |
| #THYROID | (47) | (48) | (47) |
| CYSTIC FOLLICLES | 2 (4%) | | |
| DEGENERATION, CYSTIC | 1 (2%) | | 6 (13%) |
| HYPERPLASIA, FOCAL | | 1 (2%) | |
| HYPERPLASIA, CYSTIC | | | 2 (4%) |
| HYPERPLASIA, FOLLICULAR-CELL | 3 (6%) | 1 (2%) | |
| REPRODUCTIVE SYSTEM | | | |
| *MAMMARY GLAND | (50) | (49) | (49) |
| CYSTIC DUCTS | | 3 (6%) | 15 (31%) |
| METAPLASIA, SQUAMOUS | | 1 (2%) | |
| *VAGINA | (50) | (49) | (49) |
| INFLAMMATION, ACUTE SUPPURATIVE | 3 (6%) | 2 (4%) | 5 (10%) |
| HYPERKERATOSIS | | | 1 (2%) |
| #UTERUS | (50) | (49) | (48) |
| INFLAMMATION, SUPPURATIVE | 10 (20%) | 1 (2%) | |
| INFLAMMATION, ACUTE SUPPURATIVE | 2 (4%) | 5 (10%) | 1 (2%) |
| #CERVIX UTERI | (50) | (49) | (48) |
| INFLAMMATION, ACUTE SUPPURATIVE | 3 (6%) | 5 (10%) | 16 (33%) |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
 * NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

| | CONTROL | LOW DOSE | HIGH DOSE |
|--|------------------|--------------------|-----------|
| #UTERUS/ENDOMETRIUM INFLAMMATION, SUPPURATIVE | (50) | (49) | (48) |
| HYPERPLASIA, CYSTIC | 49 (98%) | 2 (4%) 48 (98%) | 47 (98%) |
| #ENDOMETRIAL GLAND HYPERPLASIA, CYSTIC | (50) | (49) | (48) |
| | | | 1 (2%) |
| #UTERUS/MYOMETRIUM FIBROSIS, FOCAL | (50) | (49) | (48) |
| | 1 (2%) | 5 (10%) | 17 (35%) |
| #OVARY CYST, NOS | (44) | (48) | (48) |
| INFLAMMATION, SUPPURATIVE | 6 (14%) | 2 (4%) | 3 (6%) |
| INFLAMMATION, ACUTE SUPPURATIVE | 2 (5%) 1 (2%) | 1 (2%) | |
| NERVOUS SYSTEM | | | |
| #BRAIN/MENINGES LYMPHOCYTIC INFLAMMATORY INFILTR | (50) | (49) | (48) |
| | | 1 (2%) | |
| SPECIAL SENSE ORGANS | | | |
| *EYE RETINOPATHY | (50) | (49) | (49) |
| | | | 1 (2%) |
| MUSCULOSKELETAL SYSTEM | | | |
| *MUSCLE HIP/THIGH INFLAMMATION, CHRONIC FOCAL | (50) | (49) | (49) |
| | | 1 (2%) | |
| BODY CAVITIES | | | |
| *PERITONEUM INFLAMMATION, ACUTE SUPPURATIVE | (50) | (49) | (49) |
| | 1 (2%) | 1 (2%) | |
| *MESENTERY INFLAMMATION, SUPPURATIVE | (50) | (49) | (49) |
| | 2 (4%) | | |
| ALL OTHER SYSTEMS | | | |
| *MULTIPLE ORGANS LYMPHOCYTIC INFLAMMATORY INFILTR | (50) | (49) | (49) |
| | | 1 (2%) | |

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSIS OF ZEARALENONE (MIDWEST RESEARCH INSTITUTE)

APPENDIX E

A. ELEMENTAL ANALYSIS

| | Element | C | H |
|----------|------------|-------|------|
| Batch 01 | Theory | 67.90 | 6.97 |
| | Determined | 67.78 | 6.88 |
| | | 67.92 | 6.95 |
| Batch 02 | Determined | 67.76 | 6.82 |
| | | 67.87 | 6.95 |
| Batch 03 | Determined | 68.12 | 7.05 |
| | | 68.14 | 7.04 |

B. WATER ANALYSIS (Karl Fischer)

Batch 01 0.14 ± 0.01 (δ)%

Batch 03 0.05%

C. MELTING POINT

| | Determined | Literature Values |
|----------|--|--------------------------------------|
| Batch 01 | m.p.: 162° to 164°C (visual, capillary) | 164° to 165°C (Urry et al., 1977) |
| Batch 02 | m.p.: 162°-163°C (visual, capillary) | |

D. OPTICAL ROTATION

| Determined | Literature Values |
|--|---|
| Batch 01 $[\alpha]_{589}^{25} = -137.2 \pm 0.9$ (δ)° concentration = 1.0% in methanol | $[\alpha]_{546}^{25} = -170.5$ (Urry et al., 1977) concentration = 1.0% in methanol |

E. THIN-LAYER CHROMATOGRAPHY

1. Batch 01

Plates: Silica Gel 60 F-254
Amounted spotted: 100 and 300 μg

System 1: Chloroform:acetone
(90:10)

R_f: 0.53, origin (trace)
R_{st}: 2.0, origin

Ref. Standard: Resorcinol

Visualization: Ultraviolet,
254 and 366 nm and 50% sulfuric acid
System 2: Benzene:methanol
(90:10)

R_f: 0.56, origin (trace)
R_{st}: 3.5, origin

2. Batch 02

Plates: Silica gel G F-254
Amount Spotted: 10 and 30 μl ,
10 $\mu\text{g}/\mu\text{l}$ in methanol

System 1: Chloroform:acetone
(90:10)

R_f: 0.61, origin
R_{st}: 2.65, origin

Ref. Standard: Resorcinol

Visualization: Ultraviolet, 254
and 366 nm and 50% aqueous
sulfuric acid

System 2: Benzene:methanol
(90:10)

R_f: 0.63
R_{st}: 3.00

APPENDIX E

3. Batch 03

Plates: Silica gel 60 F-254
Amount Spotted: 10, 100, and 300 μg
(10 $\mu\text{g}/\mu\text{l}$ in acetone)

Ref. Standard: Resorcinol,
10 μg (10 $\mu\text{g}/\mu\text{l}$ in acetone)

Visualization: Ultraviolet (254 and
366 nm) and 50% aqueous sulfuric acid

System 1: Chloroform:acetone
(90:10)

System 2: Toluene:methanol
(90:10)

R_f: 0.58 (major); 0.20 (slight trace)
R_{st}: 3.45 (major); 1.18 (slight trace)

R_f: 0.35 (major); 0.14 (trace)
R_{st}: 3.19 (major); 1.33 (trace)

F. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1. Batch 01:

Instrument: Waters ALC 202 with Model 660 Solvent Programmer

Detection: Ultraviolet 254 nm

a. System 1:

Column: μ Bondapak C₁₈ - 300 x 4 mm ID, stainless steel

Solvent: A:B (80:20) to 100% B
A - 10% acetonitrile in water
B - 100% acetonitrile

Program: Six (linear)

Program time: 15 min

Flow: 1 ml/min

Results: Single homogeneous peak

Retention time: 14.5 min

b. System 2:

Column: μ Porsasil - 300 x 4 mm ID,

Solvent: Chloroform:tetrahydrofuran (95:5), isocratic

Flow: 1 ml/min

Results: Single homogeneous peak

Retention time: 7.0 min

2. Batch 02:

Instrument: Waters ALC 202

Detection: Ultraviolet, 280 nm

Column: μ Bondapak C₁₈, 300 mm x 4 mm ID

Solvent: Acetonitrile:water (60:40), isocratic

Flow Rate: 1 ml/min

Amount Injected: 2 μl , 1.9 $\mu\text{g}/\mu\text{l}$ in acetonitrile

Results: Single homogeneous peak, retention time 5.5 min

APPENDIX E

3. Batch 03:

Instrument: Waters ALC 202 with Waters 6000A pump with Model 660 Solvent
Programmer and Waters 440 detector

Detection: Ultraviolet, 254 nm

Column: μ Bondapak C₁₈, 300 mm x 3.9 mm ID

Guard Column: CO:PELL ODS, 72 mm x 2.3 mm ID

Solvent: Acetonitrile: water (60:40), isocratic:

Flow Rate: 1 ml/min

Samples Injected: Solution (10 μ l) of 1.0 mg/ml
Zearalenone in methanol, filtered

Results: Major peak and one impurity before the major
peak

| Peak No. | Retention Time (min) | Retention Time (Relative to Major Peak) | Area (Percent of Major Peak) |
|----------|----------------------|---|------------------------------|
| 1 | 12.8 | 0.67 | 0.04 |
| 2 | 19.2 | 1.00 | 100 |

Using isocratic solvent systems of 35% A:65% B, 30% A:70% B, and 20% A:80% B, no additional impurities were observed.

G. SPECTRAL DATA

1. Infrared

a. Batch 01:

Instrument: Beckman IR-12

Cell: Neat, melt on sodium
chloride plates

Results: Figure 5

Consistent with literature spectrum
(Mirocha et al., 1967)

b. Batch 02:

Instrument: Perkin Elmer
Model 137 Infracord

Cell: Melt on sodium
chloride plates

Results: Figure 6

Consistent with literature spectrum
(Mirocha et al., 1967)

c. Batch 03:

Instrument: Beckman IR-12

Cell: 1% in KBr pellet

Results: Figure 7

Consistent with literature spectrum
(Mirocha et al., 1967)

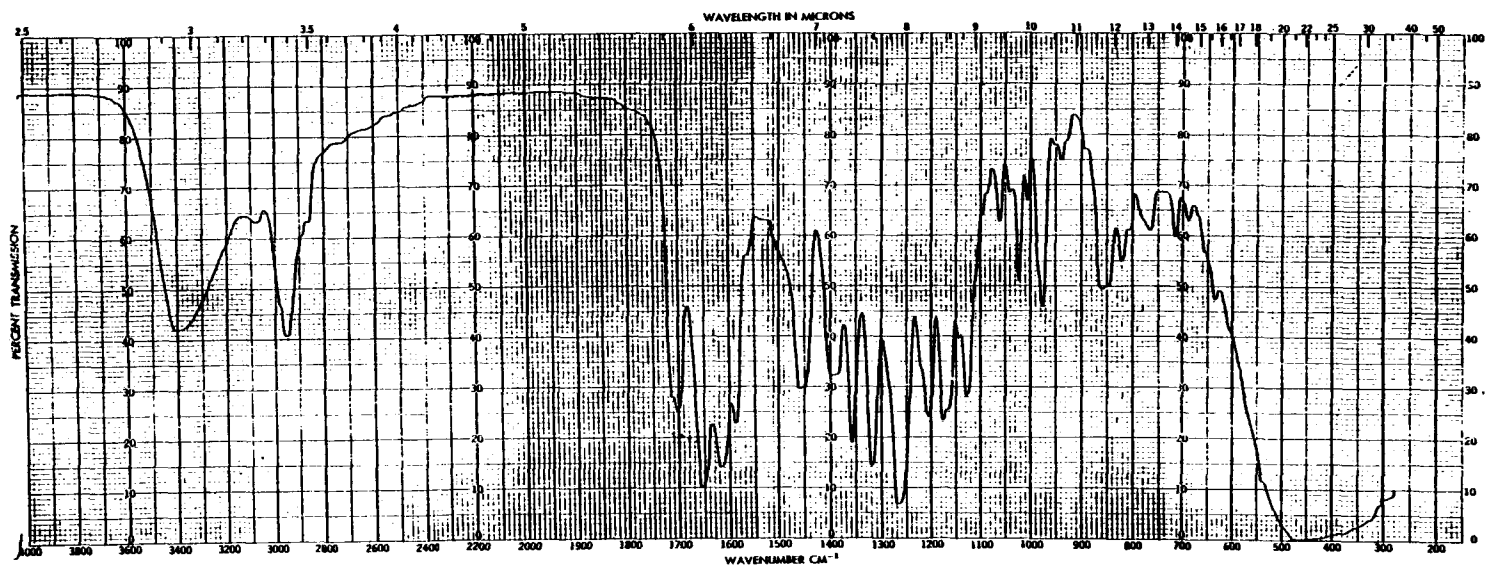


Figure 5. Infrared Absorption Spectrum of Zearalenone (Lot No. 3-75; Batch 01)

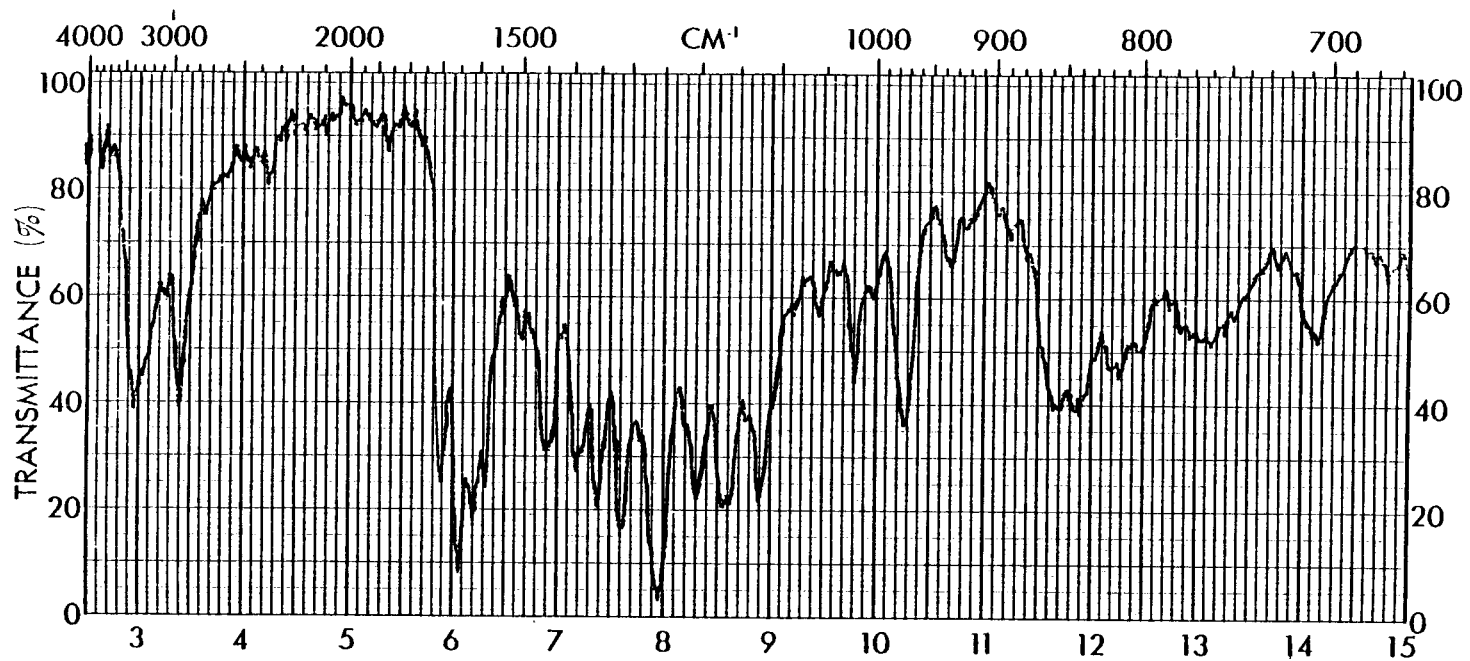


Figure 6. Infrared Absorption Spectrum of Zearalenone (Lot No. 3-75; Batch 02)

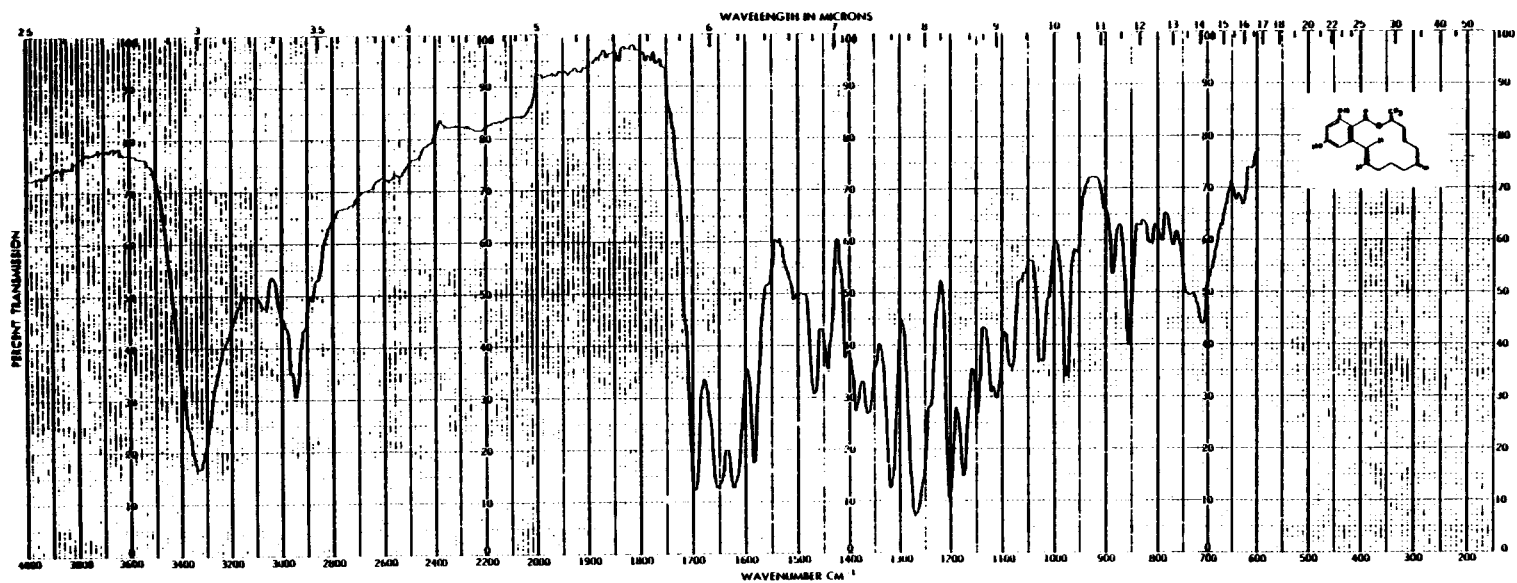


Figure 7. Infrared Absorption Spectrum of Zearalenone (Lot No. 51079; Batch 03)

APPENDIX E

2. Ultraviolet/Visible:

Instrument: Cary 118

a. Batch 01:

| Determined | | Literature Values (Urry et al., 1977) | |
|-------------------|------------------------------|--|---------------------------|
| λ max(nm) | $\epsilon \times 10^{-3}$ | λ max(nm) | $\epsilon \times 10^{-3}$ |
| 235.5 | 27.9 \pm 0.2 (δ) | 236 | 29.7 |
| 273.5 | 12.3 \pm 0.1 (δ) | 274 | 13.9 |
| 314.5 | 5.60 \pm 0.06 (δ) | 316 | 6.02 |

No absorbance between 350 and 800 nm (visible range) at a concentration of 0.1 mg/ml

Solvent: Methanol

b. Batch 03:

Instrument: Cary 118

No maxima from 800 to 350 nm (visible region) but an increase in absorbance toward 350 nm at a concentration of 0.1%.

| λ max(nm) | $\epsilon \times 10^{-3}$ | λ max(nm) | $\epsilon \times 10^{-3}$ |
|-------------------|--------------------------------|-------------------|---------------------------|
| 236 | 29.25 \pm 0.0056(δ) | 236 | 29.7 |
| 274 | 13.36 \pm 0.0077(δ) | 274 | 13.9 |
| 314 | 15.87 \pm 0.0017(δ) | 316 | 60.2 |

Solvent: Methanol

3. Nuclear Magnetic Resonance:

a. Batch 01:

Instrument: Varian HA-100

Solvent: Methanol-d-4 with internal tetramethylsilane

Assignments: Figure 8

(The proton designations are shown in Figure 10)

- (a) d, δ 1.35 ppm, $J_{af} = 6$ Hz
- (b) m, δ 1.65 ppm
- (c) m, δ 2.19 ppm
- (d) m, δ 2.48 to 3.16 ppm
- (e) m, δ 4.95 ppm, not resolved
- (f) m, δ 4.95 ppm
- (g) m, δ 5.73 ppm
- (h) d, δ 6.30 ppm, $J_{hi} = 2$ Hz
- (i) d, δ 6.44 ppm
- (j) d, δ 7.05 ppm, $J_{gi} = 16$ Hz

Experimental spectrum consistent with structure.

Literature spectrum (Urry et al., 1977) differs in the following respects:

Protons (h) and (i) are *not* resolved. The -OH protons are resolved into singlets further downfield than the other signals from zearalenone.

Solvent: CDCl₃

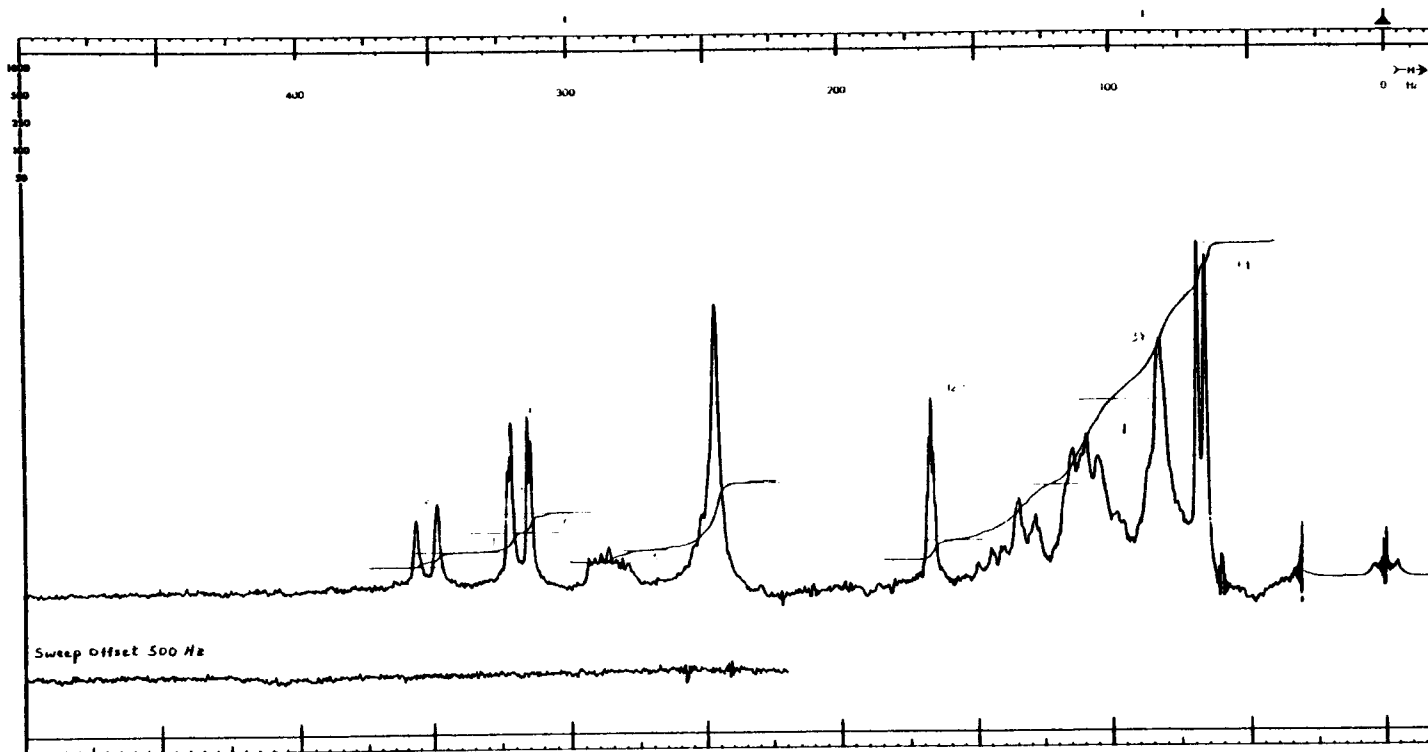


Figure 8. Nuclear Magnetic Resonance Spectrum of Zearalenone (Lot No. 3-75; Batch 01)

APPENDIX E

Integration ratios:

- (a) 2.74
- (b) 5.48
- (c) 4.47
- (d) 2.88
- (e,f) -OH from solvent and protons from zearalenone
- (g) 0.72
- (h) 1.01
- (i) 1.01
- (j) 0.72

b. Batch 02:

Instrument: Varian HA-100
Solvent: Methanol-d-4 with
internal tetramethylsilane

Assignments: Figure 9

- (a) d, δ 1.32 ppm, $J_{af} = 6$ Hz;
- (b) m, δ 1.64 ppm;
- (c) t, δ 2.14 ppm, $J_{bc} = 9$ Hz;
- (d) m, δ 2.44-2.98 ppm;
- (e,f) m, δ 4.77 ppm;
- (g) m, δ 5.64 ppm, $J_{gi} = 15$ Hz;
- (h) d, δ 6.15 ppm, $J_{gh} = 2$ Hz;
- (i) d, δ 6.30 ppm;
- (j) d, δ 6.90 ppm

Integration ratios:

- (a) 3.37
- (b) 5.28
- (c) 4.43
- (d) 1.90
- (e,f) -OH from solvent and protons from zearalenone,
- (g) 1.06
- (h) 0.95
- (i) 1.06
- (j) 0.95

c. Batch 03:

Instrument: Varian EM-360A
Solvent: Methanol-d-4 with
internal tetramethylsilane

Assignments: Figure 10

- (a) d, δ 1.37 ppm, $J_{a-f} = 6$ Hz
- (b) m, δ 1.68 ppm
- (c) m, δ 2.23 ppm
- (d) m, δ 2.50-3.18 ppm
- (e) s, δ 4.85 ppm
- (f) m, δ 4.95 ppm
- (g) m, δ 5.67 ppm
- (h) d, δ 6.23 ppm, $J_{h-i} = 2$ Hz
- (i) d, δ 6.35 ppm
- (j) d, δ 6.97 ppm, $J_{g-j} = 15$ Hz

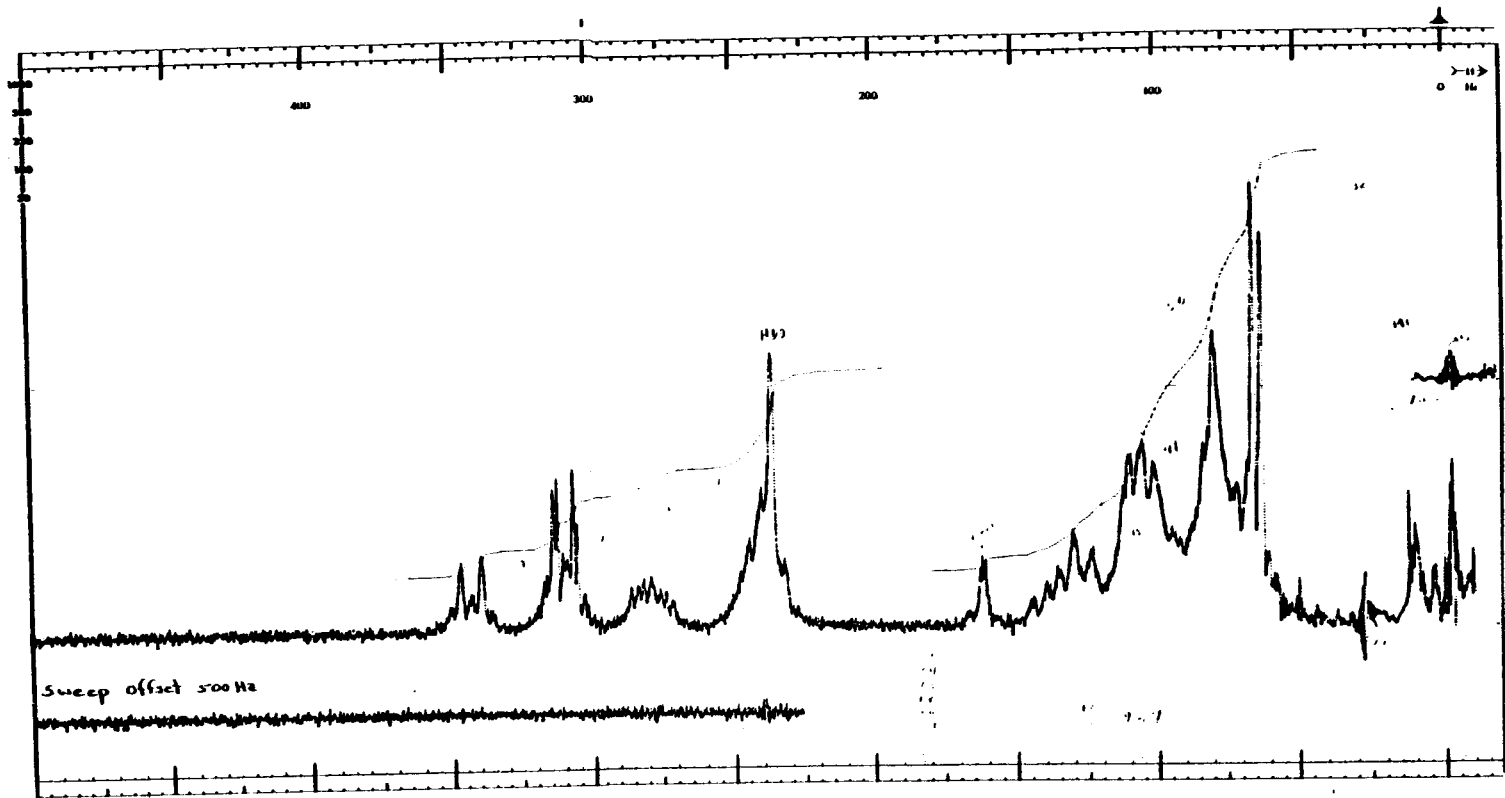


Figure 9. Nuclear Magnetic Resonance Spectrum of Zearalenone (Lot No. 3-75; Batch 02)

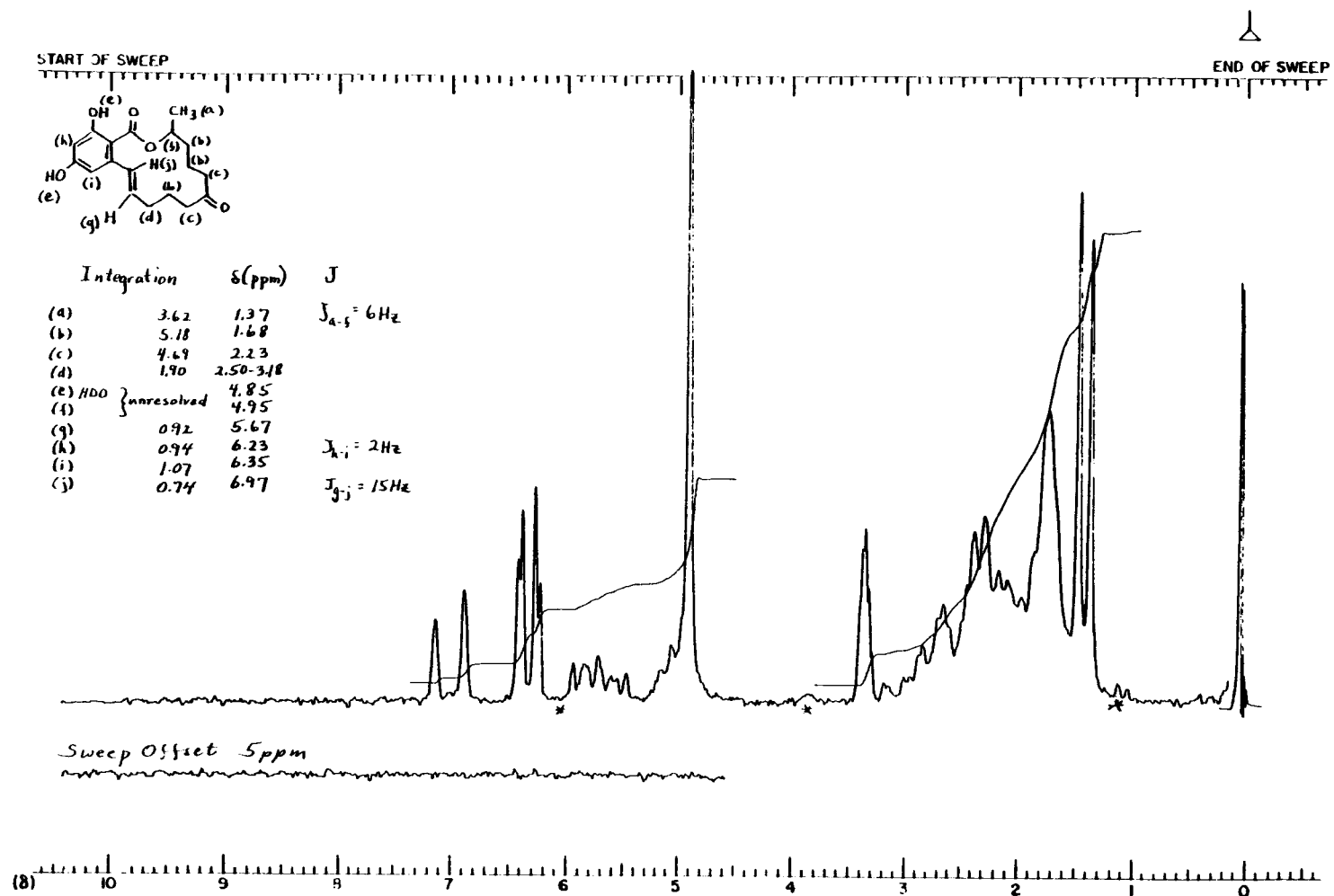


Figure 10. Nuclear Magnetic Resonance Spectrum of Zearalenone (Lot No. 51079; Batch 03)

APPENDIX E

Integration ratios:

| | |
|-----|-------------------------|
| (a) | 3.62 |
| (b) | 5.18 |
| (c) | 4.69 |
| (d) | 1.90 |
| (e) | -OH and MeOH unresolved |
| (f) | -OH and MeOH unresolved |
| (g) | 0.92 |
| (h) | 0.94 |
| (i) | 1.07 |
| (j) | 0.74 |

APPENDIX F

ANALYSIS OF FORMULATED DIETS FOR STABILITY OF ZEARELENONE (MIDWEST RESEARCH INSTITUTE)

APPENDIX F

A. Mixing and storage: Zearalenone and Wayne Lab-Blox[®] rodent feed were mixed in a mortar in two different concentrations: high (100,000 ppm) and low (1,400 ppm). Samples of the mixtures were removed and stored at 2 weeks at -20°C, 5°C, 25°C, and 45°C, respectively. The samples of high zearalenone concentration were analyzed by high-pressure liquid chromatography and those of low zearalenone concentration were analyzed by ultraviolet spectrophotometric analysis.

B. Extraction: One-gram samples of each of the above mixtures were mixed with 50 ml of methanol in an ultrasonic vibratory bath for 30 seconds and then were triturated for 1 minute using a Polytron[®] high-speed blender. The mixture was centrifuged, and the supernatant solution was decanted into a 100-ml volumetric flask. This extraction was repeated on the feed residue, and the combined supernatant solutions were brought to volume with fresh methanol.

C. Analysis

1. High zearalenone concentration (100,000 ppm \square 10.0%)

Instrument: Waters ALC 202

Column: μ -Bondapak C₁₈, 300 x 4 mm ID

Solvent: Acetonitrile:Water (60:40)

Detection: Ultraviolet, 254 nm

Flow rate: 1.0 ml/min

Results:

| Sample (°C) | Average Percent of Recovered Compound (a) |
|-------------|---|
| -20 | 10.8 \pm 0.5 |
| 5 | 10.2 \pm 0.5 |
| 25 | 10.4 \pm 0.5 |
| 45 | 9.7 \pm 0.5 |

(a) Corrected for a spiked recovery value of 99.1%; theoretical 100% value \square 10.0%.

2. Low zearalenone concentration (1,400 ppm \square 0.14%)

Instrument: Cary 118 Spectrophotometer

Absorbance wavelength: 236 nm

Results:

| Sample (°C) | Average Percent of Compound Recovered (a) |
|-------------|---|
| -20 | 0.14 \pm 0.02 |
| 5 | 0.14 \pm 0.02 |
| 25 | 0.15 \pm 0.02 |
| 45 | 0.12 \pm 0.02 |

(a) Corrected for a spiked recovery value of 100%; theoretical 100% value \square 0.14%

D. Conclusion: Zearalenone mixed with feed is stable for 2 weeks at temperatures of up to 45°C.

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF ZEARALENONE (SOUTHERN RESEARCH INSTITUTE)

A. Gas Chromatography Method

Five-gram samples of the chemical feed mixture were triturated with 50 ml of chloroform with a Polytron® high-speed blender for 90 seconds. The mixture was filtered through a glass fiber filter and the extraction tube was rinsed with two 20-ml portions of chloroform which were used to wash the filtered feed residue. The feed was washed with a final 10 ml of chloroform. The combined chloroform extract was transferred to a 150-ml beaker and evaporated to dryness. The oil-like residue was transferred to a heptane-packed florisil column, 1 cm by 25 cm, with a few ml of chloroform. The column was eluted with successive portions of solvent: 50 ml of heptane, 100 ml of chloroform, and 65 ml of ether. The ether fraction was evaporated to dryness and reserved for GC analyses of zearalenone.

The oil residue from the ether fraction was derivatized with 0.5 ml of Tri-Sil/BSA reagent. The plain feed, spiked plain feed, and chemical/feed samples were analyzed by vapor-phase chromatography.

GC Conditions:

Column: 3% OV-1 on 80/100 supelcoport,
1.8 m x 4mm ID, glass

Temperatures: Oven 260°C, isothermal
Inlet 260°C
FID 300°C

Carrier Flow: N₂, 40cc/min

Retention time: 3.3 min

Sample size: 2 µl

B. High Pressure Liquid Chromatography Method

Extraction: Ten-gram samples of the chemical/vehicle mixture were accurately weighed and transferred into large sample tubes. Acetonitrile (40 ml) was added to each sample. Plain feed blanks and spiked plain feeds were prepared by weighing undosed feed (10 g) into large sample tubes and spiking each sample with acetonitrile or zearalenone in acetonitrile. A stock solution of zearalenone in acetonitrile was prepared and used to spike samples at several concentrations in the specified dosage range of 0.001-0.05%.

The samples were extracted by triturating with acetonitrile using the Polytron® High Speed Blender. After triturating for 60 seconds, the samples were filtered through a Fiberglas® filter using a Millipore® filtering apparatus. The feed residue was then reextracted with another 40 ml aliquot of acetonitrile. The combined extracts were then brought to a 100-ml volume with acetonitrile. These extract solutions were then evaporated to an oily residue on a rotary evaporator.

Column Chromatography: Silica gel columns were prepared by first placing a ball of glass wool loosely in the bottom of the chromatographic column. Approximately 5 g of anhydrous sodium sulfate was added to give an even base for the silica gel. Chloroform was added until the columns were about one-half full; then 10 g of silica gel (ICN Pharmaceuticals, Inc., 100-200µ m, activated by drying overnight at 105°C, then adding 1% water by weight, sealing, shaking until thoroughly mixed, and stored in an airtight container). The sides of the column were washed with approximately 20 ml chloroform, and the silica gel was stirred to disperse it into the chloroform. When the rate of settling slowed, some of the chloroform was drawn off to aid in settling, leaving 2 to 3 in. of chloroform above the silica gel. Fifteen grams of anhydrous sodium sulfate was then slowly added and the chloroform was drawn off to the top of the sodium sulfate.

The oil residue of the acetonitrile extraction was mixed with 5 ml chloroform, followed by a 10-ml chloroform rinse, and transferred to 150 ml hexane. This solution was added to the chromatographic column and eluted at a maximum flow rate of 10-20 ml/min. The elution was continued with 150 ml of benzene. These hexane and benzene eluates were discarded, and the zearalenone was eluted from the column with 250 ml acetone:benzene (5:95, v/v).

The acetone:benzene fraction was evaporated on a rotary evaporator until only an oil residue was left. The residue was dissolved in 4 ml of methanol, and filtered solutions were analyzed by high-pressure liquid chromatography.

APPENDIX G

High Pressure Liquid Chromatography:

Instrument System:

Pumps: Waters 6000 A

Programmer: Waters 660

Detector: Waters 440 at x 0.1 attenuation

Injector: Waters U6K

Detection: Ultraviolet, 313 nm

Column: μ Bondapak C₁₈, 300 x 3.9 mm ID with
CO-Pell ODS guard column, 72 x 2.3 mm ID

Solvent System: Ion Pairing

A: 0.005 M tetrabutylammonium hydroxide (TBA) in
water, adjusted to pH 7.3-7.4 with 1% phosphoric
acid

B: 0.005 M tetrabutylammonium hydroxide (TBA) in
methanol, adjusted to pH 7.3-7.4 with 1% phosphoric
acid

Program: 60% B, 40% A isocratic

Flow Rate: 1 ml/min

Sample Injected: 10 μ l

Data Recorded on Hewlett-Packard 3380S Integrator

Retention Time of Zearalenone: 13 min

C. Results

See Table G1.

TABLE G1. ANALYSIS OF FORMULATED DIETS

| Week Mixed | Week Used | Concentration (a) of Zearalenone for Target Concentration of: | | |
|--|-----------|---|--------|--------|
| | | 100 ppm | 50 ppm | 25 ppm |
| Analyses Performed Using Original Procedure for Gas Chromatography | | | | |
| 02/14/78 | 02/21/78 | 96 | 50 | 19 |
| 03/21/78 | 03/28/79 | | 52 | 28 |
| 04/11/78 | 04/18/78 | 115 | 55 | |
| | | | 58 | |
| 05/09/78 | 05/16/78 | | | 38 |
| | | | | 38 |
| 06/13/78 | 06/20/78 | | 30 | 16 |
| | | | | 21 |
| 08/01/78 | 08/08/78 | 81 | 37 | 18 |
| 08/29/78 | 09/05/78 | 92 | 40 | 19 |
| 09/26/78 | 10/03/78 | 96 | 53 | 27 |
| 10/24/78 | 10/31/78 | | 57 | 27 |
| 11/28/78 | 12/05/78 | | 46 | 24 |
| | | | 45 | |
| 12/19/78 | 12/26/78 | 110 | 41 | 16 |
| <hr/> | | | | |
| Mean (ppm) | | 98.3 | 47.0 | 24.3 |
| Standard Deviation | | 12.4 | 8.7 | 7.7 |
| Coefficient of Variation (%) | | 12.6 | 18.5 | 31.7 |
| Range (ppm) | | 81-115 | 30-58 | 16-38 |
| <hr/> | | | | |
| No analysis performed on batches mixed after 12/19/78 pending development of new procedure | | | | |
| <hr/> | | | | |
| Analyses Performed Using New Procedure for High-Pressure Liquid Chromatography | | | | |
| 04/17/79 | 04/24/79 | 110 | 50 | 27 |
| 05/08/79 | 05/15/79 | | 52 | 26 |
| 06/05/79 | 06/12/79 | | 51 | 25 |
| 07/03/79 | 07/10/79 | | 52 | 25 |
| | | | 56 | |
| 07/31/79 | 08/07/79 | 95 | 52 | 23 |
| 08/28/79 | 09/04/79 | | 55 | 27 |
| | | | 52 | |
| 09/25/79 | 10/02/79 | 93 | 49 | 23 |
| 10/23/79 | 10/30/79 | | 49 | 24 |
| | | | 52 | |
| 11/27/79 | 12/04/79 | 96 | 48 | 28 |
| 12/18/79 | 12/25/79 | | 60 | 29 |
| 01/15/80 | 01/22/80 | 101 | 53 | 26 |
| <hr/> | | | | |
| Mean (ppm) | | 99.0 | 52.2 | 25.7 |
| Standard Deviation | | 6.8 | 3.1 | 2.0 |
| Coefficient of Variation (%) | | 6.9 | 5.9 | 7.8 |
| Range (ppm) | | 93-110 | 49-60 | 23-29 |

(a) The data presented are the average of the results of duplicate analyses.

APPENDIX H

CUMULATIVE MEAN BODY WEIGHT CHANGE OF RATS AND MICE FED DIETS CONTAINING ZEARALENONE

TABLE H1. CUMULATIVE MEAN BODY WEIGHT CHANGE (RELATIVE TO CONTROLS) OF RATS FED DIETS CONTAINING ZEAREALENONE

| | Week No. | Cumulative Mean Body Weight Change (a) | | | Weight Change Relative to Controls | |
|----------------|----------|--|----------|-----------|------------------------------------|-----------|
| | | Control | Low Dose | High Dose | Low Dose | High Dose |
| Males | 0 | 100 (b) | 100 (b) | 101 (b) | | |
| | 3 | 81 | 72 | 64 | -11 | -21 |
| | 22 | 246 | 228 | 207 | -7 | -16 |
| | 44 | 314 | 286 | 255 | -9 | -19 |
| | 62 | 338 | 308 | 280 | -9 | -17 |
| | 83 | 332 | 311 | 288 | -6 | -13 |
| | 104 | 308 | 305 | 288 | -1 | 6 |
| | | 408 (c) | 405 (c) | 389 (c) | -1 (d) | -5 (d) |
| Females | 0 | 88 (b) | 90 (b) | 91 (b) | | |
| | 3 | 33 | 19 | 27 | -42 | -18 |
| | 22 | 98 | 94 | 89 | -4 | -9 |
| | 44 | 129 | 124 | 115 | -4 | -11 |
| | 62 | 157 | 153 | 144 | -3 | -8 |
| | 83 | 190 | 181 | 165 | -5 | -13 |
| | 104 | 212 | 195 | 183 | -8 | 14 |
| | | 300 (c) | 285 (c) | 274 (c) | -5 (d) | -9 (d) |

(a) Weight change of the dosed group relative to that of the controls =
$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight

(c) Mean body weight at week 104.

(d) Mean body weight relative to controls.

TABLE H2. CUMULATIVE MEAN BODY WEIGHT CHANGE (RELATIVE TO CONTROLS) OF MICE FED DIETS CONTAINING ZEAREALENONE

| | Week No. | Cumulative Mean Body Weight Change (a) (grams) | | | Weight Change Relative to Controls (percent) | |
|----------------|----------|--|----------|-----------|--|-----------|
| | | Control | Low Dose | High Dose | Low Dose | High Dose |
| Males | 0 | 21 (b) | 20 (b) | 21 (b) | | |
| | 2 | 3 | 3 | 2 | 0 | -33 |
| | 21 | 13 | 13 | 10 | 0 | -23 |
| | 43 | 17 | 17 | 14 | 0 | -18 |
| | 61 | 20 | 20 | 17 | 0 | -15 |
| | 82 | 19 | 20 | 18 | +5 | 5 |
| | 104 | 19 | 18 | 18 | -5 | -5 |
| | | 40 (c) | 38 (c) | 39 (c) | -5 (d) | -3 (d) |
| Females | 0 | 17 (b) | 17 (b) | 17 (b) | | |
| | 2 | 3 | 2 | 2 | 33 | -33 |
| | 21 | 9 | 9 | 9 | 0 | 0 |
| | 43 | 13 | 11 | 14 | -15 | +8 |
| | 61 | 16 | 14 | 18 | -12 | +12 |
| | 82 | 17 | 15 | 18 | -12 | +6 |
| | 104 | 21 | 18 | 19 | -14 | -10 |
| | | 38 (c) | 35 (c) | 36 (c) | -8 (d) | -5 (d) |

(a) Weight change of the dosed group relative to that of the controls =
$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \times 100$$

(b) Initial weight

(c) Mean body weight at week 104.

(d) Mean body weight relative to controls.

APPENDIX I

FEEED CONSUMPTION BY RATS AND MICE RECEIVING ZEARALENONE IN THE CHRONIC STUDY

TABLE II. FEED CONSUMPTION BY RATS RECEIVING ZEARALENONE IN THE CHRONIC STUDY

| Week | Control | Low Dose | | High Dose | |
|----------------|---------------------------|---------------------------|------------------------|---------------------------|-------------------------|
| | Grams Feed/ Day (a) | Grams Feed/ Day (a) | Low/ Control (b) | Grams Feed/ Day (a) | High/ Control (b) |
| Males | | | | | |
| 3 | 16.0 | 16.0 | 1.0 | 13.0 | 0.8 |
| 22 | 18.0 | 20.0 | 1.1 | 15.0 | 0.8 |
| 44 | 16.0 | 15.0 | 0.9 | 14.0 | 0.9 |
| 62 | 17.0 | 17.0 | 1.0 | 16.0 | 0.9 |
| 83 | 15.7 | 16.7 | 1.1 | 15.8 | 1.0 |
| 104 | 15.0 | 15.0 | 1.0 | 15.0 | 1.0 |
| Mean | 16.3 | 16.6 | 1.0 | 14.8 | 0.9 |
| SD (c) | 1.1 | 1.9 | 0.1 | 1.1 | 0.1 |
| CV (d) | 6.7 | 11.4 | 10.0 | 7.4 | 11.1 |
| Females | | | | | |
| 3 | 13.0 | 12.0 | 0.9 | 12.0 | 0.9 |
| 22 | 10.0 | 11.0 | 1.1 | 11.0 | 1.1 |
| 44 | 10.0 | 9.0 | 0.9 | 10.0 | 1.0 |
| 62 | 11.0 | 11.0 | 1.0 | 11.0 | 1.0 |
| 83 | 12.6 | 10.5 | 0.8 | 11.6 | 0.9 |
| 104 | 11.0 | 11.0 | 1.0 | 11.0 | 1.0 |
| Mean | 11.3 | 10.8 | 1.0 | 11.1 | 1.0 |
| SD (c) | 1.3 | 1.0 | 0.1 | 0.7 | 0.1 |
| CV (d) | 11.5 | 9.3 | 10.0 | 6.3 | 10.0 |

(a) Grams of feed consumed per animal per day.

(b) Grams of feed consumed per day by the dosed group divided by that for the controls.

(c) Standard deviation.

(d) Coefficient of variation \square (Standard deviation/mean) x 100.

TABLE 12. FEED CONSUMPTION BY MICE RECEIVING ZEARALENONE IN THE CHRONIC STUDY

| Week | Control | Low Dose | | High Dose | | |
|----------------|--------------------|--------------------|-----------------|--------------------|------------------|--|
| | Grams Feed/Day (a) | Grams Feed/Day (a) | Low/Control (b) | Grams Feed/Day (a) | High/Control (b) | |
| Males | | | | | | |
| 2 | 8.6 | 8.6 | 1.0 | 7.5 | 0.9 | |
| 21 | 8.0 | 8.0 | 1.0 | 9.0 | 1.1 | |
| 43 | 6.0 | 6.0 | 1.0 | 6.0 | 1.0 | |
| 61 | 7.0 | 6.0 | 0.9 | 6.0 | 0.9 | |
| 82 | 5.1 | 5.1 | 1.0 | 5.1 | 1.0 | |
| 104 | 6.8 | 6.8 | 1.0 | 6.8 | 1.0 | |
| Mean | 6.9 | 6.8 | 1.0 | 6.7 | 1.0 | |
| SD (c) | 1.3 | 1.3 | 0.0 | 1.4 | 0.1 | |
| CV (d) | 18.8 | 19.1 | 0.0 | 20.9 | 10.0 | |
| Females | | | | | | |
| 2 | 7.5 | 7.5 | 1.0 | 7.5 | 1.0 | |
| 21 | 8.0 | 8.0 | 1.0 | 8.0 | 1.0 | |
| 43 | 7.0 | 6.0 | 0.9 | 6.0 | 0.9 | |
| 61 | 6.0 | 7.0 | 1.2 | 6.0 | 1.0 | |
| 82 | 6.0 | 5.1 | 0.9 | 5.1 | 0.9 | |
| 104 | 6.8 | 6.8 | 1.0 | 7.7 | 1.1 | |
| Mean | 6.9 | 6.7 | 1.0 | 6.7 | 1.0 | |
| SD (c) | 0.8 | 1.0 | 0.1 | 1.2 | 0.1 | |
| CV (d) | 11.6 | 14.9 | 10.0 | 17.9 | 10.0 | |

(a) Grams of feed consumed per animal per day.

(b) Grams of feed consumed per day by the dosed group divided by that for the controls.

(c) Standard deviation.

(d) Coefficient of variation = (Standard deviation/mean) x 100.

APPENDIX J

COMPOUND CONSUMPTION BY RATS AND MICE FED DIETS CONTAINING ZEARALENONE

TABLE J1. COMPOUND CONSUMPTION BY RATS FED DIETS CONTAINING ZEARALENONE

| Week No. | Low Dose | | | High Dose | | |
|----------------|-----------------|--------------------|--------------------|-----------------|--------------------|--------------------|
| | Body Weight (a) | Grams Feed/Day (b) | Dose mg/kg/day (c) | Body Weight (a) | Grams Feed/Day (b) | Dose mg/kg/day (d) |
| Males | | | | | | |
| 3 | 172 | 16.0 | 2.33 | 165 | 13.0 | 3.94 |
| 22 | 328 | 20.0 | 1.52 | 308 | 15.0 | 2.44 |
| 44 | 386 | 15.0 | 0.97 | 356 | 14.0 | 1.97 |
| 62 | 408 | 17.0 | 1.04 | 381 | 16.0 | 2.10 |
| 83 | 411 | 16.7 | 1.02 | 389 | 15.8 | 2.03 |
| 104 | 405 | 15.0 | 0.93 | 389 | 15.0 | 1.93 |
| Females | | | | | | |
| 3 | 109 | 12.0 | 2.75 | 118 | 12.0 | 5.08 |
| 22 | 184 | 11.0 | 1.49 | 180 | 11.0 | 3.06 |
| 44 | 214 | 9.0 | 1.05 | 206 | 10.0 | 2.43 |
| 62 | 243 | 11.0 | 1.13 | 235 | 11.0 | 2.34 |
| 83 | 271 | 10.5 | 0.97 | 256 | 11.6 | 2.26 |
| 104 | 285 | 11.0 | 0.96 | 274 | 11.0 | 2.01 |

(a) Group body weight average, from Table H1.

(b) From Table I1.

(c) Low Dose = 25 mg/kg of feed. Dose calculation =

$$\left[\frac{\text{Grams Feed/Day}}{\text{Body Wt. (kg)}} \times 25 \right] / 1000$$

(d) High Dose = 50 mg/kg of feed. Dose calculation =

$$\left[\frac{\text{Grams Feed/Day}}{\text{Body Wt. (kg)}} \times 50 \right] / 1000$$

TABLE J2. COMPOUND CONSUMPTION BY MICE FED DIETS CONTAINING ZEARALENONE

| Week No. | Low Dose | | | High Dose | | |
|----------------|-----------------|--------------------|--------------------|-----------------|--------------------|--------------------|
| | Body Weight (a) | Grams Feed/Day (b) | Dose mg/kg/day (c) | Body Weight (a) | Grams Feed/Day (b) | Dose mg/kg/day (d) |
| Males | | | | | | |
| 2 | 23 | 8.6 | 18.7 | 23 | 7.5 | 32.6 |
| 21 | 33 | 8.0 | 12.1 | 31 | 9.0 | 29.0 |
| 43 | 37 | 6.0 | 8.1 | 35 | 6.0 | 17.1 |
| 61 | 40 | 6.0 | 7.5 | 38 | 6.0 | 15.8 |
| 82 | 40 | 5.1 | 6.4 | 39 | 5.1 | 13.1 |
| 104 | 38 | 6.8 | 8.9 | 39 | 6.8 | 17.4 |
| Females | | | | | | |
| 2 | 19 | 7.5 | 19.7 | 19 | 7.5 | 39.5 |
| 21 | 26 | 8.0 | 15.4 | 26 | 8.0 | 30.8 |
| 43 | 28 | 6.0 | 10.7 | 31 | 6.0 | 19.4 |
| 61 | 31 | 7.0 | 11.3 | 35 | 6.0 | 17.1 |
| 82 | 32 | 5.1 | 8.0 | 35 | 5.1 | 14.6 |
| 104 | 35 | 6.8 | 9.7 | 36 | 7.7 | 21.4 |

(a) Group body weight average, from Table H2.

(b) From Table I2.

(c) Low Dose = 50 mg/kg of feed. Dose calculation =

$$\left[\frac{\text{Grams Feed/Day}}{\text{Body Wt. (kg)}} \times 50 \right] / 1000$$

(d) High Dose = 100 mg/kg of feed. Dose calculation =

$$\left[\frac{\text{Grams Feed/Day}}{\text{Body Wt. (kg)}} \times 100 \right] / 1000$$

APPENDIX K

HISTORICAL INCIDENCE OF TUMORS IN CONTROL B6C3F1 MICE

TABLE K1. HISTORICAL INCIDENCE OF LIVER TUMORS IN UNTREATED CONTROL FEMALE B6C3F1 MICE (a)

| Laboratory | Adenoma | Carcinoma | Combined |
|---------------------------------|----------------|-----------------|-----------------|
| Battelle | 5/348 (1.4%) | 21/348 (6.0%) | 25/348 (7.2%) |
| Dow | 3/98 (3.1%) | 5/98 (5.1%) | 7/98 (7.1%) |
| Frederick | 10/431 (2.3%) | 13/431 (3.0%) | 22/431 (5.1%) |
| Hazleton | 1/100 (1.0%) | 4/100 (4.0%) | 5/100 (5.0%) |
| Litton | 21/511 (4.1%) | 11/511 (2.2%) | 32/511 (6.3%) |
| Mason | 35/809 (4.3%) | 39/809 (4.8%) | 73/809 (9.0%) |
| Southern (b) | 14/498 (2.8%) | 18/498 (3.6%) | 31/498 (6.2%) |
| Total | 89/2795 (3.2%) | 111/2795 (4.0%) | 195/2795 (7.0%) |
| Overall Historical Range | | | |
| High (c) | 9/49 | 7/48 | 10/49 |
| Low (d) | 0/50 | 0/50 | 0/50 |

(a) Data as of January 17, 1981 for studies of at least 104 weeks. Range is presented for groups in which at least 35 animals were examined microscopically. Interim death (<104 weeks) animals are included.

(b) Southern Research Institute conducted the current bioassay.

(c) Highest incidence in group of 35 or more animals.

(d) Lowest incidence in group of 35 or more animals.

TABLE K2. HISTORICAL INCIDENCE OF PITUITARY TUMORS IN UNTREATED CONTROL MALE B6C3F1 MICE (a)

| Laboratory | Carcinoma NOS | Adenoma NOS | Chromophobe Adenoma | Chromophobe Carcinoma |
|---------------------------------|---------------|---------------|---------------------|-----------------------|
| Battelle | 0/276 (0.0%) | 0/276 (0.0%) | 0/276 (0.0%) | 1/276 (0.4%) |
| Dow | 0/67 (0.0%) | 0/67 (0.0%) | 0/67 (0.0%) | 0/67 (0.0%) |
| Frederick | 0/358 (0.0%) | 2/358 (0.6%) | 0/358 (0.0%) | 1/358 (0.3%) |
| Hazleton | 0/40 (0.0%) | 0/40 (0.0%) | 0/40 (0.0%) | 0/40 (0.0%) |
| Litton | 0/341 (0.0%) | 0/341 (0.0%) | 1/341 (0.3%) | 0/341 (0.0%) |
| Mason | 0/647 (0.0%) | 7/647 (1.1%) | 1/647 (0.2%) | 0/647 (0.0%) |
| Southern (b) | 0/399 (0.0%) | 0/399 (0.0%) | 0/399 (0.0%) | 0/399 (0.0%) |
| Total | 0/2128 (0.0%) | 9/2128 (0.4%) | 2/2128 (0.1%) | 2/2128 (0.1%) |
| Overall Historical Range | | | | |
| High (c) | 0/46 | 2/42 | 1/46 | 1/43 |
| Low (d) | 0/43 | 0/43 | 0/43 | 0/46 |

(a) Data as of January 17, 1981 for studies of at least 104 weeks. The range is presented for groups in which at least 35 animals were examined microscopically. Interim death (<104 weeks) animals are included.

(b) Southern Research Institute conducted the current study.

(c) Highest incidence in group of 35 or more animals.

(d) Lowest incidence in group of 35 or more animals.

TABLE K3. HISTORICAL INCIDENCE OF PITUITARY TUMORS IN UNTREATED CONTROL FEMALE B6C3F1 MICE (a)

| Laboratory | Carcinoma (Unspecified) | Adenoma (Unspecified) | Chromophobe Adenoma | Chromophobe Carcinoma |
|---------------------------------|-------------------------|-----------------------|---------------------|-----------------------|
| Battelle | 1/298 (0.3%) | 3/298 (1.0%) | 7/298 (2.3%) | 1/298 (0.3%) |
| Dow | 0/77 (0.0%) | 0/77 (0.0%) | 7/77 (9.1%) | 0/77 (0.0%) |
| Frederick | 1/403 (0.2%) | 15/403 (3.7%) | 6/403 (1.5%) | 1/403 (0.2%) |
| Hazleton | 0/93 (0.0%) | 8/93 (8.6%) | 0/93 (0.0%) | 0/93 (0.0%) |
| Litton | 0/369 (0.0%) | 5/369 (1.4%) | 11/369 (3.0%) | 2/369 (0.5%) |
| Mason | 0/666 (0.0%) | 57/666 (8.6%) | 4/666 (0.6%) | 0/666 (0.0%) |
| Southern (b) | 2/428 (0.5%) | 8/428 (1.9%) | 10/428 (2.3%) | 1/428 (0.2%) |
| Total | 4/2334 (0.2%) | 96/2334 (4.1%) | 45/2334 (1.9%) | 5/2334 (0.2%) |
| Overall Historical Range | | | | |
| High (c) | 1/43 | 11/41 | 4/38 | 2/44 |
| Low (d) | 0/49 | 0/47 | 0/49 | 0/49 |

(a) Data as of January 17, 1981 for studies of at least 104 weeks. Range is presented for groups in which at least 35 animals were examined microscopically. Interim death (< 104 weeks) animals are included.

(b) Southern Research Institute conducted the current bioassay. The combined incidence of female B6C3F1 mice at Southern with either adenomas or carcinomas in the pituitary gland is 21/428 (4.9%). The highest combined incidence in a group of 35 or more animals at Southern is 6/48 (12.5%).

(c) Highest incidence in group of 35 or more animals.

(d) Lowest incidence in group of 35 or more animals.