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National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology Studies of Indole-3-carbinol (CASRN 700-06-1) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Indole-3-carbinol in Harlan Sprague Dawley Rats and B6C3F1/N Mice (Gavage Studies)

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please email NTP or call 984-287-3211.

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Explanation of Levels of Evidence of Carcinogenic Activity

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

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

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

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

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

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

Peer Review

The draft *NTP Technical Report on the Toxicology Studies of Indole-3-carbinol (CASRN 700-06-1) in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of Indole-3-carbinol in Harlan Sprague Dawley Rats and B6C3F1/N Mice (Gavage Studies)* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Technical Report presents the experimental results and conclusions fully and clearly.

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Abstract

Indole-3-carbinol is sold as a sole ingredient in dietary supplements or as a combination nutraceutical along with a variety of herbs and/or vitamins. It is marketed for its potential ability to prevent cancer and provide other health benefits, such as detoxifying the liver and boosting the immune system. Indole-3-carbinol is a naturally formed breakdown product of glucosinolate glucobrassicin, a component found in cruciferous vegetables of the *Brassica* genus, including broccoli, brussels sprouts, cauliflower, cabbage, kale, kohlrabi, and turnips. Exposure to indole-3-carbinol occurs through the oral route through the ingestion of *Brassica* vegetables or dietary supplements. Indole-3-carbinol was nominated by the National Cancer Institute for toxicity and carcinogenicity testing because of its occurrence in natural products and for its potential use as a breast cancer chemopreventive agent. Male and female F344/N rats and Harlan Sprague Dawley rats received indole-3-carbinol in corn oil by gavage for 3 months or 2 years, respectively. Male and female B6C3F1/N mice received indole-3-carbinol in corn oil gavage for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*, rat bone marrow cells, and mouse peripheral blood erythrocytes.

Three-month Study in F344/N Rats

Groups of 10 male and 10 female core study rats were administered 0, 18.75, 37.5, 75, 150, or 300 mg indole-3-carbinol/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Groups of 10 male and 10 female clinical pathology study rats were administered the same dose for 25 days. All rats survived to the end of the study. The mean body weight gain of 300 mg/kg males was significantly less than that of the vehicle controls. The absolute and relative liver weights of all dosed groups of males and females were significantly increased compared to the vehicle controls. The relative kidney weights of 75 mg/kg or greater males and all dosed groups of females were significantly increased, as were the absolute kidney weights of 75 mg/kg males and 18.75, 37.5, and 300 mg/kg females. The absolute and relative thymus weights of 75 mg/kg or greater females were significantly decreased. There were significant and dose-dependent increases in CYP1A1-associated 7-ethoxyresorufin-*O*-deethylase (EROD) and CYP1A2-associated acetanilide-4-hydroxylase (A4H) activities in the liver of all dosed groups of male and females rats. Pulmonary EROD activity was significantly increased in males administered 75 mg/kg or greater and in all dosed groups of females. Indole-3-carbinol exhibited the potential to be a reproductive toxicant in female rats based on a significantly increased probability of extended diestrus and an increase in overall estrous cycle length (approximately 1 day) observed at 300 mg/kg. In the small intestine, significantly increased incidences of lamina propria lipidosis and lymphatic ectasia occurred in the duodenum of 150 and 300 mg/kg males and females and in the jejunum of 75 mg/kg or greater males and females. In the mesenteric lymph node, significantly increased incidences of dilatation of the lymphatic vessels associated with lipidosis occurred in 150 and 300 mg/kg males and in 300 mg/kg females.

Three-month Study in B6C3F1/N Mice

Groups of 10 male and 10 female mice were administered 0, 15.6, 31.25, 62.5, 125, or 250 mg indole-3-carbinol/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. All mice survived to the end of the study. Mean body weights of dosed groups of males and females were similar to those of the vehicle controls. Liver weights of 125 and 250 mg/kg males and all dosed groups of females were significantly increased compared to the vehicle controls. There were significant and dose-dependent increases in A4H activities in the liver of all dosed groups of

males, and hepatic EROD activities were significantly increased in males administered 31.25 mg/kg or greater. Hepatic A4H and EROD activities were significantly increased in 125 and 250 mg/kg females. Indole-3-carbinol exhibited the potential to be a reproductive toxicant in male and female mice based on significantly decreased sperm motility in all dosed groups of males and a significantly increased probability of extended diestrus in females administered 250 mg/kg. No histopathologic lesions were observed that could be attributed to the administration of indole-3-carbinol.

Two-year Study in Sprague Dawley Rats

Groups of 50 male and 50 female rats were administered 0, 75, 150, or 300 mg indole-3-carbinol/kg body weight in corn oil by gavage, 5 days per week for 104 (males) or 105 (females) weeks. Survival of dosed groups of males and females was similar to that of the vehicle controls. Mean body weights of dosed groups of males and females were similar to those of the vehicle controls throughout the study.

In the standard evaluation of the uterus, the incidences of adenocarcinoma occurred with a positive trend and were increased in all dosed groups. Extended evaluations of the uterus were conducted and additional neoplasms were identified. In the combined standard and extended evaluations, the incidence of adenocarcinoma was significantly increased in 150 mg/kg females. In the standard evaluation, the incidence of squamous metaplasia of the endometrium was significantly increased in the 150 mg/kg group.

The incidences of fibroma or fibrosarcoma (combined) in the skin occurred with a positive trend in females. An increased incidence of fibroma in the skin was observed in 300 mg/kg females. A single incidence of fibrosarcoma occurred in vehicle control and in 300 mg/kg females.

Significantly increased incidences of lymphatic ectasia in the duodenum and jejunum of the small intestine with generally increased severities occurred in 150 and 300 mg/kg males and females.

The incidences of lymphatic ectasia in the mesenteric lymph node were significantly increased in 300 mg/kg males and females compared to the vehicle controls.

In the liver, the incidences of clear cell focus in 300 mg/kg females, eosinophilic focus in 150 and 300 mg/kg females, and bile duct cyst in 300 mg/kg males were significantly increased compared to the vehicle controls.

Gene expression studies in 300 mg/kg females suggested activation of multiple xenobiotic transcription factors in rat liver with the most pronounced activation being associated with AhR and Nrf2. Consistent with these findings was the up-regulation of genes associated with xenobiotic metabolism, which suggests the potential for indole-3-carbinol to modify drug efficacy and safety. These findings are largely similar to results from other transcriptomic studies of indole-3-carbinol.

The incidences of follicular cell hypertrophy in the thyroid gland were significantly increased in all dosed groups of males, and the severities of the lesion increased with increasing dose.

Two-year Study in B6C3F1/N Mice

Groups of 50 male and 50 female mice were administered 0, 62.5, 125, or 250 mg indole-3-carbinol/kg body weight in corn oil by gavage, 5 days per week for 105 weeks. Survival of 250 mg/kg females was significantly greater than that of the vehicle controls. Mean body

weights of dosed groups of males were similar to those of the vehicle controls throughout the study; however, those of 250 mg/kg female mice were at least 10% less than those of the vehicle controls between weeks 32 and 92.

Incidences of hepatocellular adenoma occurred with a positive trend in males and the incidence was significantly increased in the 250 mg/kg group. The incidences of multiple hepatocellular adenoma were significantly increased in 62.5 and 250 mg/kg males. There were significantly increased incidences of single and multiple hepatocellular carcinoma in 125 mg/kg males compared to the vehicle controls. In males, the incidences of hepatoblastoma occurred with a positive trend, and the incidences of multiple hepatoblastoma increased with increasing dose. The incidences of hepatoblastoma and multiple hepatoblastoma were significantly increased in 250 mg/kg males. The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma occurred with a positive trend in males and were significantly increased in males administered 125 or 250 mg/kg. The combined incidences of hepatocellular carcinoma and hepatoblastoma were significantly increased in 125 and 250 mg/kg males. The incidences of clear cell focus were significantly increased in all dosed groups of males, and the incidences of eosinophilic focus were significantly increased in 62.5 and 125 mg/kg females.

In the glandular stomach, the incidences of epithelium hyperplasia, chronic inflammation, and pigmentation were significantly increased in 125 and 250 mg/kg males and all dosed groups of females compared to the vehicle controls.

In the nose, incidences of nerve atrophy in 250 mg/kg males and females, respiratory metaplasia of the olfactory epithelium in 250 mg/kg males and 125 and 250 mg/kg females, atrophy of the olfactory epithelium in 125 and 250 mg/kg males and 250 mg/kg females, necrosis of the olfactory epithelium in 250 mg/kg males, respiratory epithelium hyaline droplet accumulation in 62.5 and 125 mg/kg males, respiratory epithelium hyperplasia in 250 mg/kg males and females, and inflammation in 250 mg/kg females were significantly greater than the vehicle control incidences.

Genetic Toxicology

Indole-3-carbinol was tested in three independent bacterial mutagenicity assays, and results were varied. Two assays yielded results that were judged to be equivocal in one or more of the tester strains (*S. typhimurium* strains TA97 and TA100 and *E. coli* strain WP2 *uvrA*/pKM101). Weak positive responses were seen in a third assay in *S. typhimurium* strain TA100, both with and without exogenous metabolic activation. In vivo, no increase in the frequency of micronucleated polychromatic erythrocytes (PCEs) was seen in bone marrow of F344/N rats given three doses of indole-3-carbinol by gavage; however, a significant decrease in the percent PCEs was seen in the bone marrow of treated rats, indicating that indole-3-carbinol (500 to 2,000 mg/kg per day) was toxic to the bone marrow. In addition to the rat study, micronucleus frequencies in normochromatic erythrocytes (NCEs) of male and female mice were assessed in peripheral blood at the end of the 3-month study; no significant increases in micronucleated NCEs were seen and no significant changes in percent PCEs occurred over the dose range tested (15.6 to 250 mg/kg per day).

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; a summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in

Appendix M) of indole-3-carbinol in male Harlan Sprague Dawley rats administered 75, 150, or 300 mg/kg. There was *some evidence of carcinogenic activity* of indole-3-carbinol in female Harlan Sprague Dawley rats based on increased incidences of malignant uterine neoplasms (primarily adenocarcinoma). The occurrences of fibroma and fibrosarcoma in the skin may have been related to indole-3-carbinol administration. There was *clear evidence of carcinogenic activity* of indole-3-carbinol in male B6C3F1/N mice based on increased incidences of liver neoplasms (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma). There was *no evidence of carcinogenic activity* of indole-3-carbinol in female B6C3F1/N mice administered 62.5, 125, or 250 mg/kg.

Administration of indole-3-carbinol caused increased incidences of nonneoplastic lesions in the small intestine, mesenteric lymph node, and liver of male and female rats, the thyroid gland of male rats, the uterus of female rats, and the liver, glandular stomach, and nose of male and female mice.

Synonyms: 3-(Hydroxymethyl)indole; indole-3-methanol; 3-indolemethanol; 3-indolylcarbinol; 3-indolylmethanol

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of Indole-3-carbinol

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in Corn Oil By Gavage	0, 75, 150, or 300 mg/kg	0, 75, 150, or 300 mg/kg	0, 62.5, 125, or 250 mg/kg	0, 62.5, 125, or 250 mg/kg
Survival Rates	20/50, 13/50, 17/50, 12/50	21/50, 19/50, 20/50, 30/50	27/50, 31/50, 32/50, 32/50	33/50, 40/50, 26/50, 45/50
Body Weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	250 mg/kg group at least 10% less than the vehicle control group between weeks 32 and 92
Nonneoplastic Effects	Intestine small, duodenum: lymphatic, ectasia (0/43, 0/48, 15/47, 14/48) Intestine small, jejunum: lymphatic, ectasia (0/40, 2/39, 27/40, 41/42) Lymph node, mesenteric: lymphatic, ectasia (0/50, 0/50, 1/50, 5/50) Liver: bile duct, cyst (0/50, 0/50, 2/50, 5/50) Thyroid gland: follicular cell, hypertrophy (21/50, 34/46, 33/48, 36/47)	Uterus: endometrium, metaplasia, squamous (standard evaluation-12/50, 18/50, 20/50, 11/50) Intestine small, duodenum: lymphatic, ectasia (0/48, 0/47, 16/48, 38/47) Intestine small, jejunum: lymphatic, ectasia (0/47, 0/46, 30/48, 47/48) Lymph node, mesenteric: lymphatic, ectasia (0/50, 0/50, 1/50, 15/48) Liver: clear cell focus (6/50, 7/50, 4/50, 18/48); eosinophilic focus (0/50, 4/50, 5/50, 6/48)	Liver: clear cell focus (7/50, 17/50, 22/49, 20/50) Glandular stomach: epithelium, hyperplasia (0/50, 1/47, 22/47, 40/49); inflammation, chronic (1/50, 1/47, 18/47, 45/49); pigmentation (0/50, 1/47, 38/47, 48/49) Nose: nerve, atrophy (0/50, 0/50, 0/50, 8/50); olfactory epithelium, respiratory metaplasia (14/50, 14/50, 20/50, 27/50); olfactory epithelium, atrophy (3/50, 5/50, 11/50, 17/50); olfactory epithelium, necrosis (0/50, 0/50, 0/50, 6/50); respiratory epithelium, accumulation, hyaline droplet (18/50, 34/50, 30/50, 26/50); respiratory epithelium, hyperplasia (35/50, 40/50, 41/50, 45/50)	Liver: eosinophilic focus (16/50, 26/50, 26/50, 21/50) Glandular stomach: epithelium, hyperplasia (1/48, 7/50, 10/49, 35/50); inflammation, chronic (0/48, 15/50, 29/49, 47/50); pigmentation (0/48, 15/50, 31/49, 49/50) Nose: nerve, atrophy (0/50, 0/50, 1/50, 50/50); olfactory epithelium, respiratory metaplasia (7/50, 8/50, 16/50, 49/50); olfactory epithelium, atrophy (1/50, 2/50, 3/50, 45/50); respiratory epithelium, hyperplasia (32/50, 31/50, 38/50, 50/50); inflammation (4/50, 1/50, 8/50, 39/50)

	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Neoplastic Effects	None	Uterus: adenocarcinoma (standard evaluation-0/50, 1/50, 4/50, 4/50; standard and extended evaluations, combined-5/50, 4/50, 13/50, 10/50)	Liver: hepatocellular adenoma (26/50, 32/50, 31/49, 41/50); hepatocellular carcinoma (12/50, 11/50, 29/49, 14/50); hepatoblastoma (3/50, 4/50, 4/49, 14/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (36/50, 36/50, 44/49, 45/50)	None
Equivocal Findings	None	Skin: fibroma (1/50, 0/50, 0/50, 4/50); fibrosarcoma (1/50, 0/50, 0/50, 1/50); fibroma or fibrosarcoma (2/50, 0/50, 0/50, 5/50)	None	None
Level of Evidence of Carcinogenic Activity	No evidence	Some evidence	Clear evidence	No evidence
Genetic Toxicology				
Bacterial Gene Mutations:	Negative in *Salmonella typhimurium* strains TA98, TA102, TA104, TA1535, and TA1537 with S9 and without S9; equivocal in strain TA97 without S9; equivocal in one study in strain TA100 with 10% rat S9; weakly positive in a second study in strain TA100 with and without 30% hamster S9; equivocal in *Escherichia coli* strain WP2 *uvrA*/pKM101 without S9.			
Micronucleated Erythrocytes				
Rat Bone Marrow In Vivo:	Negative			
Mouse Peripheral Blood In Vivo:	Negative			

Introduction

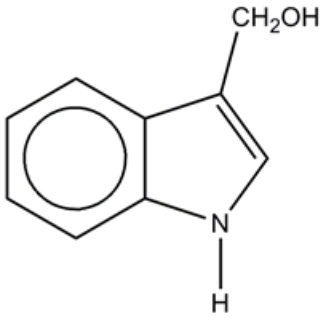

Figure 1. Indole-3-carbinol (CASRN 700-06-1; Chemical Formula: C_9H_9NO; Molecular Weight: 147.18)

Synonyms: 3-(Hydroxymethyl)indole; indole-3-methanol; 3-indolemethanol; 3-indolylcarbinol; 3-indolylmethanol.

Chemical and Physical Properties

Indole-3-carbinol is an off-white powder with a melting point of 96° to 99°C[1]. It is soluble in benzene, ethanol, and pentane[2]. Indole-3-carbinol is an unstable compound that undergoes rapid oligomerization in acid pH environments, like the stomach. At low pH, a wide variety of condensation products are formed, ranging from linear and cyclic dimers, trimers, and tetramers to extended heterocyclic compounds such as indolocarbazoles[3-6].

Production, Use, and Human Exposure

Indole-3-carbinol is a naturally formed breakdown product of glucosinolate glucobrassicin, a component found in cruciferous vegetables in the *Brassica* genus, which includes brussels sprouts, cauliflower, cabbage, kale, rape, turnips, and broccoli. Although indole-3-carbinol is not naturally occurring in these vegetables, it is readily formed from glucobrassicin via a thioglucosidase-mediated autolytic process during the simple mechanics of cutting, chewing, mashing, or cooking. Myrosinase, an enzyme that is usually compartmentalized within the plant cells, is released when the cells are physically damaged. Free myrosinase readily catalyzes the hydrolysis of glucobrassicin to indole-3-carbinol[7]. Indole-3-carbinol is also marketed and promoted as a dietary supplement that is available at health food stores, pharmacies, and via the internet. Indole-3-carbinol may be sold as the sole ingredient in products or in combination nutraceuticals that contain a variety of botanicals and/or vitamins. The health claims for indole-3-carbinol-containing supplements include cancer prevention, antioxidant protection, hormone balance, immune system enhancement, and hepatic and intestinal detoxification.

The most widespread exposure of humans to indole-3-carbinol occurs through the consumption of glucobrassicin, the indole-3-carbinol precursor found in *Brassica* vegetables. *Brassica* vegetables are frequently consumed in the diets of both Western and Eastern cultures[6]. In the United States population, the daily intake of indole-3-carbinol from cruciferous vegetables has been estimated to be 2.6 mg or less[2]. Estimated mean daily intake of glucobrassicin in the United Kingdom is approximately 12.5 and 7 mg per person from fresh and cooked sources, respectively, and the average daily consumption of indole-3-carbinol is approximately 0.1 mg/kg body weight. Based on levels of brassica consumption in the Japanese diet, the

average daily intake of indole precursors can range up to 112 mg per day, which corresponds to a daily dose of approximately 1.6 mg/kg for a 70 kg person[8; 9]. Carlson et al.[10] reported concentrations of 3-indolylmethyl glucosinolates in broccoli (42.2 to 71.7 mmol/100 g fresh weight), brussels sprouts (327.8 to 469.4 mmol/100 g), cauliflower (18.8 to 104.7 mmol/100 g), collard greens (67.2 to 165.3 mmol/100 g), kale (44.2 to 102.3 mmol/100 g), mustard greens (4.2 to 12.2 mmol/100 g), and kohlrabi (27.7 mmol/100 g).

Humans are also exposed to indole-3-carbinol as a dietary supplement. The daily dose of 200 to 400 mg that has been used to investigate the therapeutic value of indole-3-carbinol in clinical trials is also the recommended dose indicated by package labeling on indole-3-carbinol products. These daily exposures are equivalent to 2.9 to 5.7 mg/kg for a 70 kg person.

Regulatory Status

While the U.S. Food and Drug Administration (FDA) does not regulate food that contains precursors of indole-3-carbinol, compounds that are marketed as dietary supplements are regulated under the Dietary Supplement Health and Education Act of 1994. Indole-3-carbinol is not listed by the FDA as a Generally Recognized as Safe substance.

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

The disposition of indole-3-carbinol has been studied in experimental animals. In rainbow trout, 25% of an oral dose of 40 mg/kg [^{3}H]indole-3-carbinol given via feed or gavage was recovered in the water 72 hours following exposure reflecting the excretion via urine and gills[11]. Radioactivity in the liver between 48 and 72 hours following exposure accounted for 1% to 1.5% of the total administered dose. In goats, approximately 80% of the dose was recovered in urine 72 hours following a 2-hour infusion of 0.27 mmol/kg [^{3}H]indole-3-carbinol. Following exposure of F344 rats to 2,000 ppm [^{3}H]indole-3-carbinol in the diet, steady-state excretion in urine and feces was achieved at 40 hours and 112 hours, respectively, following the beginning of exposure. Excretion in these two routes accounted for approximately 75% of the dose with about 77% of the excreted dose recovered in the feces[12]. Although not demonstrated in rats, biliary excretion has been shown to be a major route of excretion in rainbow trout, suggesting that the high fecal excretion in rats could be due to biliary excretion and not due to poor absorption. Following repeated gavage administration of 1 mmol/kg indole-3-carbinol in rats (a comparable daily dose as in the feeding study above) for 6 days followed by 1 day of dosing with [^{3}H]indole-3-carbinol, indole-3-carbinol-equivalents in the liver at 1.5, 3, and 6 hours, respectively, were 0.97%, 1.34%, and 2.45% of the total administered dose. However, the concentration of indole-3-carbinol equivalents in blood, kidney, tongue, or lung changed slightly over the same time period. Of the total radioactive equivalents in the liver, the extractable radioactivity decreased from 45% to 31% from 1.5 to 6 hours. In female CD-1 mice following gavage administration of 250 mg/kg, indole-3-carbinol was rapidly absorbed, and the peak plasma concentration was reached within 15 minutes[13]. The concentration in plasma fell below the limit of detection 1 hour following administration suggesting rapid excretion. As observed in rats, indole-3-carbinol was extensively distributed to tissues including the kidney, liver, heart, and brain, with the highest levels detected in the liver.

Acidic incubation of indole-3-carbinol in vitro resulted in the formation of multiple condensation products[14-16]. The major products formed were the dimer, 3,3′-diindolylmethane (DIM), and two trimers, [2-(indol-3-yl-methyl)-indol-3-yl]indol-3-ylmethane (LTR) and 5,6,11,12,17,18-hexahydrodiindolo [2′,3′:4,5;2″,3″:7,8] cyclonona[1,2-b]indole (CTI). The formation of oligo-mers was strongly pH dependent. Incubation of indole-3-carbinol at pHs less than 3 resulted in approximately equal formation of the three oligomers, whereas incubation at pHs greater than 3 produced large amounts of DIM and LTR but not CTI; CTI was detected at pHs greater than 4.5[14].

The metabolism of indole-3-carbinol has been investigated in in vitro preparations of rat and chicken embryo liver[6]. These studies demonstrated that the first step in the metabolic route was the formation of indole-3-carboxaldehyde, via both the mixed function oxidase and alcohol dehydrogenase systems. Further metabolism by these systems resulted in the formation of 5-hydroxy-indole-carboxaldehyde and indole-3-carbox-ylic acid.

The oligomeric products of indole-3-carbinol are thought to be primarily responsible for eliciting the biological effects observed for indole-3-carbinol. In rats and fish, DIM, LTR, and 1-(3-hydroxymethyl)-indolyl-3-indolyl-methane were detected in the liver following oral administration of indole-3-carbinol; the parent indole-3-carbinol was not detected[11; 12; 14]. The pattern of condensation products in the gastric contents strongly resembled the pattern obtained after in vitro acid condensation at pHs between 4.5 and 5. In extracts from stomach tissue, small intestine, and liver, the patterns of oligomers were similar to that found in the stomach contents[14]. In addition, indolo[3,2b] carbazole (ICZ), a potent aryl hydrocarbon receptor (AhR) agonist was also identified[14]. When male Sprague Dawley rats were fed dietary indole-3-carbinol or a cabbage-supplemented diet (25% cabbage) for 5 days, ICZ levels were increased in tissues and excreta[5]. In mice as in rats, acid condensation products were detected in plasma and in some tissues; the parent was also detected in plasma, suggesting that indole-3-carbinol was not completely converted to acid condensation products in the gut. In mice, ICZ was detected only in the liver[13]. The peak plasma concentrations of DIM and LTR were approximately one sixth and one tenth that of indole-3-carbinol and the levels persisted much longer than the parent. In addition to the acid condensation products, minor oxidative metabolites of indole-3-carbinol, indol-3-carboxylic acid and indole-3-carboxalde-hyde were also detected in plasma from mice. Tissues profiles of metabolites were similar to the plasma profile suggesting that these metabolites were in equilibrium with the plasma[13]. The proposed metabolic scheme for indole-3-carbinol following oral administration in rodents is shown in Figure 2.

Humans

No published data are available regarding the absorption, distribution, metabolism, or excretion of indole-3-carbinol in humans.

Toxicity

Experimental Animals

The results of short-term studies suggest that the liver is a target organ for indole-3-carbinol. In male guinea pigs, indole-3-carbinol exposure induced steatosis in the liver, primarily in periportal hepatocytes[17]. In male CD-1 mice exposed to 500 or 750 mg/kg per day in the feed for

5 days, indole-3-carbinol significantly increased liver weight and microsomal protein content[18]. This study also showed that indole-3-carbinol alters cholesterol homeostasis. Doses of 500 and 750 mg/kg caused significant increases in hepatic acyl-CoA:cholesterol acyltransferase activity and lowered hepatic cholesterol levels. In male CD-1 mice exposed to 100 mg indole-3-carbinol/kg for 10 days, hepatotoxicity was indicated by reduced glutathione levels and dose-dependent increases in plasma alanine aminotransferase and ornithine transcarbamylase activities[19]. This dose also elicited a 16-fold increase in ethoxyresorufin-*O*-deethylase activity. Increased liver weights and hepatic cytochrome P450 (CYP) activities were also observed in male and female CD rats exposed to 50 mg indole-3-carbinol/kg body weight[20]. In Sprague Dawley rats exposed to 50 mg/kg, indole-3-carbinol significantly induced total hepatic CYP levels in both sexes[21]. Hepatic CYP1A1 and CYP1A2 were significantly induced in both sexes. Indole-3-carbinol also induced expression of hepatic CYP3A2 and CYP1A1 in the colon of both sexes of rats.

The hepatic induction of CYP1A by indole-3-carbinol is mediated via the AhR, a transcription factor that plays a critical mechanistic role in the toxicity and carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Indole-3-carbinol and its major acid condensation products are AhR agonists[22-24]. The toxicological implication of indole-3-carbinol-induced AhR activation is not clearly understood.

Studies also suggest that the nervous system is a target organ for indole-3-carbinol. In male Sprague Dawley rats treated with a single subcutaneous dose of 225, 300, or 500 mg/kg, indole-3-carbinol induced sedation ataxia and loss of righting reflex and sleep[25]. Three out of four rats died 1 to 3 hours after exposure to 500 mg/kg; animals were comatose before death. In male CD-1 mice administered doses of 100 to 500 mg/kg, indole-3-carbinol produced dose-dependent increases in neurological impairment, primarily associated with locomotion, as measured by subjective evaluation of appearance, posture, and motor activities[19]. At 500 mg/kg, animals became comatose. In male guinea pigs, clinical signs of intoxication were apparent within 48 hours of administration (per os) of 0.3 mg indole-3-carbinol/kg body weight in 10% Cremophor® castor oil[17]. Signs included moderate depression, trembling, tachypnea, polypnea, irregular breathing, and increased vesicular lung sounds. In the lung, indole-3-carbinol induced interstitial pneumonia with septal hyperemia.

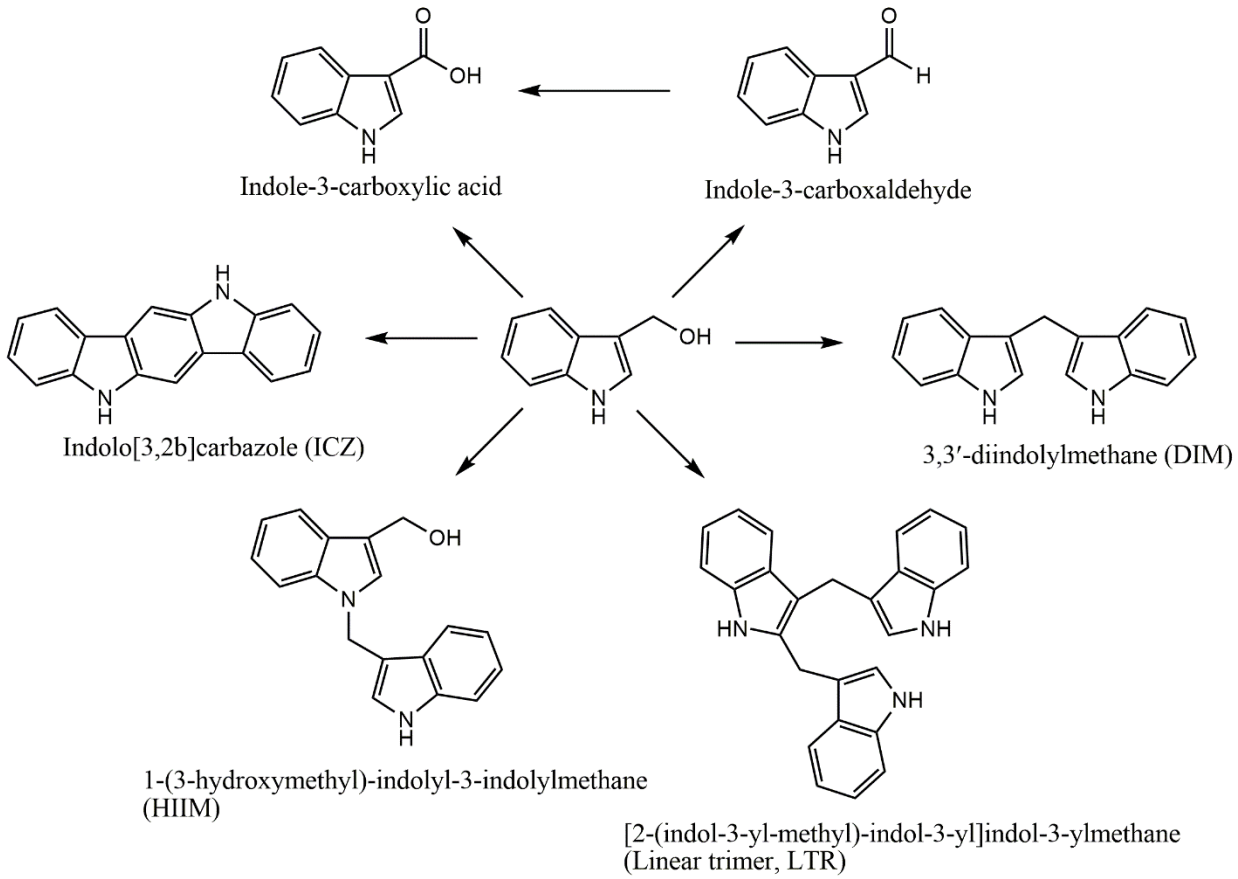

Figure 2. Proposed Metabolic Scheme for Indole-3-carbinol following Oral Administration in Rodents

Adapted from Stresser et al.[12] and Anderton et al.[13].

Humans

In general, most studies in humans have not reported overt toxicity of indole-3-carbinol. In a double-blind, placebo-controlled dose range-finding study, women at risk for breast cancer were administered up to 400 mg indole-3-carbinol for 4 weeks[26]. A slight increase in alanine aminotransferase, indicating hepatocellular membrane leakage, was observed in two participants (dose not specified). Doses of 300 mg (4.2 mg/kg for a 70 kg person) increased the ratio of 2-hydroxyestrone to 16α-hydroxyestrone in the urine, which reflects a shift in estrogen metabolism towards less estrogenic metabolites. Similar results on urinary estrogen metabolites were observed in women with cervical dysplasia administered 200 or 400 mg indole-3-carbinol/day[27] and women with systemic lupus erythematosis administered 375 mg/day for 3 months[28]. A single subject developed a skin rash that went away after cessation of treatment, and then reappeared after indole-3-carbinol was readministered[28].

Reproductive and Developmental Toxicity

Experimental Animals

Two investigative studies have reported on the results of in utero exposure to indole-3-carbinol on reproduction and development. Pregnant Harlan Sprague Dawley rats were administered indole-3-carbinol in corn oil via gavage (1 or 100 mg/kg body weight; three to five dams per dose group) on gestational day (GD) 15 and reproductive parameters were assessed in male offspring on postnatal day (PND) 62[29]. Indole-3-carbinol significantly decreased anogenital distance and crown-rump length on PND 1. When normalized to pup body weight on PND 1 however, there were no effects on these endpoints. Daily sperm production/g testicular parenchyma was significantly decreased at both doses of indole-3-carbinol; however, daily sperm production/testis was only reduced at 100 mg/kg. Exposure did not adversely affect sperm number in the total epididymis or in the head plus body or tail of the epididymis, but did increase epididymal transit time of sperm by more than 1 day in rats exposed to 1 mg/kg. Pregnant Holtzman rats (up to four dams/group) were administered either 200 or 300 mg indole-3-carbinol/kg on GDs 8 and 9[25]. Dams dosed with 300 mg/kg displayed lower body weight gain over GDs 9 to 11. Fetal weights on GD 20 were significantly depressed only in the 200 mg/kg group. There were no effects of indole-3-carbinol on embryonic death or fetal development.

Humans

No reports on the effect of indole-3-carbinol on reproduction or development in humans were found in the literature.

Carcinogenicity

Experimental Animals

No 2-year carcinogenicity studies of indole-3-carbinol in animals were found in the literature. However, several studies have investigated the effect of indole-3-carbinol on chemical-induced tumorigenesis. In initiation-promotion models of carcinogenesis, indole-3-carbinol is a tumor promoter that enhances colon tumors in rats and mice as well as pancreatic tumors in hamsters[30; 31]. Exposure to indole-3-carbinol promotes aflatoxin B_1-initiated hepatocarcinogenesis in rainbow trout[32] and thyroid gland tumors in Sprague Dawley rats[33]. In studies designed to assess inhibition, indole-3-carbinol did not induce tumors when administered without an initiator in Sprague Dawley rats, ACI/N rats, or ICR/Ha mice[34-36]. In female Donryu rats, a strain with a high incidence of endometrial cancer, exposure to 200, 500, or 1,000 ppm indole-3-carbinol in the diet for 660 days resulted in a dose-dependent decrease in the incidence of endometrial adenocarcinoma[37].

Dietary administration of indole-3-carbinol inhibited benzo[*a*]pyrene-induced neoplasia in the forestomach in ICR/Ha mice and 7,12-dimethylbenz[*a*]anthracene-induced mammary gland tumor formation in Sprague Dawley rats[34]. In female C3H/OuJ mice exposed to indole-3-carbinol in the diet, mammary gland tumor incidence and multiplicity were significantly decreased, and tumor latency was prolonged[38]. In a three-generation study in Balb/cfC3H mice, exposure to indole-3-carbinol did not affect mammary gland tumor incidence, however the latency of mammary gland tumors was increased[39]. Inhibition of tumorigenesis has been

observed for diethylnitrosamine-induced hepatocarcinogenesis in ACI/N and Sprague Dawley rats and in the infant mouse model[35; 40; 41]. Indole-3-carbinol also inhibits tumorigenesis in the lung in various animal models[42-45].

Humans

Consumption of cruciferous vegetables has been associated with a decreased risk of cancer in humans[46-49]. Based on epidemiologic evidence and results from animal studies, the National Research Council, Committee on Diet, Nutrition, and Cancer has recommended increased consumption of *Brassica* vegetables as a measure to decrease the incidence of human cancer. The anticarcinogenic activity of cruciferous vegetables has been attributed to indole-3-carbinol and its acid-condensation products[3; 50-53].

Indole-3-carbinol has been promoted and investigated for treatment and prevention of various types of common cancers. Indole-3-carbinol has been shown to inhibit tumorigenesis in multiple tissues. As a result, it has been the focus of several early-phase clinical trials to assess efficacy in the treatment of breast cancer, cervical dysplasia, systemic lupus erythematosus, and recurrent respiratory papillomatosis in adults and children[26-28; 52; 54-58]. In general, doses in these clinical studies ranged between 200 and 400 mg indole-3-carbinol per day.

Genetic Toxicity

The genotoxic potential of indole-3-carbinol has been evaluated in a limited number of bacterial mutagenicity assays and in vitro and in vivo mammalian cell tests. In the majority of tests, indole-3-carbinol gave negative results. However, indole-3-carbinol undergoes chemical reactions in the acidic environment of the stomach to produce compounds that are mutagenic or that can increase the mutagenic potential of other compounds.

Indole-3-carbinol was weakly mutagenic in *Salmonella typhimurium* strain TA100 [lowest effective dose (LED) of 312.5 µg/plate] in the presence or absence of metabolic activation (S9 mix)[59]. In contrast, no mutagenicity was detected with indole-3-carbinol in *S. typhimurium* strains TA98, TA1535, or TA1537 [highest ineffective dose (HID) of 1,250 µg/plate] or *Escherichia coli* strain WP2 *uvrA*/pKM101 (HID of 2,500 µg/plate), with or without S9 mix[59]. Additionally, in a differential survival assay, *E. coli* deficient for nucleotide excision repair and recombinational repair were no more sensitive to indole-3-carbinol (5 µg/mL) than a strain proficient for these DNA repair pathways[60].

Indole compounds present in *Brassica* vegetables (e.g., broccoli, brussels sprouts, and cabbage) may be precursors of mutagens formed during the process of digestion as, in the presence of nitrites, they can be converted to N-nitroso compounds at low pH[61-63]. One study investigated whether indole-3-carbinol became mutagenic following reaction with sodium nitrite at pH 3 at 37°C. Under those conditions, indole-3-carbinol formed a nitrosated product that was reported to be directly mutagenic to *E. coli* WP2 *uvrA*/pKM101 (LED of 12.5 nmol) and to *S. typhimurium* strains TA98 and TA100 (LEDs of 12.5 nmol and 50 nmol, respectively)[64]. Furthermore, the mutagenic effects of the nitrosated product were reduced to near-baseline levels in the presence of S9 mix, and the parent molecule, indole-3-carbinol, was not mutagenic in any of the three tester strains, with or without S9 mix[64]. However, the authors did not report the concentration range that was used to study indole-3-carbinol and no actual data were provided, thus preventing independent assessment of the findings.

No increase in mutant frequency was observed in mouse lymphoma L5178Y *tk*$^{+/-}$ cells treated with indole-3-carbinol in the presence of S9 (HID of 30 μg/mL) or in the absence of S9 (HID of 325 μg/mL)[59]. In vivo, no induction of chromosomal aberrations was observed in bone marrow cells of male Swiss albino mice following a single intraperitoneal injection of 500 mg/kg indole-3-carbinol[65] or five daily exposures to 5 mg/kg indole-3-carbinol by gavage[66]. In a micronucleus assay, male and female Swiss-Webster mice were administered single doses of indole-3-carbinol (375, 750, or 1,500 mg/kg) by oral gavage and assessed at 24 or 48 hours postexposure for the frequency of micronucleated polychromatic erythrocytes (MN-PCE) in bone marrow[59]. Although no increases in the frequency of MN-PCEs were observed in male mice, a dose-dependent increase in the frequency of MN-PCEs was observed in female mice at 24 hours (LED of 750 mg/kg)[59].

Indole-3-carbinol has been studied for antimutagenic effects in bacterial mutagenicity assays and mammalian cell in vitro and in vivo assays. No clear evidence of antimutagenic activity was seen in the bacterial mutagenicity assays and effects were mixed for in vitro mammalian cell assays. Evidence of antimutagenicity in the in vivo studies is more consistent, with decreases reported for induction of cytogenetic damage (sister chromatid exchanges and micronuclei) and adduct formation in various tissues following treatment with known genotoxicants such as cyclophosphamide, benzo[*a*] pyrene, 2-amino-3-methylimidazo[4,5-f]quinoline, and tobacco smoke[66-71].

In addition to N-nitrosation as described above, indole-3-carbinol can undergo acid condensation in the stomach to produce 3,3′-diindolylmethane. One study showed that 3,3′-diindolylmethane can increase the genotoxic effects of a known carcinogen, aflatoxin B1 (AFB1). Pre-incubating primary human hepatocytes for 48 hours with 3,3′-diindolylmethane at concentrations of 10 or 50 μM increased the formation of AFB1-DNA adducts fourfold or sixfold, respectively[72]. Furthermore, incubation with 3,3′-diindolylmethane for 48 hours increased the expression of CYP1A1 and CYP1A2 and downregulated glutathione S-transferase mu 1 at the transcriptional level[72]. Therefore, in primary human hepatocytes, 3,3′-diindolylmethane appears to increase the level of Phase I enzymes that produce the genotoxic form of AFB1 (AFB1-8,9-*exo*-epoxide) and to decrease one of the Phase II enzymes that can detoxify AFB1-8,9-*exo*-epoxide. No other studies on the genotoxic effects of 3,3′-diindolylmethane were identified.

In summary, results from the few evaluations of the genotoxicity of indole-3-carbinol that were identified in the literature were negative, except for one weakly positive bacterial mutagenicity test result and one study in which a significant increase was observed in MN-PCEs in female (but not male) mice following administration of indole-3-carbinol by oral gavage. In addition, several studies were identified that reported indole-3-carbinol-mediated inhibition of chemical-induced genotoxicity in vivo. However, indole-3-carbinol may undergo reactions in the stomach to produce genotoxic compounds or compounds that can enhance the genotoxicity of other compounds through modulation of key metabolic processes.

Study Rationale

Indole-3-carbinol was nominated by the National Cancer Institute for toxicity and carcinogenicity testing because of its occurrence in natural products and its potential use as a breast cancer chemopreventive agent. There is very little known about the toxicity of long-term exposure.

Materials and Methods

Procurement and Characterization

Indole-3-carbinol

Indole-3-carbinol was obtained from ChemPacific Corporation (Baltimore, MD) in one lot (CHP801001) that was used during the 3-month and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Support Services (Columbus, OH) and the study laboratories, Southern Research Institute (SRI) (Birmingham, AL) for the 3-month studies and Battelle Columbus Operations (Columbus, OH) for the 2-year studies (Appendix I). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the indole-3-carbinol studies are on file at the National Institute of Environmental Health Sciences.

Lot CHP801001 of the test chemical, a yellow crystalline solid, was identified as indole-3-carbinol by the analytical chemistry laboratory using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratories using IR and proton NMR (SRI only) spectroscopy. Karl Fischer titration was used to determine the water content; elemental analyses were used to determine the carbon, hydrogen, and nitrogen content; and the melting point was determined using a differential scanning calorimeter. The purity was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC) with ultraviolet detection. Karl Fischer titration indicated 1.3% water. Elemental analyses for carbon, hydrogen, and nitrogen were consistent with the theoretical values, and the melting point was 99.3°C, consistent with the manufacturer's Certificate of Analysis. HPLC analysis indicated one major peak with six impurities, each 0.1% or greater of the total peak area with a combined area of 2.8% of total peak area in the chromatogram. The overall purity of lot CHP801001 was determined to be approximately 97%.

To ensure stability, the bulk chemical was stored at −20°C, protected from light in amber glass containers sealed with Teflon®-lined lids. Periodic reanalyses of the bulk chemical were performed by the study laboratory twice during the 3-month studies and approximately every 6 months during the 2-year studies using HPLC; no degradation of the bulk chemical was detected.

Corn Oil

Corn oil was used as the vehicle for the formulations and was obtained from Red Diamond, Inc. (Birmingham, AL), in one lot that was used in the 3-month studies and from Spectrum Chemicals and Laboratory Products (Gardena, CA) in seven lots and Sigma-Aldrich (St. Louis, MO) in three lots that were used in the 2-year studies. Analysis of the corn oil for peroxides was performed by potentiometric titration, and each lot was within the acceptable range of less than or equal to 3 mEq/kg.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing the appropriate amount of milled indole-3-carbinol with corn oil to achieve the required concentrations (Table I-2). Dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5°C for up to 41 days.

Homogeneity, gavageability, and resuspendability studies were performed on 60 mg/mL dose formulations, and stability studies were performed on 1 mg/mL formulations by the analytical chemistry laboratory using HPLC. Homogeneity was confirmed, and gavageability was confirmed for a 20-gauge gavage needle. Resuspendability was confirmed for dose formulations that had been stored for 14 days at 5°C. Stability was confirmed for at least 8 days for formulations stored in amber glass vials sealed with Teflon®-lined lids at 5°C and for 3 hours under simulated animal room conditions. The study laboratory at SRI performed stability studies with 1.6 mg/mL dose formulations using HPLC, and stability was confirmed for at least 41 days for dose formulations stored in amber glass vials sealed with Teflon®-lined lids at 5°C. Prior to the 2-year studies, the study laboratory at Battelle Columbus Operations performed homogeneity studies on 6.25, 15, 25, and 60 mg/mL dose formulations and gavageability studies on 60 mg/mL dose formulations using HPLC; homogeneity and gavagability were confirmed.

Periodic analyses of the dose formulations of indole-3-carbinol were performed by the study laboratories using HPLC. During the 3-month studies, the dose formulations were analyzed three times; all 15 of the dose formulations for rats and all 15 for mice were within 10% of the target concentrations (Table I-3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months (Table I-4). Of the dose formulations analyzed, all 72 for rats and all 36 for mice were within 10% of the target concentrations; all 24 of the animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

Animal Source

Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY) for the 3-month studies. For the 2-year studies, male and female Harlan Sprague Dawley rats and B6C3F1/N mice were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN), and Taconic Farms, Inc. (Germantown, NY), respectively. In the interim between conducting the 3-month studies and the initiation of the 2-year studies, NTP discontinued use of the F344/N rat in toxicity and carcinogenicity studies.

NTP has previously conducted seven separate 2-year studies in Sprague Dawley rats based on prior observations by Kociba et al.[73] that Sprague Dawley rats are sensitive to the effects of dioxin-like compounds[74-80]. Based on the role of indole-3-carbinol as an agonist ligand for the aryl hydrocarbon receptor, a dioxin-responsive mechanism, Harlan Sprague Dawley rats were selected for the 2-year study.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to indole-3-carbinol and to determine the appropriate doses to be used in the 2-year studies. The doses selected for the 3-month studies were based on study results supplied by the National Cancer Institute (NCI) that provided sufficient information in rats to determine appropriate doses to obtain a no-observed-adverse-effect level and a possible maximum tolerated dose. On receipt, the rats and mice were 4 to 5 weeks old. Rats were quarantined for 12 (females) or 13 (males) days and mice were quarantined for 14 (males) or 15 (females) days. Rats were 5 (females only) to 6 weeks old and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female core study rats were administered 0, 18.75, 37.5, 75, 150, or 300 mg indole-3-carbinol/kg body weight in corn oil by gavage, and groups of 10 male and 10 female mice were administered 0, 15.6, 31.25, 62.5, 125, or 250 mg/kg, 5 days per week for 14 weeks. These initial doses were selected based on published literature, additional data provided from a 3-month rat study being conducted by the NCI[20], and a pilot study conducted in Sprague Dawley rats (unpublished data). Crowell et al.[20] demonstrated effects in rats at 100 mg/kg; increased incidences of centrilobular hepatocyte hypertrophy, and increased liver, kidney, and spleen weights were observed in males, and increased liver and kidney weights were observed in females at this dose. In a pilot study, exposure to 200 mg/kg indole-3-carbinol for 6 weeks induced a 10% decrease in body weight and a 20% increase in liver weight. In mice, neurological effects and increased mortality have been observed at doses of 500 mg/kg. Clinical pathology study groups of 10 male and 10 female rats were administered the same doses as the core study rats 5 days per week for 25 days. Vehicle control animals received the corn oil vehicle alone. Feed and water were available ad libitum. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings for core study rats and mice were recorded weekly. The animals were weighed initially; on day 2 (female mice), 3 (male animals), or 4 (female rats); weekly; and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Liver (median, left, and right lobes) and lung (right lobe) samples were collected from 10 male and 10 female core study rats and mice at necropsy for determination of cytochrome P450 1A1 and 1A2 activities. Liver and lung samples were weighed, minced, and frozen until analysis. CYP1A1 in the liver and lung and CYP1A2 in the liver were measured based on the activities of 7-ethoxyresorufin-*O*-deethylase (EROD) or acetanilide-4-hydroxylase (A4H), respectively. Microsomal suspensions were prepared using the $CaCl_2$ aggregation method described by Schenkman and Cinti[81]. The concentration of protein in each suspension was determined by the Lowry method[82]. EROD activities were determined by spectrofluorometric methods, including those of Chang and Waxman[83] for liver, and A4H activities were determined using HPLC[84].

Blood was collected from the retroorbital plexus of clinical pathology study rats under carbon dioxide anesthesia on days 4 and 25 and, using the same method, blood was collected from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats) analyses. For hematology analyses, blood from each animal was collected into a tube containing EDTA. Erythrocyte, platelet, and leukocyte counts, hematocrit values, hemoglobin concentration, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, and reticulocyte counts were determined using an Advia® 120 Hematology System (Bayer, Inc., Tarrytown, NY) and reagents from the instrument manufacturer. For clinical chemistry analyses, blood was collected into a tube containing no anticoagulant. Clinical chemistry analyses were performed using a Hitachi 911 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, IN). Reagents were supplied by the instrument manufacturer. The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats administered to 0, 75, 150, or 300 mg/kg and mice administered to 0, 62.5, 125, or 250 mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal euthanasia, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus of core study rats and mice were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed by the study laboratory pathologist on all vehicle control and 300 mg/kg core study rats and all vehicle control and 250 mg/kg mice. In rats, the liver, mesenteric lymph node, and small intestine were examined in all dosed groups; in mice, the liver and mesenteric lymph node (females only) were examined in all dosed groups. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any

inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[85] and Boorman et al.[86].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats were administered 0, 75, 150, or 300 mg indole-3-carbinol/kg body weight in corn oil gavage, and groups of 50 male and 50 female mice were administered 0, 62.5, 125, or 250 mg/kg, 5 days per week for 104 (male rats) or 105 weeks. To further investigate the pathogenesis of treatment-related increased incidences of dilation of lymphatics of the duodenum, jejunum, and mesenteric lymph nodes observed in the 3-month studies[87], an additional five male rats per special intestine study group were administered indole-3-carbinol for 1 or 4 weeks. To evaluate transcriptional changes in the liver, liver tissue from five vehicle control female rats and five 300 mg/kg female rats was collected and processed for microarray analyses at 3 months (Appendix L).

Rats were quarantined for 13 (males) or 14 (females) days and mice were quarantined for 18 (females) or 19 (males) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Male rats were housed up to three per cage, female rats and mice were housed up to five per cage, and male mice were housed individually. Feed and water were available ad libitum. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights of core study animals were recorded initially, weekly for the first 13 weeks, monthly thereafter, and upon study termination. Clinical findings were recorded at week 5, monthly, and upon study termination.

Complete necropsies and microscopic examinations were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except, initially, eyes were fixed in Davidson's solution and testes were fixed in modified Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. In the standard evaluation of the uterus, a transverse section through each uterine horn, approximately 0.5 cm cranial to the cervix, was collected for histopathological evaluation. In an extended review, all residual uterine, cervical and vaginal tissue stored in 10% neutral buffered formalin were opened (longitudinally) and examined for untrimmed potential gross lesions. All untrimmed potential lesions found were

collected and in addition to the remaining segments of uterus were processed histologically and sectioned longitudinally for histopathological examination. Tissues examined microscopically are listed in Table 1.

Complete necropsies were performed on all special intestine study male rats. At necropsy, the small intestines (duodenum and jejunum) were collected, fixed, and preserved in 10% neutral buffered formalin. Additionally, samples of the duodenum and jejunum were cryopreserved. Microscopic evaluations of the small intestine were performed on five special study male rats at 1 week and five special study male rats at 4 weeks. The cryosections of the small intestine (duodenum and jejunum) were stained with the Oil-red-O and Sudan Black histochemical stains that are specific for detecting fat (lipid) in tissue sections.

At 3 months, liver tissue from the left lateral lobe of five vehicle control female rats and five 300 mg/kg female rats was collected, frozen in liquid nitrogen, and transported to Battelle Biomedical Research Center (Columbus, OH) to be processed for RNA isolation and purification, cDNA synthesis, and array hybridization (Appendix L).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver of rats and mice, small intestine of rats, uterus of female rats, and nose and glandular stomach of mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[85] and Boorman et al.[86]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[88].

Table 1. Experimental Design and Materials and Methods in the Gavage Studies of Indole-3-carbinol

Three-month Studies	Two-year Studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F1/N mice	Harlan Sprague Dawley rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Rats: Harlan Sprague Dawley, Inc. (Indianapolis, IN) Mice: Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 12 (females) or 13 (males) days Mice: 14 (males) or 15 (females) days	Rats: 13 (males) or 14 (females) days Mice: 18 (females) or 19 (males) days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	6 to 7 weeks
Date of First Dose	
Core study rats: August 30 (females) or 31 (males), 2004 Mice: September 1 (males) or 2 (females), 2004	Rats: March 14 (males) or 15 (females), 2007 Mice: April 2 (females) or 3 (males), 2007
Duration of Dosing	
5 days per week for 14 weeks	5 days per week for 104 (male rats) or 105 weeks
Date of Last Dose	
Core study rats: November 30 (females) or December 1 (males), 2004 Mice: December 2 (males) or 3 (females), 2004	Rats: March 10 (males) or 12 (females), 2009 Mice: March 31 (females) or April 2 (males), 2009
Necropsy Dates	
Core study rats: December 1 (females) or 2 (males), 2004 Mice: December 3 (males) or 4 (females), 2004	Rats: March 9 to 11 (males) or 12 and 13 (females), 2009 Mice: March 30 to April 1 (females) or April 1 to 3 (males), 2009
Average Age at Necropsy	
19 to 20 weeks	Rats: 110 to 111 weeks Mice: 110 (females) to 112 weeks
Size of Study Groups	
Core study: 10 male and 10 female rats and mice	Core study: 50 males and 50 females
Clinical pathology study: 10 male and 10 female rats	Special intestine study: 10 male rats Microarray study: Five vehicle control female rats and five 300 mg/kg female rats

Three-month Studies	Two-year Studies
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed at least once weekly	Same as 3-month studies
Water	
Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available ad libitum
Cages	
Polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice weekly	Same as 3-month studies except from Lab Products, Inc., Seaford, DE; rotated every 2 weeks
Bedding	
Irradiated hardwood bedding chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice weekly	Irradiated Sani-Chips® (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice weekly
Rack Filters	
Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks
Racks	
Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks	Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks
Animal Room Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Doses	
Rats: 0, 18.75, 37.5, 75, 150, or 300 mg/kg in corn oil by gavage (dosing volume 5 mL/kg) Mice: 0, 15.6, 31.25, 62.5, 125, or 250 mg/kg in corn oil by gavage (dosing volume 10 mL/kg)	Rats: Core study rats and special intestine study male rats 0, 75, 150, or 300 mg/kg and microarray study female rats 0 or 300 mg/kg in corn oil by gavage (dosing volume 5 mL/kg) Mice: 0, 62.5, 125, or 250 mg/kg in corn oil by gavage (dosing volume 10 mL/kg)

Three-month Studies	Two-year Studies
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, day 2 (female mice), day 3 (male rats and mice), day 4 (female rats), weekly, and at the end of the studies; clinical findings for core study animals were recorded weekly.	Observed twice daily; core study animals were weighed initially, weekly for the first 13 weeks, monthly thereafter, and upon study termination; clinical findings for core study animals were recorded at week 5, monthly, and at the end of the studies.
Method of Euthanasia	
Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus of core study rats and mice.	Necropsies were performed on all core study animals and special intestine study male rats.
Clinical Pathology	
Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 25 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology	
Complete histopathology was performed on core study vehicle control rats and mice, 300 mg/kg rats, and 250 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the liver, mesenteric lymph node, and small intestine were examined in all dosed groups of rats, and the liver and mesenteric lymph node (females only) were examined in all dosed groups of mice.	Complete histopathology was performed on all core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. The small intestines were examined in five special intestine study male rats at 1 week and five special intestine study male rats at 4 weeks. Extended gross and microscopic reviews were performed on the residual uterine wet tissue from all experimental groups of female rats.

Three-month Studies	Two-year Studies
Sperm Motility and Vaginal Cytology	
At the end of the studies, spermatid and sperm samples were collected from 0, 75, 150, and 300 mg/kg core study male rats and 0, 62.5, 125, and 250 mg/kg male mice. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from 0, 75, 150, and 300 mg/kg core study female rats and 0, 62.5, 125, and 250 mg/kg female mice.	None
Cytochrome P450 Activities	
Liver (median, left, and right lobes) and lung (right lobe) samples were collected from 10 male and 10 female core study rats and mice at necropsy. CYP1A1 activity in the liver and lung and CYP1A2 activity in the liver were measured.	None
Microarray Study	
None	Liver tissue of five vehicle control female rats and five 300 mg/kg female rats was collected and processed to evaluate transcriptional changes in the liver at a 3 months (Appendix L).

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[89] and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's[90] method for testing two groups for equality and Tarone's[91] life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Table A-1, Table A-3, Table B-1, Table B-5. Table C-1, Table C-4, Table D-1, and Table D-4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Table A-2, Table B-2, Table C-2, and Table D-2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the

denominators consist of the number of animals on which a necropsy was performed. Table A-2, Table B-2, Table C-2, and Table D-2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[92-94] was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal euthanasia; if the animal died prior to terminal euthanasia and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[92]. Unless otherwise specified, a value of k = 3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[92] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344/N rats and B6C3F1/N mice[95]. Bailer and Portier[92] showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams[96].

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P = 0.99 is presented as P = 0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[97] and Williams[98; 99]. Hematology, clinical chemistry, cytochrome P450, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[100] (as modified by Williams[101]) and Dunn[102]. Jonckheere's test[103] was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[104] were examined by NTP personnel,

and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test[105]. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager[106]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors affecting the background incidences of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period[107-109]. In general, the historical control database for a given study includes studies using the same route of administration, and the overall incidences of neoplasms in controls for all routes of administration are included for comparison, including the current study. In the historical control database for Sprague Dawley rats, there are only two studies, the current study of indole-3-carbinol and PCB 118. Because both studies were corn oil gavage studies, only same route of administration historical data are presented in this Technical Report for female Sprague Dawley rats.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[110]. In addition, as records from the 3-month and 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of indole-3-carbinol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, micronucleated erythrocytes in rat bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally “small nuclei” or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of

two daughter nuclei during cell division[111; 112]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[113] and the somatic mutation theory of cancer[114; 115]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[116]. A positive response in the *Salmonella* test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens)[117; 118]. Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test[119; 120]. However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies[121]. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Three-month Study in F344/N Rats

All rats survived to the end of the study (Table 2). The mean body weight gain of males in the 300 mg/kg group was significantly less than that of the vehicle controls (Table 2; Figure 3). There were no clinical findings related to administration of indole-3-carbinol.

Table 2. Survival and Body Weights of F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	103 ± 1	309 ± 5	206 ± 4	
18.75	10/10	103 ± 1	320 ± 9	217 ± 8	103
37.5	10/10	107 ± 1*	325 ± 7	218 ± 7	105
75	10/10	105 ± 1	321 ± 8	215 ± 7	104
150	10/10	104 ± 1	309 ± 8	204 ± 8	100
300	10/10	104 ± 1	285 ± 6	182 ± 6*	92
Female					
0	10/10	91 ± 2	189 ± 2	99 ± 3	
18.75	10/10	90 ± 1	192 ± 3	102 ± 3	101
37.5	10/10	90 ± 2	195 ± 2	105 ± 2	103
75	10/10	90 ± 1	185 ± 3	95 ± 3	98
150	10/10	89 ± 2	183 ± 3	94 ± 2	97
300	10/10	91 ± 2	183 ± 3	92 ± 3	97

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test.
[a]Weights and weight changes are given as mean ± standard error.
[b]Number of animals surviving at 14 weeks/number initially in group.

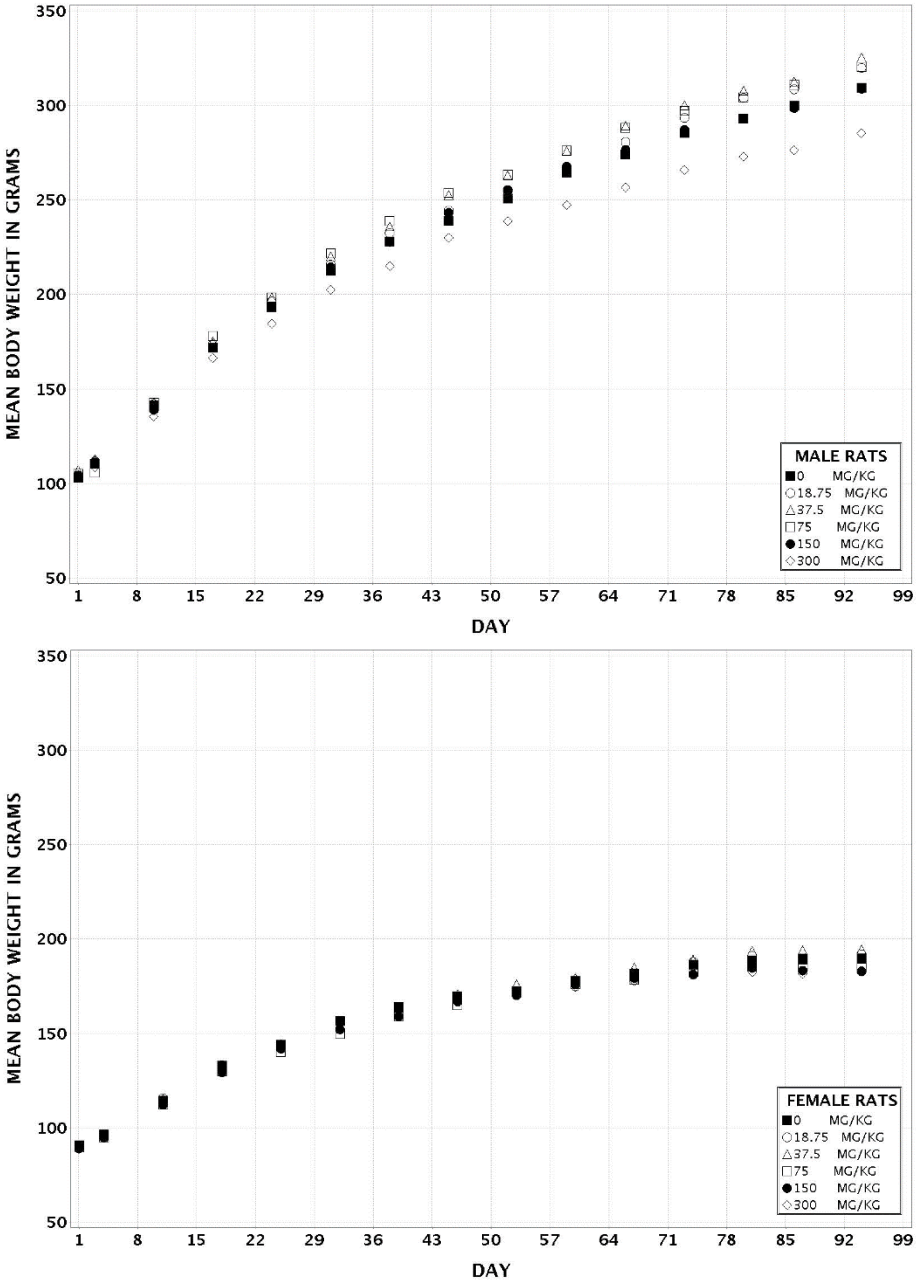

Figure 3. Growth Curves for F344/N Rats Administered Indole-3-carbinol by Gavage for Three Months

There were no changes in the hematology and clinical chemistry data of rats that were considered attributable to indole-3-carbinol administration (Table F-1).

The absolute and relative liver weights of all dosed groups of males and females were significantly increased compared to the vehicle controls (Table 3 and Table G-1). The relative kidney weights of 75 mg/kg or greater males and all dosed groups of females were significantly increased, as were the absolute kidney weights of 75 mg/kg males and 18.75, 37.5, and 300 mg/kg females. The absolute and relative thymus weights of 75 mg/kg or greater females

were significantly decreased. Microscopically, there were no histopathologic lesions that correlated with these organ weight changes.

Table 3. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	309 ± 5	320 ± 9	325 ± 7	321 ± 8	309 ± 8	285 ± 6
R. Kidney						
Absolute	0.86 ± 0.02	0.94 ± 0.02	0.93 ± 0.03	0.97 ± 0.03*	0.92 ± 0.03	0.90 ± 0.02
Relative	2.785 ± 0.038	2.940 ± 0.033	2.844 ± 0.054	3.012 ± 0.038**	2.981 ± 0.048**	3.152 ± 0.055**
Liver						
Absolute	9.98 ± 0.30	11.19 ± 0.40*	11.20 ± 0.40*	11.85 ± 0.36**	12.07 ± 0.38**	12.57 ± 0.33**
Relative	32.205 ± 0.594	34.924 ± 0.481**	34.382 ± 0.716**	36.941 ± 0.435**	39.050 ± 0.472**	44.047 ± 0.729**
Female						
Necropsy body wt	189 ± 2	192 ± 3	195 ± 2	185 ± 3	183 ± 3	183 ± 3
R. Kidney						
Absolute	0.57 ± 0.01	0.66 ± 0.01**	0.63 ± 0.01*	0.61 ± 0.01	0.62 ± 0.01	0.63 ± 0.02*
Relative	3.034 ± 0.056	3.447 ± 0.053**	3.233 ± 0.075**	3.277 ± 0.046**	3.375 ± 0.064**	3.436 ± 0.063**
Liver						
Absolute	5.08 ± 0.11	5.94 ± 0.11**	5.98 ± 0.07**	5.92 ± 0.13**	6.34 ± 0.14**	6.92 ± 0.11**
Relative	26.804 ± 0.441	30.985 ± 0.566**	30.790 ± 0.547**	32.061 ± 0.548**	34.660 ± 0.614**	37.857 ± 0.319**
Thymus						
Absolute	0.178 ± 0.005	0.173 ± 0.005	0.162 ± 0.007	0.149 ± 0.008**	0.151 ± 0.007**	0.145 ± 0.006**
Relative	0.943 ± 0.027	0.901 ± 0.022	0.836 ± 0.042*	0.805 ± 0.036**	0.825 ± 0.029**	0.792 ± 0.031**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Liver and lung samples were collected for determinations of P450 enzyme activities (Table 4). Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD) activity (a marker for CYP1A1 activity) and acetanilide-4-hydroxylase (A4H) activity (a marker for CYP1A2 activity). Microsomal samples from lung were analyzed for EROD activity only. In the liver, there were significant and dose-dependent increases in A4H and EROD activities in all dosed groups of male and female rats. Maximal inductions of A4H and EROD activities in males were greater than 5-fold and 100-fold, respectively, compared to the vehicle controls. In females, A4H and EROD activities were maximally increased 4.7-fold and 80-fold, respectively. Pulmonary EROD activity was

significantly increased in all dosed groups of females and in males administered 75 mg/kg or greater.

Table 4. Liver and Lung Cytochrome P450 Data for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Male						
Liver Microsomes						
n	9	10	10	10	10	9
A4H (nmol/minute per mg microsomal protein)						
	0.404 ± 0.027	0.627 ± 0.071*	0.999 ± 0.067**	1.187 ± 0.086**	1.546 ± 0.071*	2.045 ± 0.077**
EROD (pmol/minute per mg microsomal protein)						
	7.1 ± 1.4	40.8 ± 5.1**	160.8 ± 9.6**	303.1 ± 34.8**	485.9 ± 49.0**	774.6 ± 41.7**
Lung Microsomes						
n	8	9	10	10	10	10
EROD (pmol/minute per mg microsomal protein)						
	ND	0.033 ± 0.023	0.008 ± 0.008	0.160 ± 0.042**	1.146 ± 0.218**	1.227 ± 0.250**
Female						
n	10	10	10	10	10	10
Liver Microsomes						
A4H (nmol/minute per mg microsomal protein)						
	0.343 ± 0.026	0.652 ± 0.069**	0.826 ± 0.017**	0.973 ± 0.043**	1.408 ± 0.105**	1.621 ± 0.088**
EROD (pmol/minute per mg microsomal protein)						
	6.1 ± 1.1	70.7 ± 15.1**	172.8 ± 15.0**	236.5 ± 24.7**	440.5 ± 41.3**	495.4 ± 39.9**
Lung Microsomes						
EROD (pmol/minute per mg microsomal protein)						
	0.006 ± 0.004	1.050 ± 0.205**	2.711 ± 0.267**	2.671 ± 0.540**	2.747 ± 0.364**	3.008 ± 0.333**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test.
**$P \leq 0.01$.
ND = None detected.
[a]Data are presented as mean ± standard error. A4H = acetanilide-4-hydroxylase; EROD = 7-ethoxyresorufin-*O*-deethylase;

There were no significant differences in reproductive tissue weights or spermatid or epididymal spermatozoal measurements of males administered 75, 150, or 300 mg/kg when compared to the vehicle control group; therefore, indole-3-carbinol did not exhibit the potential to be a reproductive toxicant in male rats (Table H-1). Indole-3-carbinol did exhibit the potential to be a female reproductive toxicant based on the increased estrous cycle length (approximately 1 day), and an additional day of diestrus (Table H-2 and Table H-3; Figure H-1). This was manifested as the probability of extended diestrus being significantly higher in the 300 mg/kg group than in the vehicle control group when estrous cyclicity was analyzed using Markov transition matrix analysis.

Dose-related increased incidences and severities of minimal to mild ectasia of the lymphatic vessels and lipidosis of the lamina propria occurred in the villi of the small intestines (duodenum and jejunum) of males and females (Table 5). Compared to the vehicle controls, these incidences were significantly increased in the duodenum of 150 and 300 mg/kg males and females and in the jejunum of 75 mg/kg or greater males and females. The severity of lymphatic ectasia was minimal to moderate in the jejunum and minimal to mild in the duodenum. Affected lymphatics were variably dilated, lined by a single layer of endothelial cells, and occasionally contained fibrillar amphophilic material. Within the stroma of affected villi, there were occasional macrophages that contained clear cytoplasmic vacuoles (lamina propria) consistent with accumulated lipid (fat). Lesions of moderate severity often disrupted the architecture of the villi. In general, lesions in the jejunum were more severe than those in the duodenum. The cytoplasmic vacuoles within macrophages in the lamina propria of the villi stained positively with special histochemical stains (Oil-red-O and Sudan Black) for lipid.

Table 5. Incidences of Selected Nonneoplastic Lesions in F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Male						
Intestine Small, Duodenum[a]	10	10	10	10	10	10
Lamina Propria, Lipidosis[b]	0	0	0	1 (1.0)[c]	10** (1.2)	10** (2.2)
Lymphatic, Ectasia	0	0	0	1 (1.0)	10** (1.2)	10** (2.2)
Intestine Small, Jejunum	10	9	10	10	10	10
Lamina Propria, Lipidosis	0	1 (1.0)	1 (1.0)	5** (1.0)	10** (2.3)	10** (3.5)
Lymphatic, Ectasia	0	1 (1.0)	1 (1.0)	5** (1.0)	10** (2.3)	10** (3.5)
Lymph Node, Mesenteric	7	10	9	10	10	10
Lipidosis	0	2 (1.0)	1 (1.0)	0	5* (1.4)	10** (3.0)
Lymphatic, Ectasia	0	2 (1.0)	1 (1.0)	0	5* (1.4)	10** (3.0)
Female						
Intestine Small, Duodenum	10	10	10	10	10	10
Lamina Propria, Lipidosis	0	0	0	0	9** (1.0)	10** (2.4)
Lymphatic, Ectasia	0	0	0	0	9** (1.0)	10** (2.4)
Intestine Small, Jejunum	10	10	10	10	10	10
Lamina Propria, Lipidosis	0	0	1 (1.0)	10** (1.0)	10** (1.8)	10** (3.0)
Lymphatic, Ectasia	0	0	1 (1.0)	10** (1.0)	10** (1.8)	10** (3.0)
Lymph Node, Mesenteric	10	10	10	10	10	10
Lipidosis	0	0	0	0	3 (1.3)	9** (3.2)
Lymphatic, Ectasia	0	0	0	0	3 (1.3)	9** (3.2)

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test.
**$P \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

In the mesenteric lymph node, dose-related increased incidences and severities of lymphatic ectasia and lipidosis occurred in males and females. These incidences were significantly increased in 150 and 300 mg/kg males and 300 mg/kg females compared to the vehicle controls. These lesions occurred primarily in the subcapsular sinuses, occasionally in the cortex and paracortex, and infrequently in the medullary sinuses. Microscopically, the ectatic lymphatics were dilated and contained macrophages with foamy-appearing cytoplasm suggestive of accumulated lipid and occasional multiinucleated giant cells. Lesions of moderate or marked severity often disrupted the nodal architecture. The cytoplasmic vacuoles within macrophages stained positively with special histochemical stains (Oil-red-O and Sudan Black) for lipid.

Dose Selection Rationale: In the 3-month study, chemical-related increases in liver and kidney weights were observed in F344/N rats. Lymphatic ectasia occurred in the small intestine and mesenteric lymph node, but the lesions were not considered severe enough to compromise survival in the 2-year study. At 300 mg/kg, there were 7% and 12% decreases in body weight gains of females and males, respectively, and an 8% decrease in terminal body weight in males. Decreased body weights were not considered sufficient to exclude 300 mg/kg as the highest dose in the 2-year study. There was concern, however, that higher doses would not be well tolerated. At 300 mg/kg, the induction of hepatic EROD activity by indole-3-carbinol was greater than 100-fold in treated males and 80-fold in treated females compared to the vehicle controls. These levels of induction were equivalent to or exceeded the levels of induction at which exposure to dioxin-like aryl hydrocarbon receptor ligands such as PCB 126 and TCDD induced moderate to marked hepatotoxicity in 2-year studies[74; 75]. The only liver toxicity observed in these studies at 14 weeks was hepatocyte hypertrophy, a lesion previously observed in some studies of indole-3-carbinol. Based on the available data, the doses selected for the 2-year gavage study in Harlan Sprague Dawley rats were 75, 150, and 300 mg/kg.

Two-year Study in Sprague Dawley Rats

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 4). Survival of dosed groups was similar to that of the vehicle control groups.

Body Weights and Clinical Findings

Mean body weights of dosed groups of males and females were similar to those of the vehicle controls throughout the study (Figure 5; Table 7 and Table 8). No clinical findings related to the administration of indole-3-carbinol were observed in males or females.

Table 6. Survival of Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	14	23	15	20
Natural deaths	16	14	18	17
Animals surviving to study termination	20	13	17	12
Percent probability of survival at end of study[b]	40	26	34	25
Mean survival (days)[c]	644	617	640	615
Survival analysis[d]	P = 0.224	P = 0.084	P = 0.557	P = 0.100
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	0	1
Moribund	24	22	23	11
Natural deaths	5	8	7	8
Animals surviving to study termination	21	19[e]	20[e]	30
Percent probability of survival at end of study	42	39	38	61
Mean survival (days)	637	634	625	648
Survival analysis	P = 0.055N	P = 1.000	P = 0.837	P = 0.079N

[a]Censored in the survival analyses.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and terminal euthanasia).
[d]The result of the life table trend test[91] is in the vehicle control column, and the results of the life table pairwise comparisons[90] with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[e]Includes one animal that died during the last week of the study.

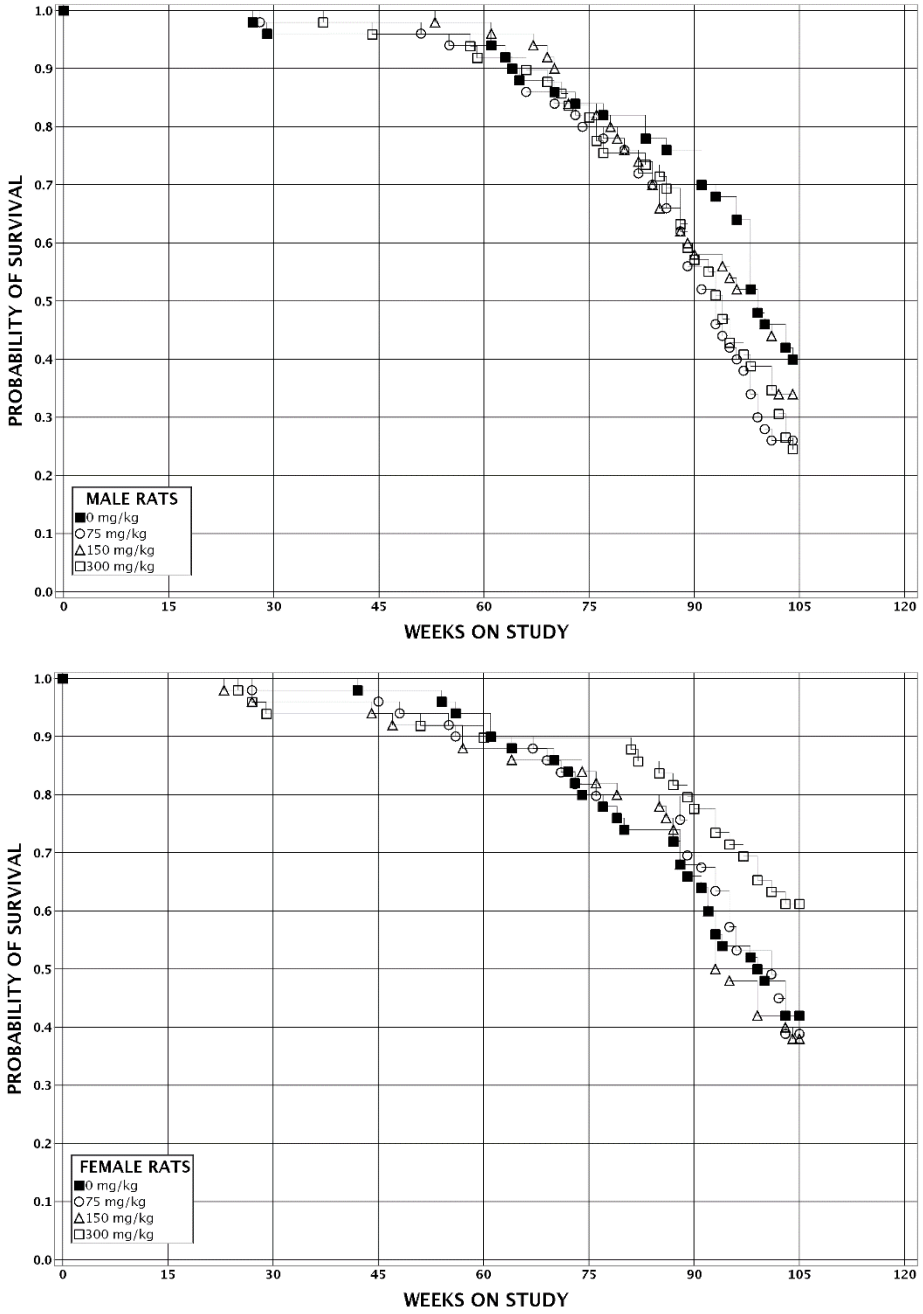

Figure 4. Kaplan-Meier Survival Curves for Sprague Dawley Rats Administered Indole-3-carbinol by Gavage for Two Years

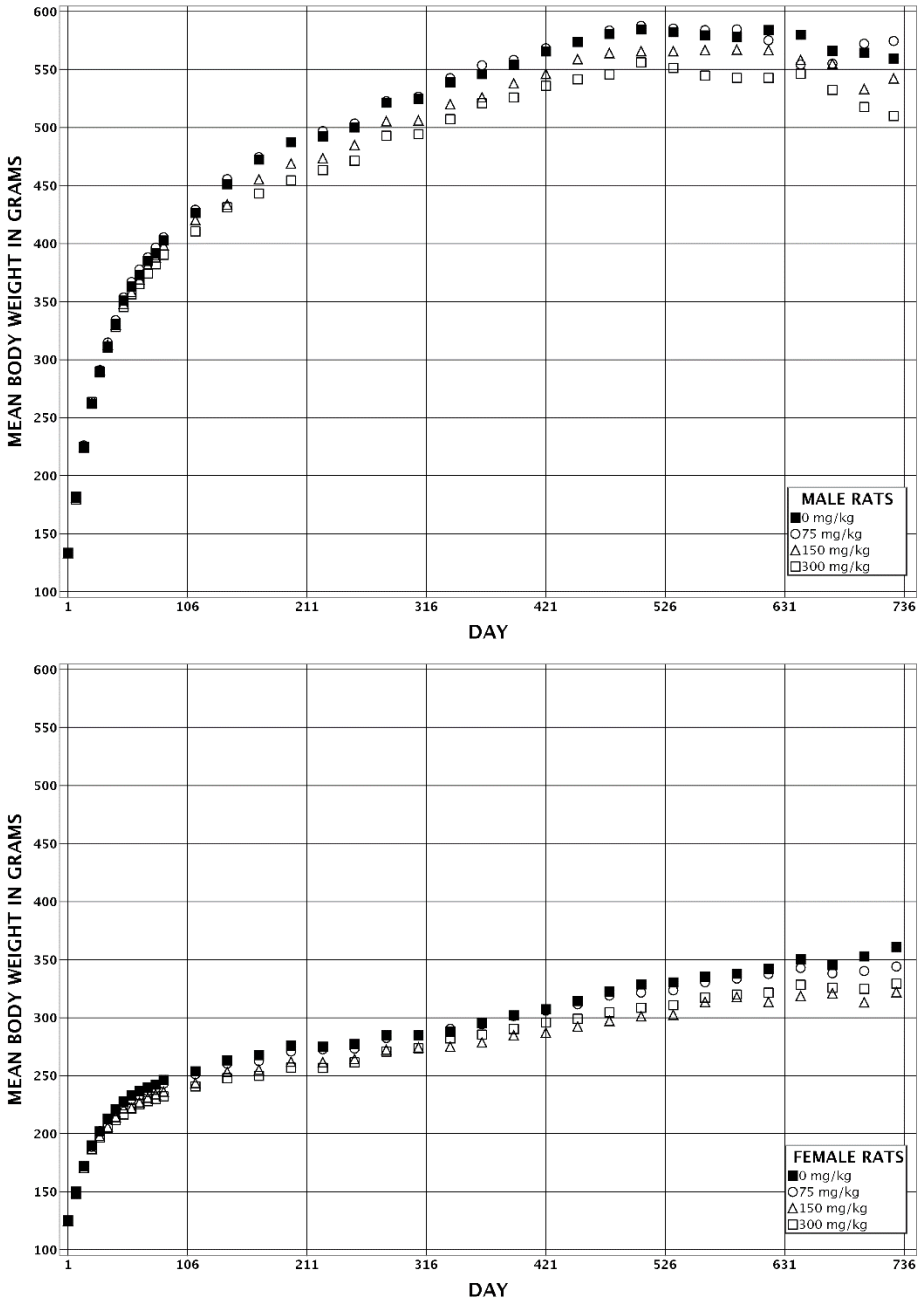

Figure 5. Growth Curves for Sprague Dawley Rats Administered Indole-3-carbinol by Gavage for Two Years

Table 7. Mean Body Weights and Survival of Male Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

Day	Vehicle Control		75 mg/kg			150 mg/kg			300 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	134	50	134	100	50	133	100	50	133	100	50
8	182	50	182	100	50	181	99	50	180	99	50
15	225	50	226	101	50	225	100	50	224	100	50
22	262	50	264	101	50	263	100	50	264	101	50
29	289	50	291	101	50	291	101	50	290	100	50
36	311	50	315	101	50	313	101	50	311	100	50
43	331	50	334	101	50	330	100	50	328	99	50
50	351	50	354	101	50	348	99	50	345	98	50
57	363	50	367	101	50	359	99	50	357	98	50
64	373	50	378	101	50	369	99	50	365	98	50
71	385	50	388	101	50	382	99	50	375	97	50
78	392	50	397	101	50	388	99	50	382	98	50
85	403	50	406	101	50	399	99	50	391	97	50
113	427	50	429	101	50	420	99	50	411	96	50
141	451	50	456	101	50	434	96	50	432	96	50
169	473	50	475	100	50	455	96	50	443	94	50
197	488	48	488	100	49	469	96	50	455	93	49
225	493	48	497	101	49	474	96	50	464	94	49
253	500	48	504	101	49	485	97	50	472	94	49
281	521	48	523	100	49	506	97	50	493	95	48
309	525	48	527	100	49	506	97	50	495	94	47
337	539	48	543	101	49	520	97	50	508	94	47
365	546	48	554	101	48	526	96	50	521	95	47
393	554	48	558	101	47	538	97	49	526	95	47
421	566	47	568	100	47	546	97	49	536	95	45
449	574	45	574	100	45	559	97	48	542	94	45
477	581	44	584	101	43	564	97	47	546	94	43
505	585	42	588	101	41	566	97	42	556	95	41
533	582	42	586	101	39	566	97	41	552	95	38
561	580	41	584	101	38	567	98	38	545	94	37
589	578	39	585	101	35	567	98	35	543	94	35
617	584	38	575	98	29	567	97	31	543	93	30
645	580	34	554	96	26	558	96	29	547	94	26
673	566	32	555	98	20	555	98	26	533	94	20
701	565	23	572	101	14	533	95	23	518	92	18
Mean for Weeks											
1–13	308		310	101		306	99		304	99	
14–52	491		494	101		474	97		464	95	
53–101	572		572	100		555	97		539	94	

Table 8. Mean Body Weights and Survival of Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

Day	Vehicle Control		75 mg/kg			150 mg/kg			300 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	126	50	125	100	50	125	99	50	125	100	50
8	150	50	150	100	50	149	99	50	148	99	50
15	172	50	172	100	50	170	99	50	171	99	50
22	190	50	189	99	50	187	98	50	187	99	50
29	202	50	203	100	50	198	98	50	197	97	50
36	213	50	212	100	50	206	97	50	205	96	50
43	221	50	220	99	50	215	97	50	212	96	50
50	228	50	227	100	50	222	98	50	217	95	50
57	233	50	230	99	50	222	95	50	222	95	50
64	237	50	233	98	50	227	96	50	225	95	50
71	240	50	237	99	50	231	96	50	228	95	50
78	243	50	239	99	50	234	97	50	230	95	50
85	246	50	243	99	50	236	96	50	233	94	50
113	254	50	251	99	50	244	96	50	241	95	50
141	263	50	260	99	50	253	96	50	248	94	50
169	268	50	263	98	50	255	95	49	250	93	50
197	276	50	271	98	49	262	95	48	257	93	46
225	275	50	273	99	49	262	95	48	257	93	46
253	277	50	273	99	49	264	95	48	262	94	46
281	285	50	283	99	49	272	96	48	271	95	46
309	285	49	285	100	49	275	96	47	274	96	46
337	288	49	290	101	47	275	96	46	282	98	46
365	295	49	294	100	47	279	94	46	286	97	45
393	302	47	301	100	45	285	94	45	290	96	45
421	307	47	306	100	44	287	93	44	296	96	44
449	315	44	312	99	44	292	93	43	299	95	44
477	323	44	319	99	43	297	92	43	305	94	44
505	329	42	322	98	41	301	92	43	309	94	44
533	331	40	324	98	39	303	92	41	311	94	44
561	335	37	330	99	39	314	94	40	317	95	44
589	338	37	334	99	39	318	94	40	320	95	42
617	342	33	338	99	37	314	92	34	322	94	40
645	350	28	343	98	31	319	91	28	329	94	36
673	346	27	338	98	26	321	93	24	326	94	35
701	353	24	340	96	26	313	89	21	325	92	31
Mean for Weeks											
1–13	208		206	99		202	97		200	96	
14–52	275		272	99		262	95		260	95	
53–101	328		323	98		303	92		310	95	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the uterus, skin, small intestine (duodenum and jejunum), mesenteric lymph node, liver, thyroid gland, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Uterus: In the standard evaluation, the incidences of adenocarcinoma occurred with a positive trend and were increased in all dosed groups (Table 9, Table B-1, and Table B-2). Although the increased incidences of adenocarcinoma were not statistically significant, the incidences in the 150 and 300 mg/kg groups exceeded the historical control range for corn oil gavage studies (Table 9 and Table B-3). In the extended evaluation, additional neoplasms and nonneoplastic proliferative lesions were diagnosed in the uteri of the vehicle control and dosed groups. In addition, previously undiagnosed types of neoplasms and nonneoplastic proliferative lesions were diagnosed in the uteri of the vehicle control and dosed groups including atypical hyperplasias, low incidences of adenomas in each of the dosed groups, and one adenosquamous carcinoma and one benign basosquamous tumor each in the 150 mg/kg group (Table 9 and Table B-2).

In the combined standard and extended evaluations, the incidence of adenocarcinoma was significantly increased in the 150 mg/kg group (Table 9). The incidences of adenoma were not significantly increased in the dosed groups; adenomas have not been observed in vehicle controls in gavage studies using female Harlan Sprague Dawley rats.

In the standard evaluation, a significantly increased incidence of squamous metaplasia of the endometrium occurred in the 150 mg/kg group (Table 9 and Table B-5). In the combined standard and extended evaluations, the incidence of squamous metaplasia of the endometrium was significantly decreased in the 300 mg/kg group (Table 9).

Uterine adenocarcinomas were large irregular, invasive masses that effaced the endometrium and commonly extended into the myometrium. They were composed of cuboidal to columnar neoplastic cells that formed irregular tubules, glandular structures or ducts that were surrounded by collagenous stroma. The neoplastic cells had scant eosinophilic cytoplasm and large vesicular nuclei and there was mild to marked pleomorphism and atypia. The neoplasm often contained areas of inflammation, hemorrhage, and/or necrosis (Figure 9 and Figure 10).

Uterine adenomas were solitary, non-invasive, nodular masses that were generally composed of a single layer of cuboidal to columnar epithelial cells typically arranged in glandular structures in which there was minimal to mild stratification or disorganized piling up of the epithelium (Figure 11 and Figure 12). The glandular structures were sometimes dilated and the neoplastic cells formed papillary infoldings or papillary projections into the lumen.

Atypical endometrial hyperplasia was observed in the luminal or glandular epithelium of the uterus. Epithelial atypia in the luminal epithelium consisted of a spectrum of changes that included increased cellular anisocytosis and anisokaryosis and increased cytoplasmic eosinophilia, epithelial blebbing, large cytoplasmic vacuoles and the presence of small papillary

projections lined by disorganized epithelial cells. Occasionally, small branching papillary projections were characterized by epithelial lined, short, slender, fibrovascular stalks that extended into the uterine lumen (Figure 13). Epithelial atypia in the glands affected single glands or clusters that were separated by minimal amounts of stroma. The glands were lined by pseudostratified to stratified, cuboidal to tall columnar epithelial cells that exhibited cytoplasmic eosinophilia or basophilia, loss of cellular polarity, karyomegaly, mitoses, and minimal to mild cellular pleomorphism (Figure 14).

Squamous metaplasia of the endometrium was of minimal to moderate severity and consisted of replacement of the normal simple columnar epithelium lining the endometrium (uterine lumen) and occasional endometrial glands by keratinized squamous epithelium. In more severe cases, the uterine lumen was dilated and filled with varying amounts of keratin.

Table 9. Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Standard Evaluation				
Number Necropsied	50	50	50	50
Endometrium, Metaplasia, Squamous[a]	12 (1.9)[b]	18 (2.2)	20* (2.1)	11 (1.8)
Adenocarcinoma, Multiple	0	0	0	1
Adenocarcinoma (includes multiple)[c]				
Overall rate[d]	0/50 (0%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate[e]	0.0%	2.7%	10.9%	9.8%
Terminal rate[f]	0/21 (0%)	0/19 (0%)	1/19 (5%)	2/30 (7%)
First incidence (days)	–[h]	645	608	645
Poly-3 test[g]	P = 0.040	P = 0.503	P = 0.059	P = 0.074
Extended Evaluation				
Number Necropsied	50	50	50	50
Endometrium, Metaplasia, Squamous	38	33	35	27**
Endometrium, Hyperplasia, Atypical	15	14	21	16
Adenoma	0	2	1	1
Basosquamous Tumor, Benign	0	0	1	0
Adenocarcinoma, Multiple	1	0	3	2
Adenocarcinoma (includes multiple)				
Overall rate	5/50 (10%)	3/50 (6%)	10/50 (20%)	8/50 (16%)
Adjusted rate	13.7%	8.2%	26.9%	19.8%
Terminal rate	3/21 (14%)	2/19 (11%)	5/19 (26%)	7/30 (23%)
First incidence (days)	719	719	615	674
Poly-3 test	P = 0.158	P = 0.351N	P = 0.127	P = 0.340

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Adenoma or Adenocarcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	11/50 (22%)	9/50 (18%)
Adjusted rate	13.7%	13.5%	29.6%	22.3%
Terminal rate	3/21 (14%)	3/19 (16%)	6/19 (32%)	8/30 (27%)
First incidence (days)	719	645	615	674
Poly-3 test	P = 0.146	P = 0.624N	P = 0.080	P = 0.247
Adenosquamous Carcinoma	0	0	1	0
Standard and Extended Evaluations (Combined)				
Number Necropsied	50	50	50	50
Endometrium, Metaplasia, Squamous	38	33	36	27**
Endometrium, Hyperplasia, Atypical	15	14	21	16
Adenoma	0	2	1	1
Basosquamous Tumor, Benign	0	0	1	0
Adenocarcinoma, Multiple	1	0	3	2
Adenocarcinoma (includes multiple)	5	4	13*	10
Adenoma or Adenocarcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	14/50 (28%)	11/50 (22%)
Adjusted rate	13.7%	13.5%	36.6%	27.0%
Terminal rate	3/21 (14%)	3/19 (16%)	6/19 (32%)	9/30 (30%)
First incidence (days)	719	645	608	645
Poly-3 test	P = 0.052	P = 0.624N	P = 0.018	P = 0.119
Adenosquamous Carcinoma	0	0	1	0
Adenocarcinoma or Adenosquamous Carcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	13/50 (26%)	10/50 (20%)
Adjusted rate	13.7%	10.8%	34.0%	24.6%
Terminal rate	3/21 (14%)	2/19 (11%)	5/19 (26%)	8/30 (27%)
First incidence (days)	719	645	608	645
Poly-3 test	P = 0.071	P = 0.491N	P = 0.033	P = 0.177

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 2/102.
[d]Number of animals with neoplasm per number of animals necropsied.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal euthanasia.
[g]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A lower incidence in a dose group is indicated by **N**.
[h]Not applicable; no neoplasms in animal group.

Skin: An increased incidence of fibroma occurred in 300 mg/kg females (Table 10, Table B-1, and Table B-2). Although the increased incidence of this neoplasm was not statistically significant, the incidence in 300 mg/kg females exceeded the historical control range for corn oil gavage studies (Table 10 and Table B-4). A single incidence each of fibrosarcoma occurred in vehicle control and 300 mg/kg females (Table 10 and Table B-1). The incidences of fibroma or fibrosarcoma (combined) occurred with a positive trend in females (Table 10 and Table B-2). Fibromas were circumscribed subcutaneous masses composed of well-differentiated fibrocytes interspersed between variable amounts of mature collagen stroma. Fibrosarcomas were large, invasive, masses that effaced the subcutaneous tissues and consisted of moderately well-differentiated neoplastic fibroblasts arranged in interlacing bundles and whorls.

Table 10. Incidences of Neoplasms of the Skin in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Number Necropsied	50	50	50	50
Fibroma[a,b]	1	0	0	4
Fibrosarcoma[c]	1	0	0	1
Fibroma or Fibrosarcoma[d]				
Overall rate[e]	2/50 (4%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rate[f]	5.4%	0.0%	0.0%	12.4%
Terminal rate[g]	1/21 (5%)	0/19 (0%)	0/19 (0%)	3/30 (10%)
First incidence (days)	645	–[i]	–	692
Poly-3 test[h]	P = 0.040	P = 0.237N	P = 0.243N	P = 0.254

[a]Number of animals with lesion.
[b]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 3/102.
[c]Historical incidence for corn oil gavage studies: 1/102.
[d]Historical incidence for corn oil gavage studies: 4/102.
[e]Number of animals with neoplasm per number of animals necropsied.
[f]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[g]Observed incidence at terminal euthanasia.
[h]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A lower incidence in a dose group is indicated by **N**.
[i]Not applicable; no neoplasms in animal group.

Small Intestine: Significantly increased incidences of lymphatic ectasia of the duodenum and jejunum with generally increased severities occurred in 150 and 300 mg/kg males and females; two incidences of lymphatic ectasia occurred in the jejunum of 75 mg/kg males (Table 11, Table A-3, and Table B-5). Lymphatic ectasia did not occur in vehicle control animals.

Lymphatic ectasia was of minimal to marked severity and characterized by dilation of lymphatic vessels in villi of the duodenum and jejunum (Figure 15 and Figure 16). The dilated lymphatics varied in size and number, and were lined by flattened endothelial cells. The lumens of the dilated lymphatics were generally empty, but a few lymphatics contained minimal amounts of lacy, amphophilic material. In general, the jejunum was more frequently and severely affected than the duodenum.

Mesenteric Lymph Node: Significantly increased incidences of lymphatic ectasia occurred in 300 mg/kg males and females (Table 11, Table A-3, and Table B-5). Single incidences of lymphatic ectasia occurred in a 150 mg/kg male and in a 150 mg/kg female. Lymphatic ectasia did not occur in vehicle control animals.

Lymphatic ectasia was of minimal to moderate severity and characterized by dilation of lymphatic vessels primarily in subcapsular and cortical regions of the nodes, but occasionally the paracortical and medullary lymphatics were affected. These dilated lymphatics varied in size and number, and were lined by flattened endothelial cells. In general, females were more frequently and severely affected than males (Figure 17 and Figure 18).

Liver: Incidences of cholangiofibrosis occurred in all dosed groups of males and in one 75 mg/kg female (Table 11, Table A-3, and Table B-5). The incidences of eosinophilic focus in 150 and 300 mg/kg females and of clear cell focus in 300 mg/kg females were significantly increased compared to the vehicle controls. The incidence of bile duct cyst was significantly increased in 300 mg/kg males.

Table 11. Incidences of Selected Nonneoplastic Lesions in Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
Intestine Small, Duodenum[a]	43	48	47	48
Lymphatic, Ectasia[b]	0	0	15** (1.5)[c]	14** (1.4)
Intestine Small, Jejunum	40	39	40	42
Lymphatic, Ectasia	0	2 (1.0)	27** (1.7)	41** (2.0)
Lymph Node, Mesenteric	50	50	50	50
Lymphatic, Ectasia	0	0	1 (3.0)	5* (1.4)
Liver	50	50	50	50
Cholangiofibrosis	0	1 (2.0)	3 (2.7)	1 (2.0)
Bile Duct, Cyst	0	0	2 (2.0)	5* (2.4)
Thyroid Gland	50	46	48	47
Follicular Cell, Hypertrophy	21 (1.8)	34** (1.9)	33** (2.1)	36** (2.6)
Female				
Intestine Small, Duodenum	48	47	48	47
Lymphatic, Ectasia	0	0	16** (1.2)	38** (1.5)
Intestine Small, Jejunum	47	46	48	48
Lymphatic, Ectasia	0	0	30** (1.7)	47** (2.5)
Lymph Node, Mesenteric	50	50	50	48
Lymphatic, Ectasia	0	0	1 (1.0)	15** (1.7)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Liver	50	50	50	48
Cholangiofibrosis	0	1 (1.0)	0	0
Clear Cell Focus	6	7	4	18**
Eosinophilic Focus	0	4	5*	6*

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with tissue examined microscopically.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Cholangiofibrosis was of minimal to marked severity and consisted of sharply demarcated, non-encapsulated, focal to locally extensive lesions that effaced the hepatic parenchyma. Frequently, cholangiofibrosis occurred as nodular lesions that protruded from the capsular surface of the liver. At necropsy, increased incidences of liver masses and cysts were grossly observed in dosed groups of males and females compared to the vehicle controls. Parenchymal lesions consisted of variable amounts of dense collagenous connective tissue that surrounded multiple, variably sized, randomly distributed, dilated, atypical bile ducts that invariably contained eosinophilic to amphophilic, mucinous material and cellular debris (Figure 19 and Figure 20). The atypical ducts were lined by low cuboidal to columnar hyperbasophilic epithelial cells among which were scattered goblet-like or mucous cells. At times, the epithelium lining the bile ducts was absent, resulting in crescent-shaped structures. Moderate numbers of inflammatory cells were scattered through the collagenous tissue. Capsular lesions were encapsulated and primarily composed of dilated atypical ducts surrounded by lesser amounts of collagenous tissue than in lesions within the hepatic parenchyma (Figure 21 and Figure 22).

Eosinophilic foci were variably-sized discrete focal areas of enlarged hepatocytes in which the cytoplasm stained more lightly or darkly eosinophilic than that of the surrounding hepatocytes; in some cases the cytoplasm appeared granular (Figure 23). Clear cell foci were discrete focal areas of normal-sized to slightly enlarged hepatocytes that had clear cytoplasmic spaces around the cell nuclei (Figure 24).

Bile duct cysts were single to multiple, variably dilated bile ducts that disrupted the hepatic parenchyma. The dilated ducts were lined by flattened to cuboidal epithelial cells and occasionally contained fibrillar eosinophilic material.

Transcriptome analysis was performed on RNA extracted from microarray study female rat livers from the 300 mg/kg and vehicle control groups after 3 months of exposure. The observed effects on transcription were consistent with induction of xenobiotic metabolism related processes that are initiated through the activation of the aryl hydrocarbon receptor and oxidative stress sensing transcription factor nuclear factor, erythroid 2-like 2. A detailed breakdown of the transcriptomic results can be found in Appendix L.

Thyroid Gland: The incidences of follicular cell hypertrophy were significantly increased in all dosed groups of males compared to the vehicle controls, and the severities of the hypertrophy increased with increasing dose (Table 11 and Table A-3).

Follicular cell hypertrophy was characterized by an increased height of follicular epithelial cells from normal cuboidal to tall columnar and a concomitant decrease in the diameter of the follicular lumens. In general, follicles contained decreased amounts of colloid and often contained aggregates of flocculent-appearing, amphophilic material. The following criteria were used when diagnosing follicular cell hypertrophy: minimal—30% to 40% of follicles affected; mild—40% to 60% of follicles affected; moderate—60% to 80% of follicles affected; marked—greater than 80% of follicles affected.

Pituitary Gland: Incidences of adenoma occurred in all groups of males and females and the incidence of this neoplasm was significantly decreased in 300 mg/kg females (males: vehicle control, 5/50; 75 mg/kg, 8/49; 150 mg/kg, 9/50; 300 mg/kg, 8/47; females: vehicle control, 18/50, 75 mg/kg, 18/50, 150 mg/kg, 19/50, 300 mg/kg, 8/49) (Table A-1, Table A-2, Table B-1, and Table B-2).

Three-month Study in Mice

All mice survived to the end of the study (Table 12). The final mean body weights and mean body weight gains of dosed groups of males and females were similar to those of the vehicle controls (Table 12; Figure 6). There were no clinical findings related to administration of indole-3-carbinol.

There were no changes in the hematology data of mice that were considered attributable to indole-3-carbinol administration (Table F-2).

Table 12. Survival and Body Weights of Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.6 ± 0.2	40.4 ± 0.6	16.8 ± 0.6	
15.6	10/10	23.6 ± 0.2	40.5 ± 1.1	17.0 ± 1.1	100
31.25	10/10	23.6 ± 0.2	41.5 ± 0.9	17.9 ± 0.8	103
62.5	10/10	23.6 ± 0.1	40.0 ± 1.1	16.4 ± 1.2	99
125	10/10	23.6 ± 0.2	42.2 ± 1.6	18.6 ± 1.6	104
250	10/10	23.7 ± 0.3	41.0 ± 0.9	17.2 ± 0.8	101
Female					
0	10/10	19.5 ± 0.2	34.2 ± 1.0	14.6 ± 1.0	
15.6	10/10	19.5 ± 0.3	33.5 ± 1.0	14.0 ± 0.9	98
31.25	10/10	19.5 ± 0.3	33.8 ± 1.6	14.3 ± 1.7	99
62.5	10/10	19.4 ± 0.3	33.7 ± 0.6	14.3 ± 0.5	99
125	10/10	19.4 ± 0.3	33.1 ± 0.7	13.7 ± 0.6	97
250	10/10	19.4 ± 0.4	31.4 ± 1.3	12.1 ± 1.3	92

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 14 weeks/number initially in group.

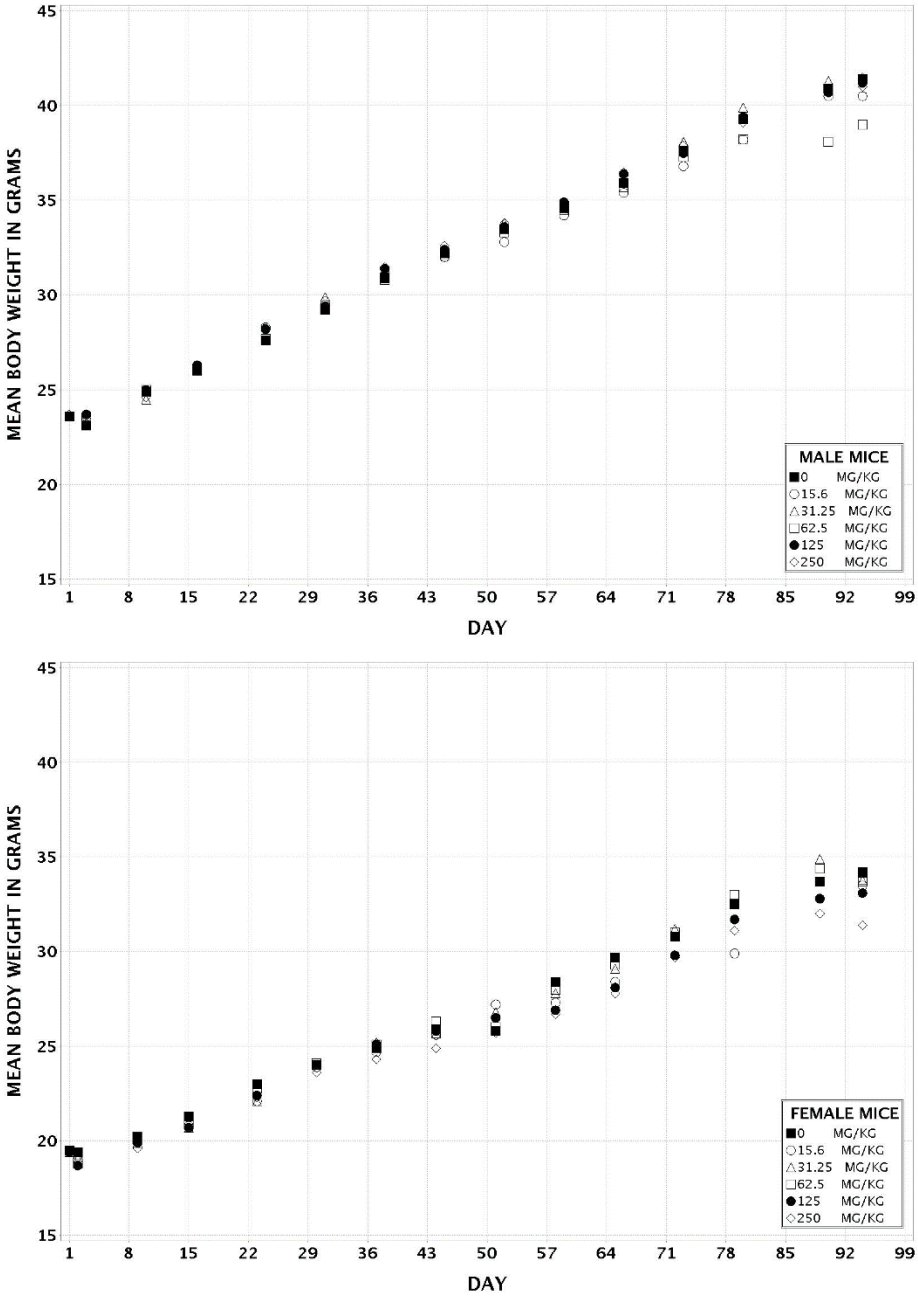

Figure 6. Growth Curves for Mice Administered Indole-3-carbinol by Gavage for Three Months

The absolute and relative liver weights of 125 and 250 mg/kg males and all dosed groups of females were significantly increased compared to the vehicle controls (Table 13 and Table G-2).

Liver and lung samples were collected for determinations of P450 enzyme activities (Table 14). Microsomal suspensions were prepared from liver samples and were assayed for 7-ethoxyresorufin-*O*-deethylase (EROD) activity (a marker for CYP1A1 activity) and acetanilide-4-hydroxylase (A4H) activity (a marker for CYP1A2 activity). Microsomal samples from lung were analyzed for EROD activity only. In the liver, there were significant and dose-dependent increases in A4H activities in all dosed groups of male mice. Hepatic EROD activities were

significantly increased in males administered 31.25 mg/kg or greater. Hepatic A4H and EROD activities were significantly increased in 125 and 250 mg/kg females. Maximal inductions of A4H and EROD activities in males were nearly 4-fold and 3-fold, respectively, compared to the vehicle controls. In females, A4H and EROD activities were maximally increased more than 2-fold and 3-fold, respectively. There were no treatment-related effects on pulmonary EROD activities in males or females.

Table 13. Liver Weights and Liver-to Body-Weight Ratios for Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	15.6 mg/kg	31.25 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	40.4 ± 0.6	40.5 ± 1.1	41.5 ± 0.9	40.0 ± 1.1	42.2 ± 1.6	41.0 ± 0.9
Liver						
Absolute	1.50 ± 0.06	1.46 ± 0.07	1.54 ± 0.05	1.54 ± 0.05	1.77 ± 0.13*	1.82 ± 0.06**
Relative	37.329 ± 1.896	35.868 ± 0.799	37.166 ± 0.702	38.520 ± 0.632	41.960 ± 2.146*	44.333 ± 0.817**
Female						
Necropsy body wt	34.2 ± 1.0	33.5 ± 1.0	33.8 ± 1.6	33.7 ± 0.6	33.1 ± 0.7	31.4 ± 1.3
Liver						
Absolute	1.04 ± 0.01	1.21 ± 0.05*	1.11 ± 0.04*	1.16 ± 0.03*	1.22 ± 0.03**	1.21 ± 0.04**
Relative	30.559 ± 0.696	36.190 ± 1.253**	32.906 ± 0.651**	34.449 ± 0.693**	36.878 ± 0.867**	38.687 ± 0.778**

*Significantly different ($P \leq 0.05$) from the vehicle control group by William's or Dunnett's test.
**$P \leq 0.01$.
[a]Liver weights (absolute weights) and body weights are given in grams; liver-weight-to-body-weight ratios (relative weights) are given as mg liver weight/g body weight (mean ± standard error).

Table 14. Liver and Lung Cytochrome P450 Data for Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	15.6 mg/kg	31.25 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	10	10	10
Male						
Liver Microsomes						
A4H (nmol/minute per mg microsomal protein)						
	0.335 ± 0.039	0.449 ± 0.011**	0.515 ± 0.024**	0.984 ± 0.037**	1.124 ± 0.087**	1.293 ± 0.109**
EROD (pmol/minute per mg microsomal protein)						
	16.7 ± 2.3	20.2 ± 1.1	24.5 ± 3.1*	36.2 ± 3.5**	45.4 ± 8.4**	43.0 ± 10.4**
Lung Microsomes						
EROD (pmol/minute per mg microsomal protein)						
	0.112 ± 0.062	0.048 ± 0.032	0.011 ± 0.011	0.000 ± 0.000	0.000 ± 0.000	0.010 ± 0.010
Female						
Liver Microsomes						
A4H (nmol/minute per mg microsomal protein)						
	0.601 ± 0.057	0.534 ± 0.063	0.635 ± 0.036	0.620 ± 0.059	1.094 ± 0.055**	1.450 ± 0.085**
EROD (pmol/minute per mg microsomal protein)						
	14.3 ± 1.0	11.4 ± 2.2	18.8 ± 1.3	12.2 ± 2.5	28.1 ± 2.5**	46.1 ± 3.8**
Lung Microsomes						
EROD (pmol/minute per mg microsomal protein)						
	0.005 ± 0.005	0.011 ± 0.011	0.059 ± 0.059	0.000 ± 0.000	0.075 ± 0.056	0.139 ± 0.071

*Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. A4H = acetanilide-4-hydroxylase; EROD = 7-ethoxyresorufin-*O*-deethylase.

Sperm motility was significantly decreased in all dosed groups of males (Table H-4). The Markov transition matrix analyses of estrous cyclicity indicated that females in the 250 mg/kg group had a significantly higher probability of extended diestrus than the vehicle control females (Table H-5 and Table H-6; Figure H-2). Based on these results, indole-3-carbinol did exhibit the potential to be a reproductive toxicant in male and female mice.

No chemical-related histopathologic lesions were observed that could be attributed to the administration of indole-3-carbinol.

Dose Selection Rationale: There were no chemical-related effects on mortality, body weights, or lesion incidences in the 3-month study in mice. Chemical-related increases in liver weights were observed, and were consistent with increased hepatic cytochrome P450 activities. In 250 mg/kg females, there was an 8% decrease in final body weight and a slightly lower overall body weight gain. These effects were not considered sufficient to exclude 250 mg/kg as the highest dose in the 2-year study. However, studies in the literature at doses of 500 mg/kg have demonstrated treatment-related effects on survival and neurotoxicity; as a result, higher doses were not

considered for the 2-year study. The doses selected for the 2-year gavage study in B6C3F1/N mice were 62.5, 125, and 250 mg/kg.

Two-year Study in Mice

Survival

Estimates of 2-year survival probabilities for male and female mice shown in Table 15 and in the Kaplan-Meier survival curves (Figure 7). Survival of 250 mg/kg females was significantly greater than that of the vehicle controls; survival of dosed groups of males was similar to that of the vehicle control group.

Body Weights and Clinical Findings

Mean body weights of dosed groups of male mice were similar to those of the vehicle controls throughout the study; however, those of 250 mg/kg female mice were at least 10% less than those of the vehicle controls between weeks 32 and 92 (Table 16 and Table 17; Figure 8). No clinical findings related to the administration of indole-3-carbinol were observed in males or females.

Table 15. Survival of Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	0	1	1
Moribund	17	12	11	9
Natural deaths	6	7	6	8
Animals surviving to study termination	27[e]	31	32	32
Percent probability of survival at end of study[b]	52	62	65	65
Mean survival (days)[c]	663	700	686	688
Survival analysis[d]	P = 0.202N	P = 0.256N	P = 0.182N	P = 0.192N
Female				
Animals initially in study	50	50	50	50
Accidental death[a]	1	0	0	0
Moribund	10	6	15	3
Natural deaths	6	4	9	2
Animals surviving to study termination	33	40	26[e]	45
Percent probability of survival at end of study	67	80	52	90
Mean survival (days)	702	704	672	713
Survival analysis	P = 0.099N	P = 0.263N	P = 0.082	P = 0.016N

[a]Censored in the survival analyses.
[b]Kaplan-Meier determinations.
[c]Mean of all deaths (uncensored, censored, and terminal euthanasia).
[d]The result of the life table trend test[91] is in the vehicle control column, and the results of the life table pairwise comparisons[90] with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[e]Includes one animal that died during the last week of the study.

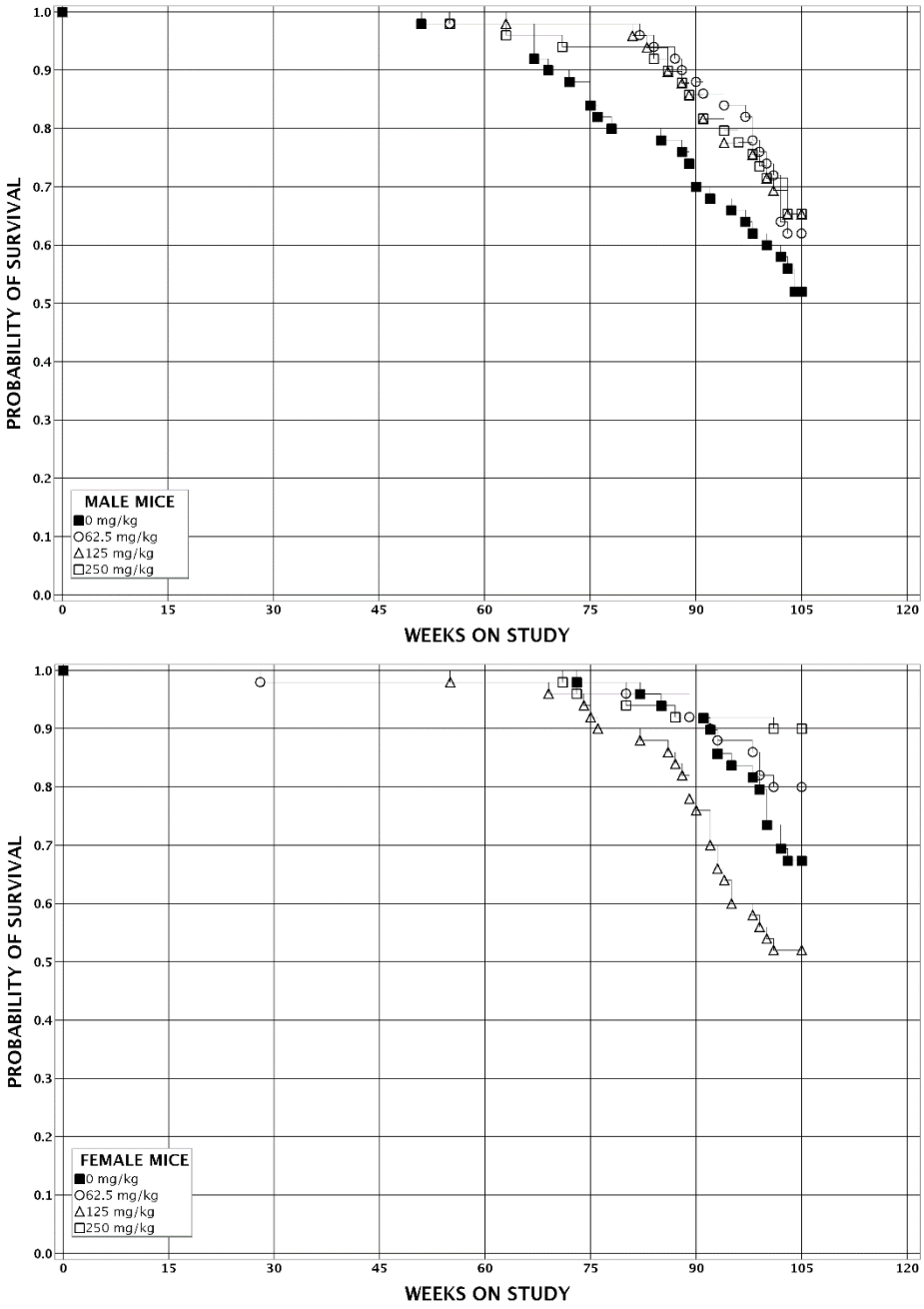

Figure 7. Kaplan-Meier Survival Curves for Mice Administered Indole-3-carbinol by Gavage for Two Years

Table 16. Mean Body Weights and Survival of Male Mice in the Two-year Gavage Study of Indole-3-carbinol

Day	Vehicle Control		62.5 mg/kg			125 mg/kg			250 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	22.8	50	22.9	101	50	22.8	100	50	22.8	100	50
8	23.9	50	24.1	101	50	24.1	101	50	24.1	101	50
15	24.7	50	25.0	101	50	24.8	101	50	24.9	101	50
22	26.4	50	26.7	101	50	26.6	101	50	26.5	101	50
29	27.0	50	27.7	103	50	27.4	101	50	27.3	101	50
36	28.8	50	29.5	103	50	29.3	102	50	28.9	100	50
43	30.0	50	30.7	102	50	30.2	101	50	29.7	99	50
50	31.7	50	32.2	101	50	31.6	100	50	31.1	98	50
57	32.9	50	33.8	103	50	33.1	101	50	32.7	99	50
64	33.7	50	34.7	103	50	33.5	100	50	33.3	99	50
71	34.5	50	35.6	103	50	34.5	100	50	34.1	99	50
78	36.4	50	37.7	104	50	36.4	100	50	36.1	99	50
85	36.4	50	37.7	103	50	36.5	100	50	36.2	99	50
113	39.8	50	42.1	106	50	40.8	103	50	40.5	102	50
141	43.6	50	45.5	104	50	44.1	101	49	43.2	99	50
169	46.1	50	48.0	104	50	47.1	102	49	45.8	99	50
197	47.8	50	48.9	102	50	48.9	102	49	46.8	98	50
225	50.8	50	51.9	102	50	51.9	102	49	49.7	98	50
253	51.4	50	52.1	101	50	52.4	102	49	50.3	98	50
281	51.9	50	52.6	101	50	52.9	102	49	51.5	99	50
309	53.8	50	54.3	101	50	54.6	102	49	52.8	98	50
337	54.0	50	54.5	101	50	54.6	101	49	52.7	98	50
365	54.5	49	55.2	101	50	55.3	101	49	53.8	99	50
393	54.5	49	55.9	103	49	55.4	102	49	54.6	100	49
421	55.2	49	56.4	102	49	55.7	101	49	55.0	100	49
449	55.6	49	56.6	102	49	55.9	101	48	55.6	100	48
477	55.5	46	57.3	103	49	56.1	101	48	55.7	100	48
505	55.6	44	57.1	103	49	55.5	100	48	56.0	101	47
533	55.4	41	57.5	104	49	55.2	100	48	56.4	102	46
561	54.7	40	56.5	103	49	53.8	98	48	55.6	102	46
589	54.5	39	56.8	104	47	53.7	99	46	55.6	102	45
617	54.6	38	55.9	102	45	53.1	97	42	55.0	101	42
645	55.0	34	54.8	100	43	52.9	96	40	53.8	98	40
673	54.4	32	53.8	99	41	53.0	98	38	52.5	97	38
701	53.3	30	53.9	101	36	52.9	99	35	50.9	96	35
Mean for Weeks											
1–13	29.9		30.6	102		30.1	101		29.8	100	
14–52	48.8		50.0	102		49.7	102		48.1	99	
53–101	54.8		56.0	102		54.5	99		54.7	100	

Table 17. Mean Body Weights and Survival of Female Mice in the Two-year Gavage Study of Indole-3-carbinol

Day	Vehicle Control		62.5 mg/kg			125 mg/kg			250 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	18.6	50	18.6	100	50	18.5	99	50	18.8	101	50
8	19.3	50	19.1	99	50	19.2	100	50	19.3	100	50
15	20.3	50	20.0	99	50	20.1	99	50	20.3	100	50
22	21.6	50	21.0	97	50	21.2	98	50	21.4	99	50
29	22.5	50	21.9	98	50	22.2	99	50	22.0	98	50
36	23.6	50	22.8	97	50	23.4	99	50	23.2	98	50
43	24.8	50	23.9	97	50	24.8	100	50	24.6	99	50
50	25.5	50	24.7	97	50	25.6	101	50	25.1	99	50
57	26.5	50	25.9	98	50	26.8	101	50	25.9	98	50
64	28.3	50	27.5	97	50	28.5	101	50	27.4	97	50
71	29.0	50	28.8	99	50	29.7	103	50	28.6	99	50
78	29.8	50	29.0	98	50	30.8	103	50	29.7	100	50
85	30.0	50	30.1	100	50	31.2	104	50	30.3	101	50
113	35.0	50	33.8	96	50	36.3	104	50	34.4	98	50
141	37.7	50	36.2	96	50	39.0	103	50	36.9	98	50
169	40.3	50	38.6	96	50	41.6	103	50	39.2	97	50
197	44.4	50	41.7	94	49	44.2	99	50	40.9	92	50
225	48.1	50	45.2	94	49	47.7	99	50	42.5	88	50
253	50.9	50	47.8	94	49	50.0	98	50	43.7	86	50
281	51.8	50	48.1	93	49	51.4	99	50	44.4	86	50
309	56.9	50	53.0	93	49	56.3	99	50	47.5	84	50
337	59.1	50	54.9	93	49	57.6	97	50	48.1	81	50
365	61.9	50	57.5	93	49	59.6	96	50	49.0	79	50
393	63.4	50	59.0	93	49	61.0	96	49	50.4	80	50
421	63.0	50	59.7	95	49	61.5	98	49	50.8	81	50
449	65.2	50	62.3	96	49	63.4	97	49	53.0	81	50
477	65.2	50	62.6	96	49	63.2	97	49	53.8	83	50
505	65.3	50	63.5	97	49	64.0	98	48	54.9	84	48
533	65.8	49	63.8	97	49	63.6	97	45	55.7	85	48
561	66.6	49	63.7	96	48	63.8	96	45	55.7	84	47
589	66.3	46	64.1	97	48	62.5	94	44	55.7	84	47
617	65.0	46	62.9	97	48	62.0	95	41	56.3	87	46
645	62.6	44	61.8	99	45	61.0	97	34	55.8	89	46
673	61.1	41	60.9	100	44	59.2	97	30	55.5	91	46
701	59.2	36	60.9	103	40	58.4	99	27	55.5	94	46
Mean for Weeks											
1–13	24.6		24.1	98		24.8	101		24.4	99	
14–52	47.1		44.4	94		47.1	100		42.0	89	
53–101	63.9		61.7	97		61.8	97		54.0	85	

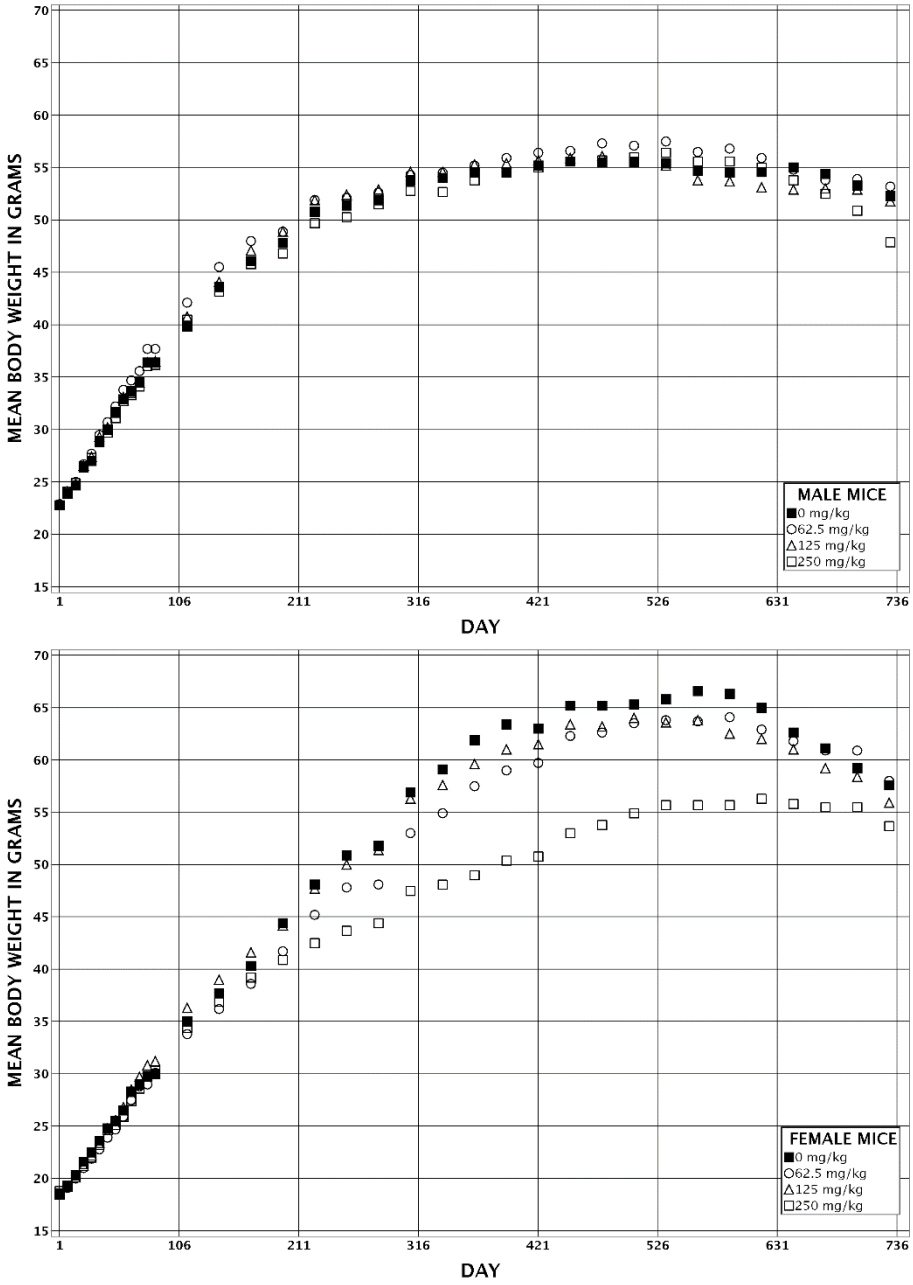

Figure 8. Growth Curves for Mice Administered Indole-3-carbinol by Gavage for Two Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, glandular stomach, and nose. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: Incidences of hepatocellular adenoma occurred with a positive trend in males and the incidence of this neoplasm was significantly increased in the 250 mg/kg group (Table 18, Table C-1, and Table C-2). In addition, the incidences of multiple hepatocellular adenoma were significantly increased in 62.5 and 250 mg/kg males.

Significantly increased incidences of hepatocellular carcinoma and multiple hepatocellular carcinoma occurred in 125 mg/kg males (Table 18, Table C-1, and Table C-2).

Incidences of hepatoblastoma occurred with a positive trend in males; the incidence of this neoplasm was significantly increased in the 250 mg/kg group and exceeded the historical control ranges for corn oil gavage studies and for all routes combined (Table 18, Table C-1, Table C-2, and Table C-3). In males, the incidences of multiple hepatoblastoma increased with increasing dose and the incidence of multiple hepatoblastoma was significantly increased in the 250 mg/kg group. One 125 mg/kg female and one 250 mg/kg female had a single hepatoblastoma (Table 18 and Table D-1).

Table 18. Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Male				
Number Examined Microscopically	50	50	49	50
Clear Cell Focus[a]	7	17*	22**	20**
Hepatocellular Adenoma, Multiple	15	25*	16	33**
Hepatocellular Adenoma (includes multiple)[b]				
Overall rate[c]	26/50 (52%)	32/50 (64%)	31/49 (63%)	41/50 (82%)
Adjusted rate[d]	62.1%	66.5%	67.9%	85.4%
Terminal rate[e]	19/26 (73%)	21/31 (68%)	23/32 (72%)	28/32 (88%)
First incidence (days)	463	381	437	385
Poly-3 test[f]	P = 0.005	P = 0.411	P = 0.360	P = 0.006
Hepatocellular Carcinoma, Multiple	5	3	18**	8
Hepatocellular Carcinoma (includes multiple)[g]				
Overall rate	12/50 (24%)	11/50 (22%)	29/49 (59%)	14/50 (28%)
Adjusted rate	27.7%	24.2%	63.4%	30.9%
Terminal rate	3/26 (12%)	8/31 (26%)	18/32 (56%)	8/32 (25%)
First incidence (days)	465	682	567	385

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Poly-3 test	P = 0.217	P = 0.449N	P < 0.001	P = 0.461
Hepatocellular Adenoma or Carcinoma[h]				
Overall rate	35/50 (70%)	36/50 (72%)	43/49 (88%)	44/50 (88%)
Adjusted rate	77.6%	74.6%	90.6%	91.1%
Terminal rate	21/26 (81%)	24/31 (77%)	29/32 (91%)	30/32 (94%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.012	P = 0.459N	P = 0.062	P = 0.049
Hepatoblastoma, Multiple	0	1	3	7*
Hepatoblastoma (Includes Multiple)[i]				
Overall rate	3/50 (6%)	4/50 (8%)	4/49 (8%)	14/50 (28%)
Adjusted rate	7.6%	8.9%	9.3%	31.6%
Terminal rate	3/26 (12%)	4/31 (13%)	3/32 (9%)	10/32 (31%)
First incidence (days)	730 (T)	730 (T)	702	617
Poly-3 test	P < 0.001	P = 0.567	P = 0.543	P = 0.005
Hepatocellular Carcinoma or Hepatoblastoma[j]				
Overall rate	15/50 (30%)	13/50 (26%)	30/49 (61%)	25/50 (50%)
Adjusted rate	34.6%	28.7%	65.6%	54.5%
Terminal rate	6/26 (23%)	10/31 (32%)	19/32 (59%)	17/32 (53%)
First incidence (days)	465	682	567	385
Poly-3 test	P = 0.005	P = 0.353N	P = 0.002	P = 0.042
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[k]				
Overall rate	36/50 (72%)	36/50 (72%)	44/49 (90%)	45/50 (90%)
Adjusted rate	79.8%	74.6%	92.7%	92.8%
Terminal rate	22/26 (85%)	24/31 (77%)	30/32 (94%)	30/32 (94%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.007	P = 0.354N	P = 0.050	P = 0.044
Hepatocholangiocarcinoma[l]				
Overall rate	0/50 (0%)	3/50 (6%)	1/49 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.6%	2.3%	0.0%
Terminal rate	0/26 (0%)	0/31 (0%)	1/32 (3%)	0/32 (0%)
First incidence (days)	–[m]	630	730 (T)	–
Poly-3 test	P = 0.319N	P = 0.146	P = 0.515	–[n]
Female				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus	16	26*	26*	21
Hepatocellular Adenoma, Multiple	0	3	2	4
Hepatocellular Adenoma, (includes multiple)[o]				
Overall rate	7/50 (14%)	14/50 (28%)	8/50 (16%)	11/50 (22%)
Adjusted rate	15.2%	30.0%	19.5%	22.9%

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Terminal rate	5/33 (15%)	13/40 (33%)	7/26 (27%)	10/45 (22%)
First incidence (days)	568	687	621	603
Poly-3 test	P = 0.390	P = 0.071	P = 0.405	P = 0.247
Hepatocellular Carcinoma, Multiple	0	2	1	0
Hepatocellular Carcinoma (includes multiple)[p]				
Overall rate	6/50 (12%)	8/50 (16%)	9/50 (18%)	4/50 (8%)
Adjusted rate	13.2%	17.1%	21.5%	8.4%
Terminal rate	5/33 (15%)	7/40 (18%)	5/26 (19%)	4/45 (9%)
First incidence (days)	709	650	606	729 (T)
Poly-3 test	P = 0.246N	P = 0.409	P = 0.228	P = 0.341N
Hepatoblastoma[q]	0	0	1	1

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
(T)Terminal euthanasia.
[a]Number of animals with lesion.
[b]Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 145/250 (58.0% ± 5.1%), range 52%–64%; all routes: 594/949 (62.6% ± 9.1%), range 48%–78%.
[c]Number of animals with neoplasm per number of animals with liver examined microscopically.
[d]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[e]Observed incidence at terminal euthanasia.
[f]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in a dose group is indicated by **N**.
[g]Historical incidence for corn oil gavage studies: 87/250 (34.8% ± 10.9%), range 22%–44%; all routes: 348/949 (36.7% ± 11.4%), range 22%–56%.
[h]Historical incidence for corn oil gavage studies: 189/250 (75.6% ± 3.3%), range 70%–78%; all routes: 742/949 (78.2% ± 7.2%), range 64%–90%.
[i]Historical incidence for corn oil gavage studies: 9/250 (3.6% ± 2.6%), range 0%–6%; all routes: 40/949 (4.2% ± 3.5%), range 0%–12%.
[j]Historical incidence for corn oil gavage studies: 93/250 (37.2% ± 10.0%), range 24%–48%; all routes: 371/949 (39.1% ± 11.6%), range 22%–58%.
[k]Historical incidence for corn oil gavage studies: 190/250 (76.0% ± 2.5%), range 72%–78%; all routes: 746/949 (78.6% ± 7.2%), range 64%–90%.
[l]Historical incidence for corn oil gavage studies: 4/250 (1.6% ± 3.6%), range 0%–8%; all routes: 10/949 (1.1% ± 2.2%), range 0%–8%.
[m]Not applicable; no neoplasms in animal group.
[n]Value of statistic cannot be computed.
[o]Historical incidence for corn oil gavage studies: 62/250 (24.8% ± 9.6%), range 14%–34%; all routes: 378/948 (39.9% ± 18.7%), range 14%–78%.
[p]Historical incidence for corn oil gavage studies: 26/250 (10.4% ± 5.6%), range 4%–18%; all routes: 152/948 (16.0% ± 10.6%), range 4%–46%.
[q]Historical incidence for corn oil gavage studies: 1/250 (0.4% ± 0.9%), range 0%–2%; all routes: 4/948 (0.4% ± 0.8%), range 0%–2%.

The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma occurred with a positive trend in males and were significantly increased in males administered 125 or 250 mg/kg (Table 18, Table C-1, and Table C-2). The combined incidences of hepatocellular carcinoma or hepatoblastoma were significantly increased in 125 and 250 mg/kg males.

In males, a single hepatocholangioma occurred in the 250 mg/kg group (Table C-1). In addition, three and one hepatocholangiocarcinomas occurred in the 62.5 and 125 mg/kg groups, respectively (Table 18, Table C-1, and Table C-2); these incidences were within the historical control ranges and were not dose-dependent (Table 18, Table C-1, and Table C-2).

The incidences of clear cell focus were significantly increased in all dosed groups of males and the incidences of eosinophilic focus were significantly increased in 62.5 and 125 mg/kg females (Table 18, Table C-4, and Table D-4).

Hepatocellular adenomas were relatively well-circumscribed, nodular masses that compressed the adjacent hepatic parenchyma (Figure 25 and Figure 26). They were composed of well-differentiated hepatocytes that formed irregular hepatic plates that often compressed sinusoids and obliquely impinged on the adjacent parenchyma. In general, there was loss of normal lobular architecture with absence of central veins and portal tracts. The cytoplasm of the neoplastic cells varied tinctorially and often appeared eosinophilic, basophilic, clear, or mixed. When more than one hepatocellular adenoma was observed in an animal, the diagnosis of multiple hepatocellular adenoma was recorded.

Hepatocellular carcinomas were generally locally invasive, compressive, irregularly-shaped masses that effaced the normal hepatic parenchyma. Neoplastic hepatocytes were often pleomorphic and in some areas formed trabeculae three or more neoplastic hepatocytes thick (Figure 27 and Figure 28). Cellular atypia and mitotic figures were frequently observed. When more than one hepatocellular carcinoma was observed in an animal, the diagnosis of multiple hepatocellular carcinoma was recorded.

Hepatoblastomas were well-demarcated, irregular-shaped, hypercellular, deeply basophilic masses that occasionally occurred within hepatocellular carcinomas or adjacent to hepatocellular adenomas or hepatocellular carcinomas (Figure 29 and Figure 30). They were composed of sheets of small, elongate to spindle-shaped cells with hyperchromatic nuclei and scant, deeply basophilic cytoplasm that were separated by scant connective tissue stroma. The cells were sometimes arranged radially around small blood vessels and formed poorly defined rosettes or pseudoglandular structures. When more than one hepatoblastoma was observed in an animal, the diagnosis of multiple hepatoblastoma was recorded; when hepatoblastoma was observed within a hepatocellular carcinoma, only the hepatoblastoma was diagnosed. Hepatocholangiocarcinomas were morphologically similar to hepatocellular carcinomas, but also contained elements of malignant biliary epithelium. The one hepatocholangioma was morphologically similar to a hepatocellular adenoma, but also contained elements of benign neoplastic biliary epithelium.

Clear cell foci consisted of foci of hepatocytes that were of normal size to slightly enlarged containing cytoplasmic clear space and centrally located nuclei. Eosinophilic foci were characterized by focal regions of enlarged hepatocytes with granular, pink cytoplasm.

Glandular Stomach: The incidences of epithelium hyperplasia, chronic inflammation, and pigmentation were significantly increased in 125 and 250 mg/kg males and all dosed groups of females compared to the vehicle controls, and the severities generally increased with increasing dose (Table 19, Table C-4, and Table D-4).

Hyperplasia of the glandular stomach epithelium consisted of multifocal proliferation of the chief cells at the base of the gastric glands in the fundic region of the stomach. This change was subtle

and of minimal severity. Compared to the glandular stomach of the vehicle controls (Figure 31), there was minimal increase in the number and disorganized crowding of the chief cells within the fundic glands; proliferating cells appeared to be slightly more basophilic than those in unaffected fundic glands (Figure 32). Chronic inflammation consisted of small aggregates of macrophages that were randomly distributed among the glands at the base of the mucosa and in the submucosa. The cytoplasm of macrophages contained a lightly staining, globular, golden yellow material presumed to be the test article and diagnosed as pigment. Multifocally, coarse aggregates of golden brown pigment similar to that within the macrophages were randomly distributed within minimally to mildly dilated gastric glands and the lamina propria. Mixed infiltrates of low numbers of lymphocytes and/or neutrophils were sparsely scattered within the lamina propria and submucosa.

Table 19. Incidences of Nonneoplastic Lesions of the Glandular Stomach in Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Male				
Number Examined Microscopically	50	47	47	49
Epithelium, Hyperplasia[a]	0	1 (1.0)[b]	22** (1.2)	40** (1.5)
Inflammation, Chronic	1 (1.0)	1 (1.0)	18** (1.0)	45** (1.0)
Pigmentation	0	1 (1.0)	38** (1.0)	48** (1.1)
Female				
Number Examined Microscopically	48	50	49	50
Epithelium, Hyperplasia	1 (2.0)	7* (1.3)	10** (1.2)	35** (1.4)
Inflammation, Chronic	0	15** (1.0)	29** (1.1)	47** (1.3)
Pigmentation	0	15** (1.0)	31** (1.2)	49** (1.9)

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Nose: Significantly increased incidences of nerve atrophy occurred in 250 mg/kg males and females compared to the vehicle controls (Table 20, Table C-4, and Table D-4). A single incidence of nerve atrophy without statistical significance occurred in a 125 mg/kg female. This lesion was most frequent in females.

Incidences of respiratory metaplasia of the olfactory epithelium were significantly increased in 250 mg/kg males and in 125 and 250 mg/kg females compared to the vehicle controls (Table 20, Table C-4, and Table D-4). The incidences of atrophy of the olfactory epithelium were significantly increased in 125 and 250 mg/kg males and 250 mg/kg females. Slightly increased incidences of mild to moderate olfactory epithelium degeneration occurred in 125 mg/kg males and in 125 and 250 mg/kg females. A significantly increased incidence of mild olfactory epithelium necrosis occurred in the 250 mg/kg males.

The incidences of respiratory epithelium hyaline droplet accumulation were significantly increased in 62.5 and 125 mg/kg males (Table 20 and Table C-4). The incidence of respiratory

epithelium hyaline droplet accumulation was significantly decreased in 62.5 mg/kg females compared to the vehicle controls; however, the incidence and severity increased in 250 mg/kg females (Table 20 and Table D-4).

Significantly increased incidences of respiratory epithelium hyperplasia occurred in 250 mg/kg males and females (Table 20, Table C-4, and Table D-4). The severity was increased in 250 mg/kg females.

The incidence of inflammation was significantly increased in 250 mg/kg females (Table 20 and Table D-4). The incidence of inflammation was also increased in 125 mg/kg females, but not significantly.

Table 20. Incidences of Nonneoplastic Lesions of the Nose in Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Nerve, Atrophy[a]	0	0	0	8** (2.0)
Olfactory Epithelium, Respiratory Metaplasia	14 (1.1)[b]	14 (1.3)	20 (1.5)	27* (1.4)
Olfactory Epithelium, Atrophy	3 (2.0)	5 (1.6)	11* (1.4)	17** (1.5)
Olfactory Epithelium, Degeneration	1 (1.0)	1 (1.0)	4 (1.8)	2 (2.0)
Olfactory Epithelium, Necrosis	0	0	0	6* (2.2)
Respiratory Epithelium, Accumulation, Hyaline Droplet	18 (1.2)	34** (1.1)	30* (1.1)	26 (1.2)
Respiratory Epithelium, Hyperplasia	35 (1.0)	40 (1.2)	41 (1.2)	45* (1.3)
Female				
Number Examined Microscopically	50	50	50	50
Nerve, Atrophy	0	0	1 (2.0)	50** (3.0)
Olfactory Epithelium, Respiratory Metaplasia	7 (1.0)	8 (1.0)	16* (1.0)	49** (2.9)
Olfactory Epithelium, Atrophy	1 (1.0)	2 (1.0)	3 (2.0)	45** (2.0)
Olfactory Epithelium, Degeneration	0	0	2 (2.0)	3 (3.0)
Respiratory Epithelium, Accumulation, Hyaline Droplet	47 (1.4)	38** (1.1)	42 (1.1)	50 (2.4)
Respiratory Epithelium, Hyperplasia	32 (1.0)	31 (1.0)	38 (1.0)	50** (3.0)
Inflammation	4 (1.5)	1 (1.0)	8 (1.1)	39** (1.2)

*Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Atrophy of olfactory epithelium was of minimal to moderate severity primarily affecting segments of the ethmoid turbinates and nasal septum in the olfactory region of the Level III nasal histologic section and occasionally in olfactory epithelium lining the dorsal meatus of the Level II nasal histologic section. In affected segments, the height of the epithelium was noticeably shorter than that of unaffected segments of comparable segments in the vehicle controls (Figure 33 and Figure 34).

Nerve atrophy occurred predominantly in the lamina propria of segments of the turbinates (ethmoid) and nasal septum in the olfactory region of the Level III nasal histologic section and occasionally in olfactory epithelium lining the dorsal meatus of the Level II nasal histologic section. In affected segments, the nerve bundles were smaller in diameter and fewer in number than in comparable sections of the vehicle controls.histologic section. In affected segments, the nerve bundles were smaller in diameter and fewer in number than in comparable sections of the vehicle controls.

Olfactory epithelium respiratory metaplasia was observed frequently and affected the olfactory epithelium lining the nasal septum and ethmoturbinates in Level III, and occasionally the dorsal meatus of Level II, in predominantly dosed animals but occasionally in vehicle controls. The incidences and severities of this lesion increased with increasing dose. Olfactory epithelium respiratory metaplasia was of minimal to moderate severity and was characterized by replacement of olfactory epithelium by cuboidal to columnar, ciliated epithelial cells similar to those lining the maxilloturbinates (Figure 35 and Figure 36). Often, the metaplastic epithelium extended into the submocosal glands replacing duct and glandular epithelium.

Degeneration of the olfactory epithelium was characterized by segmental loss of and disorganization of the olfactory epithelia cells along with increased clear intercellular spaces with or without an overall decrease in height of the epithelium.

Olfactory epithelium necrosis, of minimal to moderate severity, was observed predominantly in ethmoid turbinates and occasionally the septum of Level III. Necrosis was characterized by disorganization of olfactory epithelial cells, which had scant eosinophilic cytoplasm and pyknotic nuclei. Degenerate neutrophils and fibrinous eosinophilic material were frequently associated with necrotic olfactory epithelial cells and occasionally effaced the epithelial cell layer.

Hyaline droplet accumulation in the respiratory epithelium was primarily observed in the ventral aspects of the nasal cavity, adjacent to the squamous-respiratory junction in the Level II histologic section and to the olfactory-respiratory junction in the Level III histologic section. Hyaline droplet accumulation consisted of intracytoplasmic, homogenous, eosinophilic, globular material in respiratory epithelial cells.

Hyperplasia of the respiratory epithelium was typically observed in the Level II histologic section and less frequently at the Level I section and was characterized by proliferation of ciliated columnar cells that occasionally formed villous structures that projected into nasal passages. Frequently, the hyperplastic cells extended into the submucosal glands that were often dilated.

Inflammation was of minimal to mild severity and consisted of protein accumulations and/or infiltration of neutrophils and mononuclear inflammatory cells in the dorsal aspects of the nasal passages in the Level II and III histologic sections.

Genetic Toxicology

Indole-3-carbinol was tested in three independent bacterial mutagenicity studies, and results were varied. The first study, which employed *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 and used a concentration range of 3.3 to 3,333 μg indole-3-carbinol/plate with and without 10% or 30% rat or hamster liver S9, yielded results that were judged to be equivocal in TA97 in the absence of S9 (Table E-1). A second study, using strains TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537, and concentrations ranging from 33 to 10,000 μg/plate, yielded weak positive responses in strain TA100 without and with 30% hamster liver S9 (no mutagenicity was seen in this strain in the presence of other concentrations or species of S9) (Table E-1). Results of the third study were judged to be equivocal in *S. typhimurium* strain TA100 (concentration range 500 to 5,000 μg/plate) in the presence of 10% rat liver S9 and in *Escherichia coli* strain WP2 *uvrA*/pKM101 (concentration range of 500 to 7,500 μg/plate) in the absence of S9 activation; no mutagenic activity was seen in this assay in *S. typhimurium* strain TA98, with or without S9, tested up to 5,000 μg/plate (Table E-2).

In vivo, no increase in the frequency of micronucleated PCEs was seen in the bone marrow of male F344/N rats given three doses of indole-3-carbinol (500 to 2,000 mg/kg per day) via gavage; however, a significant decrease in the percent PCEs was seen in the bone marrow of treated rats, indicating that indole-3-carbinol was toxic to the bone marrow (Table E-3). In addition to the rat study, micronucleus frequencies in NCEs of male and female B6C3F1/N mice were assessed in peripheral blood following 3 months of daily gavage treatment with indole-3-carbinol (15.6 to 250 mg/kg per day) in corn oil; no significant increases in micronucleated NCEs were seen in either sex, and no significant changes in percent PCEs occurred over the dose range tested (Table E-4).

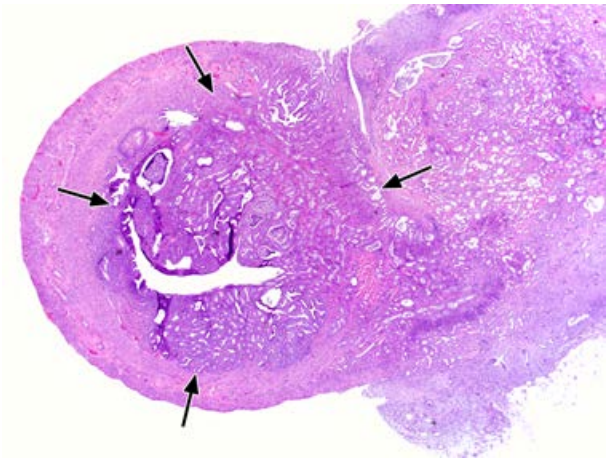

Figure 9. Adenocarcinoma of the Endometrium in the Uterus of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note the invasion through the myometrium and out to the serosal surface (arrows).

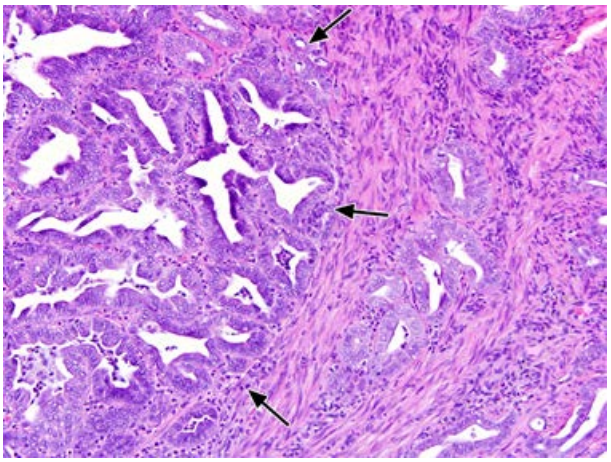

Figure 10. Higher Magnification of Figure 9 (H&E)

Note the glandular neoplastic structures (arrows) invading into the myometrium.

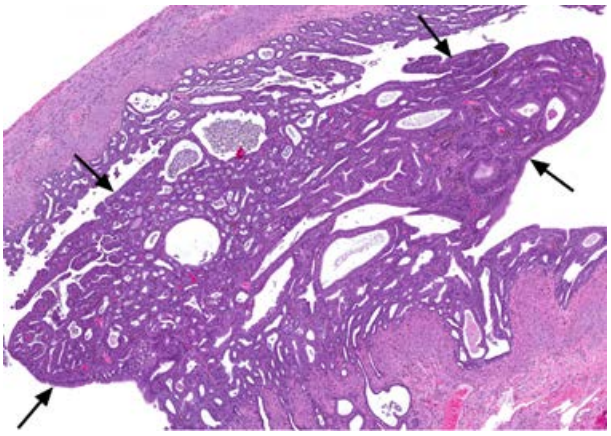

Figure 11. Adenoma (Arrows) of the Endometrium in the Uterus of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

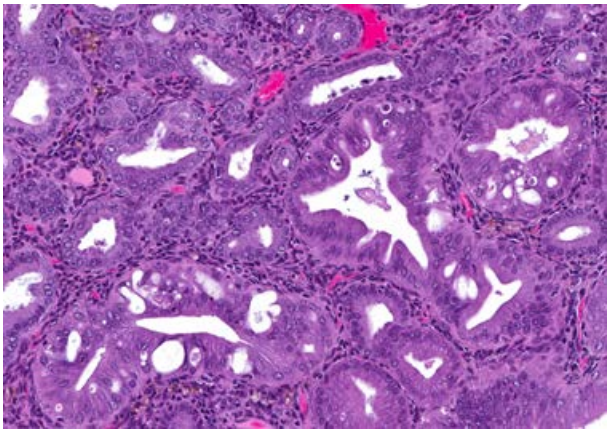

Figure 12. Higher Magnification of Figure 11 (H&E)

The adenoma is composed of irregular glandular structures lined by cuboidal to columnar epithelium that is disorganized in some areas.

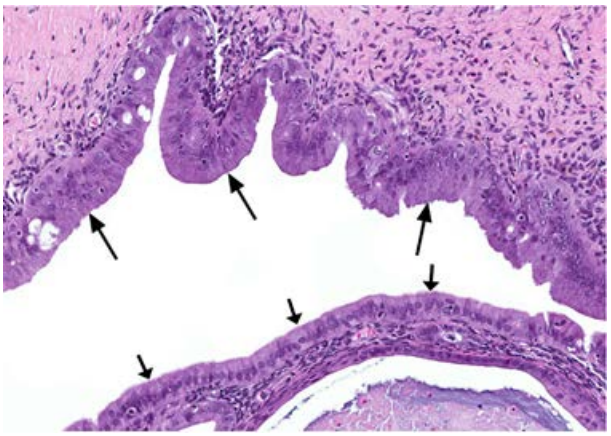

Figure 13. Atypical Hyperplasia of the Endometrium in the Uterus of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Compared to the normal appearing epithelium lining the lumen of the uterus (thin arrows), a segment of the uterine lumen is lined by disorganized atypical epithelial cells (thick arrows).

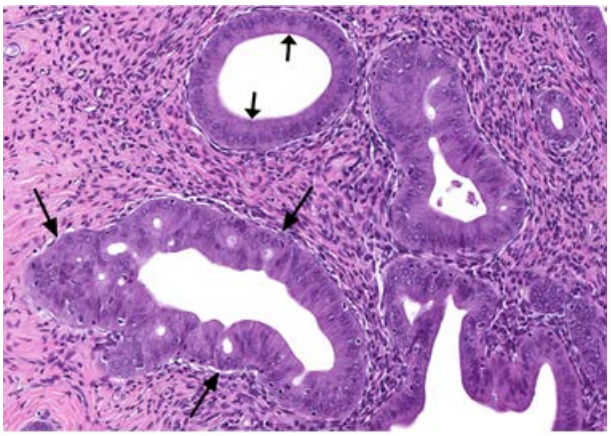

Figure 14. Atypical Hyperplasia of the Endometrium in the Uterus of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Compared to the normal appearing glands (thin arrows), the affected glands are lined by disorganized atypical epithelial cells (thick arrows).

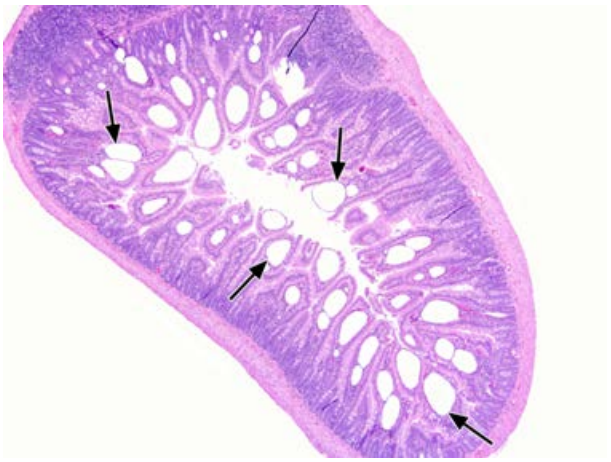

Figure 15. Lymphatic Ectasia in the Jejunum of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note multiple dilated lymphatics (arrows) in the lamina propria of the villi.

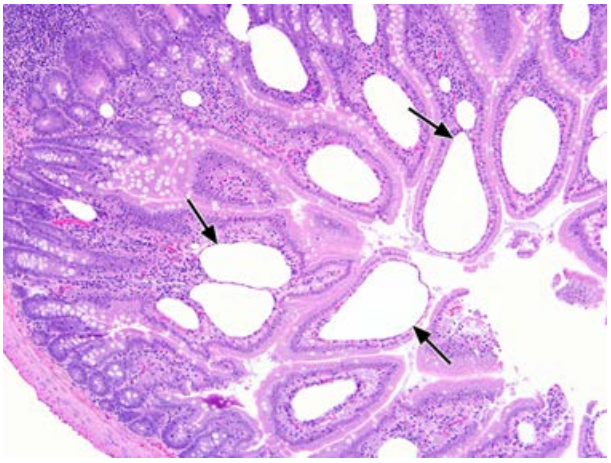

Figure 16. Higher Magnification of Figure 15 (H&E)

A single layer of endothelial cells lines the dilated lymphatics (arrows).

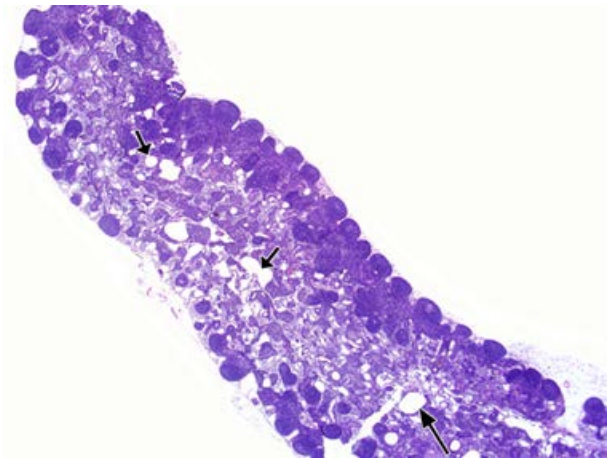

Figure 17. Lymphatic Ectasia in the Mesenteric Lymph Node of a Female Harlan Sprague Dawley Rat Administered 300 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note multiple dilated lymphatics in the medulla (arrows).

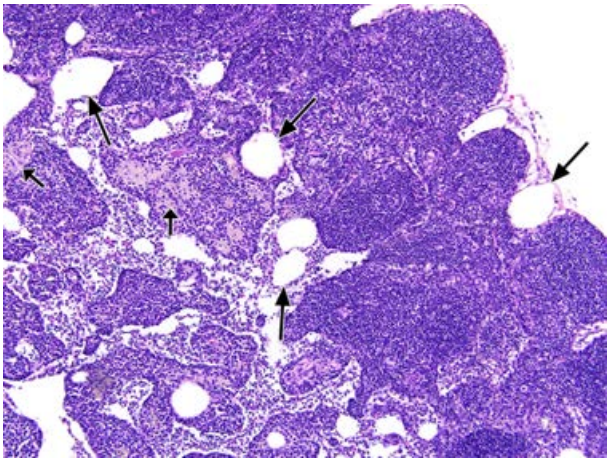

Figure 18. Higher Magnification of Figure 17 (H&E)

Note the dilated lymphatics (long arrows) and aggregates of macrophages that have golden brown cytoplasmic pigment (short arrows).

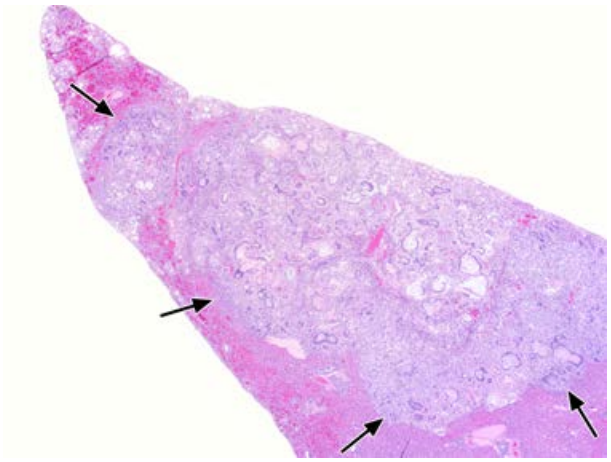

Figure 19. Cholangiofibrosis in the Liver of a Male Harlan Sprague Dawley Rat Administered 150 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note that the lesion has effaced an extensive area of the hepatic parenchyma (arrows).

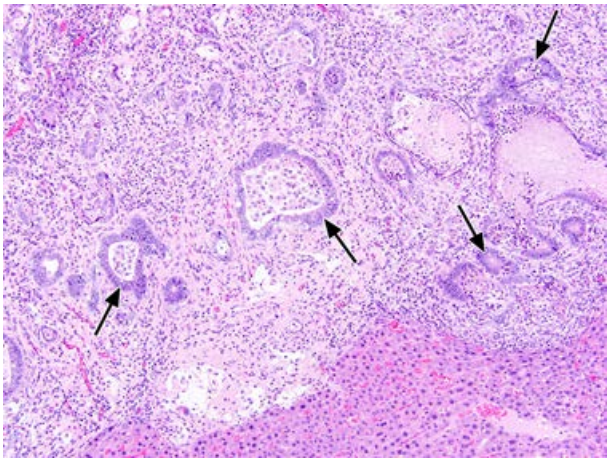

Figure 20. Higher Magnification of Figure 19 (H&E)

Cholangiofibrosis consists of multiple, variably sized, irregular, atypical bile ducts surrounded by abundant fibrous tissue infiltrated by inflammatory cells (arrows). Ducts contain mucous material, exfoliated cells, and cellular debris.

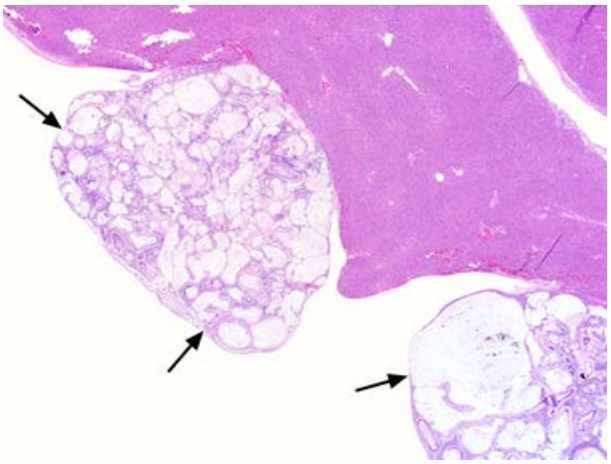

Figure 21. Cholangiofibrosis in the Liver of a Male Harlan Sprague Dawley Rat Administered 150 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note the nodular lesions (arrows) protruding from the capsular surface.

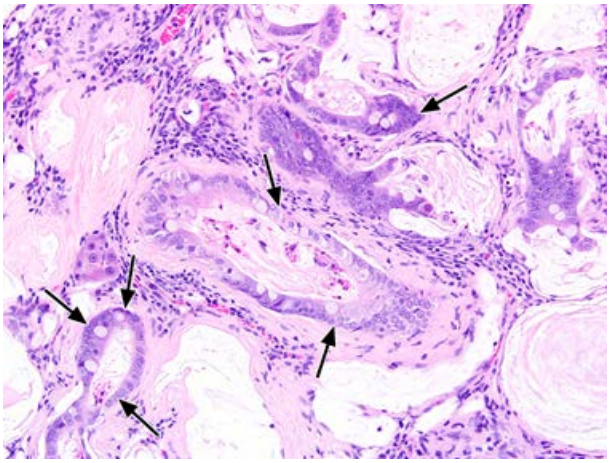

Figure 22. Higher Magnification of Figure 21 (H&E)

Note the dilated bile ducts lined by atypical epithelial cells (arrows). The ducts contain mucous and cellular debris.

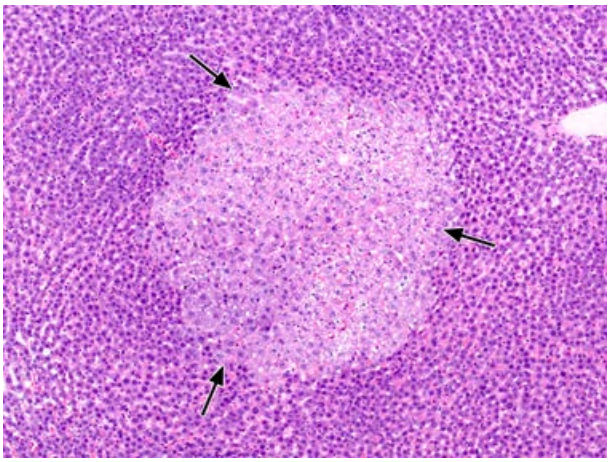

Figure 23. Eosinophilic Focus in the Liver of a Male Harlan Sprague Dawley Rat Administered 75 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note the discrete lesion (arrows) that is well-demarcated from the surrounding hepatic parenchyma.

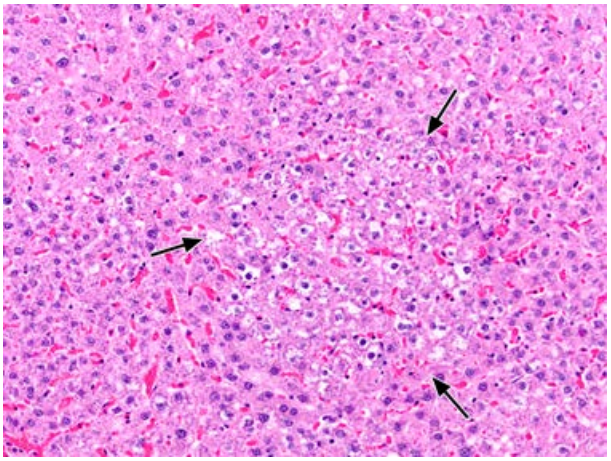

Figure 24. Clear Cell Focus (Arrows) in the Liver of a Male Harlan Sprague Dawley Rat Administered 75 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

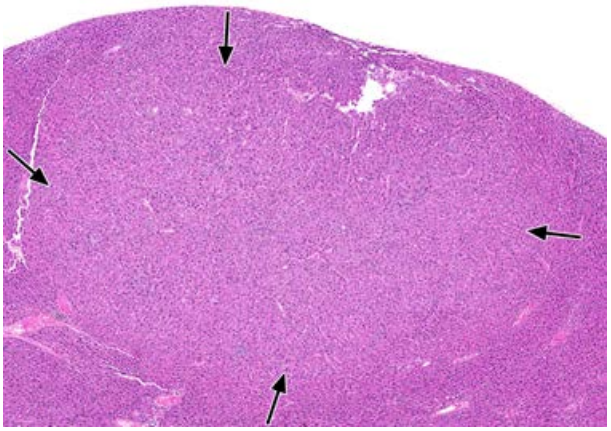

Figure 25. Hepatocellular Adenoma (Arrows) in the Liver of a Male B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

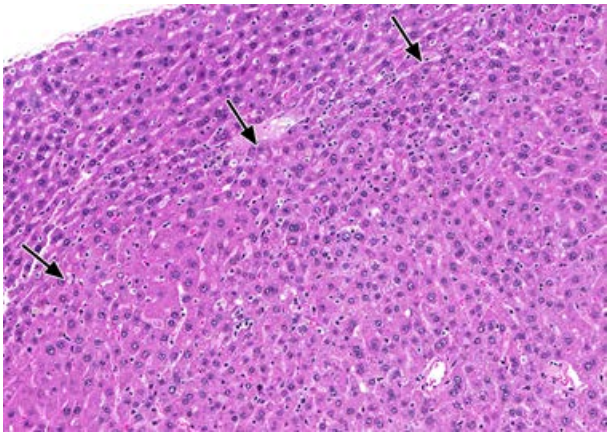

Figure 26. Higher Magnification of Figure 25 (H&E)

Note the loss of normal hepatic architecture (left of arrows) within the adenoma (right of arrows) and distorted hepatic plates obliquely impinging upon the surrounding hepatic parenchyma.

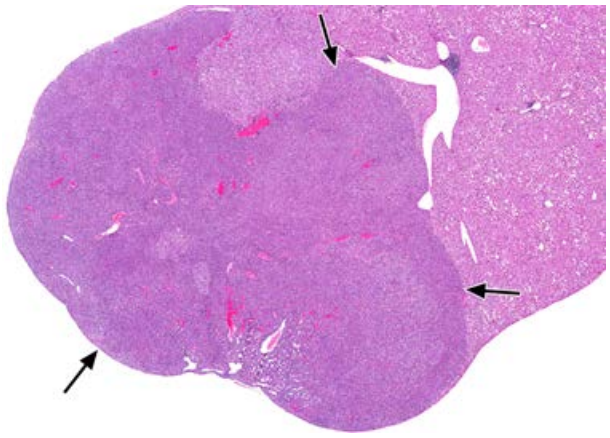

Figure 27. Large, Well-demarcated, Nodular Hepatocellular Carcinoma (Arrows) in a Male B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

The neoplasm has completely effaced the hepatic parenchyma.

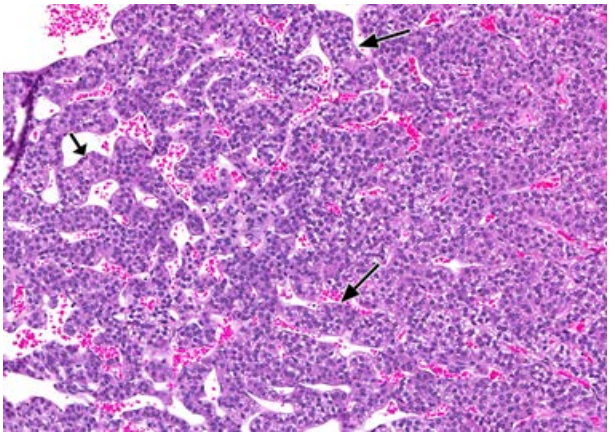

Figure 28. Higher Magnification of Figure 27 (H&E)

Note the thick trabeculae of neoplastic hepatocytes (arrows) that are a characteristic feature of hepatocellular carcinoma.

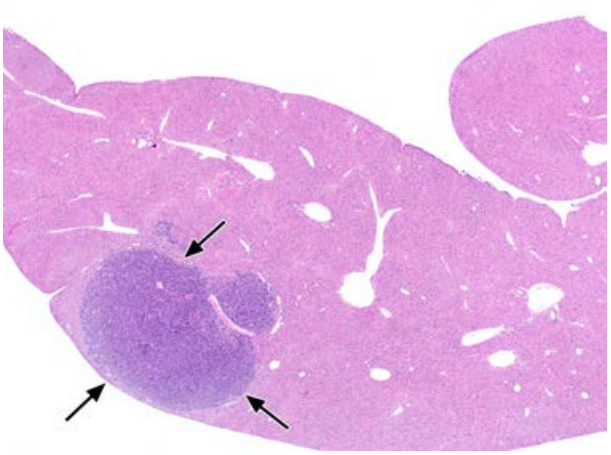

Figure 29. Hepatoblastoma (Arrows) in the Liver of a Male B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

The neoplasm is well-demarcated from the surrounding hepatic parenchyma and stains deeply basophilic.

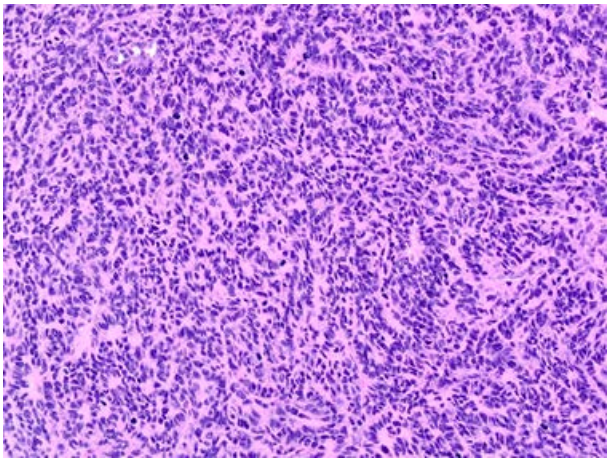

Figure 30. Higher Magnification of Figure 29 (H&E)

Cells of the hepatoblastoma are small, elongate to spindle-shaped with hyperchromatic nuclei, and are separated by scant connective tissue stroma.

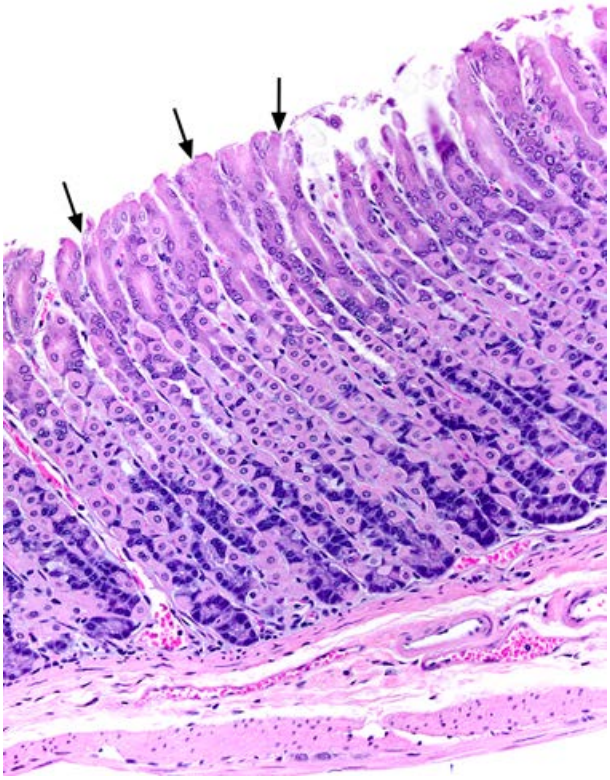

Figure 31. Normal Glandular Stomach Epithelium in a Vehicle Control Female B6C3F1/N Mouse in the Two-year Gavage Study of Indole-3-carbinol (H&E)

Note elongate gastric glands (arrows) lined by large parietal cells with granular, eosinophilic cytoplasm, and deeply basophilic chief cells at the base of the glands.

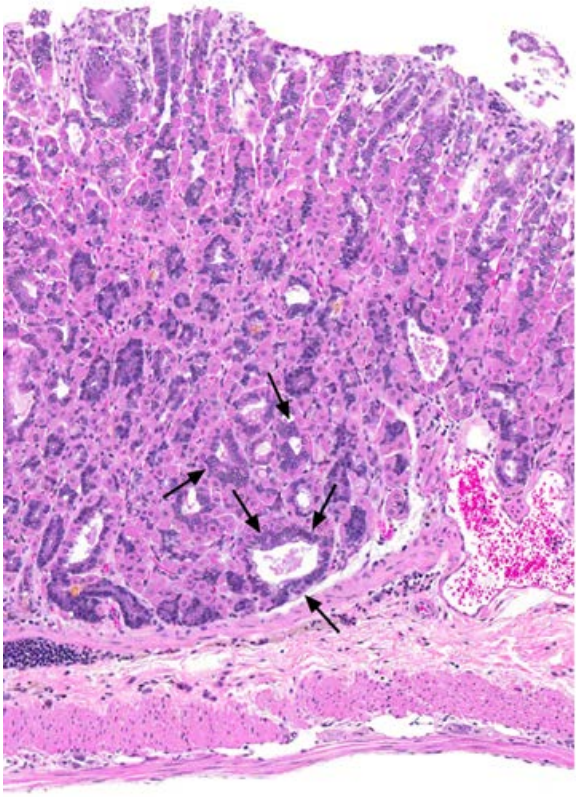

Figure 32. Hyperplasia of the Glandular Epithelium of the Stomach in a Male B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Note that there is disorganization (piling up) and a slight increase in the number of epithelial (chief) cells lining the gland (arrows).

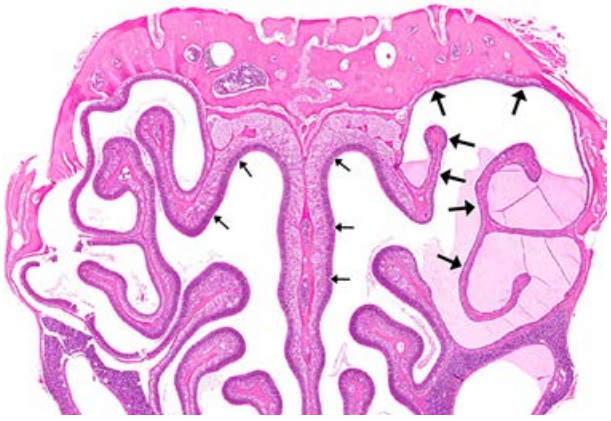

Figure 33. Low Magnification of the Ethmoid Turbinates (Level III Section) in the Nose of a Female B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years (H&E)

Histologically normal olfactory epithelium is indicated on the left side and along the nasal septum of the nasal cavity (thin arrows). Note that the olfactory epithelium lining the dorsal meatus (thick arrows) is thinner (atrophy) than the normal epithelium.

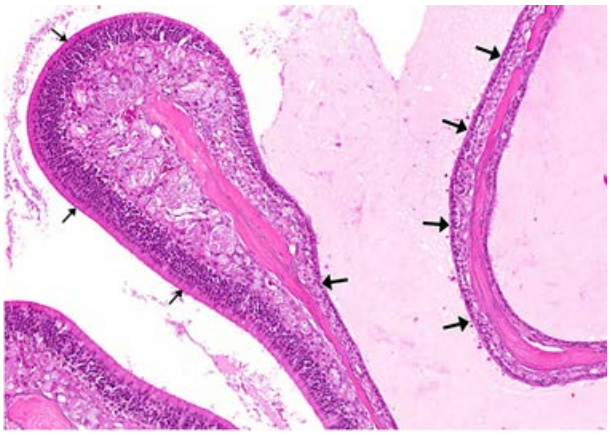

Figure 34. Higher Magnification of Figure 33 (H&E)

Note that the height of segments of the olfactory epithelium is reduced (atrophy) to a single layer of cells (thick arrows) compared to the histologically normal olfactory epithelium (thin arrows).

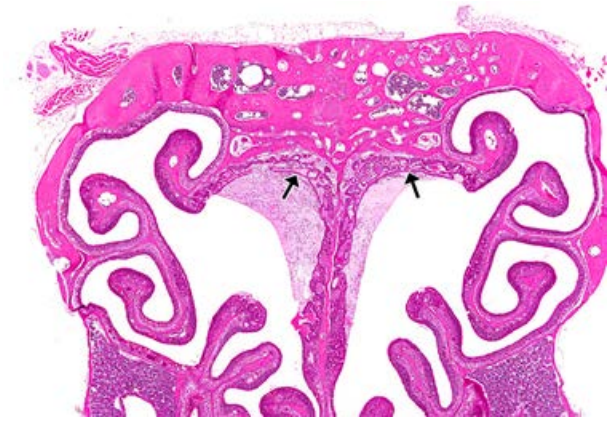

Figure 35. Low Magnification of the Ethmoid Turbinates (Level III Section) in the Nose of a Female B6C3F1/N Mouse Administered 250 mg/kg Indole-3-carbinol by Gavage for Two Years Showing Respiratory Epithelial Metaplasia in the Olfactory Epithelium (H&E)

Note that the olfactory epithelium lining the upper one-third of the nasal septum and the adjacent meatuses is bilaterally replaced by a metaplastic respiratory epithelium (arrows).

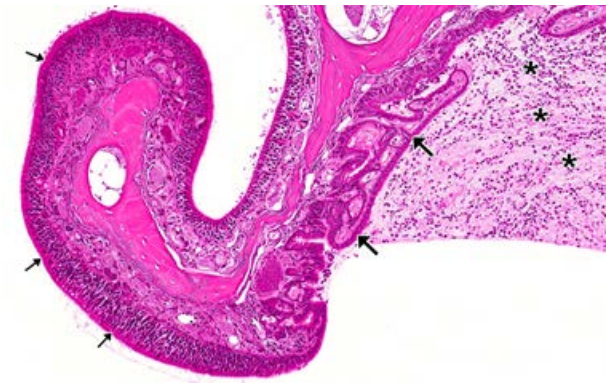

Figure 36. Higher Magnification of Figure 35 (H&E)

Compared to histologically normal olfactory epithelium (thin arrows), in the affected segment, normal olfactory epithelium is replaced by ciliated, cuboidal to tall, columnar epithelial cells (thick arrows). The ciliated epithelium extends into and replaces the epithelium of the submucosal glands. Note inflammatory cells and cellular debris in the nasal passage (asterisks).

Discussion

Indole-3-carbinol was nominated by the National Cancer Institute based on its growing use as a dietary supplement and its potential use as a therapeutic agent for the prevention of various types of cancer. While substantial evidence exists that indole-3-carbinol can reduce the risk of cancers induced by several known carcinogens when administered to animals, indole-3-carbinol can also function as an initiator and tumor promoter in certain models. The carcinogenic potential of indole-3-carbinol has not been studied in a 2-year bioassay.

The effects of gavage exposure to indole-3-carbinol for 3 months or 2 years were studied in male and female F344/N (3-month study) or Harlan Sprague Dawley (2-year study) rats and B6C3F1/N mice at doses up to 300 mg/kg for rats and 250 mg/kg for mice. In general, 3 months of exposure to indole-3-carbinol exhibited more effects in F344/N rats than in mice. Decreased body weight gains were observed in male F344/N rats at 300 mg/kg, but not in female F344/N rats or either sex of mice. In F344/N rats, changes in organ weights were observed at all administered doses of indole-3-carbinol. Increased liver and kidney weights were observed in both sexes, and decreased thymus weights were observed only in female rats. No corresponding histopathology was noted in the liver, kidney, or thymus in either sex of F344/N rats. In male and female mice, the only treatment-related effects observed were increased liver weights in males at 125 mg/kg or greater and in females at 15.6 mg/kg or greater.

Increased liver weights have been reported in rats exposed to indole-3-carbinol in the presence and absence of hepatocyte hypertrophy[20; 21]. In the current 3-month studies, changes in liver weights in F344/N rats and mice were not accompanied by hepatocyte hypertrophy. In 28-day recovery studies, Crowell et al.[20] demonstrated that the increased liver weights and hepatic hypertrophy induced by indole-3-carbinol were reversible effects.

In the liver, treatment-related increases in acetanilide-4-hydroxylase (A4H) and 7-ethoxyresorufin-*O*-deethylase (EROD) activities were observed in both sexes of F344/N rats and mice and the magnitude of induction was greater in rats than in mice. The induction of hepatic CYP1A1 and 1A2 has been widely demonstrated in repeat-dose studies of indole-3-carbinol[20; 21; 122-124]. Other studies in the literature have demonstrated that exposure to indole-3-carbinol induces other hepatic enzymes in rodents, including glutathione-S-transferase, UDP-glucuronosyl transferase, glutathione reductase, and quinone reductase[19; 125; 126]. In the current studies, increased pulmonary EROD activity was observed in F344/N rats, but not mice exposed to indole-3-carbinol.

Expression of CYP1A1 and 1A2 serves as a useful marker for activation of the aryl hydrocarbon receptor[127-130]. The induction of CYP1A1 is a very sensitive response to exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxin-like compounds[131] which are potent inducers in many tissues including the liver, lung, kidney, nasal passages, and small intestine. In fact, in many cases the relative potency for induction of CYP1A1 has provided the basis for establishing Toxic Equivalency Factors for the activity of dioxin-like compounds[132]. While increased activity or expression of CYP1A1 and 1A2 are widely accepted as sensitive markers for exposure to AhR-agonists, the potential role of the induction of hepatic CYP1A1 and 1A2 enzymes in the mechanism of toxicity and carcinogenicity of dioxin-like compounds is not fully understood. However, it has been hypothesized that AhR-dependent induction of the CYP1

family of cytochromes P450 may lead to induction of oxidative stress due to inefficient electron transfer during P450 metabolism[133]. CYP1A1 is also known to metabolize carcinogens like benzo[*a*]pyrene and aflatoxin B1 to epoxide intermediates. Therefore, the induction of these enzymes may have implications in coexposures to indole-3-carbinol and other such chemicals in humans.

In both sexes of F344/N rats, indole-3-carbinol administration for 3 months increased the incidences of lymphatic ectasia in the duodenum and jejunum, lipidosis in the lamina propria of the small intestine, and lymphatic dilatation in the mesenteric lymph node. To further clarify and elucidate the pathogenesis of these lesions, special evaluations were conducted on tissues from 1-week and 4-week euthanasia of special intestine study male Sprague Dawley rats in the 2-year bioassay[87]. Because different rat strains were evaluated in the 3-month and 2-year studies, the concordance of the development of these lesions between F344/N rats and Harlan Sprague Dawley rats was also evaluated. In both strains of rats, treatment-related dilatation of lymphatics (lymphangiectasis) of the duodenum, jejunum, and mesenteric lymph node was observed at 150 mg/kg or greater. Electron microscopy and special staining with Oil-red-O and Sudan Black confirmed extracellular lipid accumulation within the villar lamina propria, lacteals, and macrophages[87]. While lymphatic ectasia in the small intestine or mesenteric lymph node was observed in both strains of rats, it was not observed in B6C3F1/N mice administered indole-3-carbinol for 3 months or 2 years.

In the 2-year studies in Harlan Sprague Dawley rats, a positive trend in the incidences of uterine adenocarcinoma occurred in females; uterine masses observed grossly at necropsy were likely related to the increased incidences of uterine adenocarcinoma. The standard protocol for collection of uterine tissue outlined in the NTP Specifications for the Conduct of Toxicity and Carcinogenicity Studies requires that at necropsy a single transverse segment is collected from each uterine horn 0.5 cm from the cervix along with any grossly observed lesions; opening of the remaining segments of the horns is not required. The extended residual tissue review involved trimming, embedding and sectioning of the residual uterine tissue, cervix and vagina longitudinally. The reason for the residual tissue evaluation was to have a more comprehensive evaluation of the uteri in light of the occurrence of adenocarcinoma in the standard (initial) evaluation. During the extended review of the uteri, additional uterine masses were discovered mostly in uterine horns that were grossly dilated. The masses were small and would not have been discovered using the standard necropsy examination protocol unless the uterine horns were opened. During the extended microscopic evaluation, additional incidences of uterine adenocarcinomas and new incidences of adenomas were identified; adenomas were not diagnosed in the standard evaluation. In the combined standard and extended evaluations, the incidence of adenocarcinoma in the uterus was significantly increased in the 150 mg/kg group. The incidences of uterine adenoma were not significantly increased in the dosed groups.

Uterine adenocarcinomas are an uncommon background neoplasm in the Harlan Sprague Dawley rat. In the standard evaluation in the current 2-year study, no uterine adenocarcinomas were observed in vehicle control female rats. Based on the significant increase in the incidence of uterine adenocarcinomas in the 150 mg/kg group and the increased incidence in the 300 mg/kg group, these results were considered some evidence of carcinogenicity. In addition, the incidence of squamous metaplasia of the endometrium was significantly increased in 150 mg/kg female rats.

In the skin of female Sprague Dawley rats, there was a positive trend in the incidences of fibroma or fibrosarcoma (combined). Fibromas or fibrosarcomas were only observed in the vehicle control and 300 mg/kg groups. Because the incidence observed in the 300 mg/kg group was not significantly increased compared to the vehicle controls, this was an uncertain finding that may have been related to indole-3-carbinol administration.

In the thyroid gland of male Sprague Dawley rats, there were general, dose-dependent increases in the incidences and severities of follicular cell hypertrophy. While the thyroid gland has not been identified as a target organ for indole-3-carbinol exposure, indole-3-carbinol at 0.25% in the diet (total daily intake not provided) has previously been shown to promote thyroid gland follicular cell adenomas and adenocarcinomas in an initiation-promotion model[33].

In the 2-year mouse studies, body weights were decreased by greater than 10% in 250 mg/kg female mice from weeks 32 to 92, but no treatment-related decreases in survival were noted in males or females. In male mice, treatment-related neoplasms occurred only in the liver, while nonneoplastic lesions occurred in the glandular stomach and the nose. In female mice, treatment-related nonneoplastic lesions occurred in the liver, glandular stomach, and nose.

In the liver, there were generally positive trends for the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma as well as their combined incidences in indole-3-carbinol-treated male mice in the 2-year studies. Increased incidences of hepatocellular adenoma and hepatoblastoma occurred in 250 mg/kg males and included increased numbers of mice with multiple lesions. A significant increase in the incidence of multiple adenoma also occurred in 62.5 mg/kg males. An increased incidence of hepatocellular carcinoma occurred in 125 mg/kg males, but not in males administered 250 mg/kg. However, in early death animals the time to first observed incidence was 182 days less in the 250 mg/kg group than in the 125 mg/kg group, and 80 days less than in the vehicle controls. Although hepatocellular adenomas and hepatocellular carcinomas are relatively common neoplasms in male mice, the significantly increased incidences in indole-3-carbinol-treated males considerably exceeded the concurrent vehicle control and historical control rates. The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were significantly increased in 125 and 250 mg/kg males. Based on the observed increases, the incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma were considered related to indole-3-carbinol treatment and supportive of clear evidence of carcinogenicity in male mice.

Hepatic gene expression studies in 300 mg/kg females from the microarray study suggested the activation of multiple xenobiotic transcription factors in rat liver with the most pronounced activation being associated with AhR and Nrf2. Consistent with these findings was the up-regulation of genes associated with xenobiotic metabolism which suggests the potential for indole-3-carbinol to modify drug efficacy and safety. These findings are largely similar to results from other transcriptomic studies of indole-3-carbinol.

In the current 2-year study, treatment-related nonneoplastic lesions occurred in the glandular stomach and the nose of both male and female mice. Indole-3-carbinol-induced hyperplasia, pigmentation, and chronic inflammation in the glandular stomach occurred at lower doses in females (62.5 mg/kg) than in males (125 mg/kg). The spectra of nasal lesions in the olfactory and respiratory epithelia were similar in males and females. In both sexes, incidences of nerve atrophy, respiratory metaplasia and atrophy of the olfactory epithelium, and hyperplasia of the

respiratory epithelium were significantly increased in mice administered 250 mg/kg. In addition, occurrences of olfactory epithelium necrosis and respiratory epithelium hyaline droplet accumulation in males and nasal inflammation in females were considered treatment-related. Nasal lesions have not been reported in shorter-term studies of indole-3-carbinol.

As an AhR agonist, indole-3-carbinol might be expected to elicit the same responses as other AhR agonists, most notably 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxin-like compounds. However, it is important to note the absence of dioxin-like carcinogenicity in the current studies. In general, TCDD and other individual and relevant mixtures of dioxin-like compounds induce a characteristic spectrum of neoplasms in the three main target organs: liver, lung, and oral mucosa[74-80; 134]. In the liver, TCDD[75], 3,3′,4,4′,5-pentachloro-biphenyl (PCB 126)[74], 2,3,4,7,8-penta-chlorodibenzofuran (PeCDF)[76], 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118)[134], and a tertiary mixture of TCDD, PCB 126, and PeCDF[77] predominantly induce cholangiocarcinomas and hepatocellular adenomas. In addition to these neoplasms, a binary mixture of PCB 126 and PCB 153[79] and a binary mixture of PCB 126 and PCB 118[80] induce hepatocarcinomas. In general, exposure to these dioxin-like compounds induces cystic keratinizing epitheliomas in the lung[74; 75; 77-80; 134]. Gingival squamous cell carcinomas were induced by exposure to TCDD[75], PCB 126[74], PeCDF[76], a binary mixture of PCB 126 and PCB 118[80], and a binary mixture of PCB 126 and PCB 153[79]. None of these characteristic neoplasms induced by exposure to persistent dioxin-like compounds were observed in rats exposed to indole-3-carbinol, even at doses that elicited comparable fold-inductions of hepatic EROD activity, a marker of AhR activation. In addition to hepatic neoplasms, TCDD and other dioxin-like compounds induce dose-dependent increases in the incidences of a broad spectrum of nonneoplastic liver lesions, including hepatocyte hypertrophy, multinucleated hepatocytes, fatty change, bile duct hyperplasia and cyst, cholangiofibrosis, and oval cell hyperplasia[74-77; 79; 80; 134]. This broad spectrum of lesions was not observed in rats exposed to indole-3-carbinol for 2 years. Aside from increased liver weight, the only treatment-related lesions observed in the liver were increased incidences of clear cell and eosinophilic foci in female rats, and increased incidences of bile duct cysts in male rats.

Chronic exposure to dioxin-like compounds also induces nonneoplastic lesions in other tissues, including the lung, adrenal cortex, pancreas, kidney, heart, thyroid gland, and thymus[74-77; 80; 134]. The spectrum of toxicity and the specificity of target organs vary slightly between dioxin-like congeners, with exposure to some congeners affecting additional tissues. However, the general profiles of toxicity and carcinogenicity are similar. Therefore, the differences in the observed neoplastic and nonneoplastic responses between indole-3-carbinol and the dioxins demonstrate a clear difference between indole-3-carbinol and other persistent dioxin-like compounds.

Conclusions

Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity*[a] of indole-3-carbinol in male Harlan Sprague Dawley rats administered 75, 150, or 300 mg/kg. There was *some evidence of carcinogenic activity* of indole-3-carbinol in female Harlan Sprague Dawley rats based on increased incidences of malignant uterine neoplasms (primarily adenocarcinoma). The occurrences of fibroma and fibrosarcoma in the skin may have been related to indole-3-carbinol administration. There was *clear evidence of carcinogenic activity* of indole-3-carbinol in male B6C3F1/N mice based on increased incidences of liver neoplasms (hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma). There was *no evidence of carcinogenic activity* of indole-3-carbinol in female B6C3F1/N mice administered 62.5, 125, or 250 mg/kg.

Administration of indole-3-carbinol caused increased incidences of nonneoplastic lesions in the small intestine, mesenteric lymph node, and liver of male and female rats, the thyroid gland of male rats, the uterus of female rats, and the liver, glandular stomach, and nose of male and female mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in Appendix M.

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Appendix A. Summary of Lesions in Male Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

Tables

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Table A-1. Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals Initially in Study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	14	23	15	20
Natural deaths	16	14	18	17
Survivors				
Terminal euthanasia	20	13	17	12
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Lipoma	–	1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(43)	(48)	(47)	(48)
Carcinoma	–	1 (2%)	–	–
Intestine small, ileum	(35)	(43)	(42)	(38)
Intestine small, jejunum	(40)	(39)	(40)	(42)
Carcinoma	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	–	–	–	1 (2%)
Hepatocellular adenoma	1 (2%)	–	–	–
Hepatocellular carcinoma	–	–	–	1 (2%)
Mesentery	(2)	(2)	(1)	(0)
Fat, sarcoma	–	–	1 (100%)	–
Oral mucosa	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)	–	1 (2%)	1 (2%)
Pancreas	(50)	(50)	(50)	(49)
Carcinoma	–	–	–	1 (2%)
Mixed tumor malignant	–	–	1 (2%)	–
Acinus, adenoma	4 (8%)	1 (2%)	4 (8%)	4 (8%)
Acinus, adenoma, multiple	1 (2%)	3 (6%)	–	1 (2%)
Salivary glands	(50)	(50)	(49)	(50)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Schwannoma malignant	1 (2%)	–	–	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)	–	1 (2%)	–
Squamous cell papilloma	–	–	1 (2%)	–
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(0)	(1)
Squamous cell papilloma	–	–	–	1 (100%)
Tooth	(1)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, fibrous histiocytoma, metastatic, skin	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Carcinoma	–	1 (2%)	–	1 (2%)
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign	5 (10%)	5 (10%)	4 (8%)	3 (6%)
Pheochromocytoma complex	1 (2%)	–	–	–
Pheochromocytoma malignant	2 (4%)	1 (2%)	1 (2%)	–
Bilateral, pheochromocytoma benign	–	–	1 (2%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	1 (2%)	–
Parathyroid gland	(43)	(46)	(50)	(47)
Carcinoma	–	1 (2%)	–	–
Pituitary gland	(50)	(49)	(50)	(47)
Pars distalis, adenoma	5 (10%)	8 (16%)	9 (18%)	8 (17%)
Pars distalis, carcinoma	–	1 (2%)	–	–
Thyroid gland	(50)	(46)	(48)	(47)
Bilateral, c-cell, adenoma	2 (4%)	–	1 (2%)	–
C-cell, adenoma	11 (22%)	9 (20%)	8 (17%)	4 (9%)
C-cell, carcinoma	–	1 (2%)	1 (2%)	–
Follicular cell, adenoma	–	1 (2%)	–	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(49)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(49)	(50)	(50)	(50)
Testes	(49)	(50)	(50)	(50)
Interstitial cell, adenoma	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(8)	(8)	(6)
Lymph node, mandibular	(50)	(50)	(49)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Thymus	(46)	(49)	(49)	(47)
Neural crest tumor	–	–	–	1 (2%)
Thymoma benign	1 (2%)	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	1 (2%)	1 (2%)	1 (2%)	–
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	1 (2%)	–	–
Keratoacanthoma	4 (8%)	–	–	–
Squamous cell carcinoma	1 (2%)	–	1 (2%)	–
Squamous cell papilloma	1 (2%)	–	1 (2%)	1 (2%)
Sebaceous gland, adenoma	–	1 (2%)	–	–
Subcutaneous tissue, fibroma	5 (10%)	2 (4%)	5 (10%)	2 (4%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, sarcoma	–	2 (4%)	–	–
Subcutaneous tissue, schwannoma malignant	–	–	1 (2%)	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, osteoma	–	–	–	1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	–	1 (2%)	–	–
Medulloblastoma	–	–	1 (2%)	–
Meningioma malignant	–	1 (2%)	–	–
Cerebellum, glioma malignant	–	–	–	1 (2%)
Spinal cord	(0)	(2)	(1)	(0)
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	–	1 (2%)	–
Carcinoma, metastatic, adrenal cortex	–	–	–	1 (2%)
Fibrous histiocytoma, metastatic, skin	–	–	–	1 (2%)
Squamous cell carcinoma	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(49)	(49)
Harderian gland	(50)	(50)	(49)	(50)
Lacrimal gland	(1)	(2)	(4)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)	–	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia granulocytic	–	–	–	1 (2%)
Lymphoma malignant	–	–	1 (2%)	1 (2%)
Mesothelioma malignant	1 (2%)	1 (2%)	–	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	33	32	31	33
Total primary neoplasms	60	53	50	42
Total animals with benign neoplasms	27	23	26	25
Total benign neoplasms	47	37	39	29
Total animals with malignant neoplasms	12	15	10	12
Total malignant neoplasms	13	16	11	12
Total animals with metastatic neoplasms	–	–	–	3
Total metastatic neoplasms	–	–	–	4
Total animals with uncertain neoplasms —benign or malignant	–	–	–	1
Total uncertain neoplasms	–	–	–	1

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

Table A-2. Statistical Analysis of Primary Neoplasms in Male Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	5/50 (10%)	5/49 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate[b]	13.0%	14.7%	13.5%	11.7%
Terminal rate[c]	3/20 (15%)	2/13 (15%)	2/17 (12%)	3/12 (25%)
First incidence (days)	645	571	593	621
Poly-3 test[d]	P = 0.479N	P = 0.549	P = 0.605	P = 0.577N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	6/49 (12%)	6/50 (12%)	4/50 (8%)
Adjusted rate	20.5%	17.5%	16.1%	11.7%
Terminal rate	4/20 (20%)	2/13 (15%)	2/17 (12%)	3/12 (25%)
First incidence (days)	634	571	593	621
Poly-3 test	P = 0.190N	P = 0.491N	P = 0.422N	P = 0.243N
Pancreas: Adenoma				
Overall rate	5/50 (10%)	4/50 (8%)	4/50 (8%)	5/49 (10%)
Adjusted rate	13.0%	11.8%	10.9%	14.8%
Terminal rate	3/20 (15%)	2/13 (15%)	2/17 (12%)	3/12 (25%)
First incidence (days)	685	648	624	710
Poly-3 test	P = 0.474	P = 0.579N	P = 0.527N	P = 0.549

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Pancreas: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	4/50 (8%)	6/49 (12%)
Adjusted rate	13.0%	11.8%	10.9%	17.5%
Terminal rate	3/20 (15%)	2/13 (15%)	2/17 (12%)	3/12 (25%)
First incidence (days)	685	648	624	589
Poly-3 test	P = 0.337	P = 0.579N	P = 0.527N	P = 0.418
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	5/50 (10%)	8/49 (16%)	9/50 (18%)	8/47 (17%)
Adjusted rate	12.9%	23.3%	23.4%	23.3%
Terminal rate	2/20 (10%)	3/13 (23%)	1/17 (6%)	1/12 (8%)
First incidence (days)	645	617	503	589
Poly-3 test	P = 0.191	P = 0.193	P = 0.180	P = 0.193
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	9/49 (18%)	9/50 (18%)	8/47 (17%)
Adjusted rate	12.9%	25.9%	23.4%	23.3%
Terminal rate	2/20 (10%)	3/13 (23%)	1/17 (6%)	1/12 (8%)
First incidence (days)	645	614	503	589
Poly-3 test	P = 0.218	P = 0.127	P = 0.180	P = 0.193
Skin: Keratoacanthoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	10.4%	0.0%	0.0%	0.0%
Terminal rate	2/20 (10%)	0/13 (0%)	0/17 (0%)	0/12 (0%)
First incidence (days)	685	–[e]	–	–
Poly-3 test	P = 0.015N	P = 0.079N	P = 0.066N	P = 0.077N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	5/50 (10%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	13.0%	0.0%	2.8%	2.9%
Terminal rate	3/20 (15%)	0/13 (0%)	1/17 (6%)	0/12 (0%)
First incidence (days)	685	–	727 (T)	629
Poly-3 test	P = 0.075N	P = 0.043N	P = 0.112N	P = 0.129N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	5/50 (10%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Adjusted rate	13.0%	0.0%	5.4%	2.9%
Terminal rate	3/20 (15%)	0/13 (0%)	1/17 (6%)	0/12 (0%)
First incidence (days)	685	–	624	629
Poly-3 test	P = 0.104N	P = 0.043N	P = 0.233N	P = 0.129N

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	6/50 (12%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	15.5%	3.0%	5.4%	2.9%
Terminal rate	3/20 (15%)	0/13 (0%)	1/17 (6%)	0/12 (0%)
First incidence (days)	645	637	624	629
Poly-3 test	P = 0.048N	P = 0.079N	P = 0.147N	P = 0.077N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	2/50 (4%)	5/50 (10%)	2/50 (4%)
Adjusted rate	12.6%	5.8%	13.4%	5.7%
Terminal rate	1/20 (5%)	0/13 (0%)	3/17 (18%)	0/12 (0%)
First incidence (days)	575	597	500	401
Poly-3 test	P = 0.286N	P = 0.278N	P = 0.596	P = 0.267N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	5.0%	11.2%	2.7%	5.7%
Terminal rate	0/20 (0%)	1/13 (8%)	0/17 (0%)	0/12 (0%)
First incidence (days)	197	382	423	258
Poly-3 test	P = 0.485N	P = 0.286	P = 0.526N	P = 0.645
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	7/50 (14%)	6/50 (12%)	6/50 (12%)	4/50 (8%)
Adjusted rate	16.9%	16.4%	15.7%	11.1%
Terminal rate	1/20 (5%)	1/13 (8%)	3/17 (18%)	0/12 (0%)
First incidence (days)	197	382	423	258
Poly-3 test	P = 0.275N	P = 0.597N	P = 0.562N	P = 0.341N
Testes: Adenoma				
Overall rate	3/49 (6%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	7.9%	5.9%	5.4%	5.8%
Terminal rate	1/20 (5%)	1/13 (8%)	0/17 (0%)	1/12 (8%)
First incidence (days)	635	671	570	532
Poly-3 test	P = 0.446N	P = 0.553N	P = 0.506N	P = 0.543N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	13/50 (26%)	9/46 (20%)	9/48 (19%)	4/47 (9%)
Adjusted rate	32.5%	27.5%	24.2%	12.0%
Terminal rate	7/20 (35%)	4/13 (31%)	3/17 (18%)	2/12 (17%)
First incidence (days)	421	456	503	519
Poly-3 test	P = 0.024N	P = 0.416N	P = 0.289N	P = 0.033N

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	13/50 (26%)	10/46 (22%)	10/48 (21%)	4/47 (9%)
Adjusted rate	32.5%	30.2%	26.6%	12.0%
Terminal rate	7/20 (35%)	4/13 (31%)	3/17 (18%)	2/12 (17%)
First incidence (days)	421	456	503	519
Poly-3 test	P = 0.025N	P = 0.516N	P = 0.373N	P = 0.033N
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	23/50 (46%)	26/50 (52%)	25/50 (50%)
Adjusted rate	63.7%	58.1%	62.3%	62.2%
Terminal rate	12/20 (60%)	6/13 (46%)	10/17 (59%)	8/12 (67%)
First incidence (days)	421	382	482	258
Poly-3 test	P = 0.544	P = 0.378N	P = 0.540N	P = 0.537N
All Organs: Malignant Neoplasms				
Overall rate	12/50 (24%)	15/50 (30%)	10/50 (20%)	12/50 (24%)
Adjusted rate	29.3%	37.1%	25.2%	30.8%
Terminal rate	5/20 (25%)	2/13 (15%)	1/17 (6%)	2/12 (17%)
First incidence (days)	197	357	423	258
Poly-3 test	P = 0.480N	P = 0.301	P = 0.438N	P = 0.540
All Organs: Benign or Malignant Neoplasms				
Overall rate	33/50 (66%)	32/50 (64%)	31/50 (62%)	33/50 (66%)
Adjusted rate	75.2%	73.1%	71.0%	76.1%
Terminal rate	14/20 (70%)	7/13 (54%)	11/17 (65%)	9/12 (75%)
First incidence (days)	197	357	423	258
Poly-3 test	P = 0.495	P = 0.507N	P = 0.414N	P = 0.564

(T) Terminal euthanasia.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, pancreas, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal euthanasia.
[d]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	–	–	–	1
Moribund	14	23	15	20
Natural deaths	16	14	18	17
Survivors				
Terminal euthanasia	20	13	17	12
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	–	1 (2%)	–	–
Epithelium, hyperplasia	–	1 (2%)	–	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	–	1 (2%)	–	–
Necrosis	1 (2%)	1 (2%)	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Erosion	1 (2%)	–	–	–
Intestine large, rectum	(50)	(50)	(50)	(50)
Fibrosis	–	–	–	1 (2%)
Intestine small, duodenum	(43)	(48)	(47)	(48)
Lymphatic, ectasia	–	–	15 (32%)	14 (29%)
Intestine small, ileum	(35)	(43)	(42)	(38)
Intestine small, jejunum	(40)	(39)	(40)	(42)
Lymphatic, ectasia	–	2 (5%)	27 (68%)	41 (98%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Basophilic focus	3 (6%)	–	3 (6%)	1 (2%)
Cholangiofibrosis	–	1 (2%)	3 (6%)	1 (2%)
Clear cell focus	14 (28%)	13 (26%)	18 (36%)	9 (18%)
Degeneration, cystic	–	–	1 (2%)	–
Eosinophilic focus	10 (20%)	12 (24%)	10 (20%)	6 (12%)
Hepatodiaphragmatic nodule	2 (4%)	–	1 (2%)	1 (2%)
Mixed cell focus	4 (8%)	2 (4%)	1 (2%)	2 (4%)
Necrosis	3 (6%)	3 (6%)	5 (10%)	7 (14%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Artery, inflammation, chronic active	–	1 (2%)	–	–
Bile duct, cyst	–	–	1 (2%)	5 (10%)
Bile duct, cyst, multiple	–	–	1 (2%)	–
Bile duct, fibrosis	2 (4%)	–	1 (2%)	1 (2%)
Bile duct, hyperplasia	7 (14%)	4 (8%)	3 (6%)	3 (6%)
Oval cell, hyperplasia	–	–	–	1 (2%)
Periportal, inflammation, chronic active	1 (2%)	–	–	–
Mesentery	(2)	(2)	(1)	(0)
Artery, inflammation, chronic active	1 (50%)	–	–	–
Artery, mineralization	–	1 (50%)	–	–
Fat, necrosis	1 (50%)	–	–	–
Oral mucosa	(50)	(50)	(50)	(50)
Foreign body	–	1 (2%)	–	–
Hyperplasia, squamous	–	1 (2%)	2 (4%)	1 (2%)
Sebaceous gland, ectopic tissue	–	–	–	1 (2%)
Pancreas	(50)	(50)	(50)	(49)
Angiectasis	–	–	–	1 (2%)
Thrombosis	–	1 (2%)	–	–
Acinus, atrophy	1 (2%)	1 (2%)	–	2 (4%)
Acinus, hyperplasia	16 (32%)	10 (20%)	14 (28%)	10 (20%)
Artery, inflammation, chronic active	22 (44%)	32 (64%)	27 (54%)	21 (43%)
Salivary glands	(50)	(50)	(49)	(50)
Metaplasia	–	1 (2%)	–	2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion	–	–	1 (2%)	–
Hyperplasia	19 (38%)	19 (38%)	19 (38%)	22 (44%)
Inflammation	2 (4%)	–	4 (8%)	1 (2%)
Necrosis	1 (2%)	–	–	–
Ulcer	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Epithelium, amyloid deposition	–	–	1 (2%)	–
Epithelium, hyperplasia	2 (4%)	5 (10%)	7 (14%)	3 (6%)
Epithelium, inflammation	–	1 (2%)	–	–
Epithelium, inflammation, chronic active	3 (6%)	4 (8%)	2 (4%)	1 (2%)
Epithelium, mineralization	7 (14%)	12 (24%)	6 (12%)	10 (20%)
Epithelium, necrosis	1 (2%)	–	–	–
Epithelium, ulcer	–	1 (2%)	–	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Epithelium, muscularis, hyperplasia, focal	–	1 (2%)	–	–
Tongue	(0)	(0)	(0)	(1)
Tooth	(1)	(0)	(1)	(0)
Malformation	–	–	1 (100%)	–
Necrosis	1 (100%)	–	–	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Dilatation	1 (2%)	–	–	–
Inflammation, chronic active	30 (60%)	38 (76%)	30 (60%)	29 (58%)
Mineralization	–	2 (4%)	1 (2%)	1 (2%)
Thrombosis	–	–	1 (2%)	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	48 (96%)	49 (98%)	48 (96%)
Mineralization	1 (2%)	–	–	1 (2%)
Atrium, thrombosis	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Epicardium, inflammation	1 (2%)	1 (2%)	–	–
Pericardium, inflammation	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Degeneration, cystic	4 (8%)	3 (6%)	5 (10%)	4 (8%)
Degeneration, fatty	8 (16%)	13 (27%)	5 (10%)	16 (32%)
Hematopoietic cell proliferation	–	–	1 (2%)	–
Hemorrhage	–	–	1 (2%)	–
Hyperplasia	14 (28%)	19 (39%)	19 (38%)	6 (12%)
Necrosis	1 (2%)	–	–	2 (4%)
Thrombosis	–	1 (2%)	–	–
Adrenal medulla	(50)	(49)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Degeneration, fatty	–	1 (2%)	–	–
Hyperplasia	18 (36%)	22 (45%)	20 (40%)	19 (38%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)	6 (12%)	1 (2%)	3 (6%)
Parathyroid gland	(43)	(46)	(50)	(47)
Hyperplasia	3 (7%)	10 (22%)	13 (26%)	8 (17%)
Pituitary gland	(50)	(49)	(50)	(47)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Pars distalis, hyperplasia	17 (34%)	18 (37%)	19 (38%)	12 (26%)
Thyroid gland	(50)	(46)	(48)	(47)
Thrombosis	1 (2%)	–	–	–
C-cell, hyperplasia	6 (12%)	4 (9%)	4 (8%)	5 (11%)
C-cell, hypertrophy	–	–	–	1 (2%)
Follicular cell, hyperplasia	–	1 (2%)	–	–
Follicular cell, hypertrophy	21 (42%)	34 (74%)	33 (69%)	36 (77%)
General Body System				
None	–	–	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	–	–
Preputial gland	(50)	(50)	(49)	(50)
Cyst	7 (14%)	–	3 (6%)	5 (10%)
Fibrosis	1 (2%)	–	–	–
Inflammation	2 (4%)	–	–	1 (2%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	1 (2%)	–	–
Fibrosis	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Hyperplasia	3 (6%)	4 (8%)	7 (14%)	3 (6%)
Inflammation, acute	1 (2%)	6 (12%)	–	6 (12%)
Inflammation, chronic active	–	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Seminal vesicle	(49)	(50)	(50)	(50)
Hypoplasia	–	–	1 (2%)	–
Inflammation, acute	–	–	–	2 (4%)
Testes	(49)	(50)	(50)	(50)
Atrophy	30 (61%)	37 (74%)	35 (70%)	33 (66%)
Pigmentation, hemosiderin	–	–	1 (2%)	–
Artery, inflammation, chronic active	28 (57%)	34 (68%)	33 (66%)	33 (66%)
Interstitial cell, hyperplasia	1 (2%)	–	1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	4 (8%)	3 (6%)
Lymph node	(4)	(8)	(8)	(6)
Angiectasis	–	–	–	1 (17%)
Bronchial, hemorrhage	–	1 (13%)	1 (13%)	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Deep cervical, hemorrhage	–	1 (13%)	–	–
Deep cervical, hyperplasia, plasma cell	–	1 (13%)	–	–
Deep cervical, inflammation	–	1 (13%)	–	–
Iliac, infiltration cellular, histiocyte	–	–	1 (13%)	–
Lumbar, hemorrhage	–	–	2 (25%)	–
Mediastinal, ectasia	–	1 (13%)	1 (13%)	–
Mediastinal, hemorrhage	1 (25%)	2 (25%)	3 (38%)	1 (17%)
Mediastinal, hyperplasia, lymphoid	–	1 (13%)	–	–
Mediastinal, infiltration cellular, lipocyte	–	–	–	1 (17%)
Mediastinal, infiltration cellular, histiocyte	–	1 (13%)	–	–
Mediastinal, pigmentation, hemosiderin	–	–	–	1 (17%)
Pancreatic, hemorrhage	–	1 (13%)	–	1 (17%)
Renal, ectasia	2 (50%)	–	–	–
Renal, hemorrhage	1 (25%)	2 (25%)	–	–
Renal, pigmentation, hemosiderin	–	–	–	1 (17%)
Lymph node, mandibular	(50)	(50)	(49)	(50)
Ectasia	–	1 (2%)	1 (2%)	1 (2%)
Hemorrhage	–	–	1 (2%)	–
Hyperplasia, plasma cell	1 (2%)	–	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	–	–
Hyperplasia, lymphoid	1 (2%)	–	–	1 (2%)
Lymphatic, ectasia	–	–	1 (2%)	5 (10%)
Spleen	(50)	(50)	(50)	(50)
Atrophy	–	–	–	1 (2%)
Hematopoietic cell proliferation	28 (56%)	33 (66%)	28 (56%)	29 (58%)
Inflammation	1 (2%)	–	–	–
Necrosis	–	–	1 (2%)	–
Thymus	(46)	(49)	(49)	(47)
Atrophy	41 (89%)	41 (84%)	42 (86%)	41 (87%)
Hemorrhage	–	–	1 (2%)	–
Epithelial cell, hyperplasia	1 (2%)	–	–	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibrosis	–	–	1 (2%)	–
Inflammation	–	–	–	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	5 (10%)	3 (6%)	2 (4%)	2 (4%)
Inflammation	3 (6%)	2 (4%)	–	2 (4%)
Necrosis	–	2 (4%)	–	–
Epidermis, ulcer	1 (2%)	–	–	–
Sebaceous gland, hyperplasia	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	12 (24%)	19 (38%)	13 (26%)	8 (16%)
Maxilla, fracture	–	–	1 (2%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	–	–	–	1 (2%)
Gliosis	–	2 (4%)	–	–
Necrosis	–	–	–	2 (4%)
Spinal cord	(0)	(2)	(1)	(0)
Degeneration	–	2 (100%)	1 (100%)	–
Respiratory System				
Lung	(49)	(50)	(50)	(50)
Edema	–	–	1 (2%)	1 (2%)
Fibrosis	–	–	–	1 (2%)
Foreign body	–	1 (2%)	1 (2%)	–
Hemorrhage	–	–	1 (2%)	1 (2%)
Hemorrhage, multifocal	3 (6%)	3 (6%)	7 (14%)	7 (14%)
Inflammation, granulomatous	–	–	1 (2%)	–
Inflammation, chronic	17 (35%)	21 (42%)	24 (48%)	23 (46%)
Mineralization	–	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	1 (2%)	–	1 (2%)	1 (2%)
Alveolus, infiltration cellular, histiocyte	19 (39%)	22 (44%)	19 (38%)	13 (26%)
Alveolus, mineralization	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	4 (8%)	12 (24%)	11 (22%)	10 (20%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Fungus	1 (2%)	–	–	–
Inflammation	1 (2%)	–	–	–
Inflammation, chronic active	11 (22%)	15 (30%)	16 (32%)	15.(30%)
Thrombosis	–	1 (2%)	–	–
Respiratory epithelium, hyperplasia	2 (4%)	2.(4%)	4 (8%)	7 (14%)
Respiratory epithelium, metaplasia, squamous	–	–	2 (4%)	–
Trachea	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1.(2%)	1 (2%)	–
Special Senses System				
Ear	(0)	(0)	(1)	(0)
External ear, hyperplasia, squamous	–	–	1 (100%)	–
Eye	(50)	(50)	(49)	(49)
Cataract	–	–	–	1 (2%)
Anterior chamber, inflammation, acute	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Cornea, inflammation, acute	20 (40%)	25 (50%)	17 (35%)	21 (43%)
Retina, fibrosis	–	–	–	1 (2%)
Harderian gland	(50)	(50)	(49)	(50)
Angiectasis	–	–	–	1 (2%)
Pigmentation, porphyrin	1 (2%)	–	–	–
Lacrimal gland	(1)	(2)	(4)	(0)
Degeneration	1 (100%)	2 (100%)	4 (100%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	–	–	–	1 (2%)
Metaplasia, osseous	–	1 (2%)	–	–
Nephropathy	50 (100%)	49 (98%)	49 (98%)	50 (100%)
Renal tubule, dilatation	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	1 (2%)
Inflammation	–	–	–	1 (2%)

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

Appendix B. Summary of Lesions in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol B-2
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Table B-1. Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	1	–	1
Moribund	24	22	23	11
Natural deaths	5	8	7	8
Survivors				
Died last week of study	–	1	1	–
Terminal euthanasia	21	18	19	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(48)
Intestine large, colon	(50)	(50)	(50)	(48)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(48)	(47)	(48)	(47)
Intestine small, ileum	(47)	(47)	(48)	(47)
Intestine small, jejunum	(47)	(46)	(48)	(48)
Liver	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, uterus	–	–	2 (4%)	1 (2%)
Hepatocellular adenoma	2 (4%)	1 (2%)	4 (8%)	–
Hepatocellular carcinoma	–	1 (2%)	–	–
Mesentery	(0)	(2)	(2)	(1)
Fat, adenocarcinoma, metastatic, uterus	–	1 (50%)	1 (50%)	1 (100%)
Fat, squamous cell carcinoma, metastatic, uterus	–	–	1 (50%)	–
Oral mucosa	(50)	(50)	(50)	(50)
Squamous cell carcinoma	–	–	–	2 (4%)
Pancreas	(50)	(49)	(49)	(48)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	1 (2%)	1 (2%)
Sarcoma stromal, metastatic, uterus	1 (2%)	–	–	–
Acinus, adenoma	–	1 (2%)	–	1 (2%)
Salivary glands	(50)	(49)	(48)	(50)
Stomach, forestomach	(50)	(50)	(50)	(49)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Sarcoma stromal, metastatic, uterus	1 (2%)	–	–	–
Squamous cell carcinoma	1 (2%)	–	–	1 (2%)
Stomach, glandular	(50)	(49)	(50)	(49)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Carcinoma	–	1 (2%)	–	–
Tongue	(1)	(1)	(0)	(1)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, squamous cell carcinoma, metastatic, lung	–	–	1 (2%)	–
Heart	(50)	(50)	(50)	(49)
Hemangioma	–	–	1 (2%)	–
Schwannoma malignant	–	2 (4%)	–	–
Squamous cell carcinoma, metastatic, lung	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Adenoma	–	1 (2%)	1 (2%)	–
Sarcoma stromal, metastatic, uterus	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(50)	(48)
Pheochromocytoma benign	–	2 (4%)	2 (4%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Carcinoma	–	–	1 (2%)	–
Parathyroid gland	(47)	(49)	(46)	(49)
Pituitary gland	(50)	(50)	(50)	(49)
Schwannoma malignant, metastatic, peripheral nerve	–	1 (2%)	–	–
Pars distalis, adenoma	18 (36%)	18 (36%)	19 (38%)	8 (16%)
Thyroid gland	(49)	(49)	(48)	(47)
C-cell, adenoma	11 (22%)	9 (18%)	5 (10%)	10 (21%)
C-cell, carcinoma	–	1 (2%)	–	1 (2%)
Follicular cell, adenoma	–	–	1 (2%)	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Ovary	(50)	(50)	(50)	(49)
Adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Granulosa cell tumor benign	–	–	1 (2%)	–
Granulosa-theca tumor benign	1 (2%)	–	–	–
Tubulostromal carcinoma	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma	–	1 (2%)	4 (8%)	3 (6%)
Adenocarcinoma, multiple	–	–	–	1 (2%)
Polyp stromal	10 (20%)	10 (20%)	13 (26%)	9 (18%)
Sarcoma stromal	1 (2%)	–	2 (4%)	1 (2%)
Sarcoma stromal, multiple	1 (2%)	–	–	–
Squamous cell carcinoma	–	–	1 (2%)	–
Vagina	(2)	(1)	(0)	(2)
Polyp	1 (50%)	–	–	–
Sarcoma stromal, metastatic, uterus	–	–	–	1 (50%)
Schwannoma malignant	1 (50%)	1 (100%)	–	1 (50%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(7)	(6)	(4)	(3)
Pancreatic, sarcoma stromal, metastatic, uterus	1 (14%)	–	–	–
Renal, adenocarcinoma, metastatic, uterus	–	–	–	1 (33%)
Lymph node, mandibular	(50)	(49)	(49)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Sarcoma stromal, metastatic, uterus	1 (2%)	–	–	–
Spleen	(50)	(50)	(50)	(48)
Thymus	(47)	(50)	(49)	(50)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Squamous cell carcinoma, metastatic, lung	–	–	1 (2%)	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	–	2 (4%)
Carcinoma	3 (6%)	2 (4%)	2 (4%)	–
Fibroadenoma	18 (36%)	23 (46%)	20 (40%)	17 (34%)
Fibroadenoma, multiple	11 (22%)	8 (16%)	4 (8%)	6 (12%)
Myoepithelioma	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	–	–	1 (2%)	–
Fibrosarcoma	–	–	–	1 (2%)
Hemangiosarcoma	1 (2%)	–	–	–
Schwannoma malignant	1 (2%)	–	–	–
Subcutaneous tissue, fibroma	1 (2%)	–	–	4 (8%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, sarcoma	–	–	–	1 (2%)
Subcutaneous tissue, schwannoma malignant	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteoma	–	–	–	1 (2%)
Periosteum, sarcoma	–	–	–	1 (2%)
Skeletal muscle	(1)	(0)	(1)	(1)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (100%)
Sarcoma	1 (100%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma malignant	–	–	–	1 (2%)
Granular cell tumor benign	1 (2%)	–	–	–
Peripheral nerve	(0)	(2)	(0)	(0)
Trigeminal, schwannoma malignant	–	1 (50%)	–	–
Spinal cord	(0)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Adenocarcinoma, metastatic, uterus	–	1 (2%)	1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma	1 (2%)	–	1 (2%)	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Cystic keratinizing epithelioma	–	1 (2%)	–	–
Squamous cell carcinoma, multiple	–	–	1 (2%)	–
Mediastinum, sarcoma	1 (2%)	–	–	–
Nose	(49)	(50)	(49)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, peripheral nerve	–	1 (2%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(48)
Adenocarcinoma, metastatic, uterus	–	–	–	1 (2%)
Capsule, adenocarcinoma, metastatic, uterus	–	–	1 (2%)	–
Renal tubule, adenoma	–	–	1 (2%)	–
Renal tubule, adenoma, multiple	–		1 (2%)	–
Urinary bladder	(49)	(50)	(50)	(50)
Squamous cell carcinoma	–	–	1 (2%)	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	–	–
Leukemia mononuclear	–	2 (4%)	–	–
Lymphoma malignant	–	–	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	44	46	37
Total primary neoplasms	93	88	89	73
Total animals with benign neoplasms	40	40	41	35
Total benign neoplasms	79	76	74	58
Total animals with malignant neoplasms	14	11	15	15
Total malignant neoplasms	14	12	15	15
Total animals with metastatic neoplasms	1	2	4	3
Total metastatic neoplasms	5	5	11	13

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

Table B-2. Statistical Analysis of Primary Neoplasms in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Liver: Hepatocellular Adenoma				
Overall rate[a]	2/50 (4%)	1/50 (2%)	4/50 (8%)	0/48 (0%)
Adjusted rate[b]	5.5%	2.7%	11.0%	0.0%
Terminal rate[c]	2/21 (10%)	1/19 (5%)	2/19 (11%)	0/30 (0%)
First incidence (days)	729 (T)	729 (T)	596	–[e]
Poly-3 test[d]	P = 0.227N	P = 0.498N	P = 0.335	P = 0.216N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	0/48 (0%)
Adjusted rate	5.5%	5.4%	11.0%	0.0%
Terminal rate	2/21 (10%)	1/19 (5%)	2/19 (11%)	0/30 (0%)
First incidence (days)	729 (T)	616	596	–
Poly-3 test	P = 0.184N	P = 0.689N	P = 0.335	P = 0.216N
Mammary Gland: Fibroadenoma				
Overall rate	29/50 (58%)	31/50 (62%)	24/50 (48%)	23/50 (46%)
Adjusted rate	66.6%	71.5%	58.4%	53.5%
Terminal rate	11/21 (52%)	11/19 (58%)	10/19 (53%)	14/30 (47%)
First incidence (days)	288	420	329	356
Poly-3 test	P = 0.058N	P = 0.389	P = 0.283N	P = 0.147N
Mammary Gland: Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Adjusted rate	8.2%	2.7%	0.0%	5.0%
Terminal rate	1/21 (5%)	0/19 (0%)	0/19 (0%)	2/30 (7%)
First incidence (days)	681	645	–	729 (T)
Poly-3 test	P = 0.436N	P = 0.301N	P = 0.122N	P = 0.459N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	30/50 (60%)	31/50 (62%)	24/50 (48%)	25/50 (50%)
Adjusted rate	68.6%	71.5%	58.4%	58.2%
Terminal rate	11/21 (52%)	11/19 (58%)	10/19 (53%)	16/30 (53%)
First incidence (days)	288	420	329	356
Poly-3 test	P = 0.106N	P = 0.471	P = 0.217N	P = 0.210N
Mammary Gland: Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.8%	5.4%	5.6%	0.0%
Terminal rate	0/21 (0%)	1/19 (5%)	2/19 (11%)	0/30 (0%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
First incidence (days)	446	705	729 (T)	–
Poly-3 test	P = 0.077N	P = 0.522N	P = 0.536N	P = 0.111N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	15.5%	8.1%	5.6%	5.0%
Terminal rate	1/21 (5%)	1/19 (5%)	2/19 (11%)	2/30 (7%)
First incidence (days)	446	645	729 (T)	729 (T)
Poly-3 test	P = 0.084N	P = 0.261N	P = 0.158N	P = 0.119N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	32/50 (64%)	31/50 (62%)	25/50 (50%)	25/50 (50%)
Adjusted rate	70.8%	71.5%	60.8%	58.2%
Terminal rate	11/21 (52%)	11/19 (58%)	11/19 (58%)	16/30 (53%)
First incidence (days)	288	420	329	356
Poly-3 test	P = 0.076N	P = 0.566	P = 0.216N	P = 0.146N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	18/50 (36%)	18/50 (36%)	19/50 (38%)	8/49 (16%)
Adjusted rate	45.6%	45.1%	49.0%	19.8%
Terminal rate	8/21 (38%)	10/19 (53%)	11/19 (58%)	3/30 (10%)
First incidence (days)	552	420	516	571
Poly-3 test	P = 0.006N	P = 0.574N	P = 0.469	P = 0.011N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	2.7%	0.0%	0.0%	9.9%
Terminal rate	1/21 (5%)	0/19 (0%)	0/19 (0%)	3/30 (10%)
First incidence (days)	729 (T)	–	–	701
Poly-3 test	P = 0.029	P = 0.499N	P = 0.504N	P = 0.209
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Adjusted rate	5.4%	0.0%	0.0%	12.4%
Terminal rate	1/21 (5%)	0/19 (0%)	0/19 (0%)	3/30 (10%)
First incidence (days)	645	–	–	692
Poly-3 test	P = 0.040	P = 0.237N	P = 0.243N	P = 0.254
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted rate	5.4%	0.0%	0.0%	14.9%

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Terminal rate	1/21 (5%)	0/19 (0%)	0/19 (0%)	4/30 (13%)
First incidence (days)	645	–	–	692
Poly-3 test	P = 0.013	P = 0.237N	P = 0.243N	P = 0.163
Thyroid Gland (C-Cell): Adenoma				
Overall rate	11/49 (22%)	9/49 (18%)	5/48 (10%)	10/47 (21%)
Adjusted rate	29.8%	23.9%	13.9%	25.0%
Terminal rate	8/21 (38%)	6/19 (32%)	2/19 (11%)	7/30 (23%)
First incidence (days)	558	313	631	605
Poly-3 test	P = 0.385N	P = 0.375N	P = 0.082N	P = 0.413N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/49 (22%)	10/49 (20%)	5/48 (10%)	11/47 (23%)
Adjusted rate	29.8%	26.6%	13.9%	27.4%
Terminal rate	8/21 (38%)	7/19 (37%)	2/19 (11%)	7/30 (23%)
First incidence (days)	558	313	631	605
Poly-3 test	P = 0.457N	P = 0.478N	P = 0.082N	P = 0.508N
Uterus: Stromal Polyp (Standard Evaluation)				
Overall rate	10/50 (20%)	10/50 (20%)	13/50 (26%)	9/50 (18%)
Adjusted rate	26.6%	26.2%	34.7%	22.1%
Terminal rate	7/21 (33%)	5/19 (26%)	8/19 (42%)	6/30 (20%)
First incidence (days)	422	420	611	622
Poly-3 test	P = 0.379N	P = 0.589N	P = 0.300	P = 0.420N
Uterus: Stromal Polyp or Stromal Sarcoma (Standard Evaluation)				
Overall rate	12/50 (24%)	10/50 (20%)	15/50 (30%)	9/50 (18%)
Adjusted rate	31.3%	26.2%	38.6%	22.1%
Terminal rate	7/21 (33%)	5/19 (26%)	8/19 (42%)	6/30 (20%)
First incidence (days)	422	420	393	622
Poly-3 test	P = 0.260N	P = 0.405N	P = 0.330	P = 0.249N
Uterus: Adenocarcinoma (Standard Evaluation)				
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate	0.0%	2.7%	10.9%	9.8%
Terminal rate	0/21 (0%)	0/19 (0%)	1/19 (5%)	2/30 (7%)
First incidence (days)	–	645	608	645
Poly-3 test	P = 0.040	P = 0.503	P = 0.059	P = 0.074

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Uterus: Adenocarcinoma (Extended Evaluation)				
Overall rate	5/50 (10%)	3/50 (6%)	10/50 (20%)	8/50 (16%)
Adjusted rate	13.7%	8.2%	26.9%	19.8%
Terminal rate	3/21 (14%)	2/19 (11%)	5/19 (26%)	7/30 (23%)
First incidence (days)	719	719	615	674
Poly-3 test	P = 0.158	P = 0.351N	P = 0.127	P = 0.340
Uterus: Adenoma or Adenocarcinoma (Extended Evaluation)				
Overall rate	5/50 (10%)	5/50 (10%)	11/50 (22%)	9/50 (18%)
Adjusted rate	13.7%	13.5%	29.6%	22.3%
Terminal rate	3/21 (14%)	3/19 (16%)	6/19 (32%)	8/30 (27%)
First incidence (days)	719	645	615	674
Poly-3 test	P = 0.146	P = 0.624N	P = 0.080	P = 0.247
Uterus: Adenoma or Adenocarcinoma (Standard and Extended Evaluation)				
Overall rate	5/50 (10%)	5/50 (10%)	14/50 (28%)	11/50 (22%)
Adjusted rate	13.7%	13.5%	36.6%	27.0%
Terminal rate	3/21 (14%)	3/19 (16%)	6/19 (32%)	9/30 (30%)
First incidence (days)	719	645	608	645
Poly-3 test	P = 0.052	P = 0.624N	P = 0.018	P = 0.119
Uterus: Adenocarcinoma or Adenosquamous Carcinoma (Standard and Extended Evaluations)				
Overall rate	5/50 (10%)	4/50 (8%)	13/50 (26%)	10/50 (20%)
Adjusted rate	13.7%	10.8%	34.0%	24.6%
Terminal rate	3/21 (14%)	2/19 (11%)	5/19 (26%)	8/30 (27%)
First incidence (days)	719	645	608	645
Poly-3 test	P = 0.071	P = 0.491N	P = 0.033	P = 0.177
All Organs: Benign Neoplasms				
Overall rate	40/50 (80%)	40/50 (80%)	41/50 (82%)	35/50 (70%)
Adjusted rate	89.5%	88.9%	91.7%	79.1%
Terminal rate	19/21 (91%)	18/19 (95%)	18/19 (95%)	22/30 (73%)
First incidence (days)	288	313	307	356
Poly-3 test	P = 0.067N	P = 0.609N	P = 0.508	P = 0.127N
All Organs: Malignant Neoplasms				
Overall rate	14/50 (28%)	11/50 (22%)	15/50 (30%)	15/50 (30%)
Adjusted rate	33.4%	27.7%	36.7%	35.8%
Terminal rate	3/21 (14%)	4/19 (21%)	6/19 (32%)	8/30 (27%)
First incidence (days)	425	187	160	605

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Poly-3 test	P = 0.356	P = 0.374N	P = 0.464	P = 0.498
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	44/50 (88%)	46/50 (92%)	37/50 (74%)
Adjusted rate	95.3%	92.6%	96.6%	83.6%
Terminal rate	19/21 (91%)	18/19 (95%)	18/19 (95%)	24/30 (80%)
First incidence (days)	288	187	160	356
Poly-3 test	P = 0.029N	P = 0.445N	P = 0.577	P = 0.057N

(T) Terminal euthanasia.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal euthanasia.
[d]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.

Table B-3. Historical Incidence of Carcinoma of the Uterus in Control Female Sprague Dawley Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies[b]	
Indole-3-carbinol (March 2007)	0/50
PCB 118 (March 2004)	2/52
Total	2/102 (2.0%)
Mean ± standard deviation	1.9% ± 2.7%
Range	0%–4%

[a]Data as of June 2013.
[b]Same data for overall historical incidence for all routes.

Table B-4. Historical Incidence of Skin Neoplasms in Control Female Sprague Dawley Rats[a]

Study (Study Start)	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma
Historical Incidence: Corn Oil Gavage Studies[b]			
Indole-3-carbinol (March 2007)	1/50	1/50	2/50
PCB 118 (March 2004)	2/52	0/52	2/52
Total (%)	3/102 (2.9%)	1/102 (1.0%)	4/102 (3.9%)
Mean ± standard deviation	2.9% ± 1.3%	1.0% ± 1.4%	3.9% ± 0.1%
Range	2%–4%	0%–2%	4%

[a]Data as of June 2013.
[b]Same data for overall historical incidence for all routes.

Table B-5. Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	1	–	1
Moribund	24	22	23	11
Natural deaths	5	8	7	8
Survivors				
Died last week of study	–	1	1	–
Terminal euthanasia	21	18	19	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation	–	2 (4%)	–	–
Perforation	–	–	–	1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(48)
Intestine large, colon	(50)	(50)	(50)	(48)
Intestine large, rectum	(50)	(50)	(50)	(50)
Metaplasia	–	–	1 (2%)	–
Intestine small, duodenum	(48)	(47)	(48)	(47)
Lymphatic, ectasia	–	–	16 (33%)	38 (81%)
Intestine small, ileum	(47)	(47)	(48)	(47)
Intestine small, jejunum	(47)	(46)	(48)	(48)
Lymphatic, ectasia	–	–	30 (63%)	47 (98%)
Liver	(50)	(50)	(50)	(48)
Angiectasis	1 (2%)	–	2 (4%)	3 (6%)
Basophilic focus	14 (28%)	6 (12%)	11 (22%)	14 (29%)
Cholangiofibrosis	–	1 (2%)	–	–
Clear cell focus	6 (12%)	7 (14%)	4 (8%)	18 (38%)
Eosinophilic focus	–	4 (8%)	5 (10%)	6 (13%)
Hepatodiaphragmatic nodule	–	–	3 (6%)	2 (4%)
Inflammation, chronic	–	–	–	1 (2%)
Mixed cell focus	5 (10%)	1 (2%)	5 (10%)	4 (8%)
Necrosis	1 (2%)	2 (4%)	–	–
Pigmentation	1 (2%)	–	–	–

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Thrombosis	–	1 (2%)	–	–
Bile duct, cyst	2 (4%)	1 (2%)	4 (8%)	4 (8%)
Bile duct, cyst, multiple	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Bile duct, hyperplasia	2 (4%)	–	–	–
Centrilobular, degeneration	–	1 (2%)	–	–
Mesentery	(0)	(2)	(2)	(1)
Artery, inflammation, chronic active	–	1 (50%)	–	–
Oral mucosa	(50)	(50)	(50)	(50)
Hyperplasia, squamous	–	–	1 (2%)	2 (4%)
Pancreas	(50)	(49)	(49)	(48)
Acinus, hyperplasia	1 (2%)	3 (6%)	–	1 (2%)
Artery, inflammation, chronic active	1 (2%)	4 (8%)	3 (6%)	6 (13%)
Salivary glands	(50)	(49)	(48)	(50)
Inflammation	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(49)
Hyperplasia	2 (4%)	5 (10%)	7 (14%)	3 (6%)
Inflammation	1 (2%)	–	–	1 (2%)
Pigmentation, melanin	–	1 (2%)	–	–
Ulcer	1 (2%)	–	–	–
Stomach, glandular	(50)	(49)	(50)	(49)
Erosion	–	–	1 (2%)	–
Ulcer	1 (2%)	–	–	–
Epithelium, hyperplasia	1 (2%)	–	1 (2%)	–
Epithelium, mineralization	–	–	1 (2%)	–
Tongue	(1)	(1)	(0)	(1)
Cyst	–	–	–	1 (100%)
Erosion	1 (100%)	–	–	–
Inflammation	–	1 (100%)	–	–
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Degeneration, focal	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	3 (6%)	4 (8%)	7 (14%)
Thrombosis	–	–	1 (2%)	–
Heart	(50)	(50)	(50)	(49)
Cardiomyopathy	24 (48%)	24 (48%)	24 (48%)	24 (49%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Atrium, thrombosis	–	1 (2%)	–	–
Epicardium, inflammation	1 (2%)	3 (6%)	–	1 (2%)
Pericardium, inflammation	–	–	2 (4%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(48)
Degeneration, cystic	16 (32%)	12 (24%)	11 (22%)	15 (31%)
Degeneration, fatty	5 (10%)	3 (6%)	–	1 (2%)
Hyperplasia	18 (36%)	11 (22%)	18 (36%)	15 (31%)
Hypertrophy	1 (2%)	–	–	–
Inflammation	–	1 (2%)	–	1 (2%)
Necrosis	–	–	1 (2%)	1 (2%)
Thrombosis	–	1 (2%)	1 (2%)	–
Bilateral, degeneration, cystic	1 (2%)	–	–	–
Adrenal medulla	(50)	(50)	(50)	(48)
Hyperplasia	15 (30%)	13 (26%)	11 (22%)	12 (25%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(47)	(49)	(46)	(49)
Hyperplasia	–	2 (4%)	–	–
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, hyperplasia	19 (38%)	14 (28%)	18 (36%)	24 (49%)
Thyroid gland	(49)	(49)	(48)	(47)
C-cell, hyperplasia	7 (14%)	5 (10%)	6 (13%)	5 (11%)
Follicular cell, hyperplasia	1 (2%)	–	1 (2%)	1 (2%)
Follicular cell, hypertrophy	27 (55%)	23 (47%)	24 (50%)	30 (64%)
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	5 (10%)	2 (4%)	3 (6%)	4 (8%)
Inflammation	1 (2%)	–	–	2 (4%)
Ovary	(50)	(50)	(50)	(49)
Angiectasis	–	–	–	1 (2%)
Atrophy	40 (80%)	23 (46%)	37 (74%)	30 (61%)
Cyst	17 (34%)	13 (26%)	16 (32%)	16 (33%)
Inflammation, acute	–	–	1 (2%)	1 (2%)
Inflammation, chronic	–	–	–	1 (2%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Inflammation, chronic active	1 (2%)	1 (2%)	–	1 (2%)
Necrosis, fibrinoid	–	–	1 (2%)	–
Uterus	(50)	(50)	(50)	(50)
Cyst	–	–	2 (4%)	–
Dilatation	9 (18%)	9 (18%)	7 (14%)	5 (10%)
Inflammation, histiocytic, focal	1 (2%)	–	–	–
Inflammation, acute	5 (10%)	12 (24%)	6 (12%)	9 (18%)
Thrombosis	–	–	1 (2%)	1 (2%)
Endometrium, hyperplasia, cystic	29 (58%)	28 (56%)	29 (58%)	21 (42%)
Endometrium, metaplasia, squamous	12 (24%)	18 (36%)	20 (40%)	11 (22%)
Vagina	(2)	(1)	(0)	(2)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	1 (2%)	–
Hyperplasia	21 (42%)	20 (40%)	23 (46%)	17 (34%)
Lymph node	(7)	(6)	(4)	(3)
Axillary, hyperplasia, lymphoid	–	–	–	1 (33%)
Iliac, hyperplasia, lymphoid	–	1 (17%)	–	–
Iliac, infiltration cellular, histiocyte	–	–	–	1 (33%)
Inguinal, hemorrhage	–	1 (17%)	–	–
Inguinal, hyperplasia, lymphoid	1 (14%)	–	1 (25%)	–
Lumbar, hemorrhage	2 (29%)	1 (17%)	–	–
Lumbar, hyperplasia, lymphoid	1 (14%)	1 (17%)	–	–
Lumbar, infiltration cellular, histiocyte	–	1 (17%)	1 (25%)	–
Mediastinal, ectasia	–	–	1 (25%)	–
Mediastinal, hemorrhage	2 (29%)	–	1 (25%)	–
Mediastinal, infiltration cellular, histiocyte	1 (14%)	–	–	–
Lymph node, mandibular	(50)	(49)	(49)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(48)
Hemorrhage	–	–	–	1 (2%)
Pigmentation, hemosiderin	1 (2%)	–	–	–
Lymphatic, ectasia	–	–	1 (2%)	15 (31%)
Spleen	(50)	(50)	(50)	(48)
Hematopoietic cell proliferation	36 (72%)	32 (64%)	40 (80%)	33 (69%)
Thymus	(47)	(50)	(49)	(50)
Atrophy	38 (81%)	31 (62%)	34 (69%)	38 (76%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Hemorrhage	–	–	–	1 (2%)
Inflammation	–	1 (2%)	1 (2%)	–
Epithelial cell, hyperplasia	1 (2%)	4 (8%)	3 (6%)	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	1 (2%)	–	–	–
Hyperplasia	8 (16%)	3 (6%)	7 (14%)	7 (14%)
Inflammation	1 (2%)	1 (2%)	–	–
Inflammation, chronic	1 (2%)	–	–	–
Duct, dilatation	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	–	1 (2%)	–	1 (2%)
Fibrosis	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	–	1 (2%)	1 (2%)	–
Skeletal muscle	(1)	(0)	(1)	(1)
Degeneration	–	–	1 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis	–	1 (2%)	–	–
Hemorrhage	–	–	–	1 (2%)
Inflammation	–	1 (2%)	–	–
Necrosis	1 (2%)	–	–	1 (2%)
Meninges, pigmentation, lipofuscin	–	1 (2%)	–	–
Peripheral nerve	(0)	(2)	(0)	(0)
Spinal cord	(0)	(1)	(0)	(0)
Hemorrhage	–	1 (100%)	–	–
Necrosis	–	1 (100%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(49)
Atelectasis	–	1 (2%)	–	–
Foreign body	–	2 (4%)	–	–
Inflammation, granulomatous	1 (2%)	–	–	–
Inflammation, chronic	34 (68%)	30 (60%)	38 (76%)	35 (71%)
Alveolar epithelium, hyperplasia	–	–	–	1 (2%)

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Alveolar epithelium, metaplasia, squamous	–	–	–	1 (2%)
Alveolus, infiltration cellular, histiocyte	13 (26%)	16 (32%)	11 (22%)	9 (18%)
Bronchiole, hyperplasia	–	1 (2%)	1 (2%)	–
Mediastinum, inflammation	–	1 (2%)	–	–
Pleura, inflammation, chronic active	–	1 (2%)	–	–
Nose	(49)	(50)	(49)	(50)
Angiectasis	–	–	1 (2%)	–
Foreign body	3 (6%)	2 (4%)	3 (6%)	3 (6%)
Inflammation, chronic active	5 (10%)	4 (8%)	5 (10%)	6 (12%)
Glands, olfactory epithelium, hyperplasia	–	1 (2%)	–	–
Olfactory epithelium, atrophy	–	–	2 (4%)	5 (10%)
Olfactory epithelium, degeneration	1 (2%)	–	1 (2%)	–
Respiratory epithelium, hyperplasia	–	–	3 (6%)	2 (4%)
Respiratory epithelium, metaplasia, squamous	–	–	1 (2%)	1 (2%)
Trachea	(50)	(50)	(50)	(50)
Epithelium, necrosis	–	1 (2%)	–	–
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)	–	–	–
Inflammation	–	1 (2%)	1 (2%)	–
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	–	–	1 (2%)	–
Inflammation	–	1 (2%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(48)
Nephropathy	45 (90%)	41 (82%)	42 (84%)	43 (90%)
Pelvis, inflammation	–	–	–	1 (2%)
Urinary bladder	(49)	(50)	(50)	(50)
Hyperplasia	–	–	1 (2%)	–

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

Appendix C. Summary of Lesions in Male Mice in the Two-year Gavage Study of Indole-3-carbinol

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Gavage Study of Indole-3-carbinol C-2
Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Gavage Study of Indole-3-carbinol C-7
Table C-3. Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a] C-11
Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Gavage Study of Indole-3-carbinol C-12

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	–	1	1
Moribund	17	12	11	9
Natural deaths	6	7	6	8
Survivors				
Died last week of study	1	–	–	–
Terminal euthanasia	26	31	32	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(50)	(49)	(48)
Carcinoma, metastatic, pancreas	–	–	1 (2%)	–
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	–	–	1 (2%)
Carcinoma	–	–	–	2 (4%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(49)	(50)
Adenoma	1 (2%)	–	1 (2%)	–
Liver	(50)	(50)	(49)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Carcinoma, metastatic, intestine small, duodenum	–	–	–	1 (2%)
Fibrous histiocytoma	–	1 (2%)	–	–
Hemangioma	–	–	–	1 (2%)
Hemangiosarcoma	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Hemangiosarcoma, multiple	1 (2%)	2 (4%)	–	1 (2%)
Hepatoblastoma	3 (6%)	3 (6%)	1 (2%)	7 (14%)
Hepatoblastoma, multiple	–	1 (2%)	3 (6%)	7 (14%)
Hepatocellular adenoma	11 (22%)	7 (14%)	15 (31%)	8 (16%)
Hepatocellular adenoma, multiple	15 (30%)	25 (50%)	16 (33%)	33 (66%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Hepatocellular carcinoma	7 (14%)	8 (16%)	11 (22%)	6 (12%)
Hepatocellular carcinoma, multiple	5 (10%)	3 (6%)	18 (37%)	8 (16%)
Hepatocholangiocarcinoma	–	3 (6%)	1 (2%)	–
Hepatocholangioma	–	–	–	1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Mesentery	(7)	(3)	(2)	(2)
Fibrosarcoma	–	1 (33%)	–	–
Hepatoblastoma, metastatic, liver	–	1 (33%)	–	–
Hepatocellular carcinoma, metastatic, liver	1 (14%)	–	–	–
Leiomyosarcoma, metastatic, stomach, forestomach	–	1 (33%)	–	–
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (14%)	–	–	–
Oral mucosa	(0)	(1)	(0)	(2)
Pancreas	(50)	(50)	(50)	(50)
Carcinoma	–	–	1 (2%)	–
Hemangioma	–	1 (2%)	–	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Leiomyosarcoma, metastatic, stomach, glandular	–	1 (2%)	–	–
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Salivary glands	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Hemangiosarcoma	1 (2%)	–	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)	–	–	–
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	–
Squamous cell papilloma, multiple	–	1 (2%)	–	–
Stomach, glandular	(50)	(47)	(47)	(49)
Carcinoma	–	–	1 (2%)	–
Carcinoma, metastatic, intestine small, duodenum	–	–	–	1 (2%)
Carcinoma, metastatic, pancreas	–	–	1 (2%)	–
Leiomyosarcoma		1 (2%)	–	–
Tooth	(30)	(37)	(32)	(32)
Cardiovascular System				
Blood vessel	(49)	(50)	(49)	(49)
Heart	(50)	(50)	(50)	(50)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Hemangiosarcoma	–	1 (2%)	–	–
Endocrine system				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	–	–	2 (4%)	–
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	–	1 (2%)	–	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	1 (2%)
Parathyroid gland	(48)	(44)	(48)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Pars intermedia, adenoma	–	1 (2%)	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	–	1 (2%)	–	1 (2%)
Follicular cell, adenoma	–	–	1 (2%)	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Leiomyosarcoma, metastatic, stomach, glandular	–	1 (2%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	–	–	–	1 (2%)
Interstitial cell, adenoma	–	1 (2%)	–	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	–
Lymph node	(2)	(2)	(0)	(4)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mesenteric	(48)	(48)	(49)	(50)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Hemangiosarcoma	–	1 (2%)	–	–
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Thymus	(49)	(47)	(46)	(45)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	–	–	1 (2%)	–
Squamous cell carcinoma, metastatic, stomach, forestomach	1 (2%)	–	–	–
Thymoma benign	1 (2%)	–	–	–
Integumentary System				
Mammary gland	(2)	(0)	(0)	(0)
Skin	(50)	(50)	(50)	(50)
Epidermis, keratoacanthoma	–	–	1 (2%)	–
Subcutaneous tissue, fibrosarcoma	1 (2%)	1 (2%)	–	–
Subcutaneous tissue, hemangiosarcoma	–	2 (4%)	1 (2%)	–
Subcutaneous tissue, lipoma	–	–	–	1 (2%)
Subcutaneous tissue, neural crest tumor	–	–	1 (2%)	–
Subcutaneous tissue, schwannoma malignant	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(2)	(0)
Hemangiosarcoma	–	–	2 (100%)	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	1 (2%)	–	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	4 (8%)	6 (12%)	6 (12%)	8 (16%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	2 (4%)	–
Alveolar/bronchiolar carcinoma	3 (6%)	7 (14%)	5 (10%)	4 (8%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)	–	–	1 (2%)
Carcinoma, metastatic, harderian gland	–	–	–	1 (2%)
Hepatoblastoma, metastatic, liver	–	1 (2%)	1 (2%)	2 (4%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	3 (6%)	10 (20%)	4 (8%)
Hepatocholangiocarcinoma, metastatic, liver	–	1 (2%)	–	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	6 (12%)	7 (14%)	3 (6%)
Carcinoma	1 (2%)	1 (2%)	–	1 (2%)
Bilateral, adenoma	–	1 (2%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	–	–
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	1 (2%)	–	1 (2%)
Lymphoma malignant	3 (6%)	1 (2%)	1 (2%)	5 (10%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	45	48	48
Total primary neoplasms	78	95	103	107
Total animals with benign neoplasms	33	40	39	42
Total benign neoplasms	44	53	53	61
Total animals with malignant neoplasms	27	26	36	35
Total malignant neoplasms	34	42	49	46
Total animals with metastatic neoplasms	7	7	10	9
Total metastatic neoplasms	16	13	14	11
Total animals with uncertain neoplasms—benign or malignant	–	–	1	–
Total uncertain neoplasms	–	–	1	–

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

Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	7/50 (14%)	7/50 (14%)	3/50 (6%)
Adjusted rate[b]	14.5%	15.4%	15.6%	6.7%
Terminal rate[c]	2/26 (8%)	5/31 (16%)	4/32 (13%)	1/32 (3%)
First incidence (days)	463	616	437	437
Poly-3 test[d]	P = 0.144N	P = 0.571	P = 0.564	P = 0.205N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	7/50 (14%)	4/50 (8%)
Adjusted rate	16.7%	17.6%	15.6%	9.0%
Terminal rate	2/26 (8%)	5/31 (16%)	4/32 (13%)	2/32 (6%)
First incidence (days)	463	616	437	437
Poly-3 test	P = 0.152N	P = 0.568	P = 0.560N	P = 0.226N
Liver: Hemangiosarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	1/49 (2%)	3/50 (6%)
Adjusted rate	10.0%	8.8%	2.3%	6.8%
Terminal rate	2/26 (8%)	1/31 (3%)	0/32 (0%)	0/32 (0%)
First incidence (days)	626	630	596	495
Poly-3 test	P = 0.321N	P = 0.571N	P = 0.155N	P = 0.445N
Liver: Hepatocellular Adenoma				
Overall rate	26/50 (52%)	32/50 (64%)	31/49 (63%)	41/50 (82%)
Adjusted rate	62.1%	66.5%	67.9%	85.4%
Terminal rate	19/26 (73%)	21/31 (68%)	23/32 (72%)	28/32 (88%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.005	P = 0.411	P = 0.360	P = 0.006
Liver: Hepatocellular Carcinoma				
Overall rate	12/50 (24%)	11/50 (22%)	29/49 (59%)	14/50 (28%)
Adjusted rate	27.7%	24.2%	63.4%	30.9%
Terminal rate	3/26 (12%)	8/31 (26%)	18/32 (56%)	8/32 (25%)
First incidence (days)	465	682	567	385
Poly-3 test	P = 0.217	P = 0.449N	P < 0.001	P = 0.461
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	35/50 (70%)	36/50 (72%)	43/49 (88%)	44/50 (88%)
Adjusted rate	77.6%	74.6%	90.6%	91.1%

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Terminal rate	21/26 (81%)	24/31 (77%)	29/32 (91%)	30/32 (94%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.012	P = 0.459N	P = 0.062	P = 0.049
Liver: Hepatoblastoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/49 (8%)	14/50 (28%)
Adjusted rate	7.6%	8.9%	9.3%	31.6%
Terminal rate	3/26 (12%)	4/31 (13%)	3/32 (9%)	10/32 (31%)
First incidence (days)	730 (T)	730 (T)	702	617
Poly-3 test	P < 0.001	P = 0.567	P = 0.543	P = 0.005
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	15/50 (30%)	13/50 (26%)	30/49 (61%)	25/50 (50%)
Adjusted rate	34.6%	28.7%	65.6%	54.5%
Terminal rate	6/26 (23%)	10/31 (32%)	19/32 (59%)	17/32 (53%)
First incidence (days)	465	682	567	385
Poly-3 test	P = 0.005	P = 0.353N	P = 0.002	P = 0.042
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	36/50 (72%)	36/50 (72%)	44/49 (90%)	45/50 (90%)
Adjusted rate	79.8%	74.6%	92.7%	92.8%
Terminal rate	22/26 (85%)	24/31 (77%)	30/32 (94%)	30/32 (94%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.007	P = 0.354N	P = 0.050	P = 0.044
Liver: Hepatocholangiocarcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/49 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.6%	2.3%	0.0%
Terminal rate	0/26 (0%)	0/31 (0%)	1/32 (3%)	0/32 (0%)
First incidence (days)	–[e]	630	730 (T)	–
Poly-3 test	P = 0.319N	P = 0.146	P = 0.515	–[f]
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	4/50 (8%)	7/50 (14%)	8/50 (16%)	8/50 (16%)
Adjusted rate	10.1%	15.4%	17.8%	17.9%
Terminal rate	4/26 (15%)	5/31 (16%)	4/32 (13%)	5/32 (16%)
First incidence (days)	730 (T)	631	437	495
Poly-3 test	P = 0.230	P = 0.342	P = 0.241	P = 0.238
Lung: Alveolar/Bronchiolar Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	5/50 (10%)	5/50 (10%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Adjusted rate	10.0%	15.4%	11.4%	11.4%
Terminal rate	3/26 (12%)	4/31 (13%)	3/32 (9%)	4/32 (13%)
First incidence (days)	680	682	655	586
Poly-3 test	P = 0.523N	P = 0.337	P = 0.561	P = 0.560
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	8/50 (16%)	12/50 (24%)	13/50 (26%)	11/50 (22%)
Adjusted rate	20.0%	26.2%	28.6%	24.6%
Terminal rate	7/26 (27%)	7/31 (23%)	7/32 (22%)	8/32 (25%)
First incidence (days)	680	631	437	495
Poly-3 test	P = 0.420	P = 0.338	P = 0.252	P = 0.405
Small Intestine (Site Unspecified): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	7.5%	0.0%	2.3%	2.3%
Terminal rate	2/26 (8%)	0/31 (0%)	1/32 (3%)	1/32 (3%)
First incidence (days)	728	–	730 (T)	730 (T)
Poly-3 test	P = 0.308N	P = 0.098N	P = 0.273N	P = 0.274N
Small Intestine (Site Unspecified): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.5%	0.0%	2.3%	6.9%
Terminal rate	2/26 (8%)	0/31 (0%)	1/32 (3%)	3/32 (9%)
First incidence (days)	728	–	730 (T)	730 (T)
Poly-3 test	P = 0.415	P = 0.098N	P = 0.273N	P = 0.621N
All Organs: Hemangiosarcoma				
Overall rate	4/50 (8%)	5/50 (10%)	5/50 (10%)	3/50 (6%)
Adjusted rate	10.0%	10.9%	11.1%	6.8%
Terminal rate	2/26 (8%)	1/31 (3%)	1/32 (3%)	0/32 (0%)
First incidence (days)	626	630	596	495
Poly-3 test	P = 0.337N	P = 0.581	P = 0.570	P = 0.445N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	6/50 (12%)	5/50 (10%)	4/50 (8%)
Adjusted rate	10.0%	13.1%	11.1%	9.0%
Terminal rate	2/26 (8%)	2/31 (7%)	1/32 (3%)	1/32 (3%)
First incidence (days)	626	630	596	495
Poly-3 test	P = 0.430N	P = 0.454	P = 0.570	P = 0.588N

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	5/50 (10%)
Adjusted rate	7.5%	2.2%	2.3%	11.3%
Terminal rate	1/26 (4%)	0/31 (0%)	1/32 (3%)	3/32 (9%)
First incidence (days)	711	673	730 (T)	631
Poly-3 test	P = 0.164	P = 0.262N	P = 0.273N	P = 0.413
All Organs: Benign Neoplasms				
Overall rate	33/50 (66%)	40/50 (80%)	39/50 (78%)	42/50 (84%)
Adjusted rate	77.2%	81.8%	82.8%	86.1%
Terminal rate	23/26 (89%)	26/31 (84%)	28/32 (88%)	28/32 (88%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.166	P = 0.381	P = 0.331	P = 0.182
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	26/50 (52%)	36/50 (72%)	35/50 (70%)
Adjusted rate	58.2%	54.5%	75.4%	73.8%
Terminal rate	10/26 (39%)	13/31 (42%)	21/32 (66%)	23/32 (72%)
First incidence (days)	465	381	567	385
Poly-3 test	P = 0.022	P = 0.437N	P = 0.055	P = 0.078
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	45/50 (90%)	48/50 (96%)	48/50 (96%)
Adjusted rate	98.5%	90.9%	98.0%	96.8%
Terminal rate	26/26 (100%)	28/31 (90%)	31/32 (97%)	31/32 (97%)
First incidence (days)	463	381	437	385
Poly-3 test	P = 0.509	P = 0.098N	P = 0.735N	P = 0.552N

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

Table C-3. Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma	Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies				
Ginkgo biloba extract (March 2005)	31/50	22/50	39/50	3/50
Indole-3-carbinol (April 2007)	26/50	12/50	35/50	3/50
Kava kava extract (August 2004)	27/50	20/50	38/50	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	38/50	1/50
Tetrabromobisphenol A (August 2007)	32/50	11/50	39/50	2/50
Total (%)	145/250 (58.0%)	87/250 (34.8%)	189/250 (75.6%)	9/250 (3.6%)
Mean ± standard deviation	58.0% ± 5.1%	34.8% ± 10.9%	75.6% ± 3.3%	3.6% ± 2.6%
Range	52%–64%	22%–44%	70%–78%	0%–6%
Overall Historical Incidence: All Routes				
Total (%)	594/949 (62.6%)	348/949 (36.7%)	742/949 (78.2%)	40/949 (4.2%)
Mean ± standard deviation	62.6% ± 9.1%	36.7% ± 11.4%	78.2% ± 7.2%	4.2% ± 3.5%
Range	48%–78%	22%–56%	64%–90%	0%–12%

	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma	Hepatocholangio-carcinoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	24/50	39/50	0/50
Indole-3-carbinol (April 2007)	15/50	36/50	0/50
Kava kava extract (August 2004)	20/50	38/50	4/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	22/50	38/50	0/50
Tetrabromobisphenol A (August 2007)	12/50	39/50	0/50
Total (%)	93/250 (37.2%)	190/250 (76.0%)	4/250 (1.6%)
Mean ± standard deviation	37.2% ± 10.0%	76.0% ± 2.5%	1.6% ± 3.6%
Range	24%–48%	72%–78%	0%–8%
Overall Historical Incidence: All Routes			
Total (%)	371/949 (39.1%)	746/949 (78.6%)	10/949 (1.1%)
Mean ± standard deviation	39.1% ± 11.6%	78.6% ± 7.2%	1.1% ± 2.2%
Range	22%–58%	64%–90%	0%–8%

[a]Data as of June 2013.

Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	–	–	1	1
Moribund	17	12	11	9
Natural deaths	6	7	6	8
Survivors				
Died last week of study	1	–	–	–
Terminal euthanasia	26	31	32	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Mineralization	–	–	1 (2%)	–
Gallbladder	(50)	(50)	(49)	(48)
Atrophy	–	–	–	1 (2%)
Inflammation, suppurative	–	–	–	1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Ulcer	–	1 (2%)	–	1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Inflammation, chronic	–	–	–	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Inflammation, chronic	–	1 (2%)	–	–
Intestine small, duodenum	(50)	(50)	(50)	(50)
Ulcer	–	–	–	1 (2%)
Epithelium, atrophy	–	–	–	1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(49)	(50)
Atrophy	1 (2%)	–	–	–
Inflammation, suppurative	1 (2%)	–	–	–
Lymphangiectasis	1 (2%)	–	–	–
Peyer’s patch, hyperplasia	–	1 (2%)	1 (2%)	1 (2%)
Liver	(50)	(50)	(49)	(50)
Basophilic focus	2 (4%)	4 (8%)	2 (4%)	5 (10%)
Clear cell focus	7 (14%)	17 (34%)	22 (45%)	20 (40%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Eosinophilic focus	29 (58%)	35 (70%)	35 (71%)	33 (66%)
Fatty change	29 (58%)	27 (54%)	28 (57%)	37 (74%)
Hepatodiaphragmatic nodule	–	1 (2%)	–	1 (2%)
Inflammation, chronic	–	–	–	1 (2%)
Mixed cell focus	1 (2%)	6 (12%)	6 (12%)	5 (10%)
Necrosis	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Necrosis, multifocal	1 (2%)	–	–	–
Artery, thrombosis	–	–	–	1 (2%)
Bile duct, hyperplasia	–	–	–	1 (2%)
Oval cell, hyperplasia	–	–	–	1 (2%)
Periportal, fibrosis	–	–	–	1 (2%)
Mesentery	(7)	(3)	(2)	(2)
Fat, necrosis	4 (57%)	–	2 (100%)	2 (100%)
Oral mucosa	(0)	(1)	(0)	(2)
Pancreas	(50)	(50)	(50)	(50)
Inflammation, chronic	–	–	–	2 (4%)
Acinus, atrophy	1 (2%)	–	1 (2%)	–
Duct, cyst	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	23 (46%)	30 (60%)	27 (54%)	22 (44%)
Duct, sublingual gland, hyperplasia	–	–	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	–	1 (2%)	4 (8%)	1 (2%)
Ulcer	6 (12%)	5 (10%)	7 (14%)	1 (2%)
Epithelium, hyperplasia	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Stomach, glandular	(50)	(47)	(47)	(49)
Erosion	1 (2%)	–	–	–
Inflammation, chronic	1 (2%)	1 (2%)	18 (38%)	45 (92%)
Mineralization	5 (10%)	2 (4%)	–	–
Pigmentation	–	1 (2%)	38 (81%)	48 (98%)
Epithelium, atrophy	–	–	–	1 (2%)
Epithelium, hyperplasia	–	1 (2%)	22 (47%)	40 (82%)
Tooth	(30)	(37)	(32)	(32)
Dysplasia	30 (100%)	36 (97%)	32 (100%)	32 (100%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Cardiovascular System				
Blood vessel	(49)	(50)	(49)	(49)
Adventitia, pulmonary vein, infiltration cellular, polymorphonuclear	1 (2%)	–	–	–
Aorta, mineralization	–	1 (2%)	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	11 (22%)	7 (14%)	18 (36%)	8 (16%)
Inflammation, chronic	1 (2%)	–	–	–
Artery, inflammation, chronic	1 (2%)	–	–	–
Artery, mineralization	–	1 (2%)	–	–
Myocardium, mineralization	–	3 (6%)	–	1 (2%)
Valve, inflammation	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	–	1 (2%)
Hypertrophy	–	4 (8%)	2 (4%)	–
Vacuolization cytoplasmic	–	2 (4%)	–	–
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	–	1 (2%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	1 (2%)	–
Parathyroid gland	(48)	(44)	(48)	(44)
Pituitary gland	(50)	(50)	(50)	(50)
Cyst	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Pars intermedia, hyperplasia	–	1 (2%)	–	–
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation, chronic	–	–	–	1 (2%)
Follicle, cyst	1 (2%)	–	–	–
Follicle, degeneration	–	–	–	1 (2%)
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(1)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Angiectasis	–	–	–	1 (2%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Granuloma sperm	–	–	–	1 (2%)
Infiltration cellular, mononuclear cell	1 (2%)	6 (12%)	3 (6%)	5 (10%)
Inflammation, chronic	–	2 (4%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Cyst	14 (28%)	10 (20%)	6 (12%)	6 (12%)
Inflammation, suppurative	2 (4%)	–	–	–
Inflammation, granulomatous	–	2 (4%)	–	2 (4%)
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	–	1 (2%)	1 (2%)
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, chronic	1 (2%)	–	–	1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	–	–	–
Testes	(50)	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)	–	–	–
Inflammation, chronic	1 (2%)	–	–	–
Mineralization	1 (2%)	–	–	–
Germinal epithelium, atrophy	2 (4%)	–	3 (6%)	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(2)	(2)	(0)	(4)
Mediastinal, hyperplasia	–	–	–	1 (25%)
Pancreatic, inflammation	–	–	–	1 (25%)
Pancreatic, necrosis	–	1 (50%)	–	–
Renal, inflammation	–	–	–	1 (25%)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	–	1 (2%)	–	1 (2%)
Lymph node, mesenteric	(48)	(48)	(49)	(50)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hyperplasia, lymphoid	–	–	–	2 (4%)
Inflammation	–	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	–	–	1 (2%)	–
Atrophy	–	–	–	1 (2%)
Hematopoietic cell proliferation	16 (32%)	16 (32%)	24 (48%)	20 (40%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Necrosis	1 (2%)	–	–	–
Lymphoid follicle, atrophy	2 (4%)	–	–	–
Thymus	(49)	(47)	(46)	(45)
Atrophy	44 (90%)	44 (94%)	45 (98%)	39 (87%)
Ectopic parathyroid gland	–	1 (2%)	–	1 (2%)
Integumentary System				
Mammary gland	(2)	(0)	(0)	(0)
Skin	(50)	(50)	(50)	(50)
Ulcer	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Subcutaneous tissue, inflammation, acute	–	–	1 (2%)	–
Subcutaneous tissue, inflammation, chronic	–	–	2 (4%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	–	–	1 (2%)	1 (2%)
Skeletal muscle	(0)	(0)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meninges, inflammation, chronic	–	1 (2%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Infiltration cellular, histiocyte	–	2 (4%)	–	1 (2%)
Inflammation, chronic	–	1 (2%)	1 (2%)	1 (2%)
Pigmentation, hemosiderin	–	1 (2%)	–	–
Thrombosis	–	–	1 (2%)	–
Alveolar epithelium, hyperplasia	3 (6%)	4 (8%)	5 (10%)	–
Mediastinum, inflammation, granulomatous	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Foreign body	3 (6%)	1 (2%)	–	3 (6%)
Inflammation	15 (30%)	11 (22%)	12 (24%)	16 (32%)
Polyp, inflammatory	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Respiratory metaplasia	–	–	1 (2%)	–
Nerve, atrophy	–	–	–	8 (16%)
Nerve, olfactory epithelium, atrophy	–	1 (2%)	–	–

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Olfactory epithelium, accumulation, hyaline droplet	4 (8%)	4 (8%)	5 (10%)	5 (10%)
Olfactory epithelium, atrophy	3 (6%)	5 (10%)	11 (22%)	17 (34%)
Olfactory epithelium, degeneration	1 (2%)	1 (2%)	4 (8%)	2 (4%)
Olfactory epithelium, metaplasia, squamous	–	–	–	2 (4%)
Olfactory epithelium, necrosis	–	–	–	6 (12%)
Olfactory epithelium, respiratory metaplasia	14 (28%)	14 (28%)	20 (40%)	27 (54%)
Respiratory epithelium, accumulation, hyaline droplet	18 (36%)	34 (68%)	30 (60%)	26 (52%)
Respiratory epithelium, hyperplasia	35 (70%)	40 (80%)	41 (82%)	45 (90%)
Respiratory epithelium, metaplasia, squamous	–	–	–	1 (2%)
Respiratory epithelium, necrosis	–	–	–	2 (4%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Phthisis bulbi	1 (2%)	1 (2%)	–	–
Anterior chamber, inflammation, suppurative	–	1 (2%)	–	1 (2%)
Cornea, inflammation, suppurative	–	1 (2%)	–	–
Cornea, inflammation, chronic	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Optic nerve, infiltration cellular, mononuclear cell	–	–	–	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	4 (8%)	5 (10%)	3 (6%)
Inflammation, suppurative	1 (2%)	1 (2%)	–	–
Inflammation, chronic	–	1 (2%)	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	3 (6%)	2 (4%)	–	–
Inflammation, acute	–	2 (4%)	–	–
Inflammation, chronic	1 (2%)	–	–	1 (2%)
Metaplasia, osseous	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mineralization	4 (8%)	8 (16%)	1 (2%)	2 (4%)
Nephropathy	42 (84%)	50 (100%)	45 (90%)	43 (86%)
Thrombosis	–	1 (2%)	–	–

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Papilla, necrosis	1 (2%)	–	–	1 (2%)
Pelvis, inflammation, suppurative	2 (4%)	2 (4%)	1 (2%)	–
Renal tubule, cyst	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Renal tubule, hyperplasia	–	–	1 (2%)	–
Renal tubule, pigmentation	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Renal tubule, vacuolization cytoplasmic	1 (2%)	–	–	–
Urethra	(0)	(1)	(0)	(0)
Inflammation, acute	–	1 (100%)	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage	–	1 (2%)	–	–
Inflammation, chronic	1 (2%)	2 (4%)	–	–

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

Appendix D. Summary of Lesions in Female Mice in the Two-year Gavage Study of Indole-3-carbinol

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Gavage Study of Indole-3-carbinol.....D-2
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Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	1	–	–	–
Moribund	10	6	15	3
Natural deaths	6	4	9	2
Survivors				
Died last week of study	–	–	1	–
Terminal euthanasia	33	40	25	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Squamous cell carcinoma	–	–	1 (2%)	–
Gallbladder	(49)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)	–	–	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma, multiple	–	–	1 (2%)	–
Hepatoblastoma	–	–	1 (2%)	1 (2%)
Hepatocellular adenoma	7 (14%)	11 (22%)	6 (12%)	7 (14%)
Hepatocellular adenoma, multiple	–	3 (6%)	2 (4%)	4 (8%)
Hepatocellular carcinoma	6 (12%)	6 (12%)	8 (16%)	4 (8%)
Hepatocellular carcinoma, multiple	–	2 (4%)	1 (2%)	–
Mesentery	(3)	(7)	(7)	(6)
Carcinoma, metastatic, kidney	–	1 (14%)	–	–
Hemangioma	–	–	1 (14%)	–
Pancreas	(50)	(50)	(50)	(50)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Salivary glands	(50)	(49)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	–	–	–
Stomach, glandular	(48)	(50)	(49)	(50)
Tongue	(0)	(0)	(0)	(1)
Squamous cell papilloma	–	–	–	1 (100%)
Tooth	(6)	(9)	(9)	(11)
Cardiovascular System				
Blood vessel	(48)	(50)	(50)	(50)
Hemangioma	1 (2%)	–	–	–
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	1 (2%)	–	–	–
Subcapsular, adenoma	1 (2%)	–	–	–
Adrenal medulla	(49)	(50)	(50)	(50)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	–	–
Carcinoma	–	–	1 (2%)	–
Parathyroid gland	(43)	(48)	(49)	(46)
Pituitary gland	(49)	(50)	(50)	(49)
Pars distalis, adenoma	3 (6%)	4 (8%)	–	1 (2%)
Pars intermedia, adenoma	1 (2%)	–	–	–
Thyroid gland	(50)	(50)	(49)	(50)
C-cell, carcinoma	1 (2%)	–	–	–
Follicular cell, adenoma	1 (2%)	–	–	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Genital System				
Clitoral gland	(49)	(50)	(50)	(50)
Ovary	(50)	(50)	(50)	(50)
Cystadenoma	1 (2%)	4 (8%)	1 (2%)	4 (8%)
Hemangioma	–	–	–	1 (2%)
Bilateral, tubulostromal adenoma	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Carcinoma	–	1 (2%)	–	–
Polyp stromal	–	–	–	4 (8%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(9)	(5)	(6)	(1)
Lymph node, mandibular	(50)	(49)	(50)	(50)
Lymph node, mesenteric	(49)	(49)	(49)	(49)
Hemangiosarcoma	–	–	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Thymus	(49)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	1 (2%)	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Squamous cell carcinoma	–	–	1 (2%)	–
Subcutaneous tissue, fibrosarcoma	–	–	2 (4%)	–
Subcutaneous tissue, hemangioma	–	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma, multiple	–	–	1 (2%)	–
Subcutaneous tissue, sarcoma	1 (2%)	–	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(1)	(2)	(0)
Fibrosarcoma	–	–	1 (50%)	–
Rhabdomyosarcoma	–	–	1 (50%)	–
Schwannoma malignant	–	1 (100%)	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Meningioma benign	–	1 (2%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma	2 (4%)	2 (4%)	3 (6%)	–
Alveolar/bronchiolar carcinoma, multiple	–	1 (2%)	–	–
Carcinoma, metastatic, harderian gland	–	1 (2%)	–	–
Carcinoma, metastatic, thyroid gland	1 (2%)	–	–	–
Hepatocellular carcinoma, metastatic, liver	2 (4%)	2 (4%)	2 (4%)	–

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Squamous cell carcinoma, metastatic, skin	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(2)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	7 (14%)	7 (14%)	–
Carcinoma	–	1 (2%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Bilateral, renal tubule, carcinoma	–	1 (2%)	–	–
Renal tubule, carcinoma, multiple	–	–	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	–	1 (2%)
Lymphoma malignant	6 (12%)	9 (18%)	6 (12%)	3 (6%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	30	32	36	26
Total primary neoplasms	42	56	50	37
Total animals with benign neoplasms	17	21	17	20
Total benign neoplasms	22	32	20	26
Total animals with malignant neoplasms	19	19	26	9
Total malignant neoplasms	20	24	30	11
Total animals with metastatic neoplasms	4	4	4	–
Total metastatic neoplasms	4	4	4	–

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

Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Gavage Study of Indole-3-carbinol

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	7/50 (14%)	7/50 (14%)	0/50 (0%)
Adjusted rate[b]	6.6%	15.0%	16.6%	0.0%
Terminal rate[c]	3/33 (9%)	5/40 (13%)	5/26 (19%)	0/45 (0%)
First incidence (days)	729 (T)	681	381	–[e]
Poly-3 test[d]	P = 0.072N	P = 0.170	P = 0.128	P = 0.111N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	8/50 (16%)	8/50 (16%)	1/50 (2%)
Adjusted rate	6.6%	17.1%	19.0%	2.1%
Terminal rate	3/33 (9%)	6/40 (15%)	6/26 (23%)	0/45 (0%)
First incidence (days)	729 (T)	681	381	603
Poly-3 test	P = 0.134N	P = 0.108	P = 0.076	P = 0.286N
Liver: Hepatocellular Adenoma				
Overall rate	7/50 (14%)	14/50 (28%)	8/50 (16%)	11/50 (22%)
Adjusted rate	15.2%	30.0%	19.5%	22.9%
Terminal rate	5/33 (15%)	13/40 (33%)	7/26 (27%)	10/45 (22%)
First incidence (days)	568	687	621	603
Poly-3 test	P = 0.390	P = 0.071	P = 0.405	P = 0.247
Liver: Hepatocellular Carcinoma				
Overall rate	6/50 (12%)	8/50 (16%)	9/50 (18%)	4/50 (8%)
Adjusted rate	13.2%	17.1%	21.5%	8.4%
Terminal rate	5/33 (15%)	7/40 (18%)	5/26 (19%)	4/45 (9%)
First incidence (days)	709	650	606	729 (T)
Poly-3 test	P = 0.246N	P = 0.409	P = 0.228	P = 0.341N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	12/50 (24%)	19/50 (38%)	16/50 (32%)	14/50 (28%)
Adjusted rate	26.1%	40.5%	37.9%	29.2%
Terminal rate	10/33 (30%)	17/40 (43%)	11/26 (42%)	13/45 (29%)
First incidence (days)	568	650	606	603
Poly-3 test	P = 0.503N	P = 0.103	P = 0.165	P = 0.460
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	6/50 (12%)	8/50 (16%)	10/50 (20%)	4/50 (8%)
Adjusted rate	13.2%	17.1%	23.9%	8.4%
Terminal rate	5/33 (15%)	7/40 (18%)	6/26 (23%)	4/45 (9%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
First incidence (days)	709	650	606	729 (T)
Poly-3 test	P = 0.257N	P = 0.409	P = 0.154	P = 0.341N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	12/50 (24%)	19/50 (38%)	17/50 (34%)	14/50 (28%)
Adjusted rate	26.1%	40.5%	40.3%	29.2%
Terminal rate	10/33 (30%)	17/40 (43%)	12/26 (46%)	13/45 (29%)
First incidence (days)	568	650	606	603
Poly-3 test	P = 0.511N	P = 0.103	P = 0.113	P = 0.460
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	2.2%	7.3%	4.2%
Terminal rate	0/33 (0%)	1/40 (3%)	2/26 (8%)	2/45 (4%)
First incidence (days)	634	729 (T)	639	729 (T)
Poly-3 test	P = 0.343	P = 0.757N	P = 0.267	P = 0.514
Lung: Alveolar/Bronchiolar Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.4%	6.5%	7.2%	0.0%
Terminal rate	2/33 (6%)	3/40 (8%)	0/26 (0%)	0/45 (0%)
First incidence (days)	729 (T)	729 (T)	526	–
Poly-3 test	P = 0.151N	P = 0.511	P = 0.463	P = 0.227N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	4/50 (8%)	6/50 (12%)	2/50 (4%)
Adjusted rate	6.6%	8.6%	14.2%	4.2%
Terminal rate	2/33 (6%)	4/40 (10%)	2/26 (8%)	2/45 (4%)
First incidence (days)	634	729 (T)	526	729 (T)
Poly-3 test	P = 0.385N	P = 0.509	P = 0.203	P = 0.482N
Ovary: Cystadenoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	4/50 (8%)
Adjusted rate	2.2%	8.6%	2.4%	8.4%
Terminal rate	0/33 (0%)	4/40 (10%)	0/26 (0%)	4/45 (9%)
First incidence (days)	695	729 (T)	638	729 (T)
Poly-3 test	P = 0.232	P = 0.185	P = 0.737	P = 0.193
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/49 (6%)	4/50 (8%)	0/50 (0%)	1/49 (2%)
Adjusted rate	6.8%	8.6%	0.0%	2.2%
Terminal rate	3/32 (9%)	4/40 (10%)	0/26 (0%)	1/44 (2%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
First incidence (days)	729 (T)	729 (T)	–	729 (T)
Poly-3 test	P = 0.108N	P = 0.525	P = 0.135N	P = 0.288N
Uterus: Stromal Polyp				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	8.4%
Terminal rate	0/33 (0%)	0/40 (0%)	0/26 (0%)	4/45 (9%)
First incidence (days)	–	–	–	729 (T)
Poly-3 test	P = 0.003	–[f]	–	P = 0.067
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	0.0%	7.2%	4.2%
Terminal rate	0/33 (0%)	0/40 (0%)	0/26 (0%)	2/45 (4%)
First incidence (days)	709	–	621	729 (T)
Poly-3 test	P = 0.258	P = 0.496N	P = 0.274	P = 0.516
All Organs: Malignant Lymphoma				
Overall rate	6/50 (12%)	9/50 (18%)	6/50 (12%)	3/50 (6%)
Adjusted rate	12.9%	18.9%	14.4%	6.3%
Terminal rate	0/33 (0%)	6/40 (15%)	3/26 (12%)	3/45 (7%)
First incidence (days)	634	558	597	729 (T)
Poly-3 test	P = 0.116N	P = 0.301	P = 0.540	P = 0.233N
All Organs: Benign Neoplasms				
Overall rate	17/50 (34%)	21/50 (42%)	17/50 (34%)	20/50 (40%)
Adjusted rate	36.5%	44.8%	39.1%	41.7%
Terminal rate	12/33 (36%)	18/40 (45%)	11/26 (42%)	19/45 (42%)
First incidence (days)	568	681	381	603
Poly-3 test	P = 0.427	P = 0.274	P = 0.485	P = 0.382
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	19/50 (38%)	26/50 (52%)	9/50 (18%)
Adjusted rate	39.4%	39.6%	56.5%	18.3%
Terminal rate	8/33 (24%)	14/40 (35%)	10/26 (39%)	6/45 (13%)
First incidence (days)	569	558	526	491
Poly-3 test	P = 0.015N	P = 0.576	P = 0.070	P = 0.017N
All Organs: Benign or Malignant Neoplasms				
Overall rate	30/50 (60%)	32/50 (64%)	36/50 (72%)	26/50 (52%)
Adjusted rate	61.5%	66.2%	75.2%	52.8%
Terminal rate	18/33 (55%)	25/40 (63%)	17/26 (65%)	23/45 (51%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
First incidence (days)	568	558	381	491
Poly-3 test	P = 0.182N	P = 0.393	P = 0.105	P = 0.252N

(T) Terminal euthanasia.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, and pituitary gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal euthanasia.
[d]Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal euthanasia. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table D-3. Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies			
Ginkgo biloba extract (March 2005)	17/50	9/50	1/50
Indole-3-carbinol (April 2007)	7/50	6/50	0/50
Kava kava extract (August 2004)	8/50	3/50	0/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	17/50	6/50	0/50
Tetrabromobisphenol A (August 2007)	13/50	2/50	0/50
Total (%)	62/250 (24.8%)	26/250 (10.4%)	1/250 (0.4%)
Mean ± standard deviation	24.8% ± 9.6%	10.4% ± 5.6%	0.4% ± 0.9%
Range	14%–34%	4%–18%	0%–2%
Overall Historical Incidence: All Routes			
Total (%)	378/948 (39.9%)	152/948 (16.0%)	4/948 (0.4%)
Mean ± standard deviation	39.9% ± 18.7%	16.0% ± 10.6%	0.4% ± 0.8%
Range	14%–78%	4%–46%	0%–2%

[a]Data as of June 2013.

Table D-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death	1	–	–	–
Moribund	10	6	15	3
Natural deaths	6	4	9	2
Survivors				
Died last week of study	–	–	1	–
Terminal euthanasia	33	40	25	45
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(50)	(50)	(50)
Cyst	–	1 (2%)	–	–
Inflammation, chronic	1 (2%)	–	–	–
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	–	–	–
Epithelium, hyperplasia	1 (2%)	–	–	–
Intestine small, jejunum	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Basophilic focus	4 (8%)	6 (12%)	7 (14%)	5 (10%)
Clear cell focus	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Eosinophilic focus	16 (32%)	26 (52%)	26 (52%)	21 (42%)
Fatty change	36 (72%)	39 (78%)	35 (70%)	40 (80%)
Hematopoietic cell proliferation	2 (4%)	–	–	–
Hemorrhage	–	2 (4%)	–	–
Hepatodiaphragmatic nodule	–	–	–	1 (2%)
Hyperplasia, lymphoid	–	–	–	1 (2%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Inflammation, chronic	–	1 (2%)	–	–
Mixed cell focus	2 (4%)	4 (8%)	5 (10%)	1 (2%)
Necrosis	1 (2%)	1 (2%)	1 (2%)	–
Bile duct, cyst	–	1 (2%)	–	–
Centrilobular, necrosis	–	–	1 (2%)	–
Mesentery	(3)	(7)	(7)	(6)
Fat, necrosis	3 (100%)	6 (86%)	7 (100%)	5 (83%)
Pancreas	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Inflammation, chronic	1 (2%)	1 (2%)	–	–
Acinus, atrophy	–	–	–	1 (2%)
Acinus, hyperplasia	1 (2%)	2 (4%)	1 (2%)	–
Duct, cyst	1 (2%)	–	–	–
Salivary glands	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	–	–	–
Duct, cyst	–	–	–	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	–	–	–
Ulcer	9 (18%)	4 (8%)	8 (16%)	3 (6%)
Epithelium, hyperplasia	–	–	1 (2%)	–
Stomach, glandular	(48)	(50)	(49)	(50)
Erosion	–	–	–	1 (2%)
Hyperplasia, lymphoid	1 (2%)	–	–	–
Inflammation, chronic	–	15 (30%)	29 (59%)	47 (94%)
Mineralization	2 (4%)	–	3 (6%)	–
Pigmentation	–	15 (30%)	31 (63%)	49 (98%)
Epithelium, hyperplasia	1 (2%)	7 (14%)	10 (20%)	35 (70%)
Tongue	(0)	(0)	(0)	(1)
Tooth	(6)	(9)	(9)	(11)
Dysplasia	6 (100%)	9 (100%)	9 (100%)	10 (91%)
Cardiovascular System				
Blood vessel	(48)	(50)	(50)	(50)
Mineralization	–	–	1 (2%)	–
Aorta, inflammation, chronic	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Cardiomyopathy	4 (8%)	3 (6%)	1 (2%)	2 (4%)
Inflammation, chronic	–	1 (2%)	–	–
Artery, inflammation, chronic	–	–	1 (2%)	1 (2%)
Myocardium, mineralization	2 (4%)	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hyperplasia	1 (2%)	1 (2%)	–	–
Inflammation, chronic	1 (2%)	–	–	–
Necrosis	–	1 (2%)	–	1 (2%)
Vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)	–
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	1 (2%)	4 (8%)	1 (2%)
Parathyroid gland	(43)	(48)	(49)	(46)
Pituitary gland	(49)	(50)	(50)	(49)
Cyst	–	2 (4%)	–	–
Pars distalis, hyperplasia	5 (10%)	18 (36%)	6 (12%)	4 (8%)
Pars distalis, vacuolization cytoplasmic	–	–	–	1 (2%)
Pars intermedia, hyperplasia	1 (2%)	–	–	–
Thyroid gland	(50)	(50)	(49)	(50)
Inflammation, chronic	–	–	1 (2%)	–
Follicle, hyperplasia		–	–	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Inflammation, chronic	–	–	1 (100%)	–
Genital System				
Clitoral gland	(49)	(50)	(50)	(50)
Ovary	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	1 (2%)
Cyst	16 (32%)	15 (30%)	9 (18%)	8 (16%)
Inflammation, chronic	1 (2%)	–	1 (2%)	–
Mineralization	–	–	1 (2%)	–
Thrombosis	2 (4%)	–	2 (4%)	2 (4%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Uterus	(50)	(50)	(50)	(50)
Inflammation, suppurative	–	1 (2%)	–	–
Inflammation, chronic	–	4 (8%)	–	–
Metaplasia, squamous	–	–	1 (2%)	–
Necrosis	1 (2%)	–	–	–
Endometrium, hyperplasia, cystic	36 (72%)	32 (64%)	30 (60%)	34 (68%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(9)	(5)	(6)	(1)
Lumbar, ectasia	1 (11%)	–	–	–
Lumbar, hemorrhage	–	–	1 (17%)	–
Renal, ectasia	2 (22%)	1 (20%)	2 (33%)	–
Lymph node, mandibular	(50)	(49)	(50)	(50)
Lymph node, mesenteric	(49)	(49)	(49)	(49)
Spleen	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	–	–
Hematopoietic cell proliferation	30 (60%)	30 (60%)	27 (54%)	23 (46%)
Hyperplasia, granulocytic	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	1 (2%)	–	–
Hyperplasia, mast cell	–	–	–	1 (2%)
Pigmentation, hemosiderin	1 (2%)	–	–	–
Lymphoid follicle, atrophy	–	1 (2%)	–	–
Lymphoid follicle, hyperplasia	2 (4%)	–	–	–
Thymus	(49)	(50)	(50)	(50)
Atrophy	42 (86%)	40 (80%)	40 (80%)	41 (82%)
Hyperplasia	–	1 (2%)	–	–
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	–	1 (2%)	–
Subcutaneous tissue, fibrosis	1 (2%)	–	–	–
Subcutaneous tissue, hemorrhage	–	–	–	1 (2%)
Subcutaneous tissue, inflammation, chronic	–	–	1 (2%)	–

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	9 (18%)	13 (26%)	11 (22%)	17 (34%)
Fracture	1 (2%)	–	–	–
Skeletal muscle	(0)	(1)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)	–	–	–
Inflammation, granulomatous	–	–	–	1 (2%)
Hypothalamus, compression	2 (4%)	–	–	–
Meninges, inflammation, chronic	–	1 (2%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	–	–	–	1 (2%)
Hyperplasia, lymphoid	–	1 (2%)	–	–
Inflammation, chronic	–	1 (2%)	–	–
Metaplasia, osseous	–	–	1 (2%)	–
Thrombosis	1 (2%)	–	–	–
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mediastinum, inflammation, chronic	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Foreign body	–	–	1 (2%)	2 (4%)
Inflammation	4 (8%)	1 (2%)	8 (16%)	39 (78%)
Nerve, atrophy	–	–	1 (2%)	50 (100%)
Nerve, olfactory epithelium, atrophy	1 (2%)	1 (2%)	–	–
Olfactory epithelium, accumulation, hyaline droplet	18 (36%)	27 (54%)	21 (42%)	44 (88%)
Olfactory epithelium, atrophy	1 (2%)	2 (4%)	3 (6%)	45 (90%)
Olfactory epithelium, degeneration	–	–	2 (4%)	3 (6%)
Olfactory epithelium, necrosis	–	–	2 (4%)	–
Olfactory epithelium, respiratory metaplasia	7 (14%)	8 (16%)	16 (32%)	49 (98%)
Respiratory epithelium, accumulation, hyaline droplet	47 (94%)	38 (76%)	42 (84%)	50 (100%)
Respiratory epithelium, hyperplasia	32 (64%)	31 (62%)	38 (76%)	50 (100%)

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Respiratory epithelium, metaplasia, squamous	1 (2%)	–	–	–
Respiratory epithelium, necrosis	2 (4%)	–	1 (2%)	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(2)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation, chronic	1 (2%)	–	1 (2%)	1 (2%)
Lens, cataract	–	1 (2%)	1 (2%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Dilatation	–	–	–	6 (12%)
Hyperplasia	2 (4%)	3 (6%)	5 (10%)	9 (18%)
Infiltration cellular, mononuclear cell	24 (48%)	18 (36%)	8 (16%)	22 (44%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	3 (6%)	–	1 (2%)	–
Metaplasia, osseous	–	–	–	1 (2%)
Mineralization	4 (8%)	3 (6%)	4 (8%)	–
Nephropathy	24 (48%)	25 (50%)	19 (38%)	20 (40%)
Artery, inflammation, chronic	–	–	–	1 (2%)
Renal tubule, accumulation, hyaline droplet	–	–	–	1 (2%)
Renal tubule, cyst	–	–	1 (2%)	1 (2%)
Renal tubule, pigmentation	1 (2%)	–	–	–
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, mononuclear cell	3 (6%)	–	–	–
Transitional epithelium, hyperplasia	–	–	–	1 (2%)

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

Appendix E. Genetic Toxicology

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E.1. Bacterial Mutagenicity Test Protocol

Testing procedures used in the first two studies, conducted at BioReliance Corporation (Rockville, MD) followed protocols reported by Zeiger et al.[135]; in the study conducted by SITEK Research Laboratories (Rockville, MD) using the same chemical lot (CHP801001) that was used in the 3-month and 2-year bioassays, a slightly modified procedure was used, and that is described in detail below. Indole-3-carbinol was tested as a coded sample. In the studies conducted at BioReliance Corporation, indole-3-carbinol was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537 either in buffer or 10% or 30% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

The modified protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation and employed tryptophan-dependent *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with indole-3-carbinol and subsequent plating were carried out as described above for the traditional protocol with the addition of tryptophan-deficient medium for the *E. coli* tester strain.

In both studies, each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of indole-3-carbinol. At both laboratories, the highest concentration tested was limited by toxicity. All trials were repeated except TA1537 without S9.

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

E.2. Rat Bone Marrow Micronucleus Test Protocol

Indole-3-carbinol (500, 1,000, or 2,000 mg/kg per day) was administered to male F344/N rats, five per treatment group, once daily for 3 days by gavage, a protocol similar to that presented by Shelby et al.[119]. The positive control was 25 mg/kg cyclophosphamide and the vehicle control was corn oil. The rats were euthanized 24 hours after the third dosing, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained with acridine orange; 2,000 polychromatic erythrocytes (PCEs; reticulocytes) were scored for frequency of micronucleated cells in three to five rats per dose group. In addition, the percentage of PCEs among 200 erythrocytes in the bone marrow was determined for each animal as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the vehicle control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

E.3. Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[136]. At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were shipped to the genetic toxicity testing laboratory (ILS, Inc., Research Triangle Park, NC) where they were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronucleated cells in 2,000 normochromatic erythrocytes (NCEs) in each of five mice per dose group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The peripheral blood results were tabulated as described for PCEs in the rat bone marrow micronucleus test. Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained.

E.4. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

E.5. Results

Indole-3-carbinol was tested in three independent bacterial mutagenicity studies, and results were varied. The first study, which employed *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 and used a concentration range of 3.3 to 3,333 μg indole-3-carbinol/plate with and without 10% or 30% rat or hamster liver S9, yielded results that were judged to be equivocal in TA97 in the absence of S9 (Table E-1). A second study, using strains TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537, and concentrations ranging from 33 to 10,000 μg/plate, yielded weak positive responses in strain TA100 without and with 30% hamster liver S9 (no mutagenicity was seen in this strain in the presence of other concentrations or species of S9) (Table E-1). Results of the third study were judged to be equivocal in *S. typhimurium* strain TA100 (concentration range 500 to 5,000 μg/plate) in the presence of 10% rat liver S9 and in *E. coli* strain WP2 *uvrA*/pKM101 (concentration range of 500 to 7,500 μg/plate) in the absence of S9 activation; no mutagenic activity was seen in this assay in *S. typhimurium* strain TA98, with or without S9, tested up to 5,000 μg/plate (Table E-2).

In vivo, no increase in the frequency of micronucleated PCEs was seen in the bone marrow of male F344/N rats given three doses of indole-3-carbinol (500 to 2,000 mg/kg per day) via gavage; however, a significant decrease in the percent PCEs was seen in the bone marrow of treated rats, indicating that indole-3-carbinol was toxic to the bone marrow (Table E-3). In addition to the rat study, micronucleus frequencies in NCEs of male and female B6C3F1/N mice were assessed in peripheral blood following 3 months of daily gavage treatment with indole-3-carbinol (15.6 to 250 mg/kg per day) in corn oil; no significant increases in micronucleated NCEs were seen in either sex, and no significant changes in percent PCEs occurred over the dose range tested (Table E-4).

Table E-1. Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 10% Rat S9	With 10% Rat S9
Study 1							
TA100	0	98 ± 4	122 ± 10	122 ± 6	87 ± 3	117 ± 14	158 ± 9
	3.3		118 ± 7				
	33	92 ± 6	109 ± 6	121 ± 9	85 ± 5	138 ± 8	
	100	111 ± 10	127 ± 2	122 ± 5	90 ± 1	141 ± 3	163 ± 11
	333	107 ± 9	151 ± 9	147 ± 4	80 ± 7	177 ± 4	172 ± 3
	1,000	98 ± 12	163 ± 12	122 ± 17	80 ± 8	206 ± 17	162 ± 20
	1,500						139 ± 19
	2,000	34 ± 4[b]		86 ± 13[b]		148 ± 5	74 ± 7
	3,333				63 ± 8[b]		
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control		218 ± 12	327 ± 9	813 ± 96	231 ± 46	854 ± 30	635 ± 34

	Dose (µg/plate)	With 30% Rat S9
TA100 (continued)	0	84 ± 3
	33	81 ± 7
	100	76 ± 9
	333	76 ± 4
	1,000	80 ± 6[b]
	3,333	38 ± 4[b]
Trial summary		Negative
Positive control		208 ± 10

	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% Hamster S9	With 10% Hamster S9	With 30% Hamster S9
TA97	0	197 ± 10	94 ± 11	134 ± 9	164 ± 6	123 ± 4	130 ± 20
	3.3			109 ± 14			
	33	187 ± 13	92 ± 1	118 ± 4	177 ± 12		109 ± 4
	100	203 ± 10	99 ± 5	108 ± 5	98 ± 13	119 ± 7	109 ± 5
	333	249 ± 1	106 ± 2	155 ± 1	147 ± 7	103 ± 6	99 ± 15
	667		119 ± 10				
	1,000	203 ± 6	101 ± 11	159 ± 4	185 ± 14	109 ± 14	109 ± 8
	2,000	0[d]			202 ± 14	58 ± 10	123 ± 10
	2,500					0[b]	
Trial summary		Equivocal	Equivocal	Negative	Equivocal	Negative	Negative
Positive control		740 ± 78	214 ± 24	328 ± 6	958 ± 82	1,265 ± 30	536 ± 40

	Dose (µg/plate)	With 10% Rat S9	With 10% Rat S9	With 30% Rat S9			
TA97 (continued)	0	158 ± 10	125 ± 9	160 ± 11			
	33	116 ± 10		156 ± 8			
	100	166 ± 7	116 ± 4	160 ± 8			
	333	170 ± 21	111 ± 7	145 ± 8			
	1,000	193 ± 1	101 ± 11	96 ± 7			
	1,500		94 ± 7				
	2,000	122 ± 15	68 ± 5	115 ± 6			
Trial summary		Equivocal	Negative	Negative			
Positive control		1,443 ± 199	1,135 ± 37	324 ± 35			
	Dose (µg/plate)	**Without S9**	**Without S9**	**With 10% Hamster S9**	**With 30% Hamster S9**	**With 10% Rat S9**	**With 30% Rat S9**
TA98	0	26 ± 1	15 ± 1	24 ± 3	19 ± 2	21 ± 3	24 ± 2
	3.3		18 ± 0				
	33	21 ± 3	20 ± 3	28 ± 2	22 ± 1	23 ± 3	22 ± 2
	100	21 ± 2	17 ± 3	25 ± 4	17 ± 3	25 ± 2	24 ± 4
	333	24 ± 3	19 ± 0	24 ± 4	21 ± 2	30 ± 3	21 ± 2
	1,000	14 ± 2	18 ± 2	24 ± 3	21 ± 4	26 ± 1	25 ± 3
	2,000	13 ± 2		16 ± 2[b]		12 ± 1[b]	
	3,333				14 ± 2[b]		14 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		68 ± 5	90 ± 5	751 ± 22	55 ± 2	457 ± 66	82 ± 13
TA1535	0	15 ± 1	15 ± 3	16 ± 2	18 ± 1	13 ± 0	19 ± 1
	3.3		19 ± 1				
	33	19 ± 3	17 ± 2	11 ± 2	19 ± 3	18 ± 0	20 ± 3
	100	23 ± 4	20 ± 4	14 ± 3	17 ± 2	12 ± 2	12 ± 3
	333	18 ± 2	19 ± 2	13 ± 3	10 ± 3	12 ± 1	12 ± 2
	1,000	19 ± 3	18 ± 0	12 ± 4	13 ± 1	14 ± 4	10 ± 2
	2,000	Toxic		10 ± 1		9 ± 1	
	3,333				4 ± 2[e]		0[e]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		531 ± 92	364 ± 34	80 ± 13	210 ± 11	355 ± 60	79 ± 5
	Dose (µg/plate)	**Without S9**					
TA1537	0	5 ± 0					
	33	8 ± 3					
	100	5 ± 2					
	333	4 ± 1					
	1,000	7 ± 1					
	1,500	3 ± 2[b]					
Trial summary		Negative					
Positive control		13 ± 1					

	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 30% Hamster S9	With 10% Rat S9
Study 2							
TA102	0	277 ± 27	366 ± 5	347 ± 22	358 ± 9	281 ± 11	348 ± 11
	33	270 ± 28	378 ± 6	429 ± 22	357 ± 10		436 ± 24
	100	262 ± 8	362 ± 6	421 ± 14	397 ± 31	291 ± 21	405 ± 33
	333	280 ± 25	345 ± 14	394 ± 11	389 ± 23	295 ± 13	416 ± 49
	1,000	185 ± 10	255 ± 50	333 ± 7	434 ± 25	220 ± 12	320 ± 18
	1,500					217 ± 11	
	3,333	30 ± 3[d]	84 ± 5[d]	82 ± 30[d]	167 ± 32[e]	96 ± 9[e]	40 ± 7[d]
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		875 ± 17	1,435 ± 32	1,849 ± 123	1,383 ± 22	1,503 ± 46	1,714 ± 98

	Dose (µg/plate)	With 30% Rat S9	With 30% Rat S9
TA102 (continued)	0	340 ± 21	233 ± 14
	33	370 ± 22	
	100	408 ± 11	203 ± 6
	333	388 ± 8	224 ± 4
	1,000	422 ± 19	148 ± 12
	1,500		115 ± 6
	3,333	163 ± 30[e]	102 ± 8[e]
Trial summary		Equivocal	Negative
Positive control		1,107 ± 66	1,529 ± 46

	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 10% Rat S9	With 30% Rat S9
TA104	0	222 ± 11	207 ± 2	249 ± 18	225 ± 8	206 ± 13	221 ± 7
	33	205 ± 9	122 ± 18	187 ± 7	180 ± 21	184 ± 7	219 ± 13
	100	213 ± 6	163 ± 11	216 ± 19	181 ± 10	204 ± 17	244 ± 17
	333	180 ± 19	147 ± 14	175 ± 19	183 ± 19	204 ± 15	207 ± 3
	1,000	185 ± 14	89 ± 3	208 ± 23	213 ± 2	181 ± 9	237 ± 22
	3,333	0[d]	47 ± 11[d]	205 ± 5[d]	38 ± 1[d]	207 ± 26[d]	46 ± 3[d]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		705 ± 14	624 ± 6	1,080 ± 34	1,112 ± 36	1,173 ± 17	744 ± 61

	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% Hamster S9	With 10% Hamster S9	With 30% Hamster S9
TA100	0	90 ± 0	104 ± 8	149 ± 7	174 ± 7	139 ± 9	97 ± 10
	33	76 ± 10	120 ± 5	141 ± 11	180 ± 16	146 ± 7	89 ± 4
	100	114 ± 5	122 ± 7	161 ± 7	185 ± 4	138 ± 10	105 ± 6
	333	123 ± 7	144 ± 4	196 ± 23	213 ± 4	170 ± 5	144 ± 10
	1,000	133 ± 19	199 ± 7	166 ± 3	229 ± 20	183 ± 6	156 ± 5
	1,200		183 ± 9				
	1,500	46 ± 44[b]				130 ± 9[e]	149 ± 18
	3,333			0[d]	0[d]		
Trial summary		Weakly Positive	Weakly Positive	Negative	Negative	Negative	Weakly Positive
Positive control		225 ± 20	432 ± 47	594 ± 33	1,428 ± 58	845 ± 79	296 ± 25

	Dose (µg/plate)	With 30% Hamster S9	With 10% Rat S9	With 10% Rat S9	With 30% Rat S9		
TA100 (continued)	0	102 ± 2	163 ± 2	121 ± 16	101 ± 6		
	33	104 ± 2	200 ± 6	141 ± 16	105 ± 3		
	100	106 ± 2	181 ± 6	120 ± 7	94 ± 11		
	333	132 ± 11	217 ± 14	178 ± 15	107 ± 11		
	1,000	157 ± 16	239 ± 26	158 ± 16	124 ± 10		
	1,200	191 ± 8					
	1,500			132 ± 9[e]	79 ± 4[e]		
	3,333		0[d]				
Trial summary		Weakly Positive	Equivocal	Negative	Negative		
Positive control		956 ± 35	1,259 ± 205	595 ± 37	357 ± 27		
	Dose (µg/plate)	**Without S9**	**Without S9**	**With 10% Hamster S9**	**With 30% Hamster S9**	**With 30% Hamster S9**	**With 10% Rat S9**
TA97	0	114 ± 2	156 ± 15	186 ± 29	151 ± 11	132 ± 6	201 ± 12
	33	110 ± 9	148 ± 9	203 ± 13	149 ± 6		184 ± 19
	100	107 ± 8	167 ± 9	211 ± 5	157 ± 8	159 ± 8	168 ± 2
	333	126 ± 4	151 ± 6	229 ± 32	148 ± 12	160 ± 9	160 ± 4
	1,000	133 ± 4	168 ± 10	208 ± 17	162 ± 9	185 ± 6	211 ± 10
	1,500	108 ± 12[e]			210 ± 6[e]	194 ± 12	
	2,000					124 ± 13	
	3,333		0[d]	0[d]			0[d]
Trial summary		Negative	Negative	Negative	Equivocal	Equivocal	Negative
Positive control		394 ± 2	476 ± 32	1,320 ± 31	1,680 ± 75	1,458 ± 59	1,671 ± 307
	Dose (µg/plate)	**With 30% Rat S9**	**With 30% Rat S9**				
TA97 (continued)	0	151 ± 3	145 ± 8				
	33	143 ± 2					
	100	142 ± 8	132 ± 2				
	333	146 ± 6	164 ± 11				
	1,000	177 ± 5	167 ± 8				
	1,500	193 ± 12[e]	175 ± 20				
	2,000		95 ± 11				
Trial summary		Equivocal	Equivocal				
Positive control		553 ± 16	463 ± 15				
	Dose (µg/plate)	**Without S9**	**Without S9**	**With 10% Hamster S9**	**With 30% Hamster S9**	**With 10% Rat S9**	**With 30% Rat S9**
TA98	0	15 ± 2	18 ± 3	18 ± 5	17 ± 1	21 ± 3	19 ± 2
	33	13 ± 3	15 ± 1	21 ± 4	11 ± 1	18 ± 2	11 ± 1
	100	13 ± 1	16 ± 2	21 ± 5	17 ± 4	21 ± 2	13 ± 1
	333	13 ± 1	14 ± 3	21 ± 6	12 ± 1	27 ± 4	19 ± 1
	1,000	10 ± 2	9 ± 2	18 ± 3	13 ± 2	20 ± 1	17 ± 5
	1,500	0[b]			6 ± 1		9 ± 2[b]
	3,333		0[d]	0[d]		0[d]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		115 ± 2	100 ± 7	1,536 ± 136	246 ± 8	629 ± 141	129 ± 18

	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 30% Hamster S9
TA1535	0	14 ± 2	14 ± 1	18 ± 2	14 ± 1	10 ± 1	13 ± 1
	33	13 ± 2		18 ± 3	13 ± 1	13 ± 0	
	100	14 ± 1		19 ± 3	14 ± 1	10 ± 1	
	333	13 ± 1	15 ± 2	20 ± 1	13 ± 1	13 ± 1	12 ± 2
	1,000	15 ± 1	10 ± 1	18 ± 2	13 ± 1	13 ± 1	12 ± 1
	1,200	12 ± 1				14 ± 3	
	1,500		8 ± 0				9 ± 1
	3,333		0[d]	0[d]	0[d]		2[d]
	10,000		0[d]				0[d]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		130 ± 5	166 ± 4	256 ± 39	287 ± 24	66 ± 3	155 ± 23

	Dose (µg/plate)	With 10% Rat S9	With 30% Rat S9
TA1535 (continued)	0	15 ± 0	13 ± 3
	33	14 ± 2	12 ± 1
	100	17 ± 3	10[f]
	333	15 ± 1	18 ± 2
	1,000	12 ± 2	12 ± 1
	1,500		8 ± 2[e]
	3,333	0[d]	
Trial summary		Negative	Negative
Positive control		422 ± 19	95 ± 6

	Dose (µg/plate)	With 30% Hamster S9	With 30% Rat S9
TA1537	0	9 ± 2	9 ± 2
	100	8 ± 1	7 ± 1
	333	8 ± 1	10 ± 1
	1,000	9 ± 2	8 ± 1
	1,500	5 ± 1	5 ± 1
	2,000	4 ± 1[e]	5 ± 1[e]
Trial summary		Negative	Negative
Positive control		530 ± 58	143 ± 22

[a]Studies were performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger et al.[135]. 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), 4-nitro-*o*-phenylenediamine (TA98), mitomycin-C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, except 2-aminoanthracene or sterigmatocystin was used for TA102.
[d]Slight toxicity and precipitate on plate.
[e]Precipitate on plate.
[f]Contamination.

Table E-2. Mutagenicity of Indole-3-carbinol in Bacterial Tester Strains[a]

Strain	Dose (μg/plate)	Without S9	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9	With 10% Rat S9
TA100	0	44 ± 3	103 ± 7	81 ± 4	80 ± 2	61 ± 3	94 ± 2
	100		109 ± 4	90 ± 0			
	500	63 ± 2	124 ± 6	129 ± 1	109 ± 4	84 ± 2	126 ± 5
	1,500	47 ± 1	60 ± 8	75 ± 3	142 ± 13	84 ± 3	174 ± 4
	2,500	32 ± 1	20 ± 1[b]	26 ± 2	74 ± 1	84 ± 6	123 ± 5
	3,500	9 ± 1[b]	Toxic	12 ± 3[b]	45 ± 5	44 ± 8[c]	23 ± 3[b]
	5,000	Toxic			6 ± 3[b]	26 ± 8[c]	5[c]
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Equivocal
Positive control[d]		420 ± 24	844 ± 95	737 ± 40	1,108 ± 18	831 ± 93	1,086 ± 2
TA98	0	24 ± 1	21 ± 1	16 ± 2	30 ± 2	34 ± 2	25 ± 4
	100	21 ± 4		24 ± 1			
	500	17 ± 2	24 ± 1	20 ± 1	30 ± 1	37 ± 1	27 ± 4
	1,500	11 ± 2	23 ± 1	9 ± 1	30 ± 2	27 ± 2	19 ± 3
	2,500	4 ± 1[b]	15 ± 1	6 ± 1[b]	27 ± 2	17 ± 4	9 ± 2
	3,500	Toxic	Toxic	Toxic	11 ± 1[b]	11 ± 0	3 ± 1[b]
	5,000		Toxic		7 ± 1[b]	3 ± 1[c]	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		441 ± 43	654 ± 17	411 ± 41	1,533 ± 29	787 ± 4	787 ± 91
***Escherichia coli* WP2 *uvrA*/pKM101 (Analogous to TA102)**							
	0	136 ± 5	164 ± 20	188 ± 4	185 ± 4	188 ± 6	225 ± 15
	500	193 ± 5	204 ± 7	241 ± 5			
	1,500	218 ± 8	212 ± 5	279 ± 11	238 ± 9	261 ± 3	248 ± 10
	2,500	194 ± 10	199 ± 9	234 ± 11	217 ± 1	235 ± 9	355 ± 2
	3,500	139 ± 9	111 ± 2	173 ± 9	194 ± 6	178 ± 9	342 ± 2
	5,000	87 ± 8	59 ± 4[b]	128 ± 9[c]	184 ± 6	115 ± 5	98 ± 3[b]
	7,500	Toxic	Toxic		104 ± 9	63 ± 5[b]	242 ± 1[c]
	10,000				65 ± 12[b]	Toxic	
Trial summary		Equivocal	Negative	Equivocal	Negative	Negative	Equivocal
Positive control		1,992 ± 81	2,139 ± 53	1,810 ± 66	1,144 ± 12	1,080 ± 17	1,298 ± 51

[a]Study was performed at SITEK Research Laboratories using the same lot of indole-3-carbinol (CHP801001) used in the 3-month and 2-year bioassays and a modification of the protocol presented by Zeiger et al.[135]. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 μg/plate was the solvent control.
[b]Slight toxicity.
[c]Precipitate on plate.
[d]The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-3. Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male F344/N Rats Following Treatment with Indole-3-carbinol by Gavage for Three Days[a]

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]	P Value[c]	PCEs[b] (%)
Corn oil[d]	0	5	1.00 ± 0.22		51.100 ± 3.98
Indole-3-carbinol	500	5	1.22 ± 0.33	0.3227	27.560 ± 8.24
	1,000	3	1.33 ± 0.17	0.2713	41.500 ± 2.50
	2,000	4	0.83 ± 0.38	0.6306	27.375 ± 8.30
			P = 0.611[e]		
Cyclophosphamide[f]	25	4	26.17 ± 5.17	0.0000	7.075 ± 2.21

[a]Study was performed at ILS, Inc., using a protocol similar to that presented by Shelby et al.[119]; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.008$; positive control values are significant at $P \leq 0.05$.
[d]Vehicle control.
[e]Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.
[f]Positive control.

Table E-4. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Indole-3-carbinol by Gavage for Three Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	2.10 ± 0.19		3.62 ± 0.31
Indole-3-carbinol	15.6	5	1.50 ± 0.27	0.8416	3.68 ± 0.16
	31.25	5	1.80 ± 0.25	0.6847	3.74 ± 0.29
	62.5	5	2.00 ± 0.35	0.5621	3.84 ± 0.29
	125	5	1.90 ± 0.29	0.6242	3.84 ± 0.28
	250	5	1.60 ± 0.33	0.7947	3.52 ± 0.34
			P = 0.663[e]		
Female					
Corn oil	0	5	1.10 ± 0.37		3.18 ± 0.23
Indole-3-carbinol	15.6	5	1.40 ± 0.19	0.2741	2.60 ± 0.49
	31.25	5	1.60 ± 0.29	0.1678	3.78 ± 0.52
	62.5	5	1.60 ± 0.33	0.1678	3.24 ± 0.41
	125	5	1.80 ± 0.34	0.0967	3.40 ± 0.29
	250	5	1.90 ± 0.33	0.0719	3.12 ± 0.19
			P = 0.087		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[136]; NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.005$.
[d]Vehicle control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol..... F-2
Table F-2. Hematology Data for Mice in the Three-month Gavage Study of Indole-3-carbinol..... F-9

Table F-1. Hematology and Clinical Chemistry Data for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Male						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 25	9	10	9	10	9	9
Week 14	10	10	10	10	9	10
Hematocrit (auto) (%)						
Day 4	45.0 ± 0.8	45.4 ± 0.6	44.5 ± 0.4	45.1 ± 0.5	44.3 ± 0.4	47.3 ± 1.5
Day 25	45.9 ± 0.4	46.5 ± 0.5	44.6 ± 0.6	46.0 ± 0.7	45.4 ± 0.5	45.0 ± 0.2
Week 14	48.5 ± 0.3	47.8 ± 0.4	48.8 ± 0.3	48.7 ± 0.4	48.4 ± 0.5	48.5 ± 0.6
Hematocrit (spun) (%)						
Day 4	44.3 ± 0.6	44.7 ± 0.6	43.9 ± 0.3	44.3 ± 0.4	44.0 ± 0.5	46.3 ± 1.4
Day 25	46.2 ± 0.3	46.2 ± 0.5	45.0 ± 0.5	46.1 ± 0.5	45.6 ± 0.6	44.9 ± 0.3
Week 14	47.5 ± 0.3	46.8 ± 0.4	47.7 ± 0.3	47.8 ± 0.5	47.2 ± 0.4	47.3 ± 0.4
Hemoglobin (g/dL)						
Day 4	14.9 ± 0.3	14.9 ± 0.2	14.7 ± 0.1	14.9 ± 0.2	14.7 ± 0.1	15.5 ± 0.5
Day 25	15.7 ± 0.2	15.8 ± 0.2	15.2 ± 0.3	15.8 ± 0.2	15.4 ± 0.2	15.4 ± 0.1
Week 14	15.9 ± 0.1	15.7 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	15.9 ± 0.2	15.8 ± 0.2
Erythrocytes (10^6/μL)						
Day 4	7.39 ± 0.12	7.43 ± 0.11	7.30 ± 0.07	7.43 ± 0.07	7.36 ± 0.05	7.86 ± 0.25
Day 25	8.30 ± 0.09	8.38 ± 0.10	8.09 ± 0.09	8.31 ± 0.13	8.16 ± 0.09	8.25 ± 0.06
Week 14	8.49 ± 0.05	8.38 ± 0.08	8.67 ± 0.06	8.64 ± 0.07	8.63 ± 0.08	8.71 ± 0.12
Reticulocytes (10^5/μL)						
Day 4	5.49 ± 0.37	5.83 ± 0.35	5.66 ± 0.39	5.59 ± 0.33	5.13 ± 0.19	4.49 ± 0.22*
Day 25	2.87 ± 0.14	3.05 ± 0.08	2.93 ± 0.12	2.82 ± 0.14	2.78 ± 0.19	2.47 ± 0.17
Week 14	2.14 ± 0.04	2.03 ± 0.05	1.96 ± 0.04	1.98 ± 0.05	2.04 ± 0.09	1.93 ± 0.10
Reticulocytes (%)						
Day 4	7.48 ± 0.57	7.90 ± 0.55	7.77 ± 0.54	7.52 ± 0.46	6.97 ± 0.28	5.81 ± 0.41*
Day 25	3.48 ± 0.18	3.64 ± 0.12	3.63 ± 0.16	3.43 ± 0.20	3.44 ± 0.26	2.99 ± 0.22
Week 14	2.52 ± 0.05	2.43 ± 0.06	2.27 ± 0.06	2.29 ± 0.07	2.36 ± 0.10	2.24 ± 0.12
Nucleated erythrocytes/100 leukocytes						
Day 4	0.5 ± 0.2	0.9 ± 0.3	0.2 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.3
Day 25	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.2
Week 14	0.2 ± 0.2	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)						
Day 4	60.8 ± 0.3	61.2 ± 0.5	61.0 ± 0.2	60.7 ± 0.3	60.2 ± 0.3	60.2 ± 0.3
Day 25	55.4 ± 0.3	55.5 ± 0.3	55.2 ± 0.2	55.3 ± 0.3	55.6 ± 0.3	54.6 ± 0.4

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Week 14	57.1 ± 0.2	57.0 ± 0.2	56.4 ± 0.3	56.4 ± 0.2*	56.1 ± 0.2**	55.6 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	20.1 ± 0.1	20.1 ± 0.2	20.1 ± 0.1	20.0 ± 0.2	20.0 ± 0.1	19.8 ± 0.1
Day 25	18.9 ± 0.1	18.9 ± 0.1	18.7 ± 0.2	19.0 ± 0.1	18.9 ± 0.1	18.6 ± 0.1
Week 14	18.7 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.4 ± 0.1**	18.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.1 ± 0.2	32.9 ± 0.2	33.0 ± 0.2	32.9 ± 0.2	33.1 ± 0.1	32.8 ± 0.2
Day 25	34.2 ± 0.2	34.1 ± 0.2	34.0 ± 0.3	34.3 ± 0.2	34.0 ± 0.1	34.1 ± 0.2
Week 14	32.7 ± 0.1	32.8 ± 0.1	33.0 ± 0.2	33.0 ± 0.1	32.8 ± 0.1	32.6 ± 0.1
Platelets (10^3/μL)						
Day 4	972.3 ± 40.4	1,004.3 ± 20.3	934.5 ± 23.8	1,090.1 ± 47.6	1,015.7 ± 34.4	1,117.9 ± 48.0
Day 25	752.3 ± 20.6	795.3 ± 24.7	815.1 ± 47.0	775.5 ± 29.3	803.2 ± 30.0	805.4 ± 26.6
Week 14	588.3 ± 18.8	600.7 ± 11.3	604.1 ± 8.3	602.8 ± 8.1	618.3 ± 12.9	658.5 ± 15.7**
Leukocytes (10^3/μL)						
Day 4	8.87 ± 0.27	9.03 ± 0.22	8.42 ± 0.45	9.65 ± 0.36	8.91 ± 0.19	8.00 ± 0.75
Day 25	9.63 ± 0.46	9.36 ± 0.83	9.79 ± 0.61	9.67 ± 0.31	9.11 ± 0.84	9.97 ± 0.91
Week 14	7.78 ± 0.56	7.76 ± 0.47	7.94 ± 0.50	8.06 ± 0.46	8.20 ± 0.78	7.76 ± 0.70
Segmented neutrophils (10^3/μL)						
Day 4	1.08 ± 0.04	1.15 ± 0.07	1.10 ± 0.06	1.22 ± 0.07	1.08 ± 0.06	1.18 ± 0.07
Day 25	0.82 ± 0.04	0.78 ± 0.07	0.89 ± 0.06	0.85 ± 0.04	0.81 ± 0.06	0.99 ± 0.06
Week 14	1.13 ± 0.04	1.15 ± 0.06	1.11 ± 0.04	1.01 ± 0.04	1.12 ± 0.05	1.16 ± 0.05
Lymphocytes (10^3/μL)						
Day 4	7.38 ± 0.24	7.51 ± 0.19	6.96 ± 0.40	7.96 ± 0.31	7.42 ± 0.16	6.39 ± 0.67
Day 25	8.38 ± 0.44	8.15 ± 0.73	8.42 ± 0.53	8.46 ± 0.30	7.91 ± 0.74	8.50 ± 0.82
Week 14	6.32 ± 0.55	6.30 ± 0.42	6.53 ± 0.49	6.74 ± 0.45	6.74 ± 0.72	6.33 ± 0.65
Monocytes (10^3/μL)						
Day 4	0.20 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.24 ± 0.02	0.21 ± 0.01	0.22 ± 0.03
Day 25	0.15 ± 0.01	0.15 ± 0.01	0.14 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.19 ± 0.03
Week 14	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Basophils (10^3/μL)						
Day 4	0.033 ± 0.005	0.037 ± 0.004	0.028 ± 0.005	0.032 ± 0.003	0.039 ± 0.007	0.036 ± 0.008
Day 25	0.069 ± 0.010	0.066 ± 0.013	0.069 ± 0.013	0.063 ± 0.010	0.052 ± 0.012	0.066 ± 0.013
Week 14	0.059 ± 0.007	0.049 ± 0.008	0.045 ± 0.009	0.066 ± 0.005	0.066 ± 0.010	0.043 ± 0.008
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.02 ± 0.00*
Day 25	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01
Week 14	0.08 ± 0.01	0.07 ± 0.00*	0.07 ± 0.00*	0.06 ± 0.00**	0.07 ± 0.01*	0.04 ± 0.01**
Large unstained cells (10^3/μL)						
Day 4	0.142 ± 0.011	0.109 ± 0.010	0.115 ± 0.012	0.150 ± 0.008	0.137 ± 0.012	0.145 ± 0.023

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Day 25	0.150 ± 0.037	0.167 ± 0.034	0.224 ± 0.052	0.137 ± 0.018	0.162 ± 0.051	0.178 ± 0.026
Week 14	0.081 ± 0.009	0.080 ± 0.008	0.074 ± 0.008	0.091 ± 0.005	0.091 ± 0.012	0.081 ± 0.011
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 25	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	11.7 ± 0.5	13.1 ± 0.6	13.6 ± 0.6	13.4 ± 0.5	14.1 ± 0.7*	13.4 ± 0.5
Day 25	15.4 ± 0.5	14.2 ± 0.5	15.2 ± 0.5	14.4 ± 0.5	13.3 ± 0.5**	13.4 ± 0.3**
Week 14	12.2 ± 0.4	12.8 ± 0.4	13.4 ± 0.4	15.1 ± 1.1	11.9 ± 0.5	13.3 ± 0.5
Creatinine (mg/dL)						
Day 4	0.12 ± 0.01	0.10 ± 0.00	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Day 25	0.21 ± 0.02	0.23 ± 0.02	0.20 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.20 ± 0.00
Week 14	0.29 ± 0.01	0.28 ± 0.01	0.32 ± 0.01	0.30 ± 0.00	0.29 ± 0.01	0.31 ± 0.02
Glucose (mg/dL)						
Day 4	126 ± 3	123 ± 2	123 ± 1	121 ± 2	125 ± 2	116 ± 3
Day 25	127 ± 2	126 ± 4	123 ± 2	122 ± 2	126 ± 2	128 ± 3
Week 14	131 ± 3	134 ± 3	138 ± 3	135 ± 3	136 ± 3	130 ± 3
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.7 ± 0.1	5.8 ± 0.0	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Day 25	6.5 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.4 ± 0.1
Week 14	6.7 ± 0.1	6.7 ± 0.1	6.9 ± 0.0	6.8 ± 0.1	6.8 ± 0.1	6.4 ± 0.1
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.0	4.2 ± 0.1
Day 25	4.6 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.0	4.6 ± 0.0
Week 14	4.8 ± 0.1	4.7 ± 0.0	4.9 ± 0.0	4.9 ± 0.0	4.9 ± 0.0	4.6 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	77 ± 3	79 ± 3	80 ± 2	77 ± 2	79 ± 3	95 ± 3**
Day 25	64 ± 2	61 ± 2	61 ± 2	65 ± 2	58 ± 1	71 ± 1
Week 14	61 ± 3	55 ± 2	53 ± 2	59 ± 4	49 ± 1**	66 ± 4
Alkaline phosphatase (IU/L)						
Day 4	633 ± 14	631 ± 18	617 ± 12	656 ± 18	691 ± 17*	738 ± 26**
Day 25	445 ± 8	459 ± 11	437 ± 9	416 ± 13	380 ± 30**	377 ± 15**
Week 14	223 ± 3	232 ± 6	224 ± 6	206 ± 7	198 ± 4**	178 ± 4**
Creatine kinase (IU/L)						
Day 4	628 ± 61	591 ± 53	587 ± 46	622 ± 68	545 ± 42	540 ± 45
Day 25	520 ± 70	464 ± 67	368 ± 35	376 ± 41	340 ± 35	430 ± 37
Week 14	242 ± 33	256 ± 27	243 ± 34	230 ± 17	267 ± 38	284 ± 34

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Sorbitol dehydrogenase (IU/L)						
Day 4	6 ± 1[b]	9 ± 1[b]	8 ± 2[c]	8 ± 1*[b]	9 ± 1*	13 ± 2**
Day 25	9 ± 2[b]	10 ± 2	10 ± 1[b]	10 ± 2[d]	11 ± 1[b]	12 ± 2
Week 14	18 ± 1	17 ± 1	15 ± 1	17 ± 1	13 ± 2	17 ± 2
Bile acids (µmol/L)						
Day 4	18.8 ± 1.8	20.1 ± 2.0	21.7 ± 1.8	24.7 ± 1.1**	28.2 ± 2.0**	29.6 ± 1.4**
Day 25	17.8 ± 0.9	16.5 ± 1.8	14.9 ± 0.9	22.5 ± 1.8	23.4 ± 1.7	21.3 ± 1.3
Week 14	19.5 ± 2.4	20.0 ± 2.6	18.6 ± 2.6	18.0 ± 1.5	19.1 ± 1.0	21.3 ± 1.3
Female						
Hematology						
n						
Day 4	9	10	9	10	10	9
Day 25	8	10	10	8	10	9
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 4	48.8 ± 0.7	48.7 ± 0.7	50.4 ± 0.8	49.8 ± 1.0	48.8 ± 1.2	50.1 ± 1.0
Day 25	47.3 ± 0.7	46.7 ± 0.8	47.0 ± 0.5	46.8 ± 0.6	47.8 ± 0.6	47.3 ± 0.3
Week 14	47.8 ± 0.4	46.8 ± 0.3	46.2 ± 0.3*	47.0 ± 0.5	46.0 ± 0.3*	46.7 ± 0.4
Hematocrit (spun) (%)						
Day 4	48.5 ± 0.8	47.9 ± 0.6	50.0 ± 0.6	49.4 ± 0.9	48.6 ± 1.0	50.3 ± 0.9
Day 25	46.6 ± 0.5	45.9 ± 0.7	46.5 ± 0.5	46.4 ± 0.5	47.3 ± 0.5	47.2 ± 0.4
Week 14	47.3 ± 0.4	46.7 ± 0.3	46.5 ± 0.3	46.8 ± 0.4	45.8 ± 0.2*	46.6 ± 0.4
Hemoglobin (g/dL)						
Day 4	15.8 ± 0.3	15.8 ± 0.2	16.2 ± 0.3	16.4 ± 0.4	15.9 ± 0.4	16.4 ± 0.3
Day 25	15.5 ± 0.2	15.5 ± 0.2	15.7 ± 0.1	15.5 ± 0.2	15.8 ± 0.1	15.6 ± 0.1
Week 14	15.6 ± 0.1	15.4 ± 0.1	15.2 ± 0.1**	15.4 ± 0.1*	15.1 ± 0.1**	15.2 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	8.02 ± 0.10	7.99 ± 0.13	8.20 ± 0.12	8.17 ± 0.16	7.96 ± 0.19	8.35 ± 0.15
Day 25	8.56 ± 0.08	8.37 ± 0.15	8.49 ± 0.08	8.47 ± 0.12	8.63 ± 0.08	8.63 ± 0.07
Week 14	8.22 ± 0.06	8.11 ± 0.05	8.02 ± 0.07	8.24 ± 0.07	8.16 ± 0.05	8.41 ± 0.07
Reticulocytes (10^5/µL)						
Day 4	4.10 ± 0.36	4.29 ± 0.38	4.48 ± 0.43	3.82 ± 0.34	3.90 ± 0.30	3.10 ± 0.26*
Day 25	1.76 ± 0.11	2.07 ± 0.14	1.89 ± 0.09	1.96 ± 0.07	1.90 ± 0.09	1.78 ± 0.08
Week 14	1.76 ± 0.04	1.91 ± 0.08	1.88 ± 0.09	1.80 ± 0.08	1.83 ± 0.04	1.91 ± 0.06
Reticulocytes (%)						
Day 4	5.13 ± 0.48	5.38 ± 0.49	5.49 ± 0.53	4.75 ± 0.52	4.97 ± 0.48	3.74 ± 0.34*
Day 25	2.05 ± 0.14	2.49 ± 0.19	2.21 ± 0.10	2.30 ± 0.12	2.21 ± 0.12	2.07 ± 0.09
Week 14	2.14 ± 0.05	2.36 ± 0.12	2.36 ± 0.12	2.20 ± 0.09	2.23 ± 0.05	2.27 ± 0.07

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Nucleated erythrocytes/100 leukocytes						
Day 4	0.7 ± 0.3	0.3 ± 0.2	0.8 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	0.7 ± 0.4
Day 25	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.3	0.2 ± 0.1	0.1 ± 0.1
Week 14	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
Mean cell volume (fL)						
Day 4	60.9 ± 0.3	61.0 ± 0.2	61.5 ± 0.3	61.0 ± 0.2	61.2 ± 0.4	60.0 ± 0.3
Day 25	55.3 ± 0.4	55.8 ± 0.3	55.4 ± 0.3	55.3 ± 0.3	55.5 ± 0.5	54.8 ± 0.4
Week 14	58.1 ± 0.1	57.7 ± 0.1**	57.6 ± 0.2**	57.0 ± 0.1**	56.4 ± 0.2**	55.5 ± 0.1**
Mean cell hemoglobin (pg)						
Day 4	19.8 ± 0.1	19.8 ± 0.2	19.8 ± 0.1	20.0 ± 0.1	20.0 ± 0.1	19.7 ± 0.2
Day 25	18.1 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.0 ± 0.1
Week 14	19.0 ± 0.1	19.0 ± 0.0	18.9 ± 0.1*	18.7 ± 0.0**	18.5 ± 0.1**	18.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.4 ± 0.3	32.4 ± 0.2	32.2 ± 0.3	32.8 ± 0.2	32.6 ± 0.2	32.8 ± 0.2
Day 25	32.8 ± 0.2	33.1 ± 0.2	33.4 ± 0.2	33.1 ± 0.2	33.0 ± 0.2	32.9 ± 0.1
Week 14	32.8 ± 0.1	32.8 ± 0.1	32.9 ± 0.2	32.7 ± 0.1	32.8 ± 0.2	32.5 ± 0.1
Platelets (10^3/μL)						
Day 4	955.8 ± 39.5	983.1 ± 46.2	979.3 ± 58.0	962.5 ± 23.3	926.6 ± 46.6	1,077.2 ± 50.4
Day 25	755.6 ± 29.7	729.4 ± 20.9	729.0 ± 20.5	721.0 ± 27.4	787.4 ± 34.2	711.4 ± 33.7
Week 14	573.2 ± 19.6	588.1 ± 12.9	554.1 ± 18.9	621.4 ± 13.6	590.1 ± 15.0	610.7 ± 21.6
Leukocytes (10^3/μL)						
Day 4	10.37 ± 0.34	9.82 ± 0.21	9.00 ± 0.25*	10.32 ± 0.42	10.08 ± 0.35	10.75 ± 0.35
Day 25	9.64 ± 0.55	9.54 ± 0.36	9.22 ± 0.68	9.56 ± 0.81	9.93 ± 0.64	10.37 ± 0.51
Week 14	6.40 ± 0.36	6.50 ± 0.30	6.40 ± 0.44	6.49 ± 0.38	6.58 ± 0.36	7.20 ± 0.38
Segmented neutrophils (10^3/μL)						
Day 4	1.03 ± 0.04	1.05 ± 0.05	1.04 ± 0.06	1.08 ± 0.06	1.15 ± 0.08	1.30 ± 0.07**
Day 25	0.84 ± 0.06	0.98 ± 0.08	0.92 ± 0.10	0.96 ± 0.10	0.83 ± 0.07	1.08 ± 0.06
Week 14	1.08 ± 0.07	1.19 ± 0.07	1.23 ± 0.08	1.28 ± 0.07	1.03 ± 0.05	1.14 ± 0.04
Lymphocytes (10^3/μL)						
Day 4	8.89 ± 0.28	8.38 ± 0.22	7.62 ± 0.21*	8.80 ± 0.37	8.53 ± 0.30	8.96 ± 0.31
Day 25	8.40 ± 0.49	8.13 ± 0.35	7.90 ± 0.56	8.19 ± 0.70	8.63 ± 0.53	8.76 ± 0.52
Week 14	5.05 ± 0.30	5.02 ± 0.26	4.88 ± 0.40	4.94 ± 0.34	5.27 ± 0.33	5.78 ± 0.31
Monocytes (10^3/μL)						
Day 4	0.23 ± 0.02	0.20 ± 0.01	0.19 ± 0.01	0.23 ± 0.02	0.22 ± 0.02	0.27 ± 0.02
Day 25	0.16 ± 0.02	0.15 ± 0.01	0.13 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	0.19 ± 0.02
Week 14	0.09 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
Basophils (10^3/μL)						
Day 4	0.037 ± 0.004	0.031 ± 0.004	0.026 ± 0.002	0.034 ± 0.004	0.031 ± 0.004	0.037 ± 0.003
Day 25	0.050 ± 0.008	0.064 ± 0.011	0.061 ± 0.011	0.074 ± 0.010	0.070 ± 0.013	0.082 ± 0.010

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Week 14	0.045 ± 0.008	0.061 ± 0.011	0.047 ± 0.008	0.050 ± 0.006	0.050 ± 0.008	0.053 ± 0.008
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.01	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.01
Day 25	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.00**	0.05 ± 0.01*
Week 14	0.08 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00*	0.06 ± 0.01*	0.04 ± 0.00**
Large unstained cells (10^3/μL)						
Day 4	0.143 ± 0.018	0.116 ± 0.005	0.097 ± 0.008*	0.130 ± 0.012	0.120 ± 0.011	0.174 ± 0.019
Day 25	0.136 ± 0.014	0.155 ± 0.032	0.151 ± 0.024	0.141 ± 0.019	0.188 ± 0.061	0.217 ± 0.044
Week 14	0.054 ± 0.008	0.077 ± 0.020	0.079 ± 0.016	0.067 ± 0.007	0.077 ± 0.011	0.087 ± 0.023
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	9
Day 25	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	11.7 ± 0.6	12.6 ± 0.6	11.8 ± 0.6	13.2 ± 0.6	13.6 ± 0.5	12.6 ± 0.6
Day 25	16.4 ± 0.5	14.8 ± 0.3	16.4 ± 0.5	15.2 ± 0.5	15.0 ± 0.5	14.1 ± 0.4*
Week 14	12.6 ± 0.4	13.9 ± 0.3	12.8 ± 0.4	13.1 ± 0.4	12.2 ± 0.7	12.3 ± 0.5
Creatinine (mg/dL)						
Day 4	0.17 ± 0.02	0.14 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.17 ± 0.02	0.19 ± 0.01
Day 25	0.25 ± 0.02	0.19 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.22 ± 0.01
Week 14	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.02	0.30 ± 0.00	0.27 ± 0.02	0.29 ± 0.01
Glucose (mg/dL)						
Day 4	133 ± 3	135 ± 4	135 ± 7	133 ± 3	124 ± 2	124 ± 4
Day 25	125 ± 8	120 ± 4	128 ± 4	137 ± 6	116 ± 3[b]	117 ± 4
Week 14	130 ± 2	130 ± 3	131 ± 2	136 ± 4	128 ± 2	127 ± 2
Total protein (g/dL)						
Day 4	5.9 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
Day 25	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.4 ± 0.0	6.6 ± 0.1	6.4 ± 0.1
Week 14	6.6 ± 0.1	6.9 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.3 ± 0.1
Albumin (g/dL)						
Day 4	4.4 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.1
Day 25	4.7 ± 0.1	4.8 ± 0.1	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.1	4.7 ± 0.1
Week 14	4.9 ± 0.1	5.1 ± 0.1	4.9 ± 0.0	5.0 ± 0.1	5.0 ± 0.1	4.7 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	65 ± 2	66 ± 2	67 ± 3	65 ± 3	69 ± 2	80 ± 2**
Day 25	52 ± 1	48 ± 2	52 ± 1	49 ± 2	47 ± 2	58 ± 2
Week 14	62 ± 4	42 ± 2**	45 ± 2**	36 ± 1**	35 ± 1**	47 ± 1**

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
Alkaline phosphatase (IU/L)						
Day 4	553 ± 11	583 ± 15	585 ± 19	571 ± 13	576 ± 12	610 ± 18
Day 25	381 ± 8	382 ± 9	371 ± 11	348 ± 8*	327 ± 13**	328 ± 11**
Week 14	205 ± 4	180 ± 5**	189 ± 9*	190 ± 6*	164 ± 5**	155 ± 6**
Creatine kinase (IU/L)						
Day 4	447 ± 52	445 ± 46	463 ± 35	462 ± 39	395 ± 26[b]	415 ± 37
Day 25	453 ± 77	468 ± 51	438 ± 58	534 ± 91	424 ± 40	426 ± 25
Week 14	187 ± 17	229 ± 30	238 ± 20	189 ± 22	152 ± 19	187 ± 21
Sorbitol dehydrogenase (IU/L)						
Day 4	7 ± 1[b]	7 ± 1[b]	3 ± 1[b]	5 ± 1[b]	7 ± 1	8 ± 2
Day 25	10 ± 3[e]	10 ± 2	12 ± 2[d]	8 ± 2[d]	11 ± 2	10 ± 1
Week 14	15 ± 1	14 ± 1	16 ± 1	15 ± 2	12 ± 1	14 ± 1
Bile acids (µmol/L)						
Day 4	17.2 ± 3.3	16.7 ± 1.6	13.9 ± 0.7	18.2 ± 1.1	19.8 ± 2.1	20.2 ± 1.5*
Day 25	17.7 ± 2.3	16.0 ± 0.9	19.9 ± 2.6	20.2 ± 1.7	24.5 ± 2.7*	23.0 ± 1.9*
Week 14	15.8 ± 1.9	18.4 ± 3.1	16.8 ± 1.5	14.4 ± 1.2	17.2 ± 1.6	20.0 ± 2.1

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.
[c]n = 7.
[d]n = 8.
[e]n = 6.

Table F-2. Hematology Data for Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	15.6 mg/kg	31.25 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	10	10	10
Male						
Hematocrit (auto) (%)	51.0 ± 0.6	50.9 ± 0.3	51.2 ± 0.3	50.5 ± 0.7	52.6 ± 0.6	53.0 ± 0.5*
Hematocrit (spun) (%)	50.0 ± 0.5	50.0 ± 0.3	49.8 ± 0.3	49.6 ± 0.6	51.8 ± 0.6*	52.3 ± 0.5**
Hemoglobin (g/dL)	17.1 ± 0.2	17.0 ± 0.1	17.2 ± 0.1	16.9 ± 0.2	17.5 ± 0.2*	17.8 ± 0.2**
Erythrocytes (10^6/µL)	10.19 ± 0.14	10.27 ± 0.05	10.25 ± 0.07	10.14 ± 0.13	10.58 ± 0.14*	10.73 ± 0.11**
Reticulocytes (10^5/µL)	3.23 ± 0.10	3.13 ± 0.05	3.01 ± 0.04	3.15 ± 0.09	3.11 ± 0.08	3.09 ± 0.06
Reticulocytes (%)	3.19 ± 0.10	3.04 ± 0.05	2.96 ± 0.05	3.11 ± 0.11	2.93 ± 0.08*	2.88 ± 0.06*
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.1 ± 0.3	49.6 ± 0.2	49.9 ± 0.3	49.8 ± 0.2	49.7 ± 0.2	49.4 ± 0.3*
Mean cell hemoglobin (pg)	16.8 ± 0.1	16.6 ± 0.1	16.8 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.5 ± 0.1	33.5 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.4 ± 0.2	33.5 ± 0.1
Platelets (10^3/µL)	853.0 ± 22.2	906.4 ± 40.5	866.3 ± 36.4	900.1 ± 34.4	838.8 ± 35.2	900.2 ± 28.9
Leukocytes (10^3/µL)	4.26 ± 0.33	4.55 ± 0.22	4.64 ± 0.23	4.48 ± 0.35	4.74 ± 0.28	4.89 ± 0.39
Segmented neutrophils (10^3/µL)	0.55 ± 0.04	0.53 ± 0.04	0.52 ± 0.02	0.55 ± 0.05	0.58 ± 0.04	0.58 ± 0.03
Lymphocytes (10^3/µL)	3.36 ± 0.28	3.66 ± 0.18	3.82 ± 0.23	3.58 ± 0.30	3.80 ± 0.24	4.00 ± 0.35
Monocytes (10^3/µL)	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01
Basophils (10^3/µL)	0.007 ± 0.002	0.006 ± 0.002	0.011 ± 0.002	0.008 ± 0.002	0.009 ± 0.003	0.007 ± 0.002
Eosinophils (10^3/µL)	0.27 ± 0.03	0.28 ± 0.03	0.21 ± 0.02	0.27 ± 0.02	0.26 ± 0.04	0.23 ± 0.04
Large unstained cells (10^3/µL)	0.017 ± 0.004	0.015 ± 0.002	0.022 ± 0.002	0.017 ± 0.003	0.020 ± 0.004	0.022 ± 0.004
Female						
Hematocrit (auto) (%)	51.5 ± 0.4	50.3 ± 1.4	53.0 ± 0.4	51.5 ± 0.5	51.1 ± 0.6	52.1 ± 0.7
Hematocrit (spun) (%)	50.3 ± 0.5	49.5 ± 1.2	51.9 ± 0.3	50.8 ± 0.4	50.5 ± 0.6	51.0 ± 0.6
Hemoglobin (g/dL)	17.1 ± 0.2	16.6 ± 0.4	17.5 ± 0.1	17.2 ± 0.1	17.0 ± 0.2	17.3 ± 0.2
Erythrocytes (10^6/µL)	10.30 ± 0.11	9.99 ± 0.25	10.57 ± 0.10	10.31 ± 0.08	10.25 ± 0.13	10.50 ± 0.12
Reticulocytes (10^5/µL)	2.98 ± 0.16	2.64 ± 0.22	3.48 ± 0.27	2.86 ± 0.18	2.71 ± 0.10	2.64 ± 0.21
Reticulocytes (%)	2.89 ± 0.16	2.66 ± 0.22	3.31 ± 0.26	2.77 ± 0.17	2.64 ± 0.13	2.54 ± 0.20
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	50.1 ± 0.2	50.4 ± 0.4	50.2 ± 0.2	50.0 ± 0.2	49.9 ± 0.2	49.6 ± 0.2
Mean cell hemoglobin (pg)	16.6 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.7 ± 0.1	16.6 ± 0.1	16.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.1	33.0 ± 0.2	33.1 ± 0.1	33.5 ± 0.2	33.3 ± 0.1	33.2 ± 0.1
Platelets (10^3/µL)	643.3 ± 36.7	750.3 ± 49.4	734.3 ± 52.7	744.1 ± 20.8	647.2 ± 36.2	658.5 ± 21.1

	Vehicle Control	15.6 mg/kg	31.25 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
Leukocytes (10^3/µL)	2.63 ± 0.22	3.78 ± 0.60	2.84 ± 0.17	3.27 ± 0.15	2.97 ± 0.18	2.83 ± 0.18
Segmented neutrophils (10^3/µL)	0.28 ± 0.05	0.77 ± 0.44	0.34 ± 0.05	0.43 ± 0.04	0.31 ± 0.03	0.29 ± 0.04
Lymphocytes (10^3/µL)	2.11 ± 0.18	2.77 ± 0.22	2.29 ± 0.16	2.65 ± 0.13	2.44 ± 0.16	2.30 ± 0.13
Monocytes (10^3/µL)	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Basophils (10^3/µL)	0.006 ± 0.002	0.006 ± 0.002	0.007 ± 0.002	0.007 ± 0.002	0.006 ± 0.002	0.006 ± 0.002
Eosinophils (10^3/µL)	0.21 ± 0.02	0.19 ± 0.03	0.17 ± 0.03	0.14 ± 0.04	0.17 ± 0.03	0.19 ± 0.03
Large unstained cells (10^3/µL)	0.009 ± 0.002	0.017 ± 0.003	0.010 ± 0.001	0.014 ± 0.002	0.012 ± 0.001	0.010 ± 0.001

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test.
**$P \leq 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Appendix G. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol G-2
Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Indole-3-carbinol G-3

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	309 ± 5	320 ± 9	325 ± 7	321 ± 8	309 ± 8	285 ± 6
Heart						
Absolute	0.77 ± 0.02	0.82 ± 0.02	0.83 ± 0.02	0.82 ± 0.01	0.78 ± 0.02	0.75 ± 0.02
Relative	2.483 ± 0.034	2.577 ± 0.035	2.561 ± 0.030	2.562 ± 0.023	2.528 ± 0.032	2.618 ± 0.038*
R. Kidney						
Absolute	0.86 ± 0.02	0.94 ± 0.02	0.93 ± 0.03	0.97 ± 0.03*	0.92 ± 0.03	0.90 ± 0.02
Relative	2.785 ± 0.038	2.940 ± 0.033	2.844 ± 0.054	3.012 ± 0.038**	2.981 ± 0.048**	3.152 ± 0.055**
Liver						
Absolute	9.98 ± 0.30	11.19 ± 0.40*	11.20 ± 0.40*	11.85 ± 0.36**	12.07 ± 0.38**	12.57 ± 0.33**
Relative	32.205 ± 0.594	34.924 ± 0.481**	34.382 ± 0.716**	36.941 ± 0.435**	39.050 ± 0.472**	44.047 ± 0.729**
Lung						
Absolute	1.31 ± 0.06	1.43 ± 0.03	1.43 ± 0.06	1.36 ± 0.04	1.45 ± 0.08	1.28 ± 0.04
Relative	4.229 ± 0.164	4.474 ± 0.113	4.399 ± 0.196	4.240 ± 0.075	4.676 ± 0.226	4.486 ± 0.131
R. Testis						
Absolute	1.299 ± 0.020	1.311 ± 0.020	1.449 ± 0.075*	1.334 ± 0.025	1.355 ± 0.028	1.292 ± 0.029
Relative	4.201 ± 0.055	4.113 ± 0.059	4.477 ± 0.265	4.178 ± 0.099	4.396 ± 0.064	4.531 ± 0.080
Thymus						
Absolute	0.185 ± 0.008	0.187 ± 0.008	0.207 ± 0.007	0.191 ± 0.006	0.182 ± 0.006	0.170 ± 0.007
Relative	0.597 ± 0.021	0.590 ± 0.030	0.635 ± 0.019	0.597 ± 0.019	0.591 ± 0.019	0.596 ± 0.027
Female						
Necropsy body wt	189 ± 2	192 ± 3	195 ± 2	185 ± 3	183 ± 3	183 ± 3
Heart						
Absolute	0.53 ± 0.01	0.56 ± 0.01	0.54 ± 0.00	0.53 ± 0.01	0.54 ± 0.01	0.53 ± 0.01
Relative	2.784 ± 0.036	2.911 ± 0.036	2.782 ± 0.030	2.844 ± 0.050	2.926 ± 0.045*	2.913 ± 0.026
R. Kidney						
Absolute	0.57 ± 0.01	0.66 ± 0.01**	0.63 ± 0.01*	0.61 ± 0.01	0.62 ± 0.01	0.63 ± 0.02*
Relative	3.034 ± 0.056	3.447 ± 0.053**	3.233 ± 0.075**	3.277 ± 0.046**	3.375 ± 0.064**	3.436 ± 0.063**
Liver						
Absolute	5.08 ± 0.11	5.94 ± 0.11**	5.98 ± 0.07**	5.92 ± 0.13**	6.34 ± 0.14**	6.92 ± 0.11**
Relative	26.804 ± 0.441	30.985 ± 0.566**	30.790 ± 0.547**	32.061 ± 0.548**	34.660 ± 0.614**	37.857 ± 0.319**
Lung						
Absolute	0.92 ± 0.03	0.99 ± 0.03	0.93 ± 0.02	0.92 ± 0.03	0.94 ± 0.03	0.98 ± 0.02
Relative	4.874 ± 0.151	5.144 ± 0.149	4.781 ± 0.139	4.982 ± 0.182	5.121 ± 0.170	5.386 ± 0.176
Thymus						
Absolute	0.178 ± 0.005	0.173 ± 0.005	0.162 ± 0.007	0.149 ± 0.008**	0.151 ± 0.007**	0.145 ± 0.006**
Relative	0.943 ± 0.027	0.901 ± 0.022	0.836 ± 0.042*	0.805 ± 0.036**	0.825 ± 0.029**	0.792 ± 0.031**

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	15.6 mg/kg	31.25 mg/kg	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	10	10	10
Male						
Necropsy body wt	40.4 ± 0.6	40.5 ± 1.1	41.5 ± 0.9	40.0 ± 1.1	42.2 ± 1.6	41.0 ± 0.9
Heart						
Absolute	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.14 ± 0.00
Relative	3.496 ± 0.073	3.418 ± 0.066	3.453 ± 0.100	3.413 ± 0.085	3.476 ± 0.135	3.330 ± 0.088
R. Kidney						
Absolute	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
Relative	7.009 ± 0.152	6.908 ± 0.212	7.063 ± 0.204	7.137 ± 0.195	6.928 ± 0.321	6.891 ± 0.166
Liver						
Absolute	1.50 ± 0.06	1.46 ± 0.07	1.54 ± 0.05	1.54 ± 0.05	1.77 ± 0.13*	1.82 ± 0.06**
Relative	37.329 ± 1.896	35.868 ± 0.799	37.166 ± 0.702	38.520 ± 0.632	41.960 ± 2.146*	44.333 ± 0.817**
Lung						
Absolute	0.24 ± 0.02	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.23 ± 0.02
Relative	5.916 ± 0.421	5.532 ± 0.321	5.411 ± 0.341	5.406 ± 0.307	5.751 ± 0.384	5.665 ± 0.348
R. Testis						
Absolute	0.120 ± 0.002	0.121 ± 0.004	0.117 ± 0.003[b]	0.116 ± 0.003	0.118 ± 0.002	0.109 ± 0.006
Relative	2.985 ± 0.068	3.008 ± 0.122	2.828 ± 0.077[b]	2.920 ± 0.121	2.830 ± 0.135	2.656 ± 0.143
Thymus						
Absolute	0.032 ± 0.002	0.029 ± 0.002	0.031 ± 0.001	0.031 ± 0.003	0.031 ± 0.004	0.035 ± 0.002
Relative	0.779 ± 0.047	0.728 ± 0.049	0.738 ± 0.038	0.784 ± 0.066	0.743 ± 0.094	0.846 ± 0.048
Female						
Necropsy body wt	34.2 ± 1.0	33.5 ± 1.0	33.8 ± 1.6	33.7 ± 0.6	33.1 ± 0.7	31.4 ± 1.3
Heart						
Absolute	0.12 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00
Relative	3.505 ± 0.117	3.860 ± 0.158	3.606 ± 0.153	3.577 ± 0.104	3.578 ± 0.061	3.699 ± 0.130
R. Kidney						
Absolute	0.17 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.17 ± 0.01	0.17 ± 0.00	0.17 ± 0.01
Relative	4.995 ± 0.117	5.303 ± 0.119	5.069 ± 0.187	5.094 ± 0.172	5.128 ± 0.135	5.351 ± 0.178
Liver						
Absolute	1.04 ± 0.01	1.21 ± 0.05*	1.11 ± 0.04*	1.16 ± 0.03*	1.22 ± 0.03**	1.21 ± 0.04**
Relative	30.559 ± 0.696	36.190 ± 1.253**	32.906 ± 0.651**	34.449 ± 0.693**	36.878 ± 0.867**	38.687 ± 0.778**
Lung						
Absolute	0.23 ± 0.01	0.22 ± 0.01	0.20 ± 0.01*	0.21 ± 0.01	0.23 ± 0.01	0.23 ± 0.01
Relative	6.764 ± 0.244	6.673 ± 0.348	5.863 ± 0.199	6.243 ± 0.217	7.055 ± 0.265	7.408 ± 0.352
Thymus						
Absolute	0.041 ± 0.001	0.036 ± 0.001	0.038 ± 0.002	0.041 ± 0.002	0.042 ± 0.002	0.031 ± 0.002**
Relative	1.204 ± 0.039	1.087 ± 0.045	1.158 ± 0.104	1.218 ± 0.063	1.285 ± 0.068	1.019 ± 0.087

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b]n = 9.

Appendix H. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Table H-1. Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol.....H-2
Table H-2. Estrous Cycle Characterization for Female F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol.....H-2
Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/N Rats Administered Indole-3-carbinol by Gavage for Three Months.....H-3
Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Indole-3-carbinol.....H-4
Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Gavage Study of Indole-3-carbinol.....H-4
Table H-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Administered Indole-3-carbinol by Gavage for Three Months.....H-5

Figures

Figure H-1. Vaginal Cytology Plots for Female F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol.....H-6
Figure H-2. Vaginal Cytology Plots for Female Mice in the Three-month Gavage Study of Indole-3-carbinol.....H-7

Table H-1. Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	309 ± 5	321 ± 8	309 ± 8	285 ± 6*
L. Cauda epididymis	0.1657 ± 0.0055	0.1687 ± 0.0083	0.1588 ± 0.0067	0.1591 ± 0.0099
L. Epididymis	0.4403 ± 0.0125	0.4414 ± 0.0145	0.4249 ± 0.0101	0.4074 ± 0.0148
L. Testis	1.3959 ± 0.0246	1.4735 ± 0.0264	1.4580 ± 0.0235	1.4157 ± 0.0308
Spermatid measurements				
Spermatid heads (10^6/testis)	160.4 ± 6.7	168.4 ± 7.8	149.3 ± 6.4	170.9 ± 12.6
Spermatid heads (10^6/g testis)	153.4 ± 6.0	160.6 ± 9.4	143.4 ± 5.4	164.1 ± 9.5
Epididymal spermatozoal measurements				
Sperm motility (%)	84.60 ± 1.62	83.66 ± 1.85	82.23 ± 1.66	82.52 ± 1.78
Sperm (10^6/cauda epididymis)	94.03 ± 2.43	83.90 ± 2.90	89.39 ± 4.30	92.04 ± 4.57
Sperm (10^6/g cauda epididymis)	571.6 ± 19.0	505.7 ± 26.3	571.8 ± 35.7	590.9 ± 31.4

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test.
[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table H-2. Estrous Cycle Characterization for Female F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	189 ± 2	185 ± 3	183 ± 3	183 ± 3
Proportion of regular cycling females[b]	7/7	8/8	9/10	8/9
Estrous cycle length (days)	5.00 ± 0.11[c]	5.13 ± 0.13[d]	5.25 ± 0.13	5.89 ± 0.31*[e]
Estrous stages[f] (% of cycle)				
Diestrus	45.8	50.0	52.5	60.0
Proestrus	15.0	13.3	16.7	9.2
Estrus	20.8	19.2	19.2	20.0
Metestrus	6.7	6.7	11.7	10.8
Uncertain diagnoses	11.7	10.8	0.0	0.0

*Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test.
[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight). Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated a significantly higher probability of extended diestrus in the 300 mg/kg group compared to the vehicle control group.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in three of 10 animals.
[d]Estrous cycle was longer than 12 days or unclear in two of 10 animals.
[e]Estrous cycle was longer than 12 days or unclear in one of 10 animals.
[f]Evidence shows that 150 and 300 mg/kg females differ significantly (Wilkes' Criterion, $P \leq 0.05$) from the vehicle control females in the relative length of time spent in the estrous stages.

Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/N Rats Administered Indole-3-carbinol by Gavage for Three Months

Stage	Comparison[a]	P Value	Trend[b]
Overall Tests	Overall	<0.001	
Overall Tests	Low vs. Controls	0.983	–
Overall Tests	Mid vs. Controls	0.89	N
Overall Tests	High vs. Controls	<0.001	–
Extended Estrus	Overall	0.519	
Extended Estrus	Low vs. Controls	0.572	–
Extended Estrus	Mid vs. Controls	0.543	N
Extended Estrus	High vs. Controls	0.239	–
Extended Diestrus	Overall	0.001	
Extended Diestrus	Low vs. Controls	0.967	N
Extended Diestrus	Mid vs. Controls	0.861	N
Extended Diestrus	High vs. Controls	<0.001	–
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. Controls	1	–
Extended Metestrus	Mid vs. Controls	1	–
Extended Metestrus	High vs. Controls	1	–
Extended Proestrus	Overall	0.985	
Extended Proestrus	Low vs. Controls	1	–
Extended Proestrus	Mid vs. Controls	0.604	–
Extended Proestrus	High vs. Controls	1	–
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. Controls	1	–
Skipped Estrus	Mid vs. Controls	0.924	–
Skipped Estrus	High vs. Controls	1	–
Skipped Diestrus	Overall	1	
Skipped Diestrus	Low vs. Controls	1	–
Skipped Diestrus	Mid vs. Controls	1	–
Skipped Diestrus	High vs. Controls	1	–
Summary of Significant Groups			
Overall Tests	High vs. Controls	<0.001	–
Extended Diestrus	High vs. Controls	<0.001	–

[a]Controls = Vehicle Control, Low = 75 mg/kg, Mid = 150 mg/kg, High = 300 mg/kg.
[b]N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended diestrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	9
Weights (g)				
Necropsy body wt	40.4 ± 0.6	40.0 ± 1.1	42.2 ± 1.6	40.8 ± 1.0
L. Cauda epididymis	0.0245 ± 0.0010	0.0255 ± 0.0013	0.0231 ± 0.0013	0.0232 ± 0.0010
L. Epididymis	0.0583 ± 0.0019	0.0589 ± 0.0014	0.0555 ± 0.0030	0.0530 ± 0.0020
L. Testis	0.1274 ± 0.0023	0.1244 ± 0.0026	0.1254 ± 0.0014	0.1202 ± 0.0028
Spermatid measurements				
Spermatid heads (10^6/testis)	20.99 ± 1.28	22.56 ± 1.11	22.63 ± 1.60	24.81 ± 0.47
Spermatid heads (10^6/g testis)	378.9 ± 52.3	417.9 ± 28.0	478.1 ± 47.0	507.8 ± 76.5
Epididymal spermatozoal measurements				
Sperm motility (%)	83.71 ± 0.50	79.68 ± 1.09**	79.12 ± 0.96**	74.26 ± 0.80**
Sperm (10^6/cauda epididymis)	18.04 ± 0.58	18.97 ± 1.04	18.52 ± 0.90	18.73 ± 2.35
Sperm (10^6/g cauda epididymis)	742.9 ± 28.9	747.1 ± 30.7	820.0 ± 54.9	800.5 ± 94.9

**Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test.
[a]Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid measurements, sperm per cauda epididymis, and sperm per gram cauda epididymis).

Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Gavage Study of Indole-3-carbinol[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	34.2 ± 1.0	33.7 ± 0.6	33.1 ± 0.7	31.4 ± 1.3
Proportion of regular cycling females[b]	8/10	8/10	7/10	5/9
Estrous cycle length (days)	4.06 ± 0.13	4.65 ± 0.51	4.29 ± 0.17	4.17 ± 0.33[c]
Estrous stages (% of cycle)				
Diestrus	31.7	40.8	30.0	40.0
Proestrus	0.0	1.7	1.7	3.3
Estrus	46.7	38.3	46.7	37.5
Metestrus	21.7	19.2	21.7	19.2

[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated a significantly higher probability of extended diestrus in the 250 mg/kg group compared to the vehicle control group.
[b]Number of females with a regular cycle/number of females cycling.
[c]One of 10 animals was excluded from mean due to cycle length longer than 9 days.

Table H-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Administered Indole-3-carbinol by Gavage for Three Months

Stage	Comparison[a]	P Value	Trend[b]
Overall Tests	Overall	0.024	
Overall Tests	Low vs. Controls	0.437	–
Overall Tests	Mid vs. Controls	0.426	–
Overall Tests	High vs. Controls	0.004	–
Extended Estrus	Overall	0.793	
Extended Estrus	Low vs. Controls	0.77	N
Extended Estrus	Mid vs. Controls	0.351	–
Extended Estrus	High vs. Controls	0.775	–
Extended Diestrus	Overall	0.052	
Extended Diestrus	Low vs. Controls	0.065	–
Extended Diestrus	Mid vs. Controls	0.946	N
Extended Diestrus	High vs. Controls	0.031	–
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. Controls	1	–
Extended Metestrus	Mid vs. Controls	1	–
Extended Metestrus	High vs. Controls	1	–
Extended Proestrus	Overall	1	
Extended Proestrus	Low vs. Controls	1	–
Extended Proestrus	Mid vs. Controls	1	–
Extended Proestrus	High vs. Controls	1	–
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. Controls	1	–
Skipped Estrus	Mid vs. Controls	1	–
Skipped Estrus	High vs. Controls	1	–
Skipped Diestrus	Overall	1	
Skipped Diestrus	Low vs. Controls	1	–
Skipped Diestrus	Mid vs. Controls	1	–
Skipped Diestrus	High vs. Controls	1	–
Summary of Significant Groups			
Overall Tests	High vs. Controls	0.004	–
Extended Diestrus	High vs. Controls	0.031	–

[a]Controls = Vehicle Control, Low = 62.5 mg/kg, Mid = 125 mg/kg, High = 250 mg/kg.
[b]N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended diestrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

Dose (mg/kg)																					
0							D	D	E	D	D	D	D	E	M	D	D	D			
0									E	D	D	D	P	E	D	D	D	D	P	E	
0				IC	IC	D	D	D	P	E	M	D	D	P	E						
0					E	IC	D	D	IC	IC	M	D	D	P	E	M					
0									E	D	D	D	P	E	D	D	D	P	E	D	
0									P	E	D	D	D	P	E	D	D	D	P	E	
0									P	E	D	D	D	P	E	M	D	D	P	E	
0									D	E	E	D	D	P	E	M	D	D	P	E	
0						D	IC	IC	P	E	IC	D	D	P	E	M	D				
0		IC	IC	IC	IC	IC	IC	IC	M	D	D	P	E	D							
75				E	IC	D	IC	P	IC	D	D	D	P	E	M						
75		D	IC	IC	IC	IC	IC	D	P	E	M	D	D								
75				D	IC	E	D	IC	IC	M	D	D	P	E	D						
75					D	IC	IC	P	E	D	D	D	P	E	D	D					
75									E	D	D	D	P	E	M	D	D	P	E	D	
75					M	D	D	D	E	D	D	D	P	E	D	D					
75							D	E	E	D	D	D	P	E	M	D	D	P			
75							D	E	D	D	D	D	P	E	D	D	D	P			
75					D	D	E	E	D	D	D	D	P	E	D	D					
75								D	E	M	D	D	P	E	M	D	D	P	E		
150									E	D	P	D	D	E	M	D	D	P	E	D	
150				D	D	D	D	E	M	D	D	D	D	E	M						
150								P	E	D	D	D	P	E	D	D	D	P	E		
150					D	D	P	E	D	D	D	D	P	E	D	D					
150				M	D	D	P	E	M	D	D	D	P	E	M						
150					M	D	D	P	E	M	D	D	P	E	M	D					
150									E	M	D	P	P	E	D	D	D	P	E	D	
150						D	D	P	E	D	D	D	P	E	D	D	D				
150					M	D	D	P	E	M	D	D	D	E	M	D					
150							D	P	E	M	D	D	P	E	D	D	D	P			
300				D	D	D	P	E	M	D	D	D	D	E	M						
300								E	D	D	D	D	P	E	M	D	D	D	D		
300								E	D	D	D	D	P	E	D	D	D	E	M		
300					D	D	D	E	M	D	D	D	P	E	M	D					
300		M	D	D	D	D	D	D	D	D	P	E	D								
300					D	D	P	E	M	D	D	D	D	E	M	D					
300								P	E	M	D	D	D	E	D	D	D	P	E		
300				D	D	D	D	E	E	M	D	D	D	E	D						
300				M	D	D	P	E	D	D	D	D	P	E	D						
300					P	E	E	M	D	D	D	D	D	E	E	D					

Figure H-1. Vaginal Cytology Plots for Female F344/N Rats in the Three-month Gavage Study of Indole-3-carbinol

IC = Insufficient number of cells to determine stage; D = diestrus, P = proestrus, E = estrus, M = metestrus

Dose (mg/kg)																						
0										E	M	D	D	E	E	D	D	D	D	D	D	
0								M	D	E	E	M	D	E	E	M	D	E	E			
0									D	E	E	M	D	E	E	M	D	E	E	M		
0											E	M	D	E	E	M	D	E	E	M	D	E
0									D	D	E	M	D	E	E	M	D	E	E	M		
0									D	E	E	M	D	E	E	M	D	D	D	E		
0								M	D	E	E	M	D	E	E	M	D	E	E			
0											E	M	D	E	E	M	D	E	E	M	D	E
0								D	E	E	E	M	D	E	E	E	M	D	E			
0											E	M	D	E	E	M	D	E	E	M	D	E
62.5									D	E	E	M	D	E	E	M	D	D	D	D		
62.5								D	E	E	M	D	D	E	E	M	D	D	E			
62.5							M	D	E	E	M	D	P	E	E	M	D	P				
62.5							M	D	D	E	E	M	D	E	E	M	D	E				
62.5									D	E	E	M	D	E	E	M	D	D	D	D		
62.5									D	E	E	M	D	E	E	M	D	D	D	E		
62.5										E	E	M	D	E	E	E	M	D	D	E	E	
62.5							D	D	D	E	E	M	D	E	E	M	D	E				
62.5									D	D	E	M	D	E	E	M	D	E	E	M		
62.5					E	M	D	D	D	D	D	D	D	E	M	D						
125											E	M	D	E	E	M	D	E	E	M	D	E
125								M	D	E	E	M	D	E	E	M	D	E	E			
125										E	E	M	D	E	E	E	M	D	D	D	D	
125										E	M	D	P	E	E	M	D	P	E	E	M	
125										E	E	M	D	E	E	E	M	D	E	E	E	
125											E	D	D	E	E	M	D	E	E	M	D	D
125							M	D	E	E	E	M	D	E	E	M	D	D				
125								M	D	E	E	M	D	E	E	M	D	E	E			
125											E	M	D	E	E	M	D	E	E	M	D	E
125						D	D	D	D	E	E	M	D	E	E	M	D					
250										E	E	M	D	E	E	M	D	P	E	M	D	
250										E	M	D	P	E	M	D	D	D	D	E	E	
250		M	D	E	E	M	D	D	D	D	D	D	D									
250										E	E	M	D	E	E	E	M	D	E	E	E	
250								M	D	E	E	M	D	E	E	M	D	E	E			
250									E	E	E	M	D	E	E	M	D	D	D	D		
250			E	M	D	D	D	D	D	D	D	D	D	E								
250					M	D	D	D	D	D	D	E	D	E	M	D						
250										E	M	D	P	E	E	M	D	P	E	E	M	
250								M	D	E	E	M	D	E	E	M	D	E	E			

Figure H-2. Vaginal Cytology Plots for Female Mice in the Three-month Gavage Study of Indole-3-carbinol

D = diestrus, P = proestrus, E = estrus, M = metestrus

Appendix I. Chemical Characterization and Dose Formulation Studies

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I.1. Procurement and Characterization

I.1.1. Indole-3-carbinol

Indole-3-carbinol was obtained from ChemPacific Corporation (Baltimore, MD) in one lot (CHP801001) that was used during the 3-month and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Support Services (Columbus, OH) and the study laboratories, Southern Research Institute (SRI) (Birmingham, AL) for the 3-month studies and Battelle Columbus Operations (Columbus, OH) for the 2-year studies. Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the indole-3-carbinol studies are on file at the National Institute of Environmental Health Sciences.

Lot CHP801001 of the test chemical, a yellow crystalline solid, was identified as indole-3-carbinol by the analytical chemistry laboratory using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratories using IR and proton NMR (SRI only) spectroscopy. All spectra were consistent with the literature spectra[137; 138] and the structure of indole-3-carbinol. Representative IR and proton NMR spectra are presented in Figure I-1 and Figure I-2, respectively.

For lot CHP801001, Karl Fischer titration was used to determine the water content; elemental analyses were used to determine the carbon, hydrogen, and nitrogen content; and the melting point was determined using a differential scanning calorimeter. The purity was determined by the analytical chemistry laboratory using high-performance liquid chromatography (HPLC) with ultraviolet detection by systems A and B (Table I-1).

Karl Fischer titration indicated 1.3% water. Elemental analyses for carbon, hydrogen, and nitrogen were consistent with the theoretical values, and the melting point was 99.3°C, consistent with the manufacturer's Certificate of Analysis. HPLC analysis indicated one major peak with six impurities, each 0.1% or greater of the total peak area with a combined area of 2.8% of total peak area in the chromatogram. The overall purity of lot CHP801001 was determined to be approximately 97%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC by system B. These studies indicated that indole-3-carbinol was stable as a bulk chemical for at least 2 weeks when stored in sealed amber glass vials protected from light at temperatures up to 25°C. However, at 60°C, physical changes were observed in the test chemical. To ensure stability, the bulk chemical was stored at −20°C, protected from light in amber glass containers sealed with Teflon®-lined lids. Periodic reanalyses of the bulk chemical were performed by the study laboratory twice during the 3-month studies and approximately every 6 months during the 2-year studies using HPLC by a system similar to system B; no degradation of the bulk chemical was detected.

I.1.2. Corn Oil

Corn oil was used as the vehicle for the formulations and was obtained from Red Diamond, Inc. (Birmingham, AL), in one lot that was used in the 3-month studies and from Spectrum Chemicals and Laboratory Products (Gardena, CA) in seven lots and from Sigma-Aldrich (St. Louis, MO) in three lots that were used in the 2-year studies. Analysis of the corn oil for

peroxides was performed by potentiometric titration, and each lot was within the acceptable range of less than or equal to 3 mEq/kg.

I.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing the appropriate amount of milled indole-3-carbinol with corn oil to achieve the required concentrations (Table I-2). The dose formulations were prepared seven times during the 3-month studies and approximately every 4 weeks during the 2-year studies. Dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5°C for up to 41 days.

Homogeneity, gavageability, and resuspendability studies were performed on 60 mg/mL dose formulations, and stability studies were performed on 1 mg/mL formulations by the analytical chemistry laboratory using HPLC by system B (Table I-1). To achieve acceptable chromatography, all samples containing corn oil were clarified by centrifuging 1-mL aliquots of each dose formulation for approximately 5 minutes at 14,000 rpm, then diluting (1:1) with the mobile phase prior to HPLC analysis. Homogeneity was confirmed, and gavageability was confirmed for a 20-gauge gavage needle. Resuspendability was confirmed for dose formulations that had been stored for 14 days at 5°C. Stability was confirmed for at least 8 days for 1 mg/mL formulations stored in amber glass vials sealed with Teflon®-lined lids at 5°C and for 3 hours under simulated animal room conditions. The study laboratory at SRI performed stability studies with 1.6 mg/mL dose formulations using HPLC by a system similar to system B, and stability was confirmed for at least 41 days for dose formulations stored in amber glass vials sealed with Teflon®-lined lids at 5°C. Prior to the 2-year studies, the study laboratory at Battelle Columbus Operations performed homogeneity studies on 6.25, 15, 25, and 60 mg/mL dose formulations and gavageability studies on 60 mg/mL dose formulations using HPLC by a system similar to system B; homogeneity and gavagability were confirmed.

Periodic analyses of the dose formulations of indole-3-carbinol were performed by the study laboratories using HPLC by systems similar to system B. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations for rats and all 15 for mice were within 10% of the target concentrations (Table I-3). Animal room samples of these dose formulations were also analyzed; all 15 for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months (Table I-4). Of the dose formulations analyzed, all 72 for rats and all 36 for mice were within 10% of the target concentrations; all 24 of the animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

Table I-1. High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Indole-3-carbinol[a]

Detection System	Column	Solvent System
System A		
Photodiode array Ultraviolet (220 to 360 nm) light	Prodigy™ C18 ODS(3), 250 mm × 4.6 mm, 5 µm (Phenomenex, Torrance, CA)	80:20 Methanol:1% aqueous acetic acid solution, isocratic; flow rate 0.8 mL/minute
System B		
Ultraviolet (280 nm) light	Prodigy™ C18, 250 mm × 4.6 mm, 5 µm (Phenomenex)	80:20 Methanol:1.5% aqueous acetic acid solution, isocratic; flow rate 0.8 mL/minute

[a]The high-performance liquid chromatographs were manufactured by Waters, Inc. (Milford, MA) (System A), or Spectra Physics (San Jose, CA) (System B).

Table I-2. Preparation and Storage of Dose Formulations in the Gavage Studies of Indole-3--carbinol

Three-month Studies	Two-year Studies
Preparation	
Prior to the preparation of dose formulations, portions of frozen indole-3-carbinol were milled for approximately 15 seconds in an IKA Works M20 Universal mill, then passed through a 40-mesh sieve, and placed in high density polyethylene wide-mouth containers, sealed with Teflon®-lined lids, rolled and shaken for approximately 15 minutes. To prepare the dose formulations, containers of milled indole-3-carbinol were allowed to warm to room temperature. For each dose formulation, the appropriate amount of milled indole-3-carbinol was weighed into a weigh boat or beaker and transferred to a mortar; corn oil was added in increments and the mixture was ground with a pestle into a paste-like mixture, transferred to an Erlenmeyer flask with corn oil rinses, diluted to final volume and sonicated for approximately 10 minutes, and cooled by an ice water bath. A stir bar was added and the mixture was stirred for at least 2 hours to ensure homogeneity. Formulations were prepared seven times during the 3-month studies.	Same as 3-month studies except that dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number	
CHP801001	CHP801001
Maximum Storage Time	
41 days	41 days
Storage Conditions	
The dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5°C.	The dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5°C.
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Battelle Columbus Operations (Columbus, OH)

Table I-3. Results of Analyses of Dose Formulations Administered to F344/N Rats and Mice in the Three-month Gavage Studies of Indole-3-carbinol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
August 23, 2004	August 24–25, 2004	3.75	3.52 ± 0.061	−6
		7.50	7.25 ± 0.33	−3
		15	13.6 ± 0.14	−9
		30	29.1 ± 0.10	−3
		60	63.0 ± 0.41	+5
	September 13–14, 2004[b]	3.75	3.60 ± 0.090	−4
		7.50	7.33 ± 0.019	−2
		15	14.9 ± 0.0085	−1
		30	30.0 ± 0.37	0
		60	60.8 ± 0.38	+1
October 5, 2004	October 6–7, 2004	3.75	3.68 ± 0.089	−2
		7.50	7.30 ± 0.20	−3
		15	15.0 ± 0.024	0
		30	29.8 ± 0.19	−1
		60	59.2 ± 0.25	−1
	October 26–27, 2004[b]	3.75	3.60 ± 0.060	−4
		7.50	7.22 ± 0.39	−4
		15	15.0 ± 0.62	0
		30	29.4 ± 0.25	−2
		60	56.0 ± 0.27	−7
November 15, 2004	November 16–17, 2004	3.75	3.54 ± 0.074	−6
		7.50	7.03 ± 0.068	−6
		15	14.7 ± 0.42	−2
		30	29.6 ± 0.34	−1
		60	56.8 ± 0.83	−5
	December 8–9, 2004[b]	3.75	3.44 ± 0.025	−8
		7.50	6.91 ± 0.038	−8
		15	14.4 ± 0.30	−4
		30	29.9 ± 0.047	0
		60	58.7 ± 3.2	−2

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Mice				
August 23, 2004	August 24–25, 2004	1.56	1.52 ± 0.0080	−3
		3.13	3.03 ± 0.082	−3
		6.25	6.21 ± 0.081	−1
		12.5	12.1 ± 0.021	−3
		25	23.7 ± 0.46	−5
	September 13–14, 2004[b]	1.56	1.55 ± 0.00026	−1
		3.13	2.83 ± 0.039	−10
		6.25	5.86 ± 0.12	−6
		12.5	11.5 ± 0.032	−8
		25	24.2 ± 0.13	−3
October 5, 2004	October 6–7, 2004	1.56	1.55 ± 0.0013	−1
		3.13	2.86 ± 0.033	−9
		6.25	5.89 ± 0.022	−6
		12.5	11.9 ± 0.0074	−5
		25	25.1 ± 0.12	0
	October 26–27, 2004[b]	1.56	1.52 ± 0.0059	−3
		3.13	2.84 ± 0.019	−9
		6.25	5.92 ± 0.092	−5
		12.5	11.8 ± 0.63	−6
		25	25.2 ± 0.090	+1
November 15, 2004	November 16–17, 2004	1.56	1.57 ± 0.031	+1
		3.13	2.97 ± 0.0038	−5
		6.25	6.26 ± 0.017	0
		12.5	12.4 ± 0.080	−1
		25	25.1 ± 0.98	0
	December 8–9, 2004[b]	1.56	1.58 ± 0.012	+1
		3.13	3.00 ± 0.051	−4
		6.25	6.25 ± 0.080	0
		12.5	12.1 ± 0.083	−3
		25	25.2 ± 0.20	+1

[a]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 3.75 mg/mL = 18.75 mg/kg, 7.50 mg/mL = 37.5 mg/kg, 15 mg/mL = 75 mg/kg, 30 mg/mL = 150 mg/kg, 60 mg/mL = 300 mg/kg. For mice, dosing volume = 10 mL/kg; 1.56 mg/mL = 15.6 mg/kg, 3.13 mg/mL = 31.25 mg/kg, 6.25 mg/mL = 62.5 mg/kg, 12.5 mg/mL = 125 mg/kg, 25 mg/mL = 250 mg/kg.
[b]Animal room samples.

Table I-4. Results of Analyses of Dose Formulations Administered to Sprague Dawley Rats and Mice in the Two-year Gavage Studies of Indole-3-carbinol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
March 5, 2007	March 8, 2007	15	15.1 ± 0.2	+1
		15	15.0 ± 0.0	0
		30	30.2 ± 0.2	+1
		30	30.6 ± 0.2	+2
		60	61.9 ± 0.3	+3
		60	61.6 ± 0.1	+3
	April 12, 2007[b]	15	15.5 ± 0.1	+3
		15	15.4 ± 0.2	+3
		30	30.6 ± 0.1	+2
		30	30.5 ± 0.1	+2
		60	62.0 ± 0.1	+3
		60	60.7 ± 0.2	+1
May 2, 2007	May 2, 2007	15	15.5 ± 0.1	+3
		15	15.4 ± 0.1	+3
		30	31.3 ± 0.1	+4
		30	31.4 ± 0.3	+5
		60	64.3 ± 0.2	+7
		60	63.1 ± 0.1	+5
July 23, 2007	July 24, 2007	15	15.8 ± 0.1	+5
		15	15.8 ± 0.1	+5
		30	31.3 ± 0.2	+4
		30	31.8 ± 0.1	+6
		60	63.4 ± 0.4	+6
		60	63.6 ± 0.1	+6
September 17, 2007	September 19, 2007	15	15.5 ± 0.1	+3
		15	15.5 ± 0.2	+3
		30	31.3 ± 0.1	+4
		30	30.9 ± 0.2	+3
		60	63.5 ± 0.2	+6
		60	63.4 ± 0.4	+6
	October 25, 2007[b]	15	15.4 ± 0.1	+3
		15	15.6 ± 0.1	+4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
		30	29.9 ± 0.3	0
		30	31.2 ± 0.4	+4
		60	63.5 ± 0.1	+6
		60	62.2 ± 0.7	+4
November 12, 2007	November 14, 2007	15	15.4[c]	+3
		15	15.6 ± 0.2	+4
		30	30.9 ± 0.2	+3
		30	30.9 ± 0.2	+3
		60	62.9 ± 0.5	+5
		60	62.3 ± 0.4	+4
January 8, 2008	January 9, 2008	15	15.4 ± 0.1	+3
		15	15.5 ± 0.0	+3
		30	31.1 ± 0.1	+4
		30	31.1 ± 0.2	+4
		60	62.9 ± 0.1	+5
		60	63.2 ± 0.4	+5
March 31, 2008	April 2, 2008	15	15.5 ± 0.1	+3
		15	15.5 ± 0.3	+3
		30	31.7 ± 0.4	+6
		30	31.2 ± 0.2	+4
		60	63.0 ± 0.2	+5
		60	63.2 ± 0.5	+5
	May 13, 2008[b]	15	14.5 ± 0.6	−3
		15	15.2 ± 0.1	+1
		30	30.1 ± 0.4	0
		30	30.8 ± 0.6	+3
		60	62.8 ± 1.3	+5
		60	62.1 ± 0.3	+4
June 23, 2008	June 24, 2008	15	15.3 ± 0.1	+2
		15	15.6 ± 0.0	+4
		30	31.1 ± 0.2	+4
		30	30.7 ± 0.2	+2
		60	60.5 ± 0.1	+1
		60	62.1 ± 0.0	+4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
August 15, 2008	August 20, 2008	15	15.2 ± 0.1	+1
		15	15.5 ± 0.1	+3
		30	30.8 ± 0.3	+3
		30	30.7 ± 0.0	+2
		60	62.6 ± 0.3	+4
		60	61.3 ± 0.6	+2
October 13, 2008	October 14, 2008	15	15.3 ± 0.1	+2
		15	15.7 ± 0.1	+5
		30	31.2 ± 0.3	+4
		30	31.1 ± 0.1	+4
		60	61.4 ± 0.1	+2
		60	63.0 ± 0.3	+5
	November 26, 2008[b]	15	15.2 ± 0.1	+1
		15	15.7 ± 0.2	+5
		30	31.8 ± 0.7	+6
		30	31.0 ± 0.3	+3
		60	63.7 ± 1.2	+6
		60	62.4 ± 0.7	+4
December 8, 2008	December 9, 2008	15	15.1 ± 0.6	+1
		15	15.1 ± 0.5	+1
		30	30.1 ± 2.1	0
		30	30.6 ± 0.6	+2
		60	60.7 ± 1.5	+1
		60	61.6 ± 1.3	+3
February 2, 2009	February 5, 2009	15	15.2 ± 0.1	+1
		15	15.4 ± 0.1	+3
		30	30.9 ± 0.2	+3
		30	30.6 ± 0.3	+2
		60	62.5 ± 0.4	+4
		60	61.6 ± 0.3	+3

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Mice				
March 5, 2007	March 8, 2007	6.25	6.24 ± 0.51	0
		12.5	12.7 ± 0.1	+2
		25	25.1 ± 0.2	0
	April 12, 2007[b]	6.25	6.64 ± 0.09	+6
		12.5	13.0 ± 0.0	+4
		25	25.7 ± 2.9	+3
May 2, 2007	May 2, 2007	6.25	6.50 ± 0.06	+4
		12.5	12.9 ± 0.1	+3
		25	25.7 ± 0.3	+3
July 23, 2007	July 24, 2007	6.25	6.27 ± 0.01	0
		12.5	13.0 ± 0.1	+4
		25	26.2 ± 0.2	+5
September 17, 2007	September 19, 2007	6.25	6.40 ± 0.02	+2
		12.5	12.7 ± 0.1	+2
		25	25.8 ± 0.1	+3
	October 25, 2007[b]	6.25	6.43 ± 0.01	+3
		12.5	12.3 ± 0.1	−2
		25	25.2 ± 1.3	+1
November 12, 2007	November 14, 2007	6.25	6.30 ± 0.03	+1
		12.5	12.6 ± 0.0	+1
		25	25.8 ± 0.2	+3
January 8, 2008	January 9, 2008	6.25	6.46 ± 0.01	+3
		12.5	12.6 ± 0.2	+1
		25	25.7 ± 0.1	+3
March 31, 2008	April 2, 2008	6.25	6.36 ± 0.10	+2
		12.5	12.6 ± 0.1	+1
		25	26.1 ± 0.2	+4
	May 13, 2008[b]	6.25	6.55 ± 0.04	+5
		12.5	13.0 ± 0.1	+4
		25	25.7 ± 0.0	+3
June 23, 2008	June 24, 2008	6.25	6.50 ± 0.03	+4
		12.5	12.8 ± 0.0	+2
		25	26.1 ± 0.1	+4

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
August 15, 2008	August 20, 2008	6.25	6.57 ± 0.01	+5
		12.5	12.7 ± 0.0	+2
		25	26.2 ± 0.1	+5
October 13, 2008	October 14, 2008	6.25	6.61 ± 0.05	+6
		12.5	13.1 ± 0.1	+5
		25	26.2 ± 0.2	+5
	November 26, 2008[b]	6.25	6.45 ± 0.07	+3
		12.5	12.5 ± 0.2	0
		25	26.3 ± 0.2	+5
December 8, 2008	December 9, 2008	6.25	6.20 ± 0.15	−1
		12.5	12.1 ± 0.2	−3
		25	26.1 ± 0.5	+4
February 2, 2009	February 5, 2009	6.25	6.27 ± 0.03	0
		12.5	12.9 ± 0.1	+3
		25	25.9 ± 0.2	+4

[a]Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 15 mg/mL = 75 mg/kg, 30 mg/mL = 150 mg/kg, 60 mg/mL = 300 mg/kg. For mice, dosing volume = 10 mL/kg; 6.25 mg/mL = 62.5 mg/kg, 12.5 mg/mL = 125 mg/kg, 25 mg/mL = 250 mg/kg.
[b]Animal room samples.
[c]One value only.

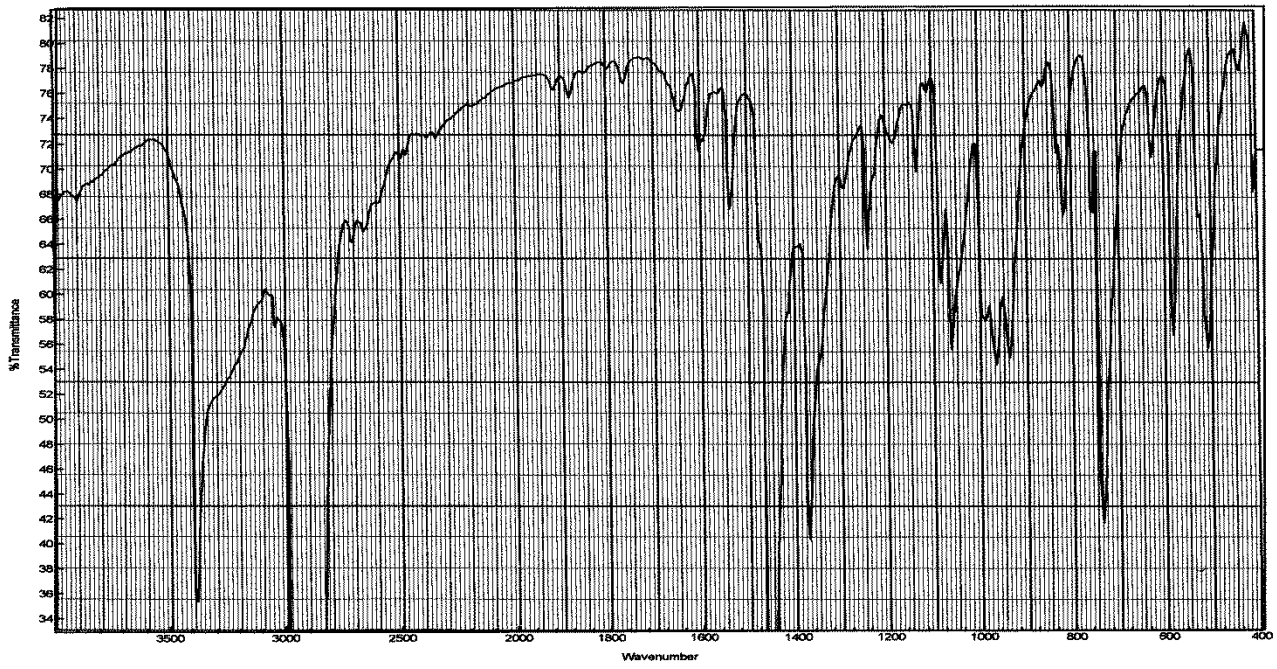

Figure I-1. Infrared Absorption Spectrum of Indole-3-carbinol

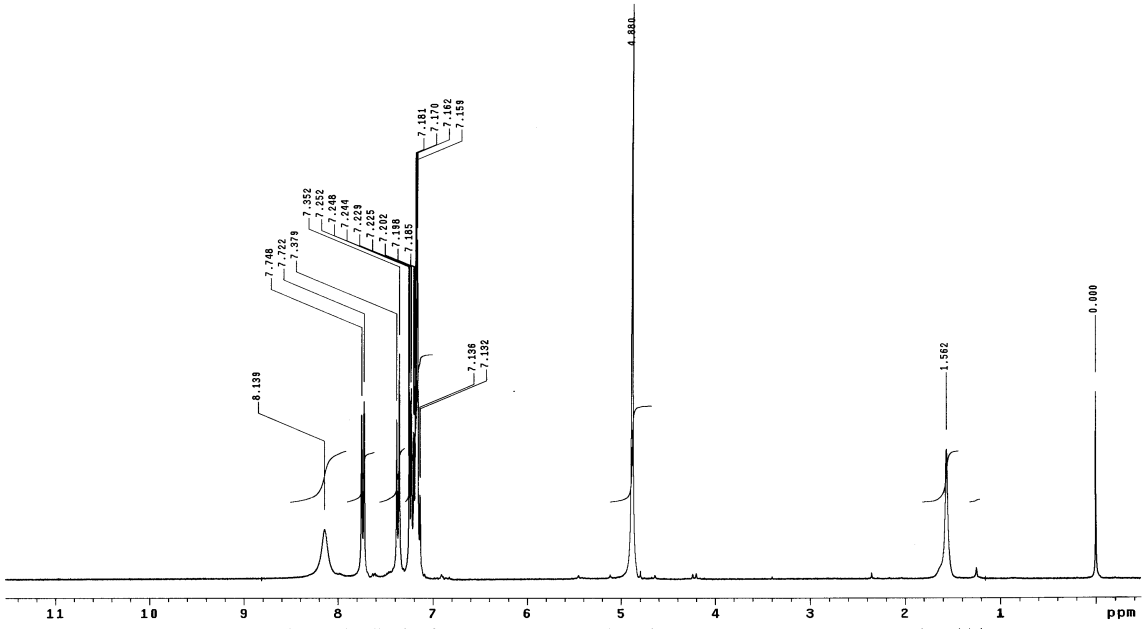

Figure I-2. Proton Nuclear Magnetic Resonance Spectrum of Indole-3-carbinol

Appendix J. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table J-1. Ingredients of NTP-2000 Rat and Mouse Ration J-2
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Table J-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table J-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	–
Niacin	23 mg	–
Folic acid	1.1 mg	–
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table J-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.71	13.7–15.9	24
Crude Fat (% by weight)	8.2 ± 0.28	7.7–8.8	24
Crude Fiber (% by weight)	9.1 ± 0.54	8.1–10.3	24
Ash (% by weight)	5.1 ± 0.25	4.4–5.4	24
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.670–0.970	23
Cystine	0.220 ± 0.024	0.150–0.250	23
Glycine	0.700 ± 0.040	0.620–0.800	23
Histidine	0.351 ± 0.076	0.270–0.680	23
Isoleucine	0.546 ± 0.043	0.430–0.660	23
Leucine	1.095 ± 0.066	0.960–1.240	23
Lysine	0.705 ± 0.116	0.310–0.860	23
Methionine	0.409 ± 0.045	0.260–0.490	23
Phenylalanine	0.628 ± 0.039	0.540–0.720	23
Threonine	0.506 ± 0.042	0.430–0.610	23
Tryptophan	0.150 ± 0.028	0.110–0.200	23
Tyrosine	0.405 ± 0.063	0.280–0.540	23
Valine	0.664 ± 0.043	0.550–0.730	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.254	3.49–4.55	23
Linolenic	0.30 ± 0.031	0.21–0.35	23
Vitamins			
Vitamin A (IU/kg)	3,601 ± 77	2,350–5,720	24
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.3 ± 21.56	27.0–124.0	23
Thiamine (ppm)[b]	6.9 ± 1.13	5.1–9.0	24
Riboflavin (ppm)	7.7 ± 2.87	4.20–17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4–98.2	23
Pantothenic acid (ppm)	27.0 ± 12.35	17.4–81.0	23
Pyridoxine (ppm)[b]	9.54 ± 1.94	6.44–13.7	23
Folic acid (ppm)	1.61 ± 0.47	1.15–3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	23
Vitamin B_{12} (ppb)	53.4 ± 38	18.3–174.0	23
Choline (ppm)[b]	2,773 ± 590	1,160–3,790	23

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.920 ± 0.048	0.808–1.020	24
Phosphorus (%)	0.555 ± 0.066	0.471–0.822	24
Potassium (%)	0.667 ± 0.030	0.626–0.733	23
Chloride (%)	0.385 ± 0.038	0.300–0.474	23
Sodium (%)	0.189 ± 0.016	0.160–0.222	23
Magnesium (%)	0.216 ± 0.061	0.185–0.490	23
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	187 ± 38.6	135–311	23
Manganese (ppm)	51.0 ± 10.19	21.0–73.1	23
Zinc (ppm)	53.6 ± 8.34	43.3–78.5	23
Copper (ppm)	7.10 ± 2.540	3.21–16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158–0.972	23
Chromium (ppm)	0.696 ± 0.269	0.330–1.380	23
Cobalt (ppm)	0.248 ± 0.163	0.094–0.864	21

[a]From formulation.
[b]As hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table J-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.24 ± 0.050	0.16–0.40	24
Cadmium (ppm)	0.06 ± 0.010	0.05–0.10	24
Lead (ppm)	0.10 ± 0.020	0.07–0.16	24
Mercury (ppm)	<0.02	–	24
Selenium (ppm)	0.30 ± 0.255	0.14–1.02	24
Aflatoxins (ppb)	<5.00	–	24
Nitrate nitrogen (ppm)[c]	17.8 ± 8.15	10.0–42.3	24
Nitrite nitrogen (ppm)[c]	<0.61	–	24
BHA (ppm)[d]	<1.0	–	24
BHT (ppm)[d]	<1.0	–	24
Aerobic plate count (CFU/g)	10 ± 0.0	10.0–10.0	24
Coliform (MPN/g)	3.0 ± 0.0	3.0–3.0	24
Escherichia coli (MPN/g)	<10	–	24
Salmonella (MPN/g)	Negative	–	24
Total nitrosoamines (ppb)[e]	8.8 ± 6.32	2.0–28.0	24
N-Nitrosodimethylamine (ppb)[e]	2.0 ± 2.27	0.9–10.3	24
N-Nitrosopyrrolidine (ppb)[e]	6.8 ± 5.03	1.0–17.7	24
Pesticides (ppm)			
α-BHC	<0.01	–	23
β-BHC	<0.02	–	23
γ-BHC	<0.01	–	23
δ-BHC	<0.01	–	23
Heptachlor	<0.01	–	23
Aldrin	<0.01	–	23
Heptachlor epoxide	<0.01	–	23
DDE	<0.01	–	23
DDD	<0.01	–	23
DDT	<0.01	–	23
HCB	<0.01	–	23
Mirex	<0.01	–	23
Methoxychlor	<0.05	–	23
Dieldrin	<0.01	–	23
Endrin	<0.01	–	23

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	23
Chlordane	<0.05	–	23
Toxaphene	<0.10	–	23
Estimated PCBs	<0.20	–	23
Ronnel	<0.01	–	23
Ethion	<0.02	–	23
Trithion	<0.05	–	23
Diazinon	<0.10	–	23
Methyl chlorpyrifos	0.059 ± 0.053	0.020–0.180	24
Methyl parathion	<0.02	–	23
Ethyl parathion	<0.02	–	23
Malathion	0.055 ± 0.044	0.020–0.194	24
Endosulfan I	<0.01	–	23
Endosulfan II	<0.01	–	23
Endosulfan sulfate	<0.03	–	23

[a]All samples were irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.
[b]For values less than the limit of detection, the detection limit is given as the mean.
[c]Sources of contamination: alfalfa, grains, and fish meal.
[d]Sources of contamination: soy oil and fish meal.
[e]All values were corrected for percent recovery.

Appendix K. Sentinel Animal Program

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Table K-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program K-2

K.1. Methods

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

Blood samples were collected from each animal and allowed to clot and the serum was separated. Additionally, fecal samples were collected and tested for *Helicobacter* species. All samples were processed appropriately and evaluated for the presence of pathogens. Samples were sent to BioReliance Corporation (Rockville, MD) or the Research Animal Diagnostic Laboratory (RADIL) at the University of Missouri (Columbia, MO). The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Table K-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
Two-year Study	
ELISA	
PVM	End of quarantine, 4 weeks, 6 months
RCV/SDA	End of quarantine, 4 weeks, 6 months
Sendai	End of quarantine, 4 weeks, 6 months
Immunofluorescence Assay	
Parvovirus	End of quarantine, 4 weeks, 6 months
RCV/SDA	4 weeks, 6 months
Multiplex Fluorescent Immunoassay	
H-1 (Toolan's H-1 virus)	12 and 18 months, study termination
KRV (Kilham rat Virus)	12 and 18 months, study termination
Mycoplasma pulmonis	12 and 18 months, study termination

Method and Test	Time of Collection
Parvo NS-1	12 and 18 months, study termination
PVM	12 and 18 months, study termination
RCV/SDA	12 and 18 months, study termination
RMV (rat minute virus)	12 and 18 months, study termination
RPV (rat parvovirus)	12 and 18 months, study termination
RTV (rat theilovirus)	12 and 18 months, study termination
Sendai	12 and 18 months, study termination
TMEV GDVII (Theiler's murine encephalomyelitis virus—mouse poliovirus, strain GDVII)	12 and 18 months, study termination
Mice	
Three-month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adeno virus-1 (Mad-1)	Study termination
MHV (mouse hepatitis virus)	Study termination
MMV VP2 (mouse minute virus)	Study termination
MPV VP2 (mouse parvovirus)	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination
TMEV GDVII	Study termination
Immunofluorescence Assay	
LCM	Study termination
Two-year Study	
ELISA	
Ectromelia virus	End of quarantine, 4 weeks, 6 months
EDIM	End of quarantine, 4 weeks, 6 months
LCM	End of quarantine, 4 weeks, 6 months
Mad-1	End of quarantine, 4 weeks, 6 months
MHV	End of quarantine, 4 weeks, 6 months
MMV VP2	End of quarantine, 4 weeks, 6 months
MPV VP2	End of quarantine, 4 weeks, 6 months
PVM	End of quarantine, 4 weeks, 6 months
Reovirus	End of quarantine, 4 weeks, 6 months

Method and Test	Time of Collection
Sendai	End of quarantine, 4 weeks, 6 months
TMEV GDVII	End of quarantine, 4 weeks, 6 months
Immunofluorescence Assay	
MHV	4 weeks
MPV	End of quarantine
LCM	4 weeks
Multiplex Fluorescent Immunoassay	
Ectromelia virus	12 and 18 months, study termination
EDIM	12 and 18 months, study termination
LCM	12 and 18 months, study termination
MHV	12 and 18 months, study termination
MMV	12 and 18 months, study termination
MNV (mouse norovirus)	12 and 18 months, study termination
MPV	12 and 18 months, study termination
M. pulmonis	12 and 18 months, study termination
Parvo NS-1	12 and 18 months, study termination
PVM	12 and 18 months, study termination
Reovirus 3	12 and 18 months, study termination
Sendai	12 and 18 months, study termination
TMEV GDVII	12 and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

K.2. Results

All test results were negative.

Appendix L. Microarray Analysis

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L.1. Objective

The objective of the microarray study was to evaluate the transcriptional changes in liver from rats exposed to 0 or 300 mg/kg indole-3-carbinol. At 3 months, livers were analyzed from female Harlan Sprague Dawley rats in the 2-year gavage study of indole-3-carbinol.

L.2. Methods

L.2.1. RNA Isolation, cDNA Synthesis, and Array Hybridization

Liver tissues were excised from five female rats gavaged with corn oil (vehicle control group) and five female rats gavaged with 300 mg/kg of indole-3-carbinol in corn oil (treated group) Monday through Friday for 3 months and on the termination day approximately 3 hours prior to tissue harvest.

Tissues were immediately frozen in liquid nitrogen at collection and transported to the Battelle Biomedical Research Center (Columbus, OH). The liver tissues were removed and added to lysis buffer. Each sample was then homogenized for 45 seconds using OmniTip™ plastic disposable probes (Omni International, Marietta, GA). Following homogenization, samples were centrifuged and the RNA was extracted from the supernatant using the Qiagen RNeasy Midi Kit (Qiagen, Valencia, CA). RNA concentration and purity were determined by ultraviolet analysis using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples were evaluated by gel electrophoresis using the FlashGel® RNA cassette system (Lonza, Rockland, ME).

Total RNA (5 μg) was used to synthesize double-stranded cDNA for each sample using Affymetrix GeneChip® 3'-Amplification One-Cycle cDNA Synthesis Reagents (Affymetrix, Inc., Santa Clara, CA) according to the manufacturer's protocol. All incubation steps were performed using a GeneAmp® 2400 thermal cycler (PerkinElmer, Waltham, MA). The cDNA served as a template to synthesize biotin-labeled antisense cRNA using an IVT Labeling Kit (Affymetrix, Inc.). Labeled cRNA was fragmented and hybridized to the Affymetrix Rat Genome 230 2.0 Array as described in the Affymetrix GeneChip® protocol. Chip hybridization, washing, and staining were performed according to the Affymetrix-recommended protocol EukGE-Ws2v5. After washing, the chips were scanned using an Affymetrix GeneChip Scanner 3000 and the acquired data was then further processed by the Affymetrix GeneChip® Operating Software (GCOS) version 1.2.

L.2.2. Microarray Analysis

The 10 .CEL files representing the 10 liver samples from the study were normalized using the robust multiarray algorithm (RMA)[139] using GeneSpring v12.6 (Agilent Technologies, Santa Clara, CA). Normalized expression values were baseline-transformed to the median of all samples. A Principal Component Analysis demonstrated clear separation of the samples by treatment group. All chips had a Gapdh 3′/5′ hybridization rate ranging from 1.1 to 1.6. Probesets were filtered to 26,253 (from 31,099) based on signal intensity values, specifically a probeset was retained if its expression values were between 20.0 and 100 percent in 1 out of 10 samples. Identification of probe sets that exhibited a statistically significant difference in intensity was determined by t-test (unpaired) with a P value of < 0.05 and the Benjamini-Hochburg post-hoc

test. Probesets that were significantly altered were further filtered using a minimum 1.5-fold difference.

L.2.3. Ingenuity Pathway Analysis™

The 343 probesets exhibiting significant ($P < 0.05$) differential expression and > 1.5-fold change (up- or down-regulation) were analyzed using the Ingenuity Pathway Analysis™ (IPA) (Ingenuity Systems®, Inc., Redwood City, CA; <www.ingenuity.com>). The probeset list when translated into genes in IPA corresponded to a total of 243 altered genes (referred to as differentially expressed genes (DEGs). The IPA Core Analysis was performed on February 12, 2014. Enrichment and activation analysis was carried out for IPA Disease or Biological Functions, Toxicity Functions, and Upstream Analysis.

Enrichment P values were calculated using a right-tailed Fisher's Exact Test combined with a Benjamini-Hochberg method of multiple testing correction. The P value ($P < 0.05$) was determined by how many DEGs overlapped genes annotated into gene groups (e.g., canonical pathways, biological processes). Activation/inhibition analysis was performed using the annotation in the Ingenuity® Knowledge Base. In order to determine if there was plausible activation/inhibition of Disease or Biological Functions, Toxicity Functions, Transcription Factors, and Chemical Signaling Signatures, a Z-score was calculated. The Z-score was determined by concordance of observed patterns of regulation (up or down) with known effects on biological functions or effects of transcription factors/chemicals (activation or inhibition of target genes) as annotated in the Ingenuity® Knowledge Base. Based on the Z-score, a potential transcription factor was deemed to be activated (Z-score > 2), inhibited (Z-score < −2), or not affected.

L.3. Results

L.3.1. Differential Gene Expression

Whole-rat genome Affymetrix 230 2.0 microarrays were used to assess the effect of 300 mg/kg per day of indole-3-carbinol for 3 months on female Sprague Dawley rat liver. Significant differential expression between treated and vehicle control animals occurred in 973 probesets (mapped to 751 Entrez Gene IDs). Of 973 altered probesets, 553 were up-regulated and 420 were down-regulated. When the 973 probesets were subjected to a > 1.5 cutoff, then 343 probesets (corresponding to 243 genes) were altered. The genes represented by the 343 probesets were used for analysis in IPA. The top five up- and down-regulated genes are shown in Table L-1.

The IPA Disease or Biological Functions and liver Toxicity Functions that were activated by indole-3-carbinol treatment primarily relate to phase I and phase 2 xenobiotic/endogenous chemical metabolism and redox pathways. The enriched/activated functions are listed in Table L-2 and Table L-3.

Consistent with the Disease or Biological Functions enrichment results, indole-3-carbinol treatment caused notable differential expression of genes involved in phase I and phase II xenobiotic/endogenous chemical metabolism along with the signaling pathways that mediate the changes in expression of these genes (e.g., Nrf2-mediated Oxidative Stress Response, Aryl Hydrocarbon Receptor Signaling, and Pxr/Rxr Activation). The enriched pathways are listed in Table L-4.

Transcription factor enrichment/activation in IPA suggests a strong activation of Nrf2 (Nfe2l2) signaling along with notable activation of a variety of hepatic chemical response transcription factors Car (Nrli3), Pxr (Nrli2) and AhR/Arnt[140]. Results of the transcription factor upstream analysis are shown in Table L-5.

Chemical response pattern activation analysis in IPA is consistent with other results above. Chemicals known to activate Nrf2 (e.g., 1,2-dithiol-3-thione, *tert*-butyl-hydroquinone, oltipraz, and butylated hydroxyanisole)[141-144], AhR/Arnt (e.g., tetrachlorodibenzodioxin, benzo(a)pyrene, 2,3,4,7,8-pentachlorodibenzofuran, and 3-methylcholanthrene)[128] and Car/Pxr signaling (e.g., phenobarbital)[145] exhibit similar patterns of gene expression compared to indole-3-carbinol. Results of the chemical response pattern activation analysis are shown in Table L-6.

L.4. Conclusions

The results strongly suggest that treatment with indole-3-carbinol strongly activates AhR and Nrf2 (Nfe2l2) signaling, but also appears to activate Car (Nrli3)/Pxr (Nrli2) signaling. These finding are most clearly demonstrated in the transcription factor enrichment analysis where Nfe2l2 has the strongest activation signal followed by Car/Pxr and then AhR/Arnt. Nfe2l2 coordinates the cellular defense against the cytotoxic effects of oxidative stress[146]. The finding that Nfe2l2 is activated is supported by the activation of glutathione conjugation and enrichment of the oxidative stress signaling and glutathione-mediated detoxification. Additionally, the gene expression pattern elicited by indole-3-carbinol treatment parallels strongly the gene expression patterns of 1,2-dithiol-3-thione, a soft electrophile known to activate Nfe2l2 signaling through effects on the redox status of cellular sulfhydryl groups. Activation of AhR/Arnt along with Car/Pxr is supported by the activation/enrichment of a number of biological functions/pathways associated with xenobiotic and endogenous chemicals and enrichment, a result consistent with hepatocyte hypertrophy/liver enlargement. Patterns of gene expression produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), benzo(a)pyrene, and 3-methylcholanthrene (prototype AhR activators) that have been curated in IPA strongly parallel patterns of gene expression produced by indole-3-carbinol. The patterns of AhR gene expression are supported by the high level of induction of Cyp1a1 and Cyp1b1. The finding that AhR signaling is activated by indole-3-carbinol treatment is consistent with previous findings that acid condensates of indole-3-carbinol formed in the gut are high affinity ligands for the AhR[23]. From the standpoint of chemical signatures, Car/Pxr activation by indole-3-carbinol is supported by the positive correlation patterns of gene expression from phenobarbital curated in IPA and the strong induction of Cyp2b1/2b2 (a documented target of Car/Pxr) by indole-3-carbinol.

Other notable findings:

1. Down-regulation of Serpina7 (gene that encodes the major thyroid hormone transport protein, TBG, in serum) may have effects on systemic thyroid hormone signaling[147].
2. Activation of Atf4 is likely related to endoplasmic reticulum stress that may be due to increased protein synthesis associated with xenobiotic metabolizing enzyme induction in combination with soft electrophile stress produced by indole-3-carbinol that can cause proteins to unfold[148].

3. Activation of Tp63 along with enrichment of p53 signaling and Gadd45 signaling is potentially a secondary effect of the soft electrophile-induced oxidative stress and subsequent change in redox status[149] that can be elicited by indole-3-carbinol[150].

Table L-1. Top Five Up- and Down-regulated Genes in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Probeset ID	Gene Symbol	Entrez Gene	Fold Change Indole-3-carbinol Treated versus Vehicle Control
Up-regulated			
1370269_at	Cyp1a1	24296	334.6
1368990_at	Cyp1b1	25426	75.1
1371076_at	Cyp2b1///Cyp2b2///LOC100362704	100362704///24300///361523	32.4
1374056_at	Rbm24	690139	29.6
1373188_at	Scn4b	315611	17.1
Down-regulated			
1369657_at	Cpa1	24269	−4.3
1370387_at	Cyp3a9	171352	−4.4
1371143_at	Serpina7	81806	−4.9
1387053_at	Fmo1	25256	−5.5
1367896_at	Car3	54232	−16.0

Table L-2. Enriched Ingenuity Pathway Analysis™ Disease or Biological Functions that Exhibit Activation/inhibition in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Disease or Function Annotation	P Value	Predicted Activation State	Activation Z-score	Number of Molecules
Metabolism of terpenoid	1.03E−08	Increased	2.726	19
Conjugation of glutathione	1.08E−09	Increased	2.605	7
Steroidogenesis of hormone	3.00E−03	Increased	2.425	6
Metabolism of hormone	2.77E−03	Increased	2.404	8
Steroid metabolism	3.98E−09	Increased	2.372	18
Joining of glutathione	2.99E−11	Increased	2.320	8
Metabolism of retinoid	4.32E−05	Increased	2.204	6
Hydroxylation of hormone	1.42E−07	Increased	2.189	5
Binding of DNA	1.27E−06	Increased	2.188	23
Oxidation of lipid	2.14E−04	Increased	2.063	10
Synthesis of fatty acid	2.04E−03	Decreased	−2.138	11
Quantity of vitamin	3.14E−06	Decreased	−2.183	8
Quantity of GPT in blood	4.54E−04	Decreased	−2.200	5
Tumorigenesis of epithelial tumor	2.93E−04	Decreased	−2.348	11
Organismal death	6.47E−04	Decreased	−2.371	54
Quantity of enzyme	1.41E−03	Decreased	−2.420	6
Inflammation of organ	4.83E−03	Decreased	−2.679	28
Hyperplasia	8.43E−03	Decreased	−2.928	15

Table L-3. Enriched Ingenuity Pathway Analysis™ Toxicity Functions that Exhibit Activation/inhibition in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Disease or Function Annotation	P Value	Predicted Activation State	Activation Z-score	Number of Molecules
Conjugation of glutathione	1.08E-09	Increased	2.605	7

Table L-4. Ingenuity Pathway Analysis™ Canonical Pathways Exhibiting Significant (P < 0.05) Enrichment in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Ingenuity Canonical Pathways	−log(P Value)
Xenobiotic metabolism signaling	16.1
Nrf2-mediated oxidative stress response	12.6
Aryl hydrocarbon receptor signaling	12.3
Glutathione-mediated detoxification	10.8
LPS/IL-1 mediated inhibition of RXR function	10
Nicotine degradation II	8.75
Nicotine degradation III	8.15
Melatonin degradation I	7.93
Superpathway of melatonin degradation	7.58
Pxr/Rxr activation	7.15
Bupropion degradation	6.39
Acetone degradation I (to methylglyoxal)	6.28
Estrogen biosynthesis	5.36
Asparagine degradation I	3.88
p53 signaling	3.86
Glutathione redox reactions II	3.41
Serine biosynthesis	3.11
Gadd45 signaling	2.83
Cell cycle: G2/M DNA damage checkpoint regulation	2.79
Superpathway of serine and glycine biosynthesis I	2.72
Atm signaling	2.3
Serotonin degradation	2.27
Hepatic cholestasis	2.26
Thyroid hormone metabolism II (via conjugation and/or degradation)	2.24
Antigen presentation pathway	2.06
Vitamin-C transport	1.96
Asparagine biosynthesis I	1.94
4-Hydroxybenzoate biosynthesis	1.94
4-Hydroxyphenylpyruvate biosynthesis	1.94
Methylglyoxal degradation III	1.9
Prostate cancer signaling	1.81
Glutathione redox reactions I	1.8
Fxr/Rxr activation	1.76
Bladder cancer signaling	1.71

Ingenuity Canonical Pathways	−log(P Value)
Glutamine biosynthesis I	1.64
Glutamine degradation I	1.64
Ppar signaling	1.63
Antioxidant action of vitamin C	1.59
Protein ubiquitination pathway	1.55
Phospholipases	1.54
Coenzyme A biosynthesis	1.47
Ascorbate recycling (cytosolic)	1.47
Hypoxia signaling in the cardiovascular system	1.39
Hereditary breast cancer signaling	1.35
1,25-Dihydroxyvitamin D3 biosynthesis	1.34

Table L-5. Enriched Ingenuity Pathway Analysis™ Transcription Factors that Exhibit Activation/inhibition in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Upstream Regulator	Fold Change[a]	Molecule Type	Predicted Activation State	Activation Z-score	P Value of Overlap
Nfe212	2.075	Transcription regulator	Activated	4.399	2.31E−23
Nrli3	2.492	Ligand-dependent nuclear receptor	Activated	4.155	2.60E−20
Atf4	1.684	Transcription regulator	Activated	3.085	3.20E−07
Tp63	–	Transcription regulator	Activated	2.743	2.46E−04
Nr1i2	–	Ligand-dependent nuclear receptor	Activated	2.366	5.49E−16
Brca1	–	Transcription regulator	Activated	2.359	1.35E−07
Nupr1	–	Transcription regulator	Activated	2.309	5.10E−03
Xbp1	–	Transcription regulator	Activated	2.207	1.70E−02
Arnt	–	Transcription regulator	Activated	2.165	8.36E−10
AhR	2.026	Ligand-dependent nuclear receptor	Activated	2.062	1.43E−11
Trim24	–	Transcription regulator	Activated	2.000	1.75E−02

[a]Indicates whether the gene encoding the transcription factor was differentially expressed by indole-3-carbinol. Blank cells indicate that the transcription factor was not differentially expressed.

Table L-6. Enriched Ingenuity Pathway Analysis™ Chemical Signaling Patterns that Exhibit Activation/inhibition in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for Three Months

Upstream Regulator	Molecule Type	Predicted Activation State	Activation Z-score	P Value of Overlap
1,2-Dithiol-3-thione	Chemical reagent	Activated	4.182	1.12E−19
Tetrachlorodibenzodioxin	Chemical toxicant	Activated	3.728	3.66E−14
Benzo(a)pyrene	Chemical toxicant	Activated	3.722	5.51E−16
Phenobarbital	Chemical drug	Activated	3.640	7.04E−18
3,4,5,3′,4′-Pentachlorobiphenyl	Chemical toxicant	Activated	3.356	3.86E−15
2,3,4,7,8-Pentachlorodibenzofuran	Chemical toxicant	Activated	3.080	4.15E−15
1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene	Chemical toxicant	Activated	3.064	2.41E−12
Pirinixic acid	Chemical toxicant	Activated	3.056	5.26E−13
3-Methylcholanthrene	Chemical toxicant	Activated	3.009	9.62E−16
Tert-butyl-hydroquinone	Chemical reagent	Activated	2.908	3.16E−10
Tunicamycin	Chemical—endogenous non-mammalian	Activated	2.782	1.35E−07
Thapsigargin	Chemical toxicant	Activated	2.781	4.06E−06
Ethoxyquin	Chemical toxicant	Activated	2.779	2.34E−12
Beta-naphthoflavone	Chemical toxicant	Activated	2.749	8.28E−14
Troglitazone	Chemical drug	Activated	2.734	5.67E−04
Bardoxolone	Chemical drug	Activated	2.621	1.36E−08
Tosedostat	Chemical drug	Activated	2.619	1.76E−08
Pxr ligand-Pxr-Retinoic Acid-Rxr α	Complex	Activated	2.593	1.76E−07
Oltipraz	Chemical drug	Activated	2.576	4.33E−09
Arsenic trioxide	Chemical drug	Activated	2.504	9.92E−07
Diallyl disulfide	Chemical—endogenous non-mammalian	Activated	2.425	3.15E−09
8-Bromo-camp	Chemical reagent	Activated	2.407	7.91E−03
Allyl sulfide	Chemical drug	Activated	2.388	7.79E−09
Cholic acid	Chemical—endogenous mammalian	Activated	2.359	1.28E−04
Bilirubin	Chemical—endogenous mammalian	Activated	2.236	8.16E−07
Sulforafan	Chemical drug	Activated	2.212	2.12E−14
Pregnenolone carbonitrile	Chemical drug	Activated	2.193	2.23E−06
Polycyclic aromatic hydrocarbons	Chemical toxicant	Activated	2.188	7.93E−08
Benz[a]anthracene	Chemical toxicant	Activated	2.156	6.31E−10
Cigarette smoke	Chemical toxicant	Activated	2.154	7.73E−13
15-Deoxy-*delta*-12,14 -PGJ 2	Chemical—endogenous non-mammalian	Activated	2.136	5.22E−04
Butylated hydroxyanisole	Chemical toxicant	Activated	2.050	1.96E−10
Cephaloridine	Chemical drug	Activated	2.025	1.04E−07
Capsaicin	Chemical drug	Activated	2.000	1.95E−03

Appendix M. Summary of Peer Review Panel Comments

On May 22, 2014, the draft Technical Report on the toxicology and carcinogenesis studies of indole-3-carbinol received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.E. Wyde, NIEHS, introduced the toxicology and carcinogenesis studies of indole-3-carbinol, a commercially available dietary supplement and natural product by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats and mice. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* in male Sprague Dawley rats, *some evidence of carcinogenic activity* in female Sprague Dawley rats, *clear evidence of carcinogenic activity* in male B6C3F1/N mice, and *no evidence of carcinogenic activity* in female B6C3F1/N mice.

Dr. Carpenter noted that there were no written or oral public comments concerning this report.

Dr. Perdew, the first primary reviewer, noted the study was well done. He questioned if high concentrations of indole-3-carbinol were insoluble in corn oil, and if the insolubility might affect the results. He asked whether any inflammatory markers were examined in tissues or serum. Dr. Wyde said all doses used in the studies were suspensions, and no inflammatory markers were looked at in tissue or serum. Dr. Perdew asked if it was standard practice to not look at inflammatory markers, and Dr. Wyde replied it was. Dr. Perdew questioned a statement in the report regarding comparative affinity for the aryl hydrocarbon receptor (AhR). Dr. Wyde said he could adjust the language accordingly. Dr. Perdew recommended more careful wording for the discussion concerning the mechanism of toxicity for indole-3-carbinol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Dr. Wyde said National Toxicology Program staff had discussed how much to write about the AhR and dioxins, versus just the mechanism associated with indole-3-carbinol.

Dr. Regan, the second primary reviewer, said the standard and extended uterine examinations should be presented together, as that is the basis of the carcinogenicity conclusion. She suggested less emphasis on the findings from the standard and extended examinations alone in the report, focusing instead on positive trends and statistical significance seen when the two are combined. She noted there was no observable increase in atypical endometrial hyperplasia in the uterine neoplasms. She suggested clarifying the estrous cycle data in cycling F344/N rats. Regarding nose inflammation, she said the animals with foreign material present in the nasal cavity should be shown separately in the incidence table, as inflammation could be attributed to the foreign material. She asked if assays for cytochrome P450 (CYP) induction were conducted on the nose.

Dr. Wyde, responding to Dr. Regan's comments, said the carcinogenicity call in female rats was based on the combined data, both the standard and extended examinations of the uterus. Dr. Regan recommended limiting discussion in the text to the combined incidences. Dr. J.R. Bucher, NIEHS, noted this was presented based on a historical precedent, with substantial historical control information available. Because there was no substantial historical database in this study, he understood Dr. Regan's suggestion. Dr. Wyde said he could clarify the estrous cycle characterization data for female F344/N rats. He stated that NTP staff had not conducted assays

for CYP induction in the nose. Dr. R.A. Herbert, the NIEHS study pathologist, said foreign bodies are often diagnosed in the nose and often associated with inflammation. NTP diagnoses the foreign body, not the inflammatory change, because the inflammatory change is considered secondary to the foreign body. He described how this issue was approached in the indole-3-carbinol studies. He said in female mice there was only one diagnosis of foreign body in the mid dose group with no associated evidence of inflammation and two diagnoses in the high dose group, both of which were in animals that had a treatment-related diagnosis of inflammation. Dr. Regan suggested adding more information in the report reflective of Dr. Herbert's statement.

Dr. Mirsalis, the third primary reviewer, said lower doses may not impact the overall conclusions, but it would be good to see if there was a threshold by using lower doses. He said the increases in liver weights were equally striking in rats and mice and recommended bringing the table for mice into the main body of the text from the appendix. The report has an extended discussion about how indole-3-carbinol is an AhR agonist, and compares the responses to those seen with dioxin. The increases in mouse liver tumors could be explained by induction of CYPs resulting in increased liver size, which has been shown to be a significant risk factor for mouse liver tumors; therefore, he recommended removing the dioxin discussion.

Dr. Perdew asked Dr. Mirsalis to clarify his point, because the CYP induction is through the AhR in the liver. Dr. Mirsalis said the response in the mice is different than that seen with dioxin, and noted that in the report, after extensive discussion about dioxin, the conclusion was that the response was not similar to dioxin. Dr. Bucher noted the nomination of this substance was related to the question about how there could be an AhR agonist that was unlike dioxin, and perhaps there was overemphasis in the discussion about dioxin. Dr. Wyde said it would be remiss not to mention the dioxin comparison at all. Dr. Fanucchi recommended adding language to the discussion related to Dr. Bucher's comments. Dr. Wyde agreed with Dr. Mirsalis that the liver weight data in mice could be brought forward in the report.

Dr. Mirsalis moved to accept the proposed conclusions as written. Dr. Perdew seconded the motion. Dr. Regan recommended changing the conclusion in the female rats from *some evidence* to *equivocal evidence* because there was no dose-related increase in malignant uterine neoplasms at the high dose or positive trend test, and because no preneoplastic changes were noted in the uterus. Dr. N.J.Walker, NIEHS, explained that in the level of evidence criteria, *some evidence* is used when there are treatment-related increases; in this case, there was a pairwise significance against the concurrent vehicle control. Dr. Conner disagreed with mentioning an increase that did not reach statistical significance; thus, he agreed that the call should be *equivocal evidence* rather than *some evidence*. Dr. Mirsalis said with the multiple adenocarcinomas involved, the *some evidence* call was appropriate. Dr. G.E. Kissling, NIEHS, stated statistical significance is only one piece of evidence used to make calls, and that there are cases where a biological increase is seen without reaching statistical significance. Dr. D.E. Malarkey, NIEHS, said it could be difficult to interpret preneoplastic lesions. Dr. Regan said even if the preneoplastic lesions were added in this case, it would not change the numbers. Dr. Carpenter called for a vote on the motion. The panel voted 4 to 2 in favor of the motion, so the conclusions were accepted as written. Drs. Regan and Connor cited their previous comments regarding the uterine neoplasms in rats as their reasons for voting against the motion.

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