

**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY STUDIES**  
**OF INDOLE-3-CARBINOL**  
**(CAS NO. 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)**

**Scheduled Peer Review Date: May 22, 2014**

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**NTP TR 584**

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**National Toxicology Program**

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

## 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, the 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 the 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.

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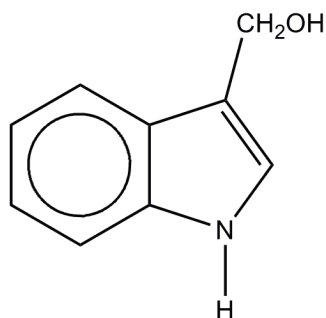
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## ABSTRACT



### INDOLE-3-CARBINOL

CAS No. 700-06-1

Chemical Formula: C<sub>9</sub>H<sub>9</sub>NO      Molecular Weight: 147.18

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

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.

### 3-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 female 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 increased estrous cycle length (approximately 1 day) and an additional day of diestrus in females administered 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. Indole-3-carbinol exhibited the potential to be a reproductive toxicant in female rats based on an increase in estrous cycle length (approximately 1 day) and the significantly increased probability of extended diestrus observed at 300 mg/kg.

### **3-MONTH STUDY IN B6C3F1 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.

### **2-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 adenocarcinomas 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 incidences of adenocarcinoma were 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.

## **2-YEAR STUDY IN B6C3F1 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 incidence 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 (*Salmonella typhimurium* strains TA97 and TA100 and *Escherichia 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 this 2-year gavage study, there was *no evidence of carcinogenic activity*\* 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.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 15.

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**Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Indole-3-carbinol**


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	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
<b>Doses in corn oil by gavage</b>	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
<b>Body weights</b>	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
<b>Survival rates</b>	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
<b>Nonneoplastic effects</b>	<u>Intestine small, duodenum:</u> lymphatic, ectasia (0/43, 0/48, 15/47, 14/48) <u>Intestine small, jejunum:</u> lymphatic, ectasia (0/40, 2/39, 27/40, 41/42) <u>Lymph node, mesenteric:</u> lymphatic, ectasia (0/50, 0/50, 1/50, 5/50) <u>Liver:</u> bile duct, cyst (0/50, 0/50, 2/50, 5/50) <u>Thyroid gland:</u> follicular cell, hypertrophy (21/50, 34/46, 33/48, 36/47)	<u>Uterus:</u> endometrium, metaplasia, squamous (standard evaluation-12/50, 18/50, 20/50, 11/50) <u>Intestine small, duodenum:</u> lymphatic, ectasia (0/48, 0/47, 16/48, 38/47) <u>Intestine small, jejunum:</u> lymphatic, ectasia (0/47, 0/46, 30/48, 47/48) <u>Lymph node, mesenteric:</u> lymphatic, ectasia (0/50, 0/50, 1/50, 15/48) <u>Liver:</u> clear cell focus (6/50, 7/50, 4/50, 18/48); eosinophilic focus (0/50, 4/50, 5/50, 6/48)	<u>Liver:</u> clear cell focus (7/50, 17/50, 22/49, 20/50) <u>Glandular stomach:</u> 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) <u>Nose:</u> 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)	<u>Liver:</u> eosinophilic focus (16/50, 26/50, 26/50, 21/50) <u>Glandular stomach:</u> 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) <u>Nose:</u> 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)
<b>Neoplastic effects</b>	None	<u>Uterus:</u> adenocarcinoma (standard evaluation-0/50, 1/50, 4/50, 4/50; standard and extended evaluations, combined-5/50, 4/50, 12/50, 10/50)	<u>Liver:</u> 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
<b>Equivocal findings</b>	None	<u>Skin:</u> 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
<b>Level of evidence of carcinogenic activity</b>	No evidence	Some evidence	Clear evidence	No evidence

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**Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Indole-3-carbinol**


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	Male Sprague Dawley Rats	Female Sprague Dawley Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
<b>Genetic toxicology</b>				
Bacterial gene mutations:	Negative in <i>Salmonella typhimurium</i> 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 <i>Escherichia coli</i> strain WP2 <i>uvrA</i> /pKM101 without S9.			
Micronucleated erythrocytes				
Rat bone marrow <i>in vivo</i> :	Negative			
Mouse peripheral blood <i>in vivo</i> :	Negative			

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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 the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

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

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

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

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

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

**NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS  
PEER REVIEW PANEL**

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on indole-3-carbinol on May 22, 2014, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

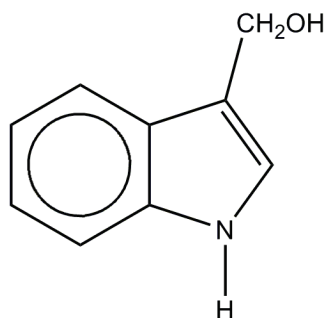
- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance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

**SUMMARY OF PEER REVIEW PANEL COMMENTS**

**NOTE:** A summary of the Peer Review Panel's remarks will appear in a future draft of this report.



## INTRODUCTION



### INDOLE-3-CARBINOL

CAS No. 700-06-1

Chemical Formula: C<sub>9</sub>H<sub>9</sub>NO    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 (*Aldrich*, 1996). It is soluble in benzene, ethanol, and pentane (Broadbent and Broadbent, 1998). 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 (Bradfield and Bjeldanes, 1991; Wortelboer *et al.*, 1992; Kwon *et al.*, 1994; Jongen, 1996).

### 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 (Holst and Williamson, 2004). 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 (Jongen, 1996). 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 (Broadbent and Broadbent, 1998). 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 (McDanell *et al.*, 1988; Heaney and Fenwick, 1995). Carlson *et al.* (1987) 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 [<sup>3</sup>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 (Dashwood *et al.*, 1989). 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 [<sup>3</sup>H]indole-3-carbinol. Following exposure of F344 rats to 2,000 ppm [<sup>3</sup>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 (Stresser *et al.*, 1995). 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 [<sup>3</sup>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 (Anderton *et al.*, 2004). 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 (De Kruif *et al.*, 1991; Chang *et al.*, 1999; Riby *et al.*, 2000). 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 oligomers 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 (De Kruif *et al.*, 1991).

The metabolism of indole-3-carbinol has been investigated in *in vitro* preparations of rat and chicken embryo liver (Jongen, 1996). 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-carboxylic 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-indolylmethane were detected in the liver following oral administration of indole-3-carbinol; the parent indole-3-carbinol was not detected (Dashwood *et al.*, 1989; De Kruif *et al.*, 1991; Stresser *et al.*, 1995). 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 (De Kruif *et al.*, 1991). In addition, indolo[3,2b]carbazole (ICZ), a potent aryl hydrocarbon receptor agonist was also identified (De Kruif *et al.*, 1991). 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 (Kwon *et al.*, 1994). 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



(Anderton *et al.*, 2004). 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-carboxaldehyde 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 (Anderton *et al.*, 2004). The proposed metabolic scheme for indole-3-carbinol following oral administration in rodents is shown in Figure 1.

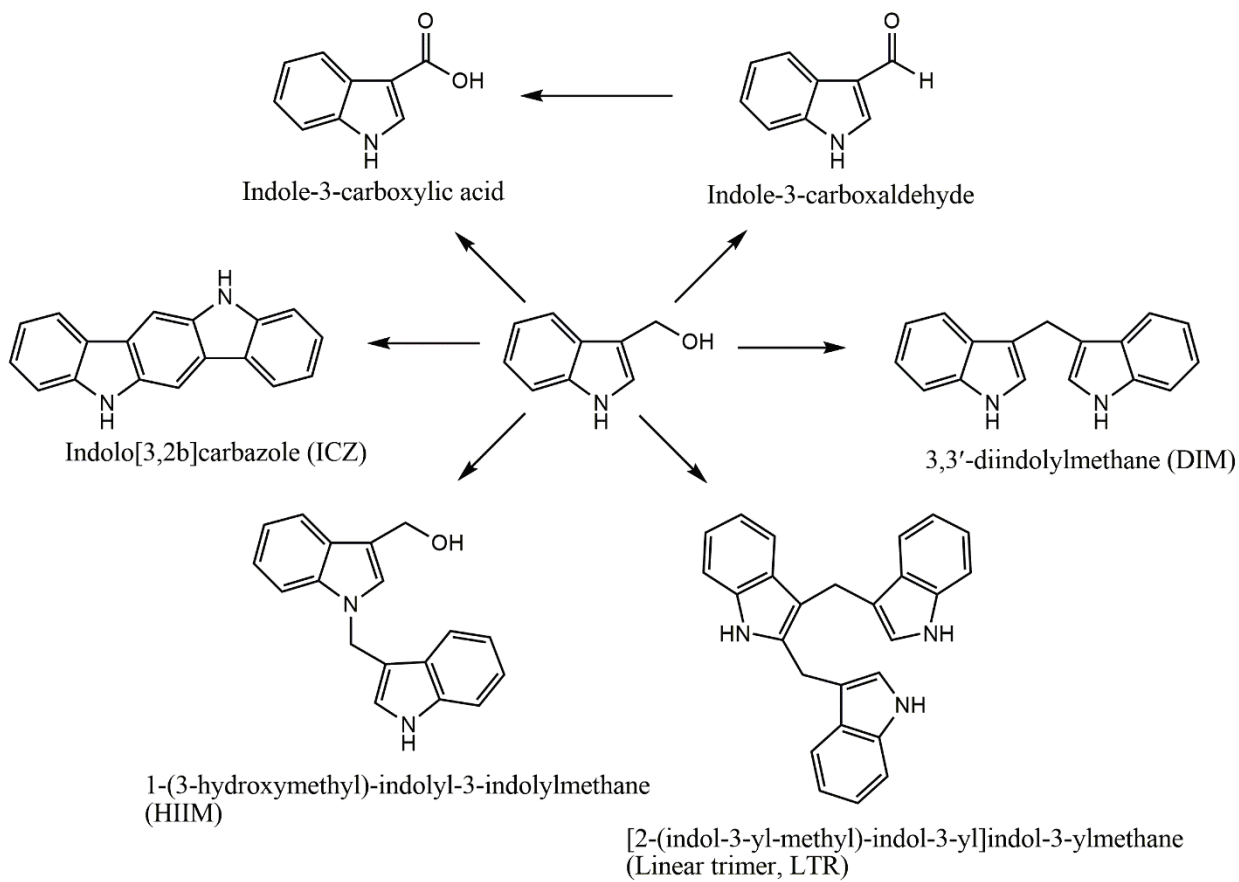
## Humans

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 (Gonzalez *et al.*, 1986). 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 (LeBlanc *et al.*, 1994). This study also showed that indole-3-carbinol alters hepatic 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 (Shertzer and Sainsbury, 1991). 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 (Crowell *et al.*, 2006). In Sprague Dawley rats exposed to 50 mg/kg, indole-3-carbinol significantly induced total hepatic CYP levels in both sexes (Leibelt *et al.*, 2003). 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.



**FIGURE 1**  
**Proposed Metabolic Scheme for Indole-3-carbinol following Oral Administration in Rodents**  
 (Adapted from Stresser *et al.*, 1995 and Anderton *et al.*, 2004)

The hepatic induction of CYP1A by indole-3-carbinol is mediated via the aryl hydrocarbon receptor (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 with significantly lower binding affinities than the most potent AhR ligand, TCDD (Johansson *et al.*, 1982; Bjeldanes *et al.*, 1991; Jellinck *et al.*, 1993). 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 (Nishie and Daxenbichler, 1980). 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 (Shertzer and Sainsbury, 1991). 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<sup>®</sup> castor oil (Gonzalez *et al.*, 1986). 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.

## 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 (Wong *et al.*, 1997). 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 16 $\alpha$ -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 (Bell *et al.*, 2000) and women with systemic lupus erythematosus administered 375 mg/day for 3 months

(McAlindon *et al.*, 2001). A single subject developed a skin rash that went away after cessation of treatment, and then reappeared after indole-3-carbinol was readministered (McAlindon *et al.*, 2001).

## 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 (Wilker *et al.*, 1996). 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 (Nishie and Daxenbichler, 1980). 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 (Pence *et al.*, 1986; Dashwood, 1998). Exposure to indole-3-carbinol promotes aflatoxin B<sub>1</sub>-initiated hepatocarcinogenesis in rainbow trout (Bailey *et al.*, 1987) and thyroid gland tumors in Sprague Dawley rats (Kim *et al.*, 1997). 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 (Wattenberg and Loub, 1978; Tanaka *et al.*, 1992; Grubbs *et al.*, 1995). 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 (Kojima *et al.*, 1994).

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 (Wattenberg and Loub, 1978). 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 (Bradlow *et al.*, 1991). 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 (Malloy *et al.*, 1997). Inhibition of tumorigenesis has been observed for diethylnitrosamine-induced hepatocarcinogenesis in ACI/N and Sprague Dawley rats and in the infant mouse model (Tanaka *et al.*, 1992; Kim *et al.*, 1994; Organesian *et al.*, 1997). Indole-3-carbinol also inhibits tumorigenesis in the lung in various animal models (Morse *et al.*, 1990; Chung *et al.*, 1993; Stoner *et al.*, 1993; el-Bayoumy *et al.*, 1996).

## Humans

Consumption of cruciferous vegetables has been associated with a decreased risk of cancer in humans (Jain *et al.*, 1999; Kolonel *et al.*, 2000; Voorrips *et al.*, 2000; Terry *et al.*, 2001). 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 (Young and Wolf, 1988; Bradfield and Bjeldanes, 1991; Arnao *et al.*, 1996; Kelloff *et al.*, 1996a; Keck and Finley, 2004).

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 (Kelloff *et al.*, 1996a,b; Wong *et al.*, 1997; Rosen *et al.*, 1998; Bell *et al.*, 2000; McAlindon *et al.*, 2001; Rosen and Bryson, 2004; Reed *et al.*, 2005; Naik *et al.*, 2006). 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) (Doppalapudi *et al.*, 2007). 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 (Doppalapudi *et al.*, 2007). 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 (Kassie *et al.*, 1996).

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 (Ochiai *et al.*, 1986; Tiedink *et al.*, 1988, 1991). 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)

(Sasagawa and Matsushima, 1991). 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 (Sasagawa and Matsushima, 1991). 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*<sup>+/−</sup> 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) (Doppalapudi *et al.*, 2007). *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 (Agrawal and Kumar, 1999) or five daily exposures to 5 mg/kg indole-3-carbinol by gavage (Shukla *et al.*, 2003). 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 (Doppalapudi *et al.*, 2007). 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) (Doppalapudi *et al.*, 2007).

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 (Schut and Dashwood, 1995; Xu *et al.*, 1996, 1997; Schut *et al.*, 1997; Arif *et al.*, 2000; Shukla *et al.*, 2003).

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  $\mu$ M increased the formation of AFB1-DNA adducts fourfold or sixfold, respectively (Gross-Steinmeyer *et al.*, 2009). 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 (Gross-Steinmeyer *et al.*, 2009). 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  $-20^{\circ}\text{C}$ , protected from light in amber glass containers sealed with Teflon<sup>®</sup>-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 I2). Dose formulations were stored in amber glass vials sealed with Teflon<sup>®</sup>-lined lids at  $5^{\circ}\text{C}$  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  $5^{\circ}\text{C}$ . Stability was confirmed for at least 8 days for formulations stored in amber glass vials sealed with Teflon<sup>®</sup>-lined lids at  $5^{\circ}\text{C}$  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<sup>®</sup>-lined lids at  $5^{\circ}\text{C}$ . 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 I3). 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 I4). 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, the NTP discontinued use of the F344/N rat in toxicity and carcinogenicity studies.

The NTP has previously conducted seven separate 2-year studies in Sprague-Dawley rats based on prior observations by Kociba *et al.* (1978) that Sprague-Dawley rats are sensitive to the effects of dioxin-like compounds (NTP 2006a,b,c,d,e,f,g). Based on the role of indole-3-carbinol as an agonist ligand for the AhR, 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.

## 3-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 (Crowell *et al.*, 2006), and a pilot study conducted in Sprague Dawley rats (unpublished data). Crowell *et al.* (2006) 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<sub>2</sub> aggregation method described by Schenkman and Cinti (1978). The concentration of protein in each suspension was determined by the Lowry method (Lowry *et al.*, 1951). EROD activities were determined by spectrofluorometric methods, including those of Chang and Waxman (1998) for liver, and A4H activities were determined using HPLC (Hamm *et al.*, 1998).

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<sup>®</sup> 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 kill, 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 was 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 (1982) and Boorman *et al.* (1985).

## 2-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 (Boyle *et al.*, 2012), 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 19 (females) or 20 (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  $\mu\text{m}$ , 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 (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

**TABLE 1**  
**Experimental Design and Materials and Methods in the Gavage Studies of Indole-3-carbinol**

3-Month Studies	2-Year Studies
<b>Study Laboratory</b> Southern Research Institute (Birmingham, AL)	Battelle Columbus Operations (Columbus, OH)
<b>Strain and Species</b> F344/N rats B6C3F1/N mice	Harlan Sprague Dawley rats B6C3F1/N mice
<b>Animal Source</b> Taconic Farms, Inc. (Germantown, NY)	Rats: Harlan Sprague Dawley, Inc. (Indianapolis, IN) Mice: Taconic Farms, Inc. (Germantown, NY)
<b>Time Held Before Studies</b> Rats: 12 (females) or 13 (males) days Mice: 14 (males) or 15 (females) days	Rats: 13 (males) or 14 (females) days Mice: 19 (females) or 20 (males) days
<b>Average Age When Studies Began</b> Rats: 5 to 6 weeks Mice: 6 to 7 weeks	6 to 7 weeks
<b>Date of First Dose</b> 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
<b>Duration of Dosing</b> 5 days per week for 14 weeks	5 days per week for 104 (male rats) or 105 weeks
<b>Date of Last Dose</b> 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
<b>Necropsy Dates</b> 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
<b>Average Age at Necropsy</b> 19 to 20 weeks	Rats: 110 to 111 weeks Mice: 110 (females) to 112 weeks
<b>Size of Study Groups</b> Core study: 10 male and 10 female rats and mice Clinical pathology study: 10 male and 10 female rats	Core study: 50 males and 50 females Special intestine study: 10 male rats Microarray study: Five vehicle control female rats and five 300 mg/kg female rats
<b>Method of Distribution</b> Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
<b>Animals per Cage</b> Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
<b>Method of Animal Identification</b> Tail tattoo	Tail tattoo
<b>Diet</b> Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available <i>ad libitum</i> , changed at least once weekly	Same as 3-month studies

**TABLE 1**  
**Experimental Design and Materials and Methods in the Gavage Studies of Indole-3-carbinol**

3-Month Studies	2-Year Studies
<p><b>Water</b> Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available <i>ad libitum</i></p>	<p>Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available <i>ad libitum</i></p>
<p><b>Cages</b> Polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice weekly</p>	<p>Same as 3-month studies except from Lab Products, Inc., Seaford, DE; rotated every 2 weeks</p>
<p><b>Bedding</b> Irradiated hardwood bedding chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice weekly</p>	<p>Irradiated Sani-Chips® (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice weekly</p>
<p><b>Rack Filters</b> Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks</p>	<p>Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks</p>
<p><b>Racks</b> Stainless steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks</p>	<p>Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks</p>
<p><b>Animal Room Environment</b> Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour</p>	<p>Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour</p>
<p><b>Doses</b> 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)</p>	<p>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)</p>
<p><b>Type and Frequency of Observation</b> 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.</p>	<p>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.</p>
<p><b>Method of Kill</b> Carbon dioxide asphyxiation</p>	<p>Same as 3-month studies</p>
<p><b>Necropsy</b> 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.</p>	<p>Necropsies were performed on all core study animals and special intestine study male rats.</p>
<p><b>Clinical Pathology</b> 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). <b>Hematology:</b> 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 <b>Clinical chemistry:</b> urea nitrogen, creatinine, glucose, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids</p>	<p>None</p>

**TABLE 1**  
**Experimental Design and Materials and Methods in the Gavage Studies of Indole-3-carbinol**

3-Month Studies	2-Year Studies
<p><b>Histopathology</b>            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, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, 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 was examined in all dosed groups of mice.</p>	<p>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, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, 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.</p>
<p><b>Sperm Motility and Vaginal Cytology</b>            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.</p>	None
<p><b>Cytochrome P450 activities</b>            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.</p>	None
<p><b>Microarray Study</b>            None</p>	<p>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).</p>

## STATISTICAL METHODS

### Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) 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 (1972) method for testing two groups for equality and Tarone's (1975) 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 Tables A1, A3, B1, B4, C1, C4, D1, and D4 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 (Tables A2, B2, C2, and D2) 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. Tables A2, B2, C2, and D2 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 kill.

## Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) 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 kill; if the animal died prior to terminal kill 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 (Bailer and Portier, 1988). 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 (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific

neoplasms in control F344/N rats and B6C3F1/N mice (Portier *et al.*, 1986). Bailer and Portier (1988) 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 (1993).

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 (1955) and Williams (1971, 1972). 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 (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) 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 (1957) 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 (Gart *et al.*, 1979). 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 (1987). 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 (Haseman, 1992, 1995; Haseman and Rao, 1992). 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 (21 CFR, Part 58). 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 (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

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

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

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 (Shelby *et al.*, 1993; Shelby and Witt, 1995). 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 (Witt *et al.*, 2000). 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

### 3-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 2). There were no clinical findings related to administration of indole-3-carbinol.

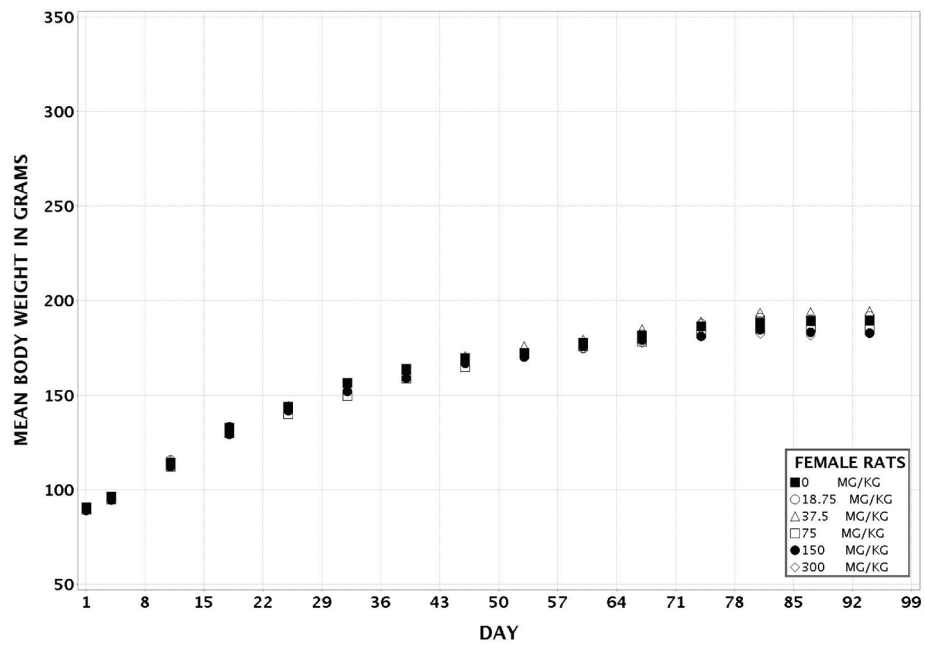
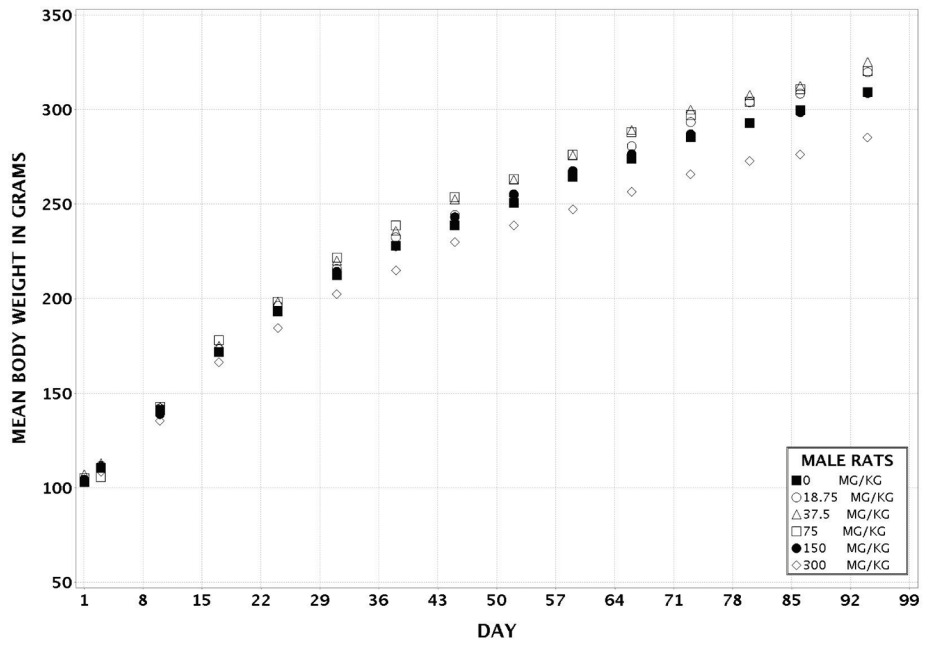
**TABLE 2**  
**Survival and Body Weights of F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

Dose (mg/kg)	Survival <sup>b</sup>	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
<b>Male</b>					
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
<b>Female</b>					
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

<sup>a</sup> Weights and weight changes are given as mean ± standard error.

<sup>b</sup> Number of animals surviving at 14 weeks/number initially in group



**FIGURE 2**  
**Growth Curves for F344/N Rats Administered Indole-3-carbinol by Gavage for 3 Months**

There were no changes in the hematology and clinical chemistry data of rats that were considered attributable to indole-3-carbinol administration (Table F1).

The absolute and relative liver weights of all dosed groups of males and females were significantly increased compared to the vehicle controls (Tables 3 and G1). 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 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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
<b>Male</b>						
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**
<b>Female</b>						
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<0.05) from the vehicle control group by Williams' or Dunnett's test

\*\* P<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 4.5-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.

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 H1). 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 (Tables H2 and H3; Figure H1). 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 analyses.

**TABLE 4**  
**Liver and Lung Cytochrome P450 Data for F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
<b>Male</b>						
<b>Liver Microsomes</b>						
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**
<b>Lung Microsomes</b>						
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**
<b>Female</b>						
n	10	10	10	10	10	10
<b>Liver Microsomes</b>						
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**	1.545 ± 0.123**
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**
<b>Lung Microsomes</b>						
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

\*\*  $P \leq 0.01$

<sup>a</sup> Data are presented as mean ± standard error. A4H = acetanilide-4-hydroxylase; EROD = 7-ethoxyresorufin-*O*-deethylase; ND = None detected

Dose-related increased incidences and severities of minimal to mild ectasia of the lymphatic vessels and lipodosis 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

**TABLE 5**  
**Incidences of Selected Nonneoplastic Lesions in F344/N Rats in the 3-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
<b>Male</b>						
Intestine Small, Duodenum <sup>a</sup>	10	10	10	10	10	10
Lamina Propria, Lipidosis <sup>b</sup>	0	0	0	1 (1.0) <sup>c</sup>	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)
<b>Female</b>						
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

\*\*  $P \leq 0.01$

<sup>a</sup> Number of animals with tissue examined microscopically

<sup>b</sup> Number of animals with lesion

<sup>c</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

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.

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 multinucleated 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 AhR ligands such as PCB 126 and TCDD induced moderate to marked hepatotoxicity in 2-year studies (NTP, 2006a,b). 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.

## 2-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 3). Survival of dosed groups was similar to that of the vehicle control groups.

**TABLE 6**  
**Survival of Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Male</b>				
Animals initially in study	50	50	50	50
Accidental death <sup>a</sup>	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 <sup>b</sup>	40	26	34	25
Mean survival (days) <sup>c</sup>	644	617	640	615
Survival analysis <sup>d</sup>	P=0.224	P=0.084	P=0.557	P=0.100
<b>Female</b>				
Animals initially in study	50	50	50	50
Accidental deaths <sup>a</sup>	0	1	0	1
Moribund	24	22	23	11
Natural deaths	5	8	7	8
Animals surviving to study termination	21	19 <sup>e</sup>	20 <sup>e</sup>	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

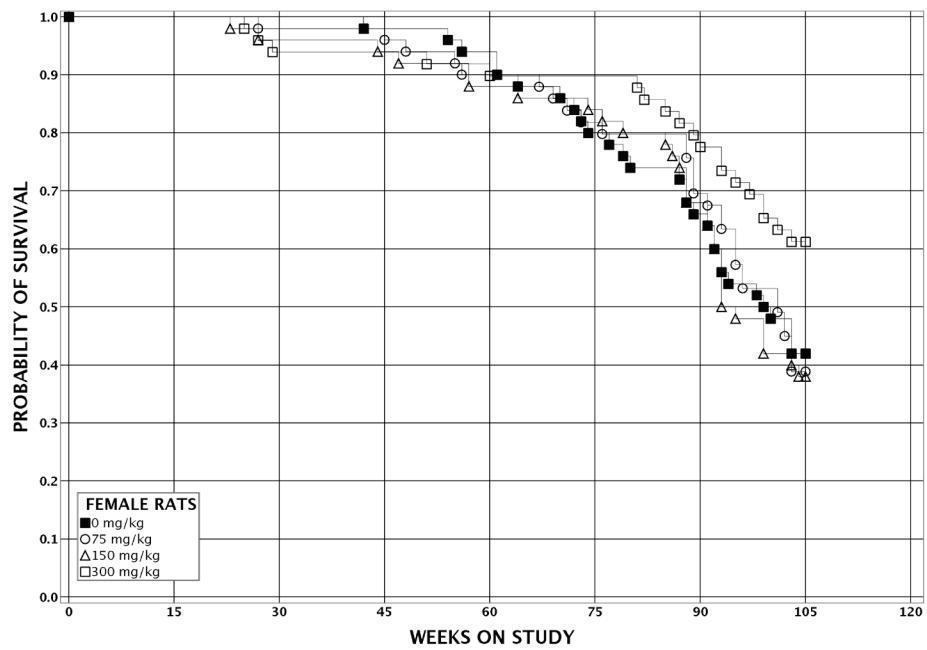
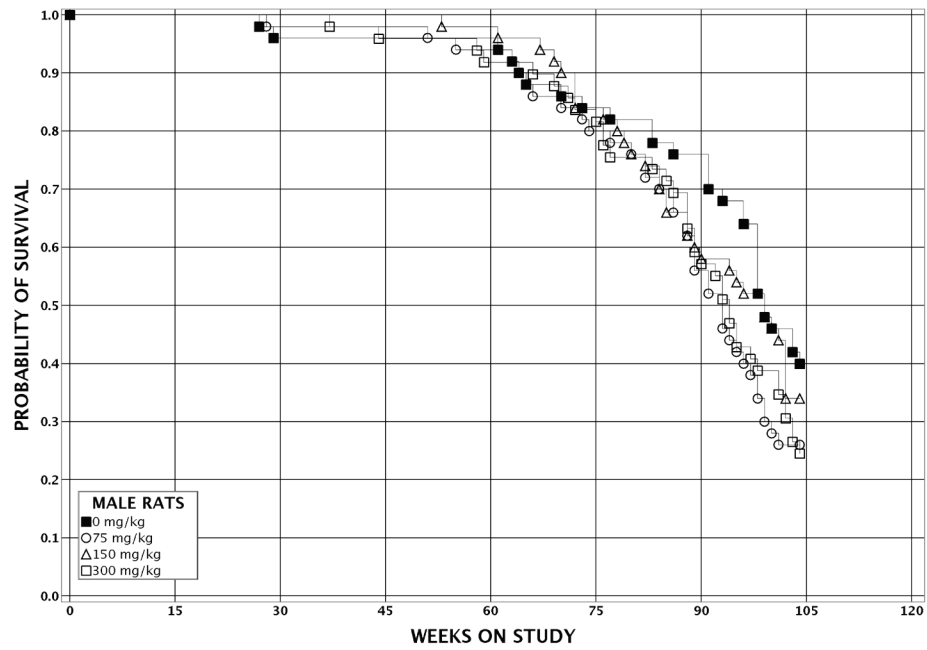
<sup>a</sup> Censored from survival analyses

<sup>b</sup> Kaplan-Meier determinations

<sup>c</sup> Mean of all deaths (uncensored, censored, and terminal kill).

<sup>d</sup> The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.

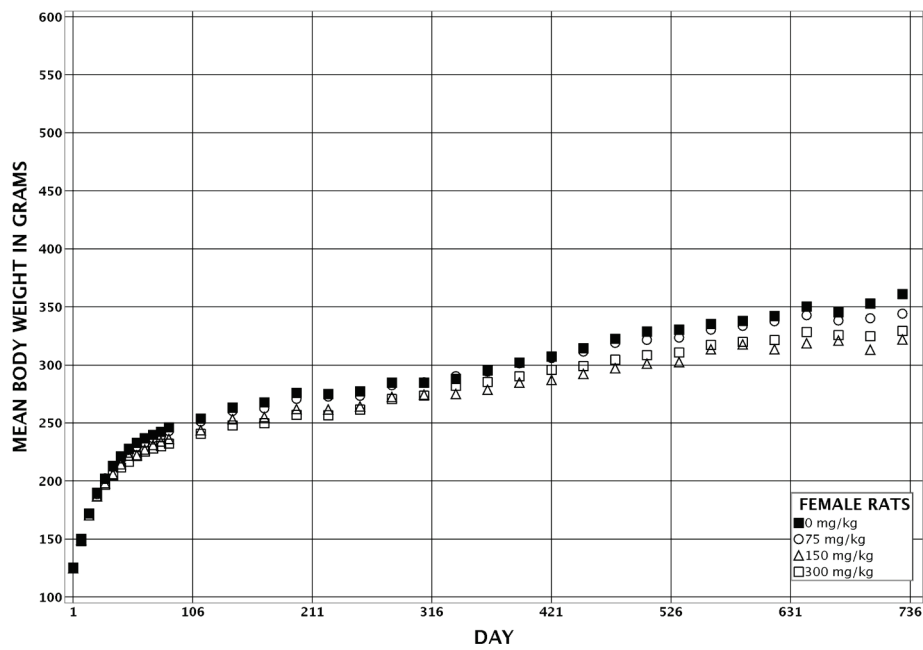
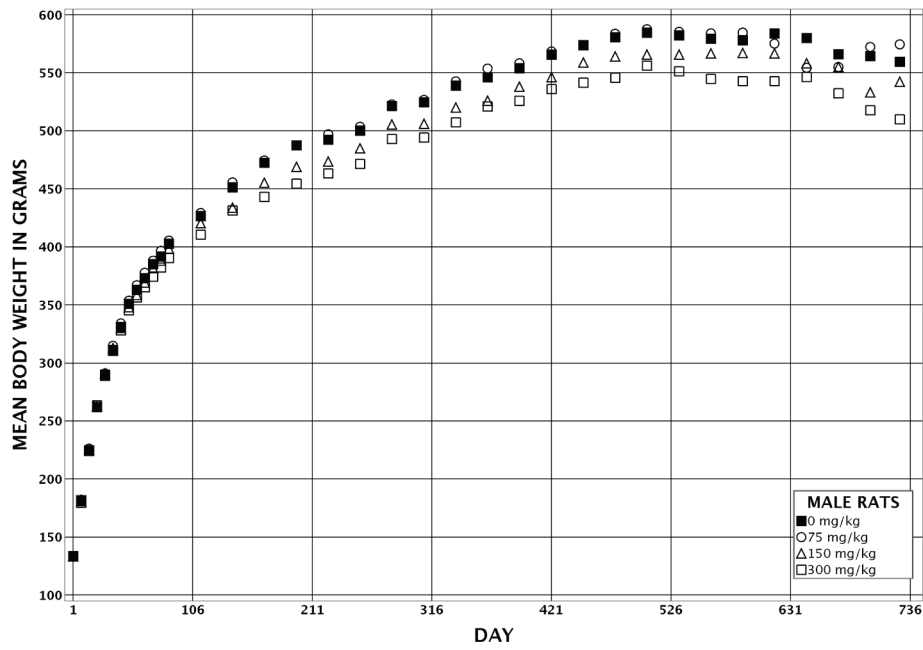
<sup>e</sup> Includes one animal that died during the last week of the study



**FIGURE 3**  
**Kaplan-Meier Survival Curves for Sprague Dawley Rats Administered Indole-3-carbinol by Gavage for 2 Years**

### **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 4; Tables 7 and 8). No clinical findings related to the administration of indole-3-carbinol were observed in males or females.



**Figure 4**  
Growth Curves for Sprague Dawley Rats Administered Indole-3-carbinol by Gavage for 2 Years

**TABLE 7**  
**Mean Body Weights and Survival of Male Sprague Dawley Rats in the 2-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
<b>Mean for Weeks</b>											
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 2-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
<b>Mean for Weeks</b>											
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 (Tables 9, B1, and B2). 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 (Tables 9 and B3a). 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 (Tables 9 and B2).

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 (Tables 9 and B4). 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).



**TABLE 9**  
**Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Standard Evaluations</b>				
Number Necropsied	50	50	50	50
Endometrium, Metaplasia, Squamous <sup>a</sup>	12 (1.9) <sup>b</sup>	18 (2.2)	20* (2.1)	11 (1.8)
Adenocarcinoma, Multiple	0	0	0	1
Adenocarcinoma (includes multiple) <sup>c</sup>				
Overall rate <sup>d</sup>	0/50 (0%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate <sup>e</sup>	0.0%	2.7%	10.9%	9.8%
Terminal rate <sup>f</sup>	0/21 (0%)	0/19 (0%)	1/19 (5%)	2/30 (7%)
First incidence (days)	— <sup>h</sup>	645	608	645
Poly-3 test <sup>g</sup>	P=0.040	P=0.503	P=0.059	P=0.074
<b>Extended Evaluation</b>				
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
Adenosquamous Carcinoma	0	0	1	0

**TABLE 9**  
**Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Standard and Extended Evaluations (Combined)</b>				
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	12*	10
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

\*\*  $P \leq 0.01$

<sup>a</sup> Number of animals with lesion

<sup>b</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

<sup>c</sup> Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean  $\pm$  standard deviation): 2/102

<sup>d</sup> Number of animals with neoplasm per number of animals necropsied

<sup>e</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>f</sup> Observed incidence at terminal kill

<sup>g</sup> 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 kill.

<sup>h</sup> Not applicable; no neoplasms in animal group

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 (Plates 1 and 2).

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 (Plates 3 and 4). 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 (Plate 5). 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 (Plate 6).

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.

*Skin:* An increased incidence of fibroma occurred in 300 mg/kg females (Tables 10, B1, and B2). 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 (Tables 10 and B3b). A single incidence each of fibrosarcoma occurred in vehicle control and 300 mg/kg females (Tables 10 and B1). The incidences of fibroma or fibrosarcoma (combined) occurred with a positive trend in females (Tables 10 and B2).

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 2-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 <sup>a,b</sup>	1	0	0	4
Fibrosarcoma <sup>c</sup>	1	0	0	1
Fibroma or Fibrosarcoma <sup>d</sup>				
Overall rate <sup>e</sup>	2/50 (4%)	0/50 (8%)	0/50 (0%)	5/50 (10%)
Adjusted rate <sup>f</sup>	5.4%	0.0%	0.0%	12.4%
Terminal rate <sup>g</sup>	1/21 (5%)	0/19 (0%)	0/19 (0%)	3/30 (10%)
First incidence (days)	645	— <sup>i</sup>	—	692
Poly-3 test <sup>h</sup>	P=0.040	P=0.237N	P=0.243N	P=0.254

<sup>a</sup> Number of animals with lesion

<sup>b</sup> Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 3/102

<sup>c</sup> Historical incidence for corn oil gavage studies: 1/102

<sup>d</sup> Historical incidence for corn oil gavage studies: 4/102

<sup>e</sup> Number of animals with neoplasm per number of animals necropsied

<sup>f</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>g</sup> Observed incidence at terminal kill

<sup>h</sup> 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 kill.

<sup>i</sup> 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 (Tables 11, A3, and B4). 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 (Plates 7 and 8). 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.

**TABLE 11**  
**Incidences of Selected Nonneoplastic Lesions in Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Male</b>				
Intestine Small, Duodenum <sup>a</sup>	43	48	47	48
Lymphatic, Ectasia <sup>b</sup>	0	0	15** (1.5) <sup>c</sup>	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)
<b>Female</b>				
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)
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

\*\*  $P \leq 0.01$

<sup>a</sup> Number of animals with tissue examined microscopically

<sup>b</sup> Number of animals with lesion

<sup>c</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

*Mesenteric Lymph Node:* Significantly increased incidences of lymphatic ectasia occurred in 300 mg/kg males and females (Tables 11, A3, and B4). 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 (Plates 9 and 10).

*Liver:* Incidences of cholangiofibrosis occurred in all dosed groups of males and in one 75 mg/kg female (Tables 11, A3, and B4). 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.

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 (Plates 11 and 12). 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 (Plates 13 and 14).

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 (Plate 15). Clear cell foci were discrete focal areas of normal-sized to slightly enlarged hepatocytes that had clear cytoplasmic spaces around the cell nuclei (Plate 16).

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 (Tables 11 and A3).

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: 5/50, 8/49, 9/50, 8/47; females: 18/50, 18/50, 19/50, 8/49) (Tables A1, A2, B1, and B2).

## MICE

### 3-MONTH STUDY

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 5). There were no clinical findings related to administration of indole-3-carbinol.

**TABLE 12**  
**Survival and Body Weights of Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

Dose (mg/kg)	Survival <sup>b</sup>	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
<b>Male</b>					
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
<b>Female</b>					
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

<sup>a</sup> Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

<sup>b</sup> Number of animals surviving at 14 weeks/number initially in group



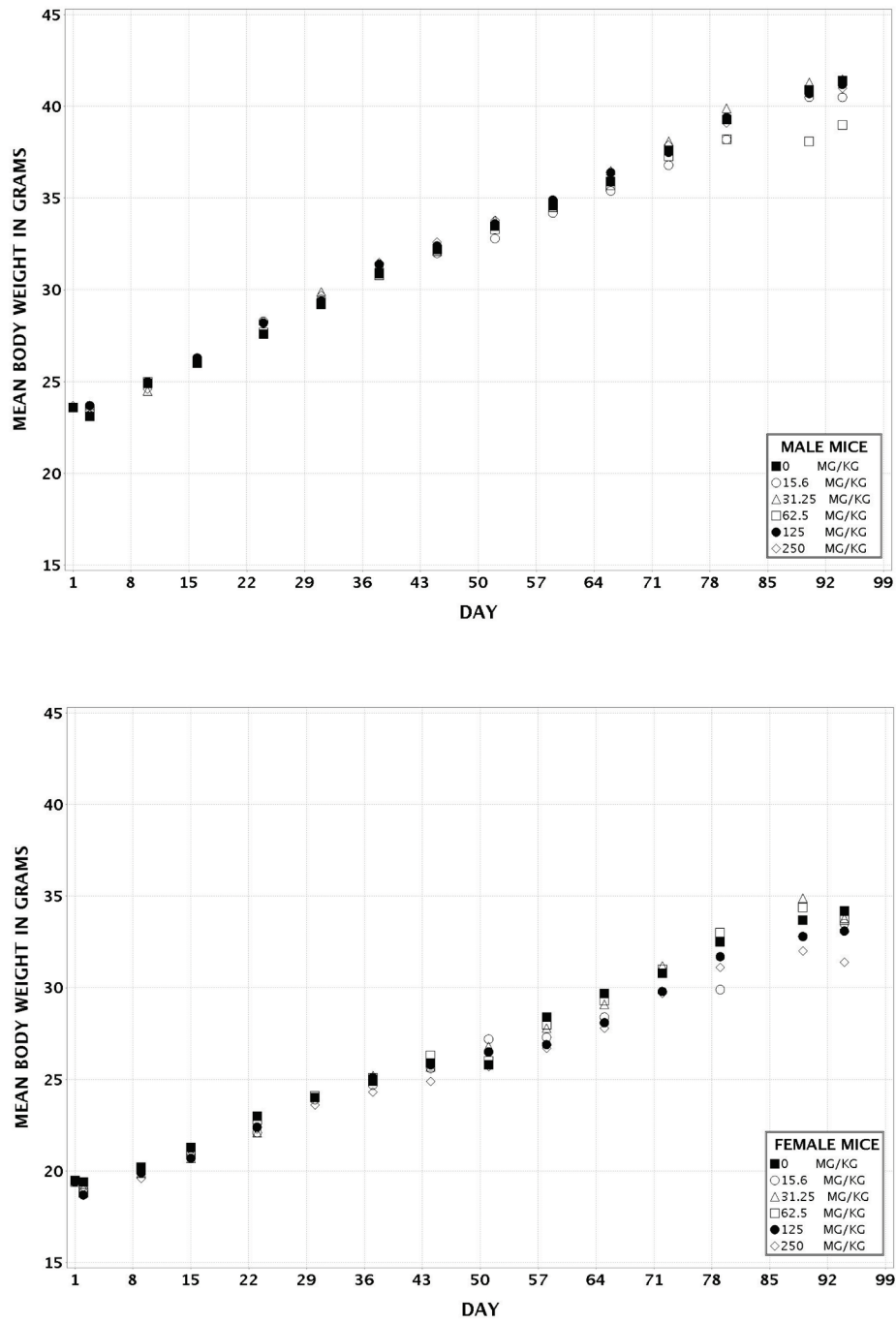


FIGURE 5  
Growth Curves for Mice Administered Indole-3-carbinol by Gavage for 3 Months

There were no changes in the hematology data of mice that were considered attributable to indole-3-carbinol administration (Table F2).

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 G2).

Liver and lung samples were collected for determinations of P450 enzyme activities (Table 13). 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.

Sperm motility was significantly decreased in all dosed groups of males (Table H4). The Markov transition matrix analyses of estrous cyclicity indicated that females in the 250 mg/kg groups had a significantly higher probability of extended diestrus than the vehicle control females (Tables H5 and H6; Figure H2). 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.

**TABLE 13**  
**Liver and Lung Cytochrome P450 Data for Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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
<b>Male</b>						
<b>Liver Microsomes</b>						
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**
<b>Lung Microsomes</b>						
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
<b>Female</b>						
<b>Liver Microsomes</b>						
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**
<b>Lung Microsomes</b>						
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≤0.05) from the vehicle control group by Shirley's test

\*\* P≤0.01

<sup>a</sup> Data are presented as mean ± standard error. A4H = acetanilide-4-hydroxylase; EROD = 7-ethoxyresorufin-*O*-deethylase

*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.

## 2-YEAR STUDY

### Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 6). 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.

**TABLE 14**  
**Survival of Mice in the 2-Year Gavage Study of Indole-3-carbinol**

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

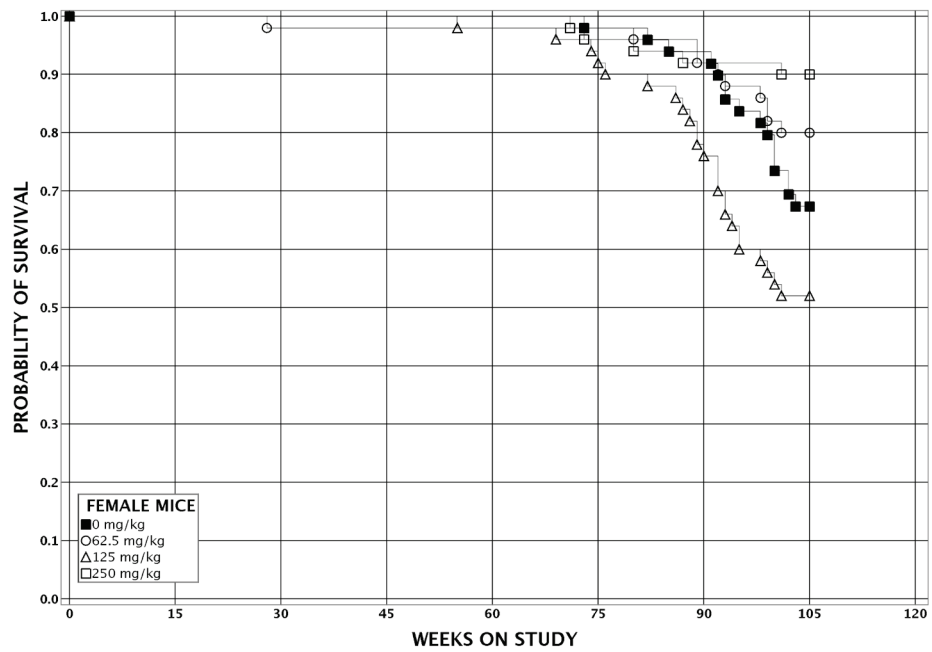
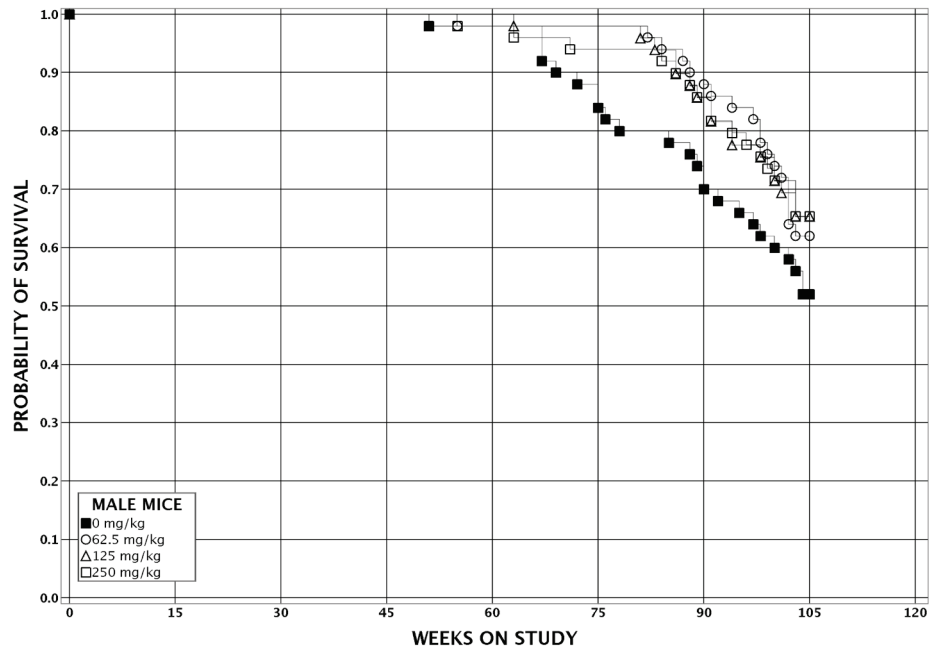
<sup>a</sup> Censored from survival analyses

<sup>b</sup> Kaplan-Meier determinations

<sup>c</sup> Mean of all deaths (uncensored, censored, and terminal kill).

<sup>d</sup> The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.

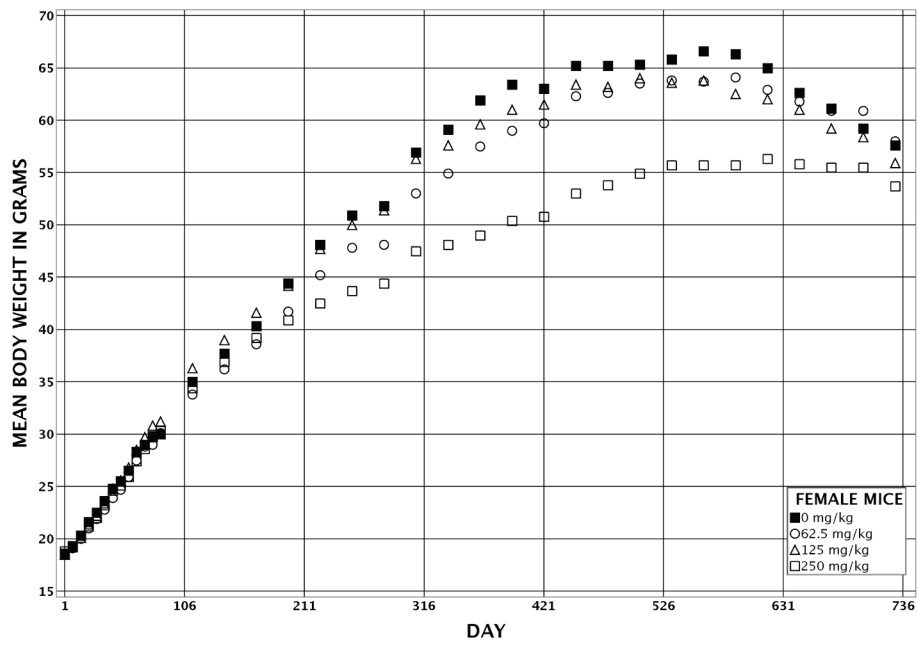
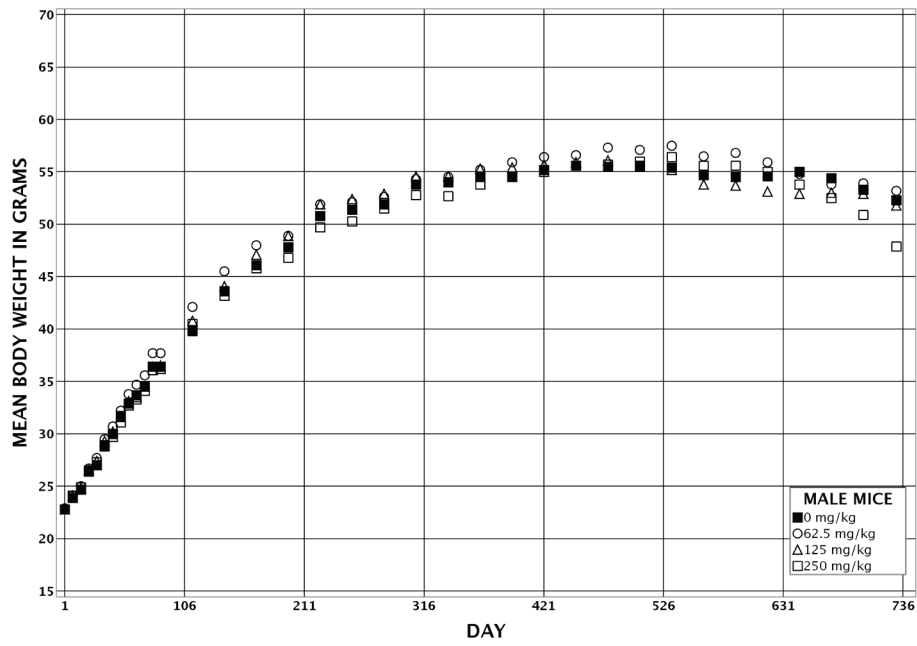
<sup>e</sup> Includes one animal that died during the last week of the study



**FIGURE 6**  
**Kaplan-Meier Survival Curves for Mice Administered Indole-3-carbinol by Gavage for 2 Years**

### **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 (Figure 7; Tables 15 and 16). No clinical findings related to the administration of indole-3-carbinol were observed in males or females.



**FIGURE 7**  
**Growth Curves for Mice Administered Indole-3-carbinol by Gavage for 2 Years**

**TABLE 15**  
**Mean Body Weights and Survival of Male Mice in the 2-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
<b>Mean for Weeks</b>											
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 16**  
**Mean Body Weights and Survival of Female Mice in the 2-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
<b>Mean for Weeks</b>											
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	

## 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 (Tables 17, C1, and C2). 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 (Tables 17, C1, and C2).

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 (Tables 17, C1, C2, and C3). 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 (Tables 17 and D1).

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 (Tables 17, C1, and C2). The combined incidences of hepatocellular carcinoma or hepatoblastoma were significantly increased in 125 and 250 mg/kg males.

**TABLE 17**  
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Male</b>				
Number Examined Microscopically	50	50	49	50
Clear Cell Focus <sup>a</sup>	7	17*	22**	20**
Hepatocellular Adenoma, Multiple	15	25*	16	33**
Hepatocellular Adenoma (includes multiple) <sup>b</sup>				
Overall rate <sup>c</sup>	26/50 (52%)	32/50 (64%)	31/49 (63%)	41/50 (82%)
Adjusted rate <sup>d</sup>	62.1%	66.5%	67.9%	85.4%
Terminal rate <sup>e</sup>	19/26 (73%)	21/31 (68%)	23/32 (72%)	28/32 (88%)
First incidence (days)	463	381	437	385
Poly-3 test <sup>f</sup>	P=0.005	P=0.411	P=0.360	P=0.006
Hepatocellular Carcinoma, Multiple	5	3	18**	8
Hepatocellular Carcinoma (includes multiple) <sup>g</sup>				
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
Hepatocellular Adenoma or Carcinoma <sup>h</sup>				
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) <sup>i</sup>				
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 <sup>j</sup>				
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 <sup>k</sup>				
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

**TABLE 17**  
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Male (continued)</b>				
Hepatocholangiocarcinoma <sup>l</sup>				
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)	— <sup>m</sup>	630	730 (T)	—
Poly-3 test	P=0.319N	P=0.146	P=0.515	— <sup>n</sup>
<b>Female</b>				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus	16	26*	26*	21
Hepatocellular Adenoma, Multiple	0	3	2	4
Hepatocellular Adenoma (includes multiple) <sup>o</sup>				
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
Hepatocellular Carcinoma, Multiple	0	2	1	0
Hepatocellular Carcinoma (includes multiple) <sup>p</sup>				
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 <sup>q</sup>	0	0	1	1

\* Significantly different (P<0.05) from the vehicle control group by the Poly-3 test

\*\* P<0.01

(T) Terminal kill

<sup>a</sup> Number of animals with lesion

<sup>b</sup> 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%

<sup>c</sup> Number of animals with neoplasm per number of animals with liver examined microscopically

<sup>d</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>e</sup> Observed incidence at terminal kill

<sup>f</sup> 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 kill. A negative trend or a lower incidence in a dose group is indicated by N.

<sup>g</sup> 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%

<sup>h</sup> 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%

<sup>i</sup> 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%

<sup>j</sup> 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%

<sup>k</sup> 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%

<sup>l</sup> 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%

<sup>m</sup> Not applicable; no neoplasms in animal group

**TABLE 17**  
**Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Indole-3-carbinol**

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<sup>n</sup> Value of statistic cannot be computed.

<sup>o</sup> 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%

<sup>p</sup> 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%

<sup>q</sup> 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%

In males, a single hepatocholangioma occurred in the 250 mg/kg group (Table C1). In addition, three and one hepatocholangiocarcinomas occurred in the 62.5 and 125 mg/kg groups, respectively (Tables 17, C1, and C2); these incidences were within the historical control ranges and were not dose-dependent (Tables 17, C2, and C3).

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 (Tables 17, C4, and D4).

Hepatocellular adenomas were relatively well-circumscribed, nodular masses that compressed the adjacent hepatic parenchyma (Plates 17 and 18). 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 (Plates 19 and 20). 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

(Plates 21 and 22). 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 (Tables 18, C4, and D4).

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 (Plate 23), 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 (Plate 24). 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

**TABLE 18**  
**Incidences of Nonneoplastic Lesions of the Glandular Stomach in Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Male</b>				
Number Examined Microscopically	50	47	47	49
Epithelium, Hyperplasia <sup>a</sup>	0	1 (1.0) <sup>b</sup>	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)
<b>Female</b>				
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

\*\*  $P \leq 0.01$

<sup>a</sup> Number of animals with lesion

<sup>b</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

propria. Mixed infiltrates of low numbers of lymphocytes and/or neutrophils were sparsely scattered within the lamina propria and submucosa.

*Nose:* Significantly increased incidences of nerve atrophy occurred in 250 mg/kg males and females compared to the vehicle controls (Tables 19, C4, and D4). 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 (Tables 19, C4, and D4). 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.

**TABLE 19**  
**Incidences of Nonneoplastic Lesions of the Nose in Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Male</b>				
Number Examined Microscopically	50	50	50	50
Nerve, Atrophy <sup>a</sup>	0	0	0	8** (2.0)
Olfactory Epithelium, Respiratory Metaplasia	14 (1.1) <sup>b</sup>	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)
<b>Female</b>				
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

\*\*  $P \leq 0.01$

<sup>a</sup> Number of animals with lesion

<sup>b</sup> Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The incidences of respiratory epithelium hyaline droplet accumulation were significantly increased in 62.5 and 125 mg/kg males (Tables 19 and C4). 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 (Tables 19 and D4).

Significantly increased incidences of respiratory epithelium hyperplasia occurred in 250 mg/kg males and females (Tables 19, C4, and D4). The severities generally increased with increasing dose in males and the severity was increased in 250 mg/kg females.



The incidence of inflammation was significantly increased in 250 mg/kg females (Tables 19 and D4). The incidence of inflammation was also increased in 125 mg/kg females, but not significantly.

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 (Plates 25 and 26).

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.

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 (Plates 27 and 28). Often, the metaplastic epithelium extended into the submucosal 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. Inflammation was occasionally associated with foreign material (plant, food, hair) in the ventral portions of the Level I and II sections.

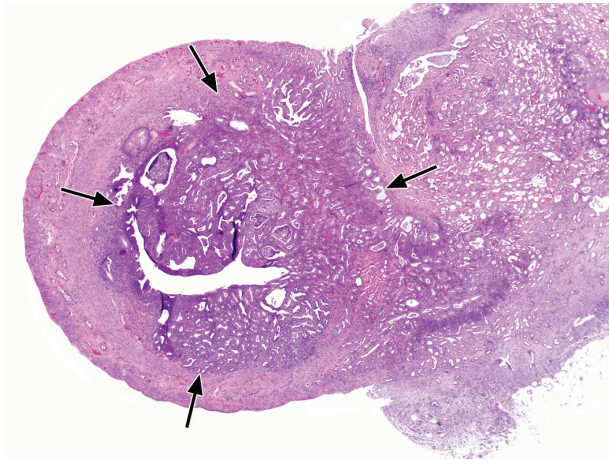
## GENETIC TOXICOLOGY

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 E1). A second study, using strains TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537, and concentrations ranging from 33 to 3,333 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 E1). Results of the third study were judged to be equivocal in *S. typhimurium* strain TA100 (concentration range 500 to

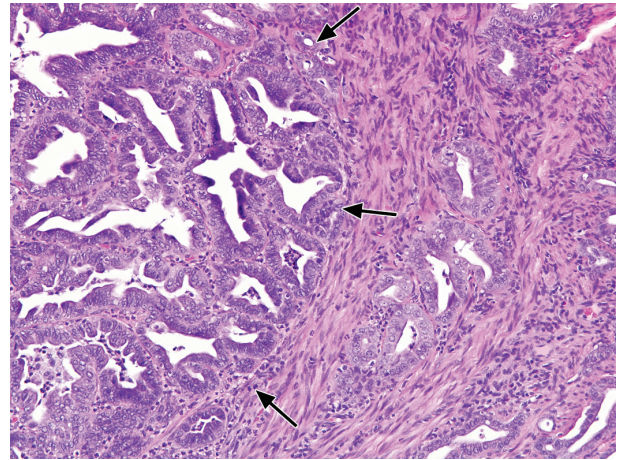
5,000 µg/plate) in the presence of 10% rat liver S9 and in *Escherichia coli* strain WP2 *uvrA*/pKM101 (concentration range of 500 to 7,500 µg/plate) in the absence of S9 activation; no mutagenic activity was seen in this assay in *S. typhimurium* strain TA98, with or without S9, tested up to 5,000 µg/plate (Table E2).

*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-carbinole was toxic to the bone marrow (Table E3). 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 E4).

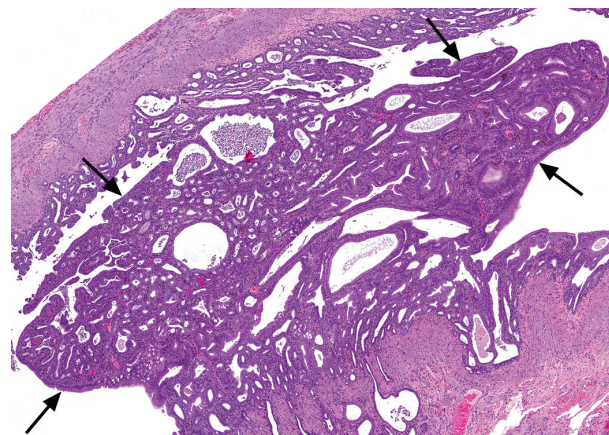




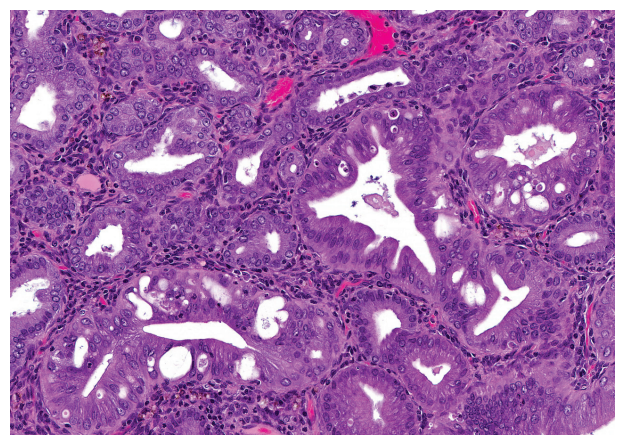
**PLATE 1**  
Adenocarcinoma of the endometrium in the uterus of a female Harlan Sprague-Dawley rat administered 300 mg/kg indole-3-carbinol by gavage for 2 years. Note the invasion through the myometrium and out to the serosal surface (arrows). H&E



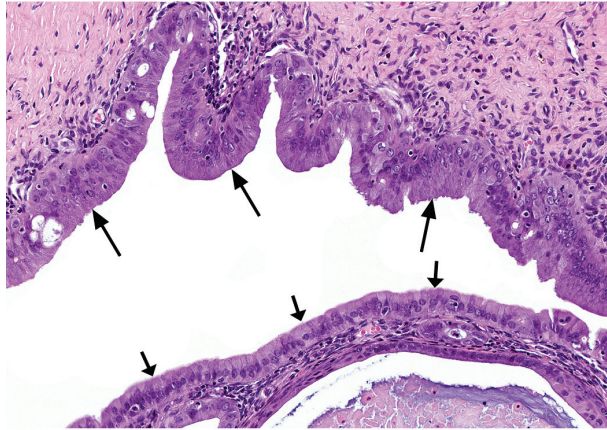
**PLATE 2**  
Higher magnification of Plate 1. Note the glandular neoplastic structures (arrows) invading into the myometrium. H&E



**PLATE 3**  
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 2 years. H&E

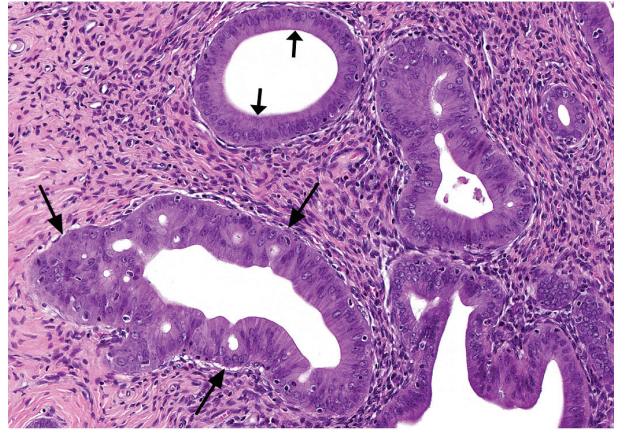


**PLATE 4**  
Higher magnification of Plate 3. The adenoma is composed of irregular glandular structures lined by cuboidal to columnar epithelium that is disorganized in some areas. H&E



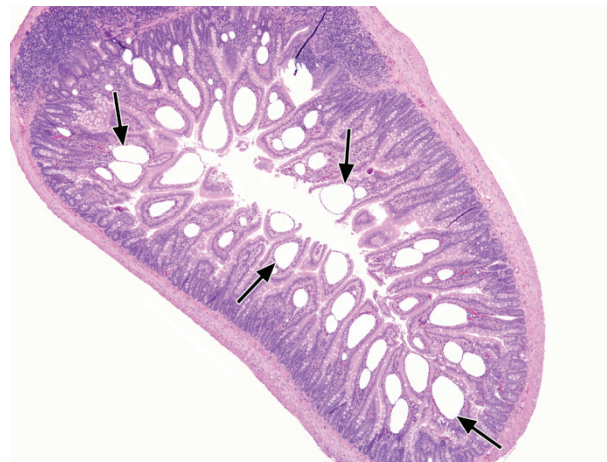
**PLATE 5**

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 2 years. 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). H&E



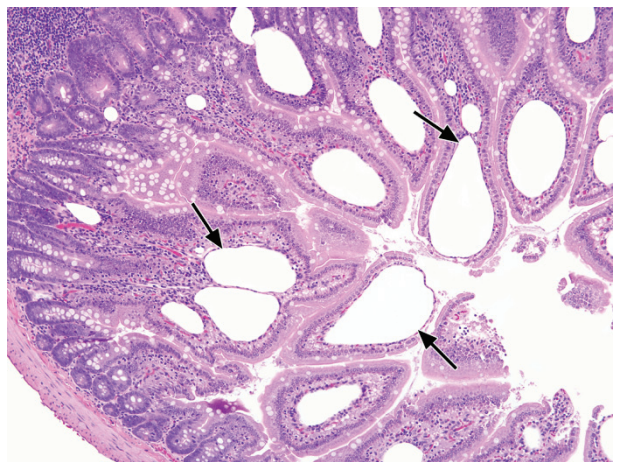
**PLATE 6**

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



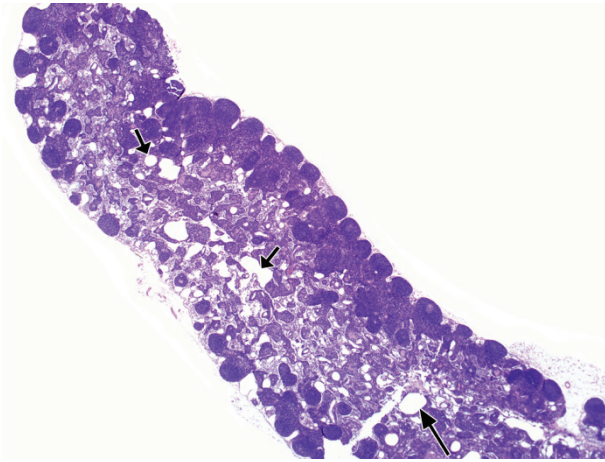
**PLATE 7**

Lymphatic ectasia in the jejunum of a female Harlan Sprague-Dawley rat administered 300 mg/kg indole-3-carbinol by gavage for 2 years. Note multiple dilated lymphatics (arrows) in the lamina propria of the villi. H&E

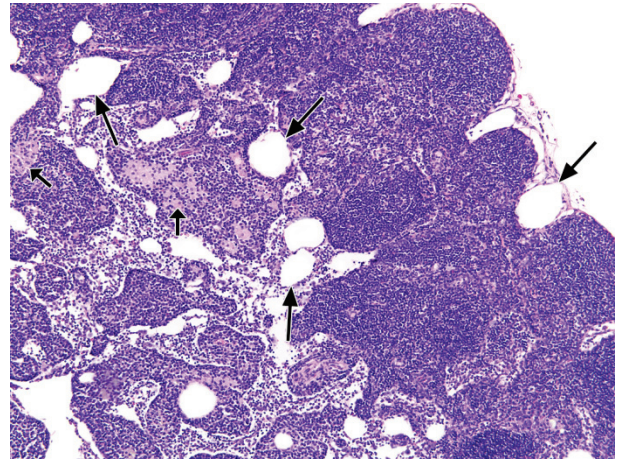


**PLATE 8**

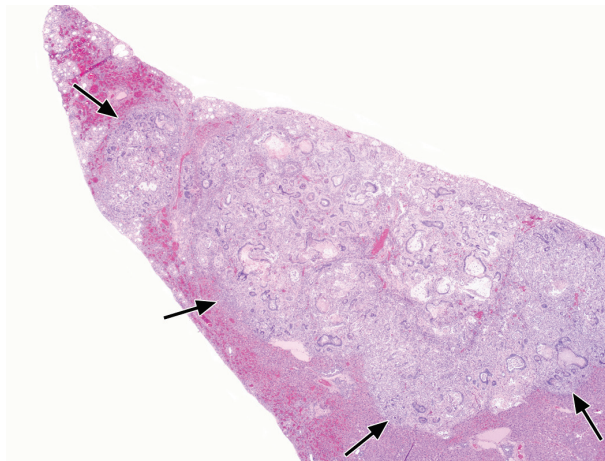
Higher magnification of Plate 7. A single layer of endothelial cells lines the dilated lymphatics (arrows). H&E



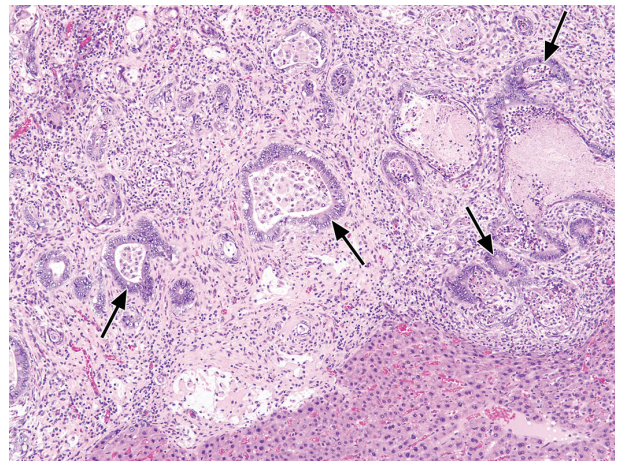
**PLATE 9**  
Lymphatic ectasia in the mesenteric lymph node of a female Harlan Sprague-Dawley rat administered 300 mg/kg indole-3-carbinol by gavage for 2 years. Note multiple dilated lymphatics in the medulla (arrows). H&E



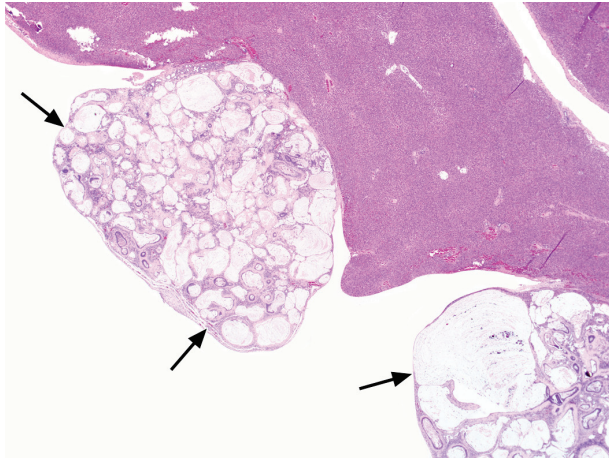
**PLATE 10**  
Higher magnification of Plate 9. Note the dilated lymphatics (long arrows) and aggregates of macrophages that have golden brown cytoplasmic pigment (short arrows). H&E



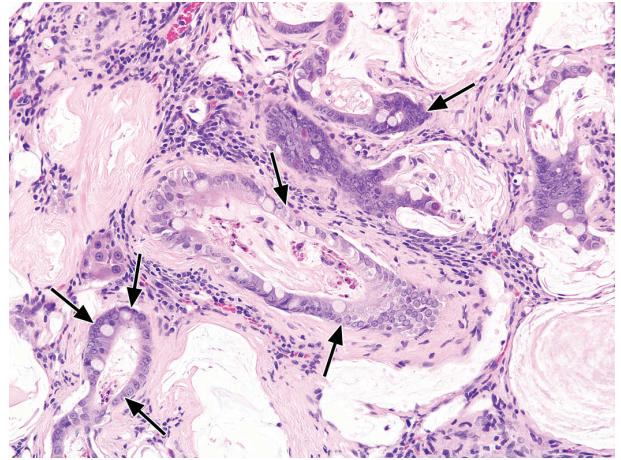
**PLATE 11**  
Cholangiofibrosis in the liver of a male Harlan Sprague-Dawley rat administered 150 mg/kg indole-3-carbinol by gavage for 2 years. Note that the lesion has effaced an extensive area of the hepatic parenchyma (arrows). H&E



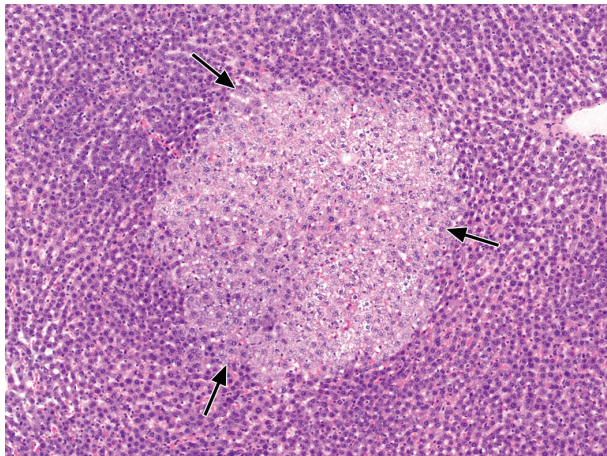
**PLATE 12**  
Higher magnification of Plate 11. Cholangiofibrosis consists of multiple, variably sized, irregular, atypical bile ducts surrounded by abundant fibrous tissue infiltrated by inflammatory cells (arrows). Ducts contain mucous material, exfoliated cells, and cellular debris. H&E



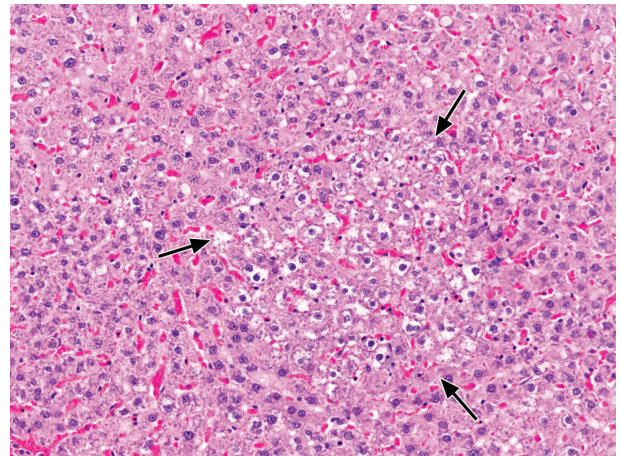
**PLATE 13**  
 Cholangiofibrosis in the liver of a male Harlan Sprague-Dawley rat administered 150 mg/kg indole-3-carbinol by gavage for 2 years. Note the nodular lesions (arrows) protruding from the capsular surface. H&E



**PLATE 14**  
 Higher magnification of Plate 13. Note the dilated bile ducts lined by atypical epithelial cells (arrows). The ducts contain mucous and cellular debris. H&E

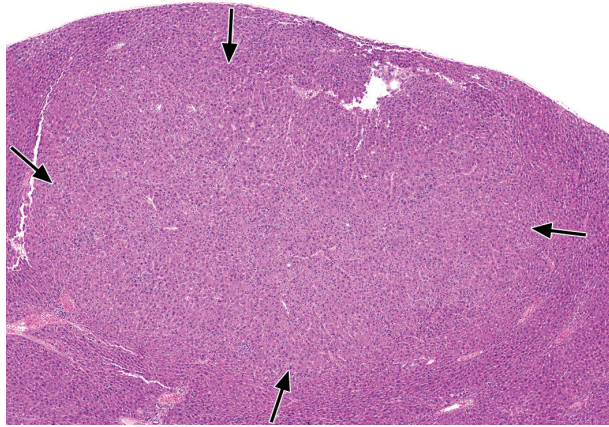


**PLATE 15**  
 Eosinophilic focus in the liver of a male Harlan Sprague-Dawley rat administered 75 mg/kg indole-3-carbinol by gavage for 2 years. Note the discrete lesion (arrows) that is well-demarcated from the surrounding hepatic parenchyma. H&E

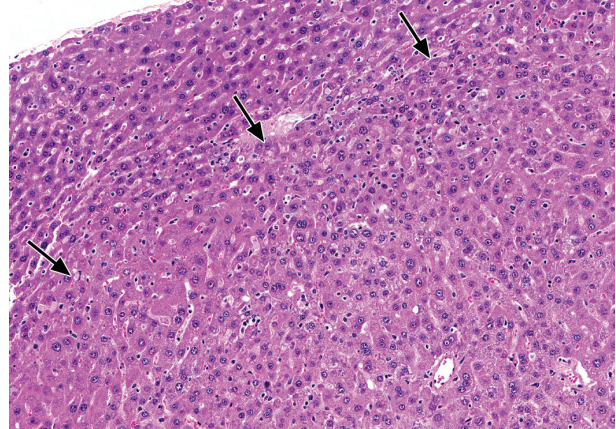


**PLATE 16**  
 Clear cell focus (arrows) in the liver of a male Harlan Sprague-Dawley rat administered 75 mg/kg indole-3-carbinol by gavage for 2 years. H&E

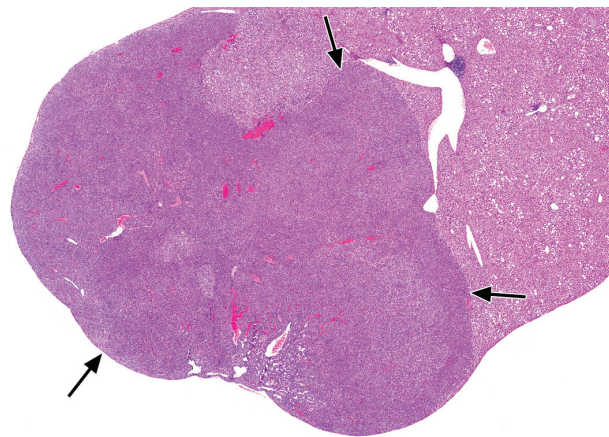




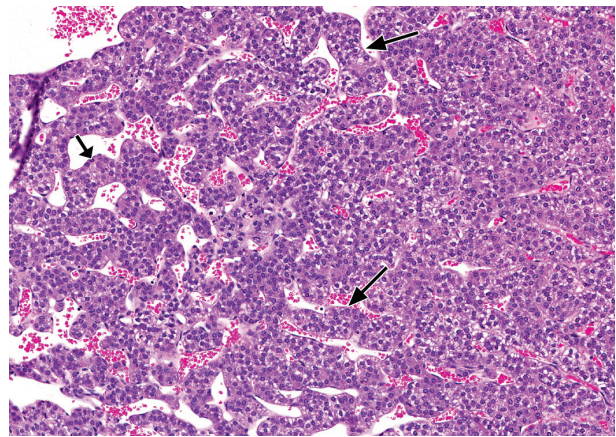
**PLATE 17**  
Hepatocellular adenoma (arrows) in the liver of a male B6C3F1/N mouse administered 250 mg/kg indole-3-carbinol by gavage for 2 years. H&E



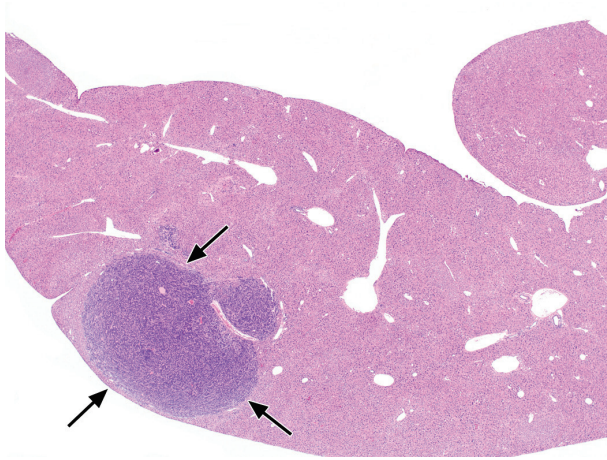
**PLATE 18**  
Higher magnification of Plate 17. Note the loss of normal hepatic architecture (left of arrows) within the adenoma (right of arrows) and distorted hepatic plates obliquely impinging upon the surrounding hepatic parenchyma. H&E



**PLATE 19**  
Large, well-demarcated, nodular hepatocellular carcinoma (arrows) in a male B6C3F1/N mouse administered 250 mg/kg indole-3-carbinol by gavage for 2 years. The neoplasm has completely effaced the hepatic parenchyma. H&E

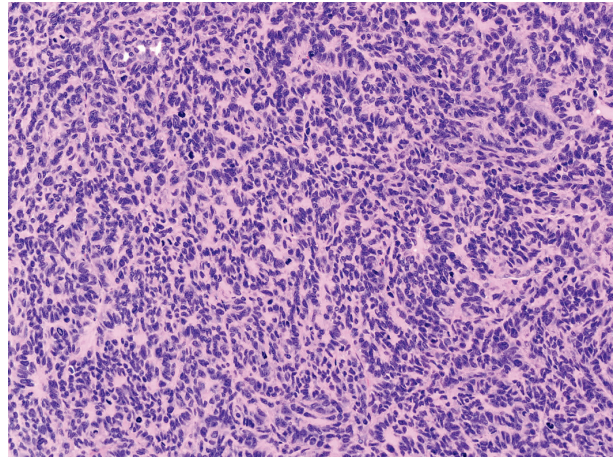


**PLATE 20**  
Higher magnification of Plate 19. Note the thick trabeculae of neoplastic hepatocytes (arrows) that are a characteristic feature of hepatocellular carcinoma. H&E



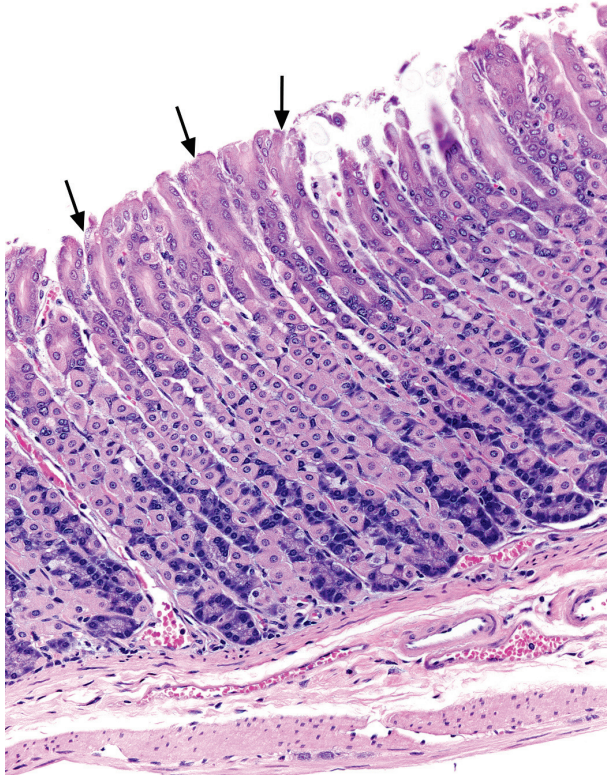
**PLATE 21**

Hepatoblastoma (arrows) in the liver of a male B6C3F1/N mouse administered 250 mg/kg indole-3-carbinol by gavage for 2 years. The neoplasm is well-demarcated from the surrounding hepatic parenchyma and stains deeply basophilic. H&E

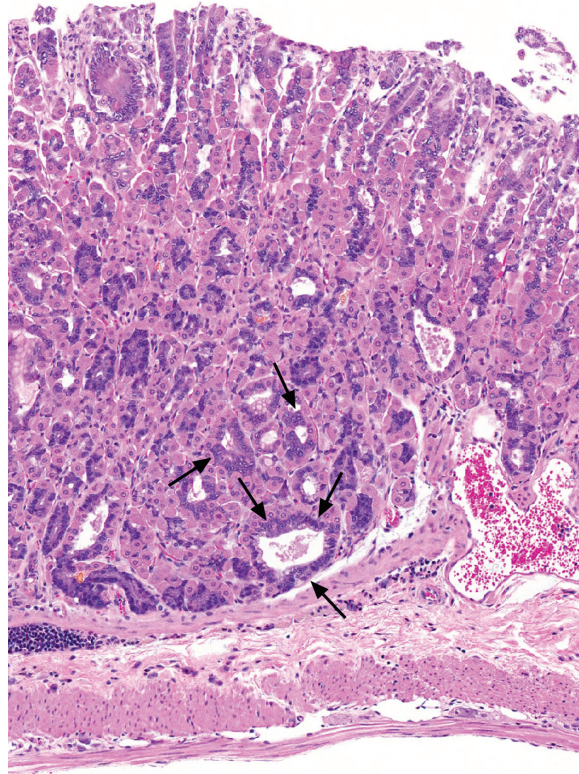


**PLATE 22**

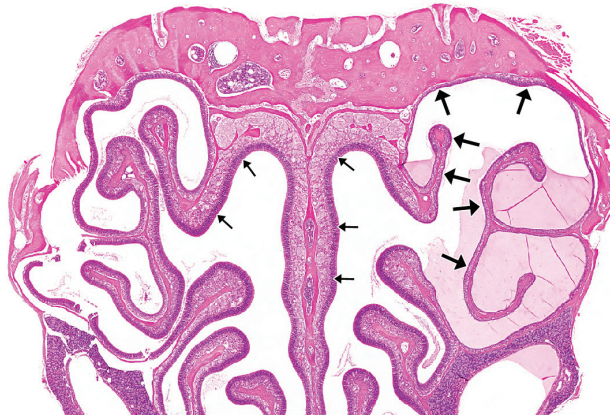
Higher magnification of Plate 21. Cells of the hepatoblastoma are small, elongate to spindle-shaped with hyperchromatic nuclei, and are separated by scant connective tissue stroma. H&E



**PLATE 23**  
Normal glandular stomach epithelium in a vehicle control female B6C3F1/N mouse in the 2-year gavage study of indole-3-carbinol. 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. H&E

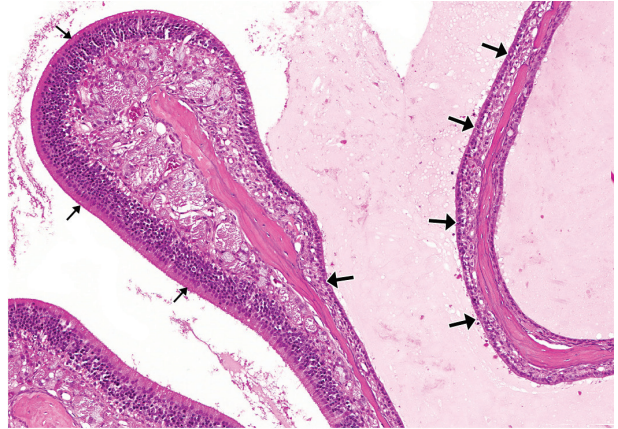


**PLATE 24**  
Hyperplasia of the glandular epithelium of the stomach in a male B6C3F1/N mouse administered 250 mg/kg indole-3-carbinol by gavage for 2 years. Note that there is disorganization (piling up) and a slight increase in the number of epithelial (chief) cells lining the gland (arrows). H&E



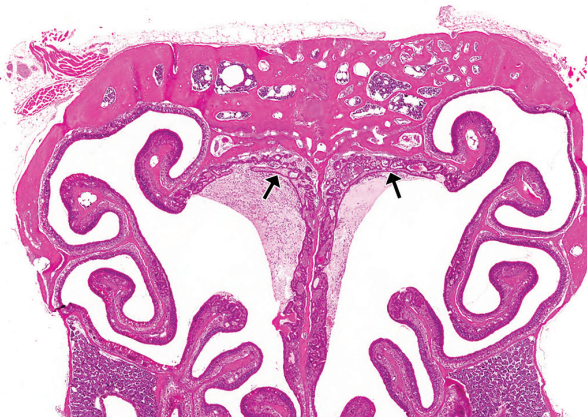
**PLATE 25**

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 2 years. 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. H&E



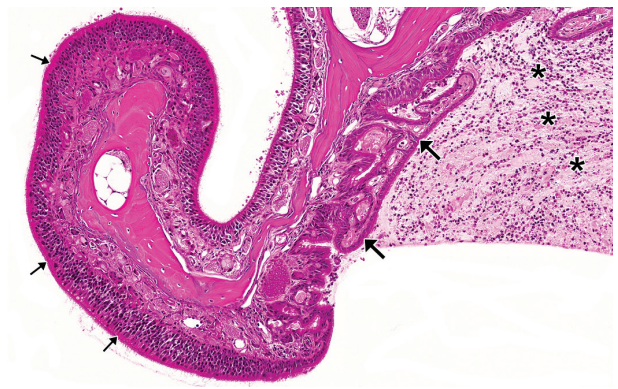
**PLATE 26**

Higher magnification of Plate 25. 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). H&E



**PLATE 27**

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 2 years showing respiratory epithelial metaplasia in the olfactory epithelium. 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). H&E



**PLATE 28**

Higher magnification of Plate 27. 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). H&E

## DISCUSSION AND CONCLUSIONS

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 (Leibelt *et al.*, 2003; Crowell, *et al.*, 2006). 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.* (2006) 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 (Baldwin and LeBlanc, 1992; Horn *et al.*, 2002; Leibel *et al.*, 2003; Crowell, *et al.*, 2006; Szaefer *et al.*, 2012). 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 (Sparnins *et al.*, 1982; Bradfield and Bjeldanes, 1984; Shertzer and Sainsbury, 1991). 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 AhR (Whitlock, 1999; Denison and Nagy, 2003; Mimura and Fujii-Kuriyama, 2003; Hankinson, 2005). 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 (Whitlock, 1993) 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 (Van den Berg *et al.*, 1998).

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 (Park *et al.*, 1996). 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 kills of special intestine study male Sprague Dawley rats in the 2-year bioassay (Boyle *et al.*, 2012). Since 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 (Boyle *et al.*, 2012). 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. Since 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 (Kim *et al.*, 1997).

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 (NTP, 2006a,b,c,d,e,f,g; 2010). In the liver, TCDD (NTP, 2006b), 3,3',4,4',5-pentachlorobiphenyl (PCB 126) (NTP, 2006a), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) (NTP, 2006c), 2,3',4,4',5-pentachlorobiphenyl (PCB 118) (NTP, 2010), and a tertiary mixture of TCDD, PCB 126, and PeCDF (NTP, 2006d) predominantly induce cholangiocarcinomas and hepatocellular adenomas. In addition to these neoplasms, a binary mixture of PCB126 and PCB 153 (NTP, 2006f) and a binary mixture of PCB126 and PCB 118 (NTP, 2006g) induce hepatocarcinomas. In general, exposure to these dioxin-like compounds induces cystic keratinizing epitheliomas in the lung (NTP, 2006a,b,d,e,f,g; 2010). Gingival squamous cell carcinomas were induced by exposure to TCDD (NTP, 2006b), PCB 126 (NTP, 2006a), PeCDF (NTP, 2006c), a binary mixture of PCB 126 and PCB 118 (NTP, 2006g), and a binary mixture of PCB 126 and PCB 153 (NTP, 2006f). 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 (NTP, 2006a,b,c,d,f,g; 2010). 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 (NTP, 2006a,b,c,d,g; 2010). 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 this 2-year gavage study, there was *no evidence of carcinogenic activity*\* 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.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 15.



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**APPENDIX A**  
**SUMMARY OF LESIONS**  
**IN MALE SPRAGUE DAWLEY RATS**  
**IN THE 2-YEAR GAVAGE STUDY**  
**OF INDOLE-3-CARBINOL**

<b>TABLE A1</b>	<b>Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>A-2</b>
<b>TABLE A2</b>	<b>Statistical Analysis of Primary Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>A-6</b>
<b>TABLE A3</b>	<b>Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>A-9</b>

**TABLE A1**  
**Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Disposition Summary</b>				
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 kill	20	13	17	12
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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)
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)
<b>Cardiovascular System</b>				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, fibrous histiocytoma, metastatic, skin				1 (2%)
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)	1 (2%)		
<b>Endocrine System</b>				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma		1 (2%)		1 (2%)



**TABLE A1**  
**Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Endocrine System</b> (continued)				
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%)		
<b>General Body System</b>				
None				
<b>Genital System</b>				
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%)
<b>Hematopoietic System</b>				
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%)		
<b>Integumentary System</b>				
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%)		

**TABLE A1**  
**Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Integumentary System (continued)</b>				
Skin (continued)	(50)	(50)	(50)	(50)
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%)	
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Vertebra, osteoma				1 (2%)
<b>Nervous System</b>				
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)
<b>Respiratory System</b>				
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)
<b>Special Senses System</b>				
Ear	(0)	(0)	(1)	(0)
Eye	(50)	(50)	(49)	(49)
Harderian gland	(50)	(50)	(49)	(50)
Lacrimal gland	(1)	(2)	(4)	(0)
<b>Urinary System</b>				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)			1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
<b>Systemic Lesions</b>				
Multiple organs <sup>b</sup>	(50)	(50)	(50)	(50)
Leukemia granulocytic				1 (2%)
Lymphoma malignant			1 (2%)	1 (2%)
Mesothelioma malignant	1 (2%)	1 (2%)		

**TABLE A1**  
**Summary of the Incidence of Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Neoplasm Summary</b>				
Total animals with primary neoplasms <sup>c</sup>	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

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with neoplasm

<sup>b</sup> Number of animals with any tissue examined microscopically

<sup>c</sup> Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE A2**  
**Statistical Analysis of Primary Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Adrenal Medulla: Benign Pheochromocytoma</b>				
Overall rate <sup>a</sup>	5/50 (10%)	5/49 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate <sup>b</sup>	13.0%	14.7%	13.5%	11.7%
Terminal rate <sup>c</sup>	3/20 (15%)	2/13 (15%)	2/17 (12%)	3/12 (25%)
First incidence (days)	645	571	593	621
Poly-3 test <sup>d</sup>	P=0.479N	P=0.549	P=0.605	P=0.577N
<b>Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma</b>				
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
<b>Pancreas: Adenoma</b>				
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
<b>Pancreas: Adenoma or Carcinoma</b>				
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
<b>Pituitary Gland (Pars Distalis): Adenoma</b>				
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
<b>Pituitary Gland (Pars Distalis): Adenoma or Carcinoma</b>				
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
<b>Skin: Keratoacanthoma</b>				
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	— <sup>e</sup>	—	—
Poly-3 test	P=0.015N	P=0.079N	P=0.066N	P=0.077N
<b>Skin: Squamous Cell Papilloma or Keratoacanthoma</b>				
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

**TABLE A2**  
**Statistical Analysis of Primary Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma</b>				
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
<b>Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Carcinoma, or Squamous Cell Carcinoma</b>				
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
<b>Skin (Subcutaneous Tissue): Fibroma</b>				
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
<b>Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</b>				
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
<b>Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</b>				
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
<b>Testes: Adenoma</b>				
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
<b>Thyroid Gland (C-Cell): Adenoma</b>				
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
<b>Thyroid Gland (C-Cell): Adenoma or Carcinoma</b>				
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

**TABLE A2**  
**Statistical Analysis of Primary Neoplasms in Male Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>All Organs: Benign Neoplasms</b>				
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
<b>All Organs: Malignant Neoplasms</b>				
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
<b>All Organs: Benign or Malignant Neoplasms</b>				
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 kill

<sup>a</sup> 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.

<sup>b</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> 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 kill. A negative trend or a lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Disposition Summary</b>				
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 kill	20	13	17	12
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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%)
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%)

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with lesion

**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Alimentary System (continued)</b>				
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%)		
Epithelium, muscularis, hyperplasia, focal		1 (2%)		
Tongue	(0)	(0)	(0)	(1)
Tooth	(1)	(0)	(1)	(0)
Malformation			1 (100%)	
Necrosis	1 (100%)			
<b>Cardiovascular System</b>				
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%)	
<b>Endocrine System</b>				
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%)



**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Endocrine System</b> (continued)				
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)
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%)
<b>General Body System</b>				
None				
<b>Genital System</b>				
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%)

**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Hematopoietic System</b>				
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%)	
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%)			
<b>Integumentary System</b>				
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%)
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy	12 (24%)	19 (38%)	13 (26%)	8 (16%)
Maxilla, fracture			1 (2%)	

**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Nervous System</b>				
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%)	
<b>Respiratory System</b>				
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%)
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%)	
<b>Special Senses System</b>				
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%)	

**TABLE A3**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Urinary System</b>				
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%)

**APPENDIX B**  
**SUMMARY OF LESIONS**  
**IN FEMALE SPRAGUE DAWLEY RATS**  
**IN THE 2-YEAR GAVAGE STUDY**  
**OF INDOLE-3-CARBINOL**

<b>TABLE B1</b>	<b>Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>B-2</b>
<b>TABLE B2</b>	<b>Statistical Analysis of Primary Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>B-6</b>
<b>TABLE B3a</b>	<b>Historical Incidence of Carcinoma of the Uterus in Control Female Sprague Dawley Rats .....</b>	<b>B-9</b>
<b>TABLE B3b</b>	<b>Historical Incidence of Skin Neoplasms in Control Female Sprague Dawley Rats.....</b>	<b>B-9</b>
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**TABLE B1**  
**Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Disposition Summary</b>				
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 kill	21	18	19	30
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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)
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)
<b>Cardiovascular System</b>				
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%)	

**TABLE B1**  
**Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Endocrine System</b>				
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%)	
<b>General Body System</b>				
None				
<b>Genital System</b>				
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%)
<b>Hematopoietic System</b>				
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%)	

**TABLE B1**  
**Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Integumentary System</b>				
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%)	
<b>Musculoskeletal System</b>				
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%)			
<b>Nervous System</b>				
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)
<b>Respiratory System</b>				
Lung	(50)	(50)	(50)	(49)
Adenocarcinoma, metastatic, uterus		1 (2%)	1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma	1 (2%)		1 (2%)	
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)
<b>Special Senses System</b>				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Schwannoma malignant, metastatic, peripheral nerve		1 (2%)		



**TABLE B1**  
**Summary of the Incidence of Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Urinary System</b>				
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%)	
<b>Systemic Lesions</b>				
Multiple organs <sup>b</sup>	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear		2 (4%)		
Lymphoma malignant			1 (2%)	1 (2%)
<b>Neoplasm Summary</b>				
Total animals with primary neoplasms <sup>c</sup>	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

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with neoplasm

<sup>b</sup> Number of animals with any tissue examined microscopically

<sup>c</sup> Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE B2**  
**Statistical Analysis of Primary Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Liver: Hepatocellular Adenoma</b>				
Overall rate <sup>a</sup>	2/50 (4%)	1/50 (2%)	4/50 (8%)	0/48 (0%)
Adjusted rate <sup>b</sup>	5.5%	2.7%	11.0%	0.0%
Terminal rate <sup>c</sup>	2/21 (10%)	1/19 (5%)	2/19 (11%)	0/30 (0%)
First incidence (days)	729 (T)	729 (T)	596	— <sup>e</sup>
Poly-3 test <sup>d</sup>	P=0.227N	P=0.498N	P=0.335	P=0.216N
<b>Liver: Hepatocellular Adenoma or Carcinoma</b>				
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
<b>Mammary Gland: Fibroadenoma</b>				
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
<b>Mammary Gland: Adenoma</b>				
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
<b>Mammary Gland: Fibroadenoma or Adenoma</b>				
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
<b>Mammary Gland: Carcinoma</b>				
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%)
First incidence (days)	446	705	729 (T)	—
Poly-3 test	P=0.077N	P=0.522N	P=0.536N	P=0.111N
<b>Mammary Gland: Adenoma or Carcinoma</b>				
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
<b>Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma</b>				
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

**TABLE B2**  
**Statistical Analysis of Primary Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Pituitary Gland (Pars Distalis): Adenoma</b>				
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
<b>Skin (Subcutaneous Tissue): Fibroma</b>				
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
<b>Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma</b>				
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
Overall rate	P=0.040	P=0.237N	P=0.243N	P=0.254
<b>Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma</b>				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	6/50 (12%)
Adjusted rate	5.4%	0.0%	0.0%	14.9%
Terminal rate	1/21 (5%)	0/19 (0%)	0/19 (0%)	4/30 (13%)
First incidence (days)	645	—	—	692
Overall rate	P=0.013	P=0.237N	P=0.243N	P=0.163
<b>Thyroid Gland (C-Cell): Adenoma</b>				
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
<b>Thyroid Gland (C-Cell): Adenoma or Carcinoma</b>				
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
<b>Uterus: Stromal Polyp (Standard Evaluation)</b>				
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
<b>Uterus: Stromal Polyp or Stromal Sarcoma (Standard Evaluation)</b>				
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

**TABLE B2**  
**Statistical Analysis of Primary Neoplasms in Female Sprague Dawley Rats in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Uterus: Adenocarcinoma (Standard Evaluation)</b>				
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
<b>Uterus: Adenocarcinoma (Extended Evaluation)</b>				
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
<b>Uterus: Adenocarcinoma or Adenosquamous Carcinoma (Standard and Extended Evaluations)</b>				
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
<b>All Organs: Benign Neoplasms</b>				
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
<b>All Organs: Malignant Neoplasms</b>				
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
Poly-3 test	P=0.356	P=0.374N	P=0.464	P=0.498
<b>All Organs: Benign or Malignant Neoplasms</b>				
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 kill

<sup>a</sup> 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.

<sup>b</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> 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 kill. A negative trend or a lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE B3a**  
**Historical Incidence of Carcinoma of the Uterus in Control Female Sprague Dawley Rats<sup>a</sup>**

Study (Study Start)	Incidence in Controls
<b>Historical Incidence: Corn Oil Gavage Studies<sup>b</sup></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%

<sup>a</sup> Data as of June 2013

<sup>b</sup> Same data for overall historical incidence for all routes

**TABLE B3b**  
**Historical Incidence of Skin Neoplasms in Control Female Sprague Dawley Rats<sup>a</sup>**

Study (Study Start)	Fibroma	Fibrosarcoma	Fibroma or Fibrosarcoma
<b>Historical Incidence: Corn Oil Gavage Studies<sup>b</sup></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%

<sup>a</sup> Data as of June 2013

<sup>b</sup> Same data for overall historical incidence for all routes

**TABLE B4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Disposition Summary</b>				
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 kill	21	18	19	30
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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%)			
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%)			

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with lesion

**TABLE B4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Alimentary System (continued)</b>				
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)
<b>Cardiovascular System</b>				
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%)
Atrium, thrombosis		1 (2%)		
Epicardium, inflammation	1 (2%)	3 (6%)		1 (2%)
Pericardium, inflammation			2 (4%)	
<b>Endocrine System</b>				
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%)
<b>General Body System</b>				
None				
<b>Genital System</b>				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	5 (10%)	2 (4%)	3 (6%)	4 (8%)
Inflammation	1 (2%)			2 (4%)

**TABLE B4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Genital System (continued)</b>				
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%)
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)
<b>Hematopoietic System</b>				
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%)
Hemorrhage				1 (2%)
Inflammation		1 (2%)	1 (2%)	
Epithelial cell, hyperplasia	1 (2%)	4 (8%)	3 (6%)	
<b>Integumentary System</b>				
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%)			



**TABLE B4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Integumentary System (continued)</b>				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		1 (2%)
Fibrosis				1 (2%)
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy		1 (2%)	1 (2%)	
Skeletal muscle	(1)	(0)	(1)	(1)
Degeneration			1 (100%)	
<b>Nervous System</b>				
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%)		
<b>Respiratory System</b>				
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%)
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%)		

**TABLE B4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Sprague Dawley Rats**  
**in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
<b>Special Senses System</b>				
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%)		
<b>Urinary System</b>				
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%)	

**APPENDIX C**  
**SUMMARY OF LESIONS IN MALE MICE**  
**IN THE 2-YEAR GAVAGE STUDY**  
**OF INDOLE-3-CARBINOL**

<b>TABLE C1</b>	<b>Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>C-2</b>
<b>TABLE C2</b>	<b>Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>C-6</b>
<b>TABLE C3</b>	<b>Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice .....</b>	<b>C-9</b>
<b>TABLE C4</b>	<b>Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>C-10</b>

**TABLE C1**  
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Disposition Summary</b>				
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 kill	26	31	32	32
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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%)
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%)			

**TABLE C1**  
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Alimentary System (continued)</b>				
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)
<b>Cardiovascular System</b>				
Blood vessel	(49)	(50)	(49)	(49)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Hemangiosarcoma		1 (2%)		
<b>Endocrine System</b>				
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%)
<b>General Body System</b>				
None				

**TABLE C1**  
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Genital System</b>				
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%)
<b>Hematopoietic System</b>				
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)
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%)			
<b>Integumentary System</b>				
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%)	
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(2)	(0)
Hemangiosarcoma			2 (100%)	
<b>Nervous System</b>				
Brain	(50)	(50)	(50)	(50)
Granular cell tumor benign	1 (2%)			

**TABLE C1**  
**Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Respiratory System</b>				
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)
<b>Special Senses System</b>				
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%)		
<b>Urinary System</b>				
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)
<b>Systemic Lesions</b>				
Multiple organs <sup>b</sup>	(50)	(50)	(50)	(50)
Histiocytic sarcoma	2 (4%)	1 (2%)		1 (2%)
Lymphoma malignant	3 (6%)	1 (2%)	1 (2%)	5 (10%)
<b>Neoplasm Summary</b>				
Total animals with primary neoplasms <sup>c</sup>	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	

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with neoplasm

<sup>b</sup> Number of animals with any tissue examined microscopically

<sup>c</sup> Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE C2**  
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Harderian Gland: Adenoma</b>				
Overall rate <sup>a</sup>	6/50 (12%)	7/50 (14%)	7/50 (14%)	3/50 (6%)
Adjusted rate <sup>b</sup>	14.5%	15.4%	15.6%	6.7%
Terminal rate <sup>c</sup>	2/26 (8%)	5/31 (16%)	4/32 (13%)	1/32 (3%)
First incidence (days)	463	616	437	437
Poly-3 test <sup>d</sup>	P=0.144N	P=0.571	P=0.564	P=0.205N
<b>Harderian Gland: Adenoma or Carcinoma</b>				
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
<b>Liver: Hemangiosarcoma</b>				
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
<b>Liver: Hepatocellular Adenoma</b>				
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
<b>Liver: Hepatocellular Carcinoma</b>				
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
<b>Liver: Hepatocellular Adenoma or Carcinoma</b>				
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
<b>Liver: Hepatoblastoma</b>				
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
<b>Liver: Hepatocellular Carcinoma or Hepatoblastoma</b>				
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



**TABLE C2**  
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</b>				
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
<b>Liver: Hepatocholangiocarcinoma</b>				
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)	— <sup>e</sup>	630	730 (T)	—
Poly-3 test	P=0.319N	P=0.146	P=0.515	— <sup>f</sup>
<b>Lung: Alveolar/bronchiolar Adenoma</b>				
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
<b>Lung: Alveolar/bronchiolar Carcinoma</b>				
Overall rate	4/50 (8%)	7/50 (14%)	5/50 (10%)	5/50 (10%)
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
<b>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</b>				
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
<b>Small Intestine (Site Unspecified): Adenoma</b>				
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
<b>Small Intestine (Site Unspecified): Adenoma or Carcinoma</b>				
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
<b>All Organs: Hemangiosarcoma</b>				
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

**TABLE C2**  
**Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>All Organs: Hemangioma or Hemangiosarcoma</b>				
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
<b>All Organs: Malignant Lymphoma</b>				
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
<b>All Organs: Benign Neoplasms</b>				
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
<b>All Organs: Malignant Neoplasms</b>				
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
<b>All Organs: Benign or Malignant Neoplasms</b>				
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 kill

<sup>a</sup> 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.

<sup>b</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> 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 kill. A negative trend or a lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

<sup>f</sup> Value of statistic cannot be computed.

**TABLE C3**  
**Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma	Hepatoblastoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>				
<i>Ginkgo biloba</i> 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
<i>N,N</i> -Dimethyl- <i>p</i> -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%
<b>Overall Historical Incidence: All Routes</b>				
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%
		<b>Hepatocellular Carcinoma or Hepatoblastoma</b>	<b>Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</b>	<b>Hepatocholeangioma</b>
<b>Historical Incidence: Corn Oil Gavage Studies</b>				
<i>Ginkgo biloba</i> 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
<i>N,N</i> -Dimethyl- <i>p</i> -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%
<b>Overall Historical Incidence: All Routes</b>				
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%

<sup>a</sup> Data as of June 2013

**TABLE C4**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Disposition Summary</b>				
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 kill	26	31	32	32
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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%)
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%)		

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with lesion

**TABLE C4**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Alimentary System</b> (continued)				
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%)
<b>Cardiovascular System</b>				
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%)
<b>Endocrine System</b>				
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%)
<b>General Body System</b>				
None				

**TABLE C4**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Genital System</b>				
Coagulating gland	(1)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
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%)
<b>Hematopoietic System</b>				
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%)
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%)
<b>Integumentary System</b>				
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%)	

**TABLE C4**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Hyperostosis			1 (2%)	1 (2%)
Skeletal muscle	(0)	(0)	(2)	(0)
<b>Nervous System</b>				
Brain	(50)	(50)	(50)	(50)
Meninges, inflammation, chronic		1 (2%)		
<b>Respiratory System</b>				
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%)		
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)
<b>Special Senses System</b>				
Eye	(50)	(50)	(50)	(50)
Phthisis bulbi	1 (2%)	1 (2%)		
Anterior chamber, inflammation, suppurative				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%)		

**TABLE C4**  
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Urinary System</b>				
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%)		
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%)		



**APPENDIX D**  
**SUMMARY OF LESIONS IN FEMALE MICE**  
**IN THE 2-YEAR GAVAGE STUDY**  
**OF INDOLE-3-CARBINOL**

<b>TABLE D1</b>	<b>Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>D-2</b>
<b>TABLE D2</b>	<b>Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>D-5</b>
<b>TABLE D3</b>	<b>Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice.....</b>	<b>D-8</b>
<b>TABLE D4</b>	<b>Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol.....</b>	<b>D-9</b>

**TABLE D1**  
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Disposition Summary</b>				
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 kill	33	40	25	45
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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)
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)
<b>Cardiovascular System</b>				
Blood vessel	(48)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Heart	(50)	(50)	(50)	(50)
<b>Endocrine System</b>				
Adrenal cortex	(49)	(50)	(50)	(50)
Adenoma	1 (2%)			
Subcapsular, adenoma	1 (2%)			
Adrenal medulla	(49)	(50)	(50)	(50)

**TABLE D1**  
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Endocrine System</b> (continued)				
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%)
<b>General Body System</b>				
Tissue NOS	(0)	(0)	(1)	(0)
<b>Genital System</b>				
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%)
<b>Hematopoietic System</b>				
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%)	
<b>Integumentary System</b>				
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%)			
<b>Musculoskeletal System</b>				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(1)	(2)	(0)
Fibrosarcoma			1 (50%)	
Rhabdomyosarcoma			1 (50%)	
Schwannoma malignant		1 (100%)		

**TABLE D1**  
**Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Nervous System</b>				
Brain	(50)	(50)	(50)	(50)
Meningioma benign		1 (2%)		
<b>Respiratory System</b>				
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%)	
Squamous cell carcinoma, metastatic, skin			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
<b>Special Senses System</b>				
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%)
<b>Urinary System</b>				
Kidney	(50)	(50)	(50)	(50)
Bilateral, renal tubule, carcinoma		1 (2%)		
Renal tubule, carcinoma, multiple				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
<b>Systemic Lesions</b>				
Multiple organs <sup>b</sup>	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymphoma malignant	6 (12%)	9 (18%)	6 (12%)	3 (6%)
<b>Neoplasm Summary</b>				
Total animals with primary neoplasms <sup>c</sup>	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	

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with neoplasm

<sup>b</sup> Number of animals with any tissue examined microscopically

<sup>c</sup> Primary neoplasms: all neoplasms except metastatic neoplasms

**TABLE D2**  
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Harderian Gland: Adenoma</b>				
Overall rate <sup>a</sup>	3/50 (6%)	7/50 (14%)	7/50 (14%)	0/50 (0%)
Adjusted rate <sup>b</sup>	6.6%	15.0%	16.6%	0.0%
Terminal rate <sup>c</sup>	3/33 (9%)	5/40 (13%)	5/26 (19%)	0/45 (0%)
First incidence (days)	729 (T)	681	381	— <sup>e</sup>
Poly-3 test <sup>d</sup>	P=0.072N	P=0.170	P=0.128	P=0.111N
<b>Harderian Gland: Adenoma or Carcinoma</b>				
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
<b>Liver: Hepatocellular Adenoma</b>				
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
<b>Liver: Hepatocellular Carcinoma</b>				
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
<b>Liver: Hepatocellular Adenoma or Carcinoma</b>				
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
<b>Liver: Hepatocellular Carcinoma or Hepatoblastoma</b>				
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%)
First incidence (days)	709	650	606	729 (T)
Poly-3 test	P=0.257N	P=0.409	P=0.154	P=0.341N
<b>Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</b>				
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
<b>Lung: Alveolar/bronchiolar Adenoma</b>				
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

**TABLE D2**  
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Lung: Alveolar/bronchiolar Carcinoma</b>				
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
<b>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</b>				
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
<b>Ovary: Cystadenoma</b>				
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
<b>Pituitary Gland (Pars Distalis): Adenoma</b>				
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%)
First incidence (days)	729 (T)	729 (T)	—	729 (T)
Poly-3 test	P=0.108N	P=0.525	P=0.135N	P=0.288N
<b>Uterus: Stromal Polyp</b>				
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	— <sup>f</sup>	—	P=0.067
<b>All Organs: Hemangioma or Hemangiosarcoma</b>				
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
<b>All Organs: Malignant Lymphoma</b>				
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
<b>All Organs: Benign Neoplasms</b>				
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

**TABLE D2**  
**Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>All Organs: Malignant Neoplasms</b>				
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
<b>All Organs: Benign or Malignant Neoplasms</b>				
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%)
First incidence (days)	568	558	381	491
Poly-3 test	P=0.182N	P=0.393	P=0.105	P=0.252N

(T) Terminal kill

<sup>a</sup> 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.

<sup>b</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> 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 kill. A negative trend or a lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

<sup>f</sup> Value of statistic cannot be computed.

**TABLE D3**  
**Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice<sup>a</sup>**

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma
<b>Historical Incidence: Corn Oil Gavage Studies</b>			
<i>Ginkgo biloba</i> 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
<i>N,N</i> -Dimethyl- <i>p</i> -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%
<b>Overall Historical Incidence: All Routes</b>			
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%

<sup>a</sup> Data as of June 2013



**TABLE D4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Disposition Summary</b>				
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 kill	33	40	25	45
Animals examined microscopically	50	50	50	50
<b>Alimentary System</b>				
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%)
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%)	

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with lesion

**TABLE D4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Alimentary System (continued)</b>				
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%)
<b>Cardiovascular System</b>				
Blood vessel	(48)	(50)	(50)	(50)
Mineralization			1 (2%)	
Aorta, inflammation, chronic				1 (2%)
Heart	(50)	(50)	(50)	(50)
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%)
<b>Endocrine System</b>				
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%)
<b>General Body System</b>				
Tissue NOS	(0)	(0)	(1)	(0)
Inflammation, chronic			1 (100%)	

**TABLE D4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Genital System</b>				
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%)
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%)
<b>Hematopoietic System</b>				
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%)		
<b>Integumentary System</b>				
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%)	
<b>Musculoskeletal System</b>				
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)

**TABLE D4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Nervous System</b>				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Inflammation, granulomatous				1 (2%)
Hypothalamus, compression	2 (4%)			
Meninges, inflammation, chronic		1 (2%)		
<b>Respiratory System</b>				
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%)
Respiratory epithelium, metaplasia, squamous	1 (2%)			
Respiratory epithelium, necrosis	2 (4%)		1 (2%)	
Trachea	(50)	(50)	(50)	(50)
<b>Special Senses System</b>				
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%)

**TABLE D4**  
**Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study**  
**of Indole-3-carbinol**

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
<b>Urinary System</b>				
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%)



## APPENDIX E

# GENETIC TOXICOLOGY

<b>BACTERIAL MUTAGENICITY TEST PROTOCOL</b> .....	<b>E-2</b>
<b>RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL</b> .....	<b>E-2</b>
<b>MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL</b> .....	<b>E-3</b>
<b>EVALUATION PROTOCOL</b> .....	<b>E-3</b>
<b>RESULTS</b> .....	<b>E-3</b>
<b>TABLE E1</b> Mutagenicity of Indole-3-carbinol in <i>Salmonella typhimurium</i> .....	<b>E-4</b>
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<b>TABLE E3</b> Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male F344/N Rats Following Treatment with Indole-3-carbinol by Gavage for 3 Days .....	<b>E-11</b>
<b>TABLE E4</b> Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Indole-3-carbinol by Gavage for 3 Months .....	<b>E-12</b>

## GENETIC TOXICOLOGY

### 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.* (1992); 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.

### 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.* (1993). The positive control was 25 mg/kg cyclophosphamide and the vehicle control was corn oil. The rats were killed 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.

## MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). 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.

## 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.

## 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 E1). A second study, using strains TA97, TA98, TA100, TA102, TA104, TA1535, and TA1537, and concentrations ranging from 33 to 3,333 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 E1). 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 E2).

*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-carbinole was toxic to the bone marrow (Table E3). 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 E4).

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium*<sup>a</sup>**

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 10% rat S9
<b>Study 1</b>							
<b>TA100</b>	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 <sup>b</sup>		86 ± 13 <sup>b</sup>		148 ± 5	74 ± 7
	3,333				63 ± 8 <sup>b</sup>		
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control <sup>c</sup>		218 ± 12	327 ± 9	813 ± 96	231 ± 46	854 ± 30	635 ± 34
<b>With 30% rat S9</b>							
<b>TA100 (continued)</b>	0	84 ± 3					
	33	81 ± 7					
	100	76 ± 9					
	333	76 ± 4					
	1,000	80 ± 6 <sup>b</sup>					
	3,333	38 ± 4 <sup>b</sup>					
Trial summary		Negative					
Positive control		208 ± 10					
<b>Without S9      Without S9      Without S9      With 10% hamster S9      With 10% hamster S9      With 30% hamster S9</b>							
<b>TA97</b>	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 <sup>d</sup>			202 ± 14	58 ± 10	123 ± 10
	2,500					0 <sup>b</sup>	
Trial summary		Equivocal	Equivocal	Negative	Equivocal	Negative	Negative
Positive control		740 ± 78	214 ± 24	328 ± 6	958 ± 82	1,265 ± 30	536 ± 40

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium***

		With 10% rat S9	With 10% rat S9	With 30% rat S9			
<b>Study 1 (continued)</b>							
<b>TA97 (continued)</b>	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			
		Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
<b>TA98</b>	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 <sup>b</sup>		12 ± 1 <sup>b</sup>	
	3,333				14 ± 2 <sup>b</sup>		14 ± 1 <sup>b</sup>
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		68 ± 5	90 ± 5	751 ± 22	55 ± 2	457 ± 66	82 ± 13
<b>TA1535</b>	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 <sup>e</sup>		0 <sup>e</sup>
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		531 ± 92	364 ± 34	80 ± 13	210 ± 11	355 ± 60	79 ± 5
		Without S9					
<b>TA1537</b>	0	5 ± 0					
	33	8 ± 3					
	100	5 ± 2					
	333	4 ± 1					
	1,000	7 ± 1					
	1,500	3 ± 2 <sup>b</sup>					
Trial summary		Negative					
Positive control		13 ± 1					

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium***

Strain	Dose ( $\mu\text{g}/\text{plate}$ )	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 30% hamster S9	With 10% rat S9
<b>Study 2</b>							
<b>TA102</b>	0	277 $\pm$ 27	366 $\pm$ 5	347 $\pm$ 22	358 $\pm$ 9	281 $\pm$ 11	348 $\pm$ 11
	33	270 $\pm$ 28	378 $\pm$ 6	429 $\pm$ 22	357 $\pm$ 10		436 $\pm$ 24
	100	262 $\pm$ 8	362 $\pm$ 6	421 $\pm$ 14	397 $\pm$ 31	291 $\pm$ 21	405 $\pm$ 33
	333	280 $\pm$ 25	345 $\pm$ 14	394 $\pm$ 11	389 $\pm$ 23	295 $\pm$ 13	416 $\pm$ 49
	1,000	185 $\pm$ 10	255 $\pm$ 50	333 $\pm$ 7	434 $\pm$ 25	220 $\pm$ 12	320 $\pm$ 18
	1,500					217 $\pm$ 11	
	3,333	30 $\pm$ 3 <sup>d</sup>	84 $\pm$ 5 <sup>d</sup>	82 $\pm$ 30 <sup>d</sup>	167 $\pm$ 32 <sup>e</sup>	96 $\pm$ 9 <sup>e</sup>	40 $\pm$ 7 <sup>d</sup>
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Negative
Positive control		875 $\pm$ 17	1,435 $\pm$ 32	1,849 $\pm$ 123	1,383 $\pm$ 22	1,503 $\pm$ 46	1,714 $\pm$ 98
		<b>With 30% rat S9</b>	<b>With 30% rat S9</b>				
<b>TA102 (continued)</b>	0	340 $\pm$ 21	233 $\pm$ 14				
	33	370 $\pm$ 22					
	100	408 $\pm$ 11	203 $\pm$ 6				
	333	388 $\pm$ 8	224 $\pm$ 4				
	1,000	422 $\pm$ 19	148 $\pm$ 12				
	1,500		115 $\pm$ 6				
	3,333	163 $\pm$ 30 <sup>e</sup>	102 $\pm$ 8 <sup>e</sup>				
Trial summary		Equivocal	Negative				
Positive control		1,107 $\pm$ 66	1,529 $\pm$ 46				
		<b>Without S9</b>	<b>Without S9</b>	<b>With 10% hamster S9</b>	<b>With 30% hamster S9</b>	<b>With 10% rat S9</b>	<b>With 30% rat S9</b>
<b>TA104</b>	0	222 $\pm$ 11	207 $\pm$ 2	249 $\pm$ 18	225 $\pm$ 8	206 $\pm$ 13	221 $\pm$ 7
	33	205 $\pm$ 9	122 $\pm$ 18	187 $\pm$ 7	180 $\pm$ 21	184 $\pm$ 7	219 $\pm$ 13
	100	213 $\pm$ 6	163 $\pm$ 11	216 $\pm$ 19	181 $\pm$ 10	204 $\pm$ 17	244 $\pm$ 17
	333	180 $\pm$ 19	147 $\pm$ 14	175 $\pm$ 19	183 $\pm$ 19	204 $\pm$ 15	207 $\pm$ 3
	1,000	185 $\pm$ 14	89 $\pm$ 3	208 $\pm$ 23	213 $\pm$ 2	181 $\pm$ 9	237 $\pm$ 22
	3,333	0 <sup>d</sup>	47 $\pm$ 11 <sup>d</sup>	205 $\pm$ 5 <sup>d</sup>	38 $\pm$ 1 <sup>d</sup>	207 $\pm$ 26 <sup>d</sup>	46 $\pm$ 3 <sup>d</sup>
	Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		705 $\pm$ 14	624 $\pm$ 6	1,080 $\pm$ 34	1,112 $\pm$ 36	1,173 $\pm$ 17	744 $\pm$ 61
		<b>Without S9</b>	<b>Without S9</b>	<b>Without S9</b>	<b>With 10% hamster S9</b>	<b>With 10% hamster S9</b>	<b>With 30% hamster S9</b>
<b>TA100</b>	0	90 $\pm$ 0	104 $\pm$ 8	149 $\pm$ 7	174 $\pm$ 7	139 $\pm$ 9	97 $\pm$ 10
	33	76 $\pm$ 10	120 $\pm$ 5	141 $\pm$ 11	180 $\pm$ 16	146 $\pm$ 7	89 $\pm$ 4
	100	114 $\pm$ 5	122 $\pm$ 7	161 $\pm$ 7	185 $\pm$ 4	138 $\pm$ 10	105 $\pm$ 6
	333	123 $\pm$ 7	144 $\pm$ 4	196 $\pm$ 23	213 $\pm$ 4	170 $\pm$ 5	144 $\pm$ 10
	1,000	133 $\pm$ 19	199 $\pm$ 7	166 $\pm$ 3	229 $\pm$ 20	183 $\pm$ 6	156 $\pm$ 5
	1,200		183 $\pm$ 9				
	3,333	46 $\pm$ 44 <sup>b</sup>		0 <sup>d</sup>	0 <sup>d</sup>	130 $\pm$ 9 <sup>e</sup>	149 $\pm$ 18
Trial summary		Weakly Positive	Weakly Positive	Negative	Negative	Negative	Weakly Positive
Positive control		225 $\pm$ 20	432 $\pm$ 47	594 $\pm$ 33	1,428 $\pm$ 58	845 $\pm$ 79	296 $\pm$ 25

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium***

Strain	Dose (µg/plate)	With 30% hamster S9	With 10% rat S9	With 10% rat S9	With 30% rat S9		
<b>Study 2 (continued)</b>							
<b>TA100 (continued)</b>	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 <sup>e</sup>	79 ± 4 <sup>e</sup>		
	3,333		0 <sup>d</sup>				
Trial summary		Weakly Positive	Equivocal	Negative	Negative		
Positive control		956 ± 35	1,259 ± 205	595 ± 37	357 ± 27		
		<b>Without S9</b>	<b>Without S9</b>	<b>With 10% hamster S9</b>	<b>With 30% hamster S9</b>	<b>With 30% hamster S9</b>	<b>With 10% rat S9</b>
<b>TA97</b>	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 <sup>e</sup>			210 ± 6 <sup>e</sup>	194 ± 12	
	2,000					124 ± 13	
	3,333		0 <sup>d</sup>	0 <sup>d</sup>			0 <sup>d</sup>
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
		<b>With 30% rat S9</b>	<b>With 30% rat S9</b>				
<b>TA97 (continued)</b>	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 <sup>e</sup>	175 ± 20				
2,000		95 ± 11					
Trial summary		Equivocal	Equivocal				
Positive control		553 ± 16	463 ± 15				

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium***

Strain	Dose ( $\mu\text{g}/\text{plate}$ )	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
<b>Study 2 (continued)</b>							
<b>TA98</b>	0	15 $\pm$ 2	18 $\pm$ 3	18 $\pm$ 5	17 $\pm$ 1	21 $\pm$ 3	19 $\pm$ 2
	33	13 $\pm$ 3	15 $\pm$ 1	21 $\pm$ 4	11 $\pm$ 1	18 $\pm$ 2	11 $\pm$ 1
	100	13 $\pm$ 1	16 $\pm$ 2	21 $\pm$ 5	17 $\pm$ 4	21 $\pm$ 2	13 $\pm$ 1
	333	13 $\pm$ 1	14 $\pm$ 3	21 $\pm$ 6	12 $\pm$ 1	27 $\pm$ 4	19 $\pm$ 1
	1,000	10 $\pm$ 2	9 $\pm$ 2	18 $\pm$ 3	13 $\pm$ 2	20 $\pm$ 1	17 $\pm$ 5
	1,500	0 <sup>b</sup>			6 $\pm$ 1		9 $\pm$ 2 <sup>b</sup>
	3,333		0 <sup>d</sup>	0 <sup>d</sup>		0 <sup>d</sup>	
	Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		115 $\pm$ 2	100 $\pm$ 7	1,536 $\pm$ 136	246 $\pm$ 8	629 $\pm$ 141	129 $\pm$ 18
		Without S9	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 30% hamster S9
<b>TA1535</b>	0	14 $\pm$ 2	14 $\pm$ 1	18 $\pm$ 2	14 $\pm$ 1	10 $\pm$ 1	13 $\pm$ 1
	33	13 $\pm$ 2		18 $\pm$ 3	13 $\pm$ 1	13 $\pm$ 0	
	100	14 $\pm$ 1		19 $\pm$ 3	14 $\pm$ 1	10 $\pm$ 1	
	333	13 $\pm$ 1	15 $\pm$ 2	20 $\pm$ 1	13 $\pm$ 1	13 $\pm$ 1	12 $\pm$ 2
	1,000	15 $\pm$ 1	10 $\pm$ 1	18 $\pm$ 2	13 $\pm$ 1	13 $\pm$ 1	12 $\pm$ 1
	1,200	12 $\pm$ 1				14 $\pm$ 3	
	1,500		8 $\pm$ 0				9 $\pm$ 1
	3,333		0 <sup>d</sup>	0 <sup>d</sup>	0 <sup>d</sup>		2 <sup>d</sup>
10,000		0 <sup>d</sup>				0 <sup>d</sup>	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		130 $\pm$ 5	166 $\pm$ 4	256 $\pm$ 39	287 $\pm$ 24	66 $\pm$ 3	155 $\pm$ 23
		With 10% rat S9	With 30% rat S9				
<b>TA1535 (continued)</b>	0	15 $\pm$ 0	13 $\pm$ 3				
	33	14 $\pm$ 2	12 $\pm$ 1				
	100	17 $\pm$ 3	10 <sup>f</sup>				
	333	15 $\pm$ 1	18 $\pm$ 2				
	1,000	12 $\pm$ 2	12 $\pm$ 1				
	1,500		8 $\pm$ 2 <sup>e</sup>				
3,333	0 <sup>d</sup>						
Trial summary		Negative	Negative				
Positive control		422 $\pm$ 19	95 $\pm$ 6				

**TABLE E1**  
**Mutagenicity of Indole-3-carbinol in *Salmonella typhimurium***

Strain	Dose ( $\mu\text{g}/\text{plate}$ )	With 30% hamster S9	With 30% rat S9
<b>Study 2 (continued)</b>			
TA1537	0	9 $\pm$ 2	9 $\pm$ 2
	100	8 $\pm$ 1	7 $\pm$ 1
	333	8 $\pm$ 1	10 $\pm$ 1
	1,000	9 $\pm$ 2	8 $\pm$ 1
	1,500	5 $\pm$ 1	5 $\pm$ 1
	2,000	4 $\pm$ 1 <sup>e</sup>	5 $\pm$ 1 <sup>e</sup>
Trial summary		Negative	Negative
Positive control		530 $\pm$ 58	143 $\pm$ 22

<sup>a</sup> Studies were performed at BioReliance Corporation. Data are presented as revertants/plate (mean  $\pm$  standard error) from three plates. The detailed protocol is presented by Zeiger *et al.* (1992). 0  $\mu\text{g}/\text{plate}$  was the solvent control.

<sup>b</sup> Slight toxicity

<sup>c</sup> 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.

<sup>d</sup> Slight toxicity and precipitate on plate

<sup>e</sup> Precipitate on plate

<sup>f</sup> Contamination

**TABLE E2**  
**Mutagenicity of Indole-3-carbinol in Bacterial Tester Strains<sup>a</sup>**

Strain	Dose (µg/plate)	Without S9	Without S9	Without S9	With 10% rat S9	With 10% rat S9	With 10% rat S9
<b>TA100</b>	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 <sup>b</sup>	26 ± 2	74 ± 1	84 ± 6	123 ± 5
	3,500	9 ± 1 <sup>b</sup>	Toxic	12 ± 3 <sup>b</sup>	45 ± 5	44 ± 8 <sup>c</sup>	23 ± 3 <sup>b</sup>
	5,000	Toxic			6 ± 3 <sup>b</sup>	26 ± 8 <sup>c</sup>	5 <sup>c</sup>
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Equivocal
Positive control <sup>d</sup>		420 ± 24	844 ± 95	737 ± 40	1,108 ± 18	831 ± 93	1,086 ± 2
<b>TA98</b>	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 <sup>b</sup>	15 ± 1	6 ± 1 <sup>b</sup>	27 ± 2	17 ± 4	9 ± 2
	3,500	Toxic	Toxic	Toxic	11 ± 1 <sup>b</sup>	11 ± 0	3 ± 1 <sup>b</sup>
	5,000		Toxic		7 ± 1 <sup>b</sup>	3 ± 1 <sup>c</sup>	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		441 ± 43	654 ± 17	411 ± 41	1,533 ± 29	787 ± 4	787 ± 91
<b><i>Escherichia coli</i> WP2 <i>uvrA</i>/pKM101 (analogous to TA102)</b>							
	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 <sup>b</sup>	128 ± 9 <sup>c</sup>	184 ± 6	115 ± 5	98 ± 3 <sup>b</sup>
	7,500	Toxic	Toxic		104 ± 9	63 ± 5 <sup>b</sup>	242 ± 1 <sup>c</sup>
	10,000				65 ± 12 <sup>b</sup>	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

<sup>a</sup> 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.* (1992). Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

<sup>b</sup> Slight toxicity

<sup>c</sup> Precipitate on plate

<sup>d</sup> 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 E3**  
**Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male F344/N Rats**  
**Following Treatment with Indole-3-carbinol by Gavage for 3 Days<sup>a</sup>**

	Dose (mg/kg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs <sup>b</sup>	P Value <sup>c</sup>	PCEs <sup>b</sup> (%)
Corn oil <sup>d</sup>	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 <sup>e</sup>		
Cyclophosphamide <sup>f</sup>	25	4	26.17 ± 5.17	0.0000	7.075 ± 2.21

<sup>a</sup> Study was performed at ILS, Inc., using a protocol similar to that presented by Shelby *et al.* (1993); PCE=polychromatic erythrocyte

<sup>b</sup> Mean ± standard error

<sup>c</sup> Pairwise comparison with the vehicle control group; dosed group values are significant at  $P \leq 0.008$ ; positive control values are significant at  $P \leq 0.05$ .

<sup>d</sup> Vehicle control

<sup>e</sup> Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test; significant at  $P \leq 0.025$

<sup>f</sup> Positive control

**TABLE E4**  
**Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Indole-3-carbinol by Gavage for 3 Months<sup>a</sup>**

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs <sup>b</sup>	P Value <sup>c</sup>	PCEs <sup>b</sup> (%)
<b>Male</b>					
Corn oil <sup>d</sup>	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 <sup>e</sup>		
<b>Female</b>					
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		

<sup>a</sup> Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990); NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

<sup>b</sup> Mean ± standard error

<sup>c</sup> Pairwise comparison with the vehicle control group; dosed group values are significant at  $P \leq 0.005$

<sup>d</sup> Vehicle control

<sup>e</sup> 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

TABLE F1	Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol.....	F-2
TABLE F2	Hematology Data for Mice in the 3-Month Gavage Study of Indole-3-carbinol.....	F-8

**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	Vehicle Control	18.75 mg/kg	37.5 mg/kg	75 mg/kg	150 mg/kg	300 mg/kg
<b>Male</b>						
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 <sup>6</sup> /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 <sup>5</sup> /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
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 <sup>3</sup> /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 <sup>3</sup> /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

**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-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
<b>Male (continued)</b>						
Hematology (continued)						
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
Segmented neutrophils (10 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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
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

**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-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
<b>Male (continued)</b>						
Clinical Chemistry (continued)						
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
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
Sorbitol dehydrogenase (IU/L)						
Day 4	6±1 <sup>b</sup>	9±1 <sup>b</sup>	8±2 <sup>c</sup>	8±1 <sup>*b</sup>	9±1*	13±2**
Day 25	9±2 <sup>b</sup>	10±2	10±1 <sup>b</sup>	10±2 <sup>d</sup>	11±1 <sup>b</sup>	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
<b>Female</b>						
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**

**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-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
<b>Female (continued)</b>						
Hematology (continued)						
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
Erythrocytes (10 <sup>6</sup> /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 <sup>5</sup> /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
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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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

**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-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
<b>Female (continued)</b>						
Hematology (continued)						
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
Basophils (10 <sup>3</sup> /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
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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>b</sup>	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**
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**



**TABLE F1**  
**Hematology and Clinical Chemistry Data for F344/N Rats in the 3-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
<b>Female (continued)</b>						
Clinical Chemistry (continued)						
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
Creatine kinase (IU/L)						
Day 4	447 ± 52	445 ± 46	463 ± 35	462 ± 39	395 ± 26 <sup>b</sup>	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 <sup>b</sup>	7 ± 1 <sup>b</sup>	3 ± 1 <sup>b</sup>	5 ± 1 <sup>b</sup>	7 ± 1	8 ± 2
Day 25	10 ± 3 <sup>c</sup>	10 ± 2	12 ± 2 <sup>d</sup>	8 ± 2 <sup>d</sup>	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≤0.05) from the vehicle control group by Dunn's or Shirley's test

\*\* P≤0.01

<sup>a</sup> Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

<sup>b</sup> n=9

<sup>c</sup> n=7

<sup>d</sup> n=8

<sup>e</sup> n=6

**TABLE F2**  
**Hematology Data for Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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
<b>Male</b>						
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 <sup>6</sup> /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 <sup>5</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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
<b>Female</b>						
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 <sup>6</sup> /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 <sup>5</sup> /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 <sup>3</sup> /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
Leukocytes (10 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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 <sup>3</sup> /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<0.05) from the vehicle control group by Dunn's or Shirley's test

\*\* P<0.01

<sup>a</sup> 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**

<b>TABLE G1</b>	<b>Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>G-2</b>
<b>TABLE G2</b>	<b>Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>G-3</b>

**TABLE G1**  
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 3-Month Gavage Study**  
**of Indole-3-carbinol<sup>a</sup>**

	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
<b>Male</b>						
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
<b>Female</b>						
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<0.05) from the vehicle control group by Williams' or Dunnett's test

\*\* P<0.01

<sup>a</sup> 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 G2**  
**Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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
<b>Male</b>						
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 <sup>b</sup>	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 <sup>b</sup>	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
<b>Female</b>						
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

\*\*  $P \leq 0.01$

<sup>a</sup> 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).

<sup>b</sup> n=9



## **APPENDIX H**

# **REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION**

<b>TABLE H1</b>	<b>Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-2</b>
<b>TABLE H2</b>	<b>Estrous Cycle Characterization for Female F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-2</b>
<b>FIGURE H1</b>	<b>Vaginal Cytology Plots for Female F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-3</b>
<b>TABLE H3</b>	<b>Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/N Rats Administered Indole-3-carbinol by Gavage for 3 Months.....</b>	<b>H-4</b>
<b>TABLE H4</b>	<b>Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-5</b>
<b>TABLE H5</b>	<b>Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-5</b>
<b>FIGURE H2</b>	<b>Vaginal Cytology Plots for Female Mice in the 3-Month Gavage Study of Indole-3-carbinol.....</b>	<b>H-6</b>
<b>TABLE H6</b>	<b>Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice Administered Indole-3-carbinol by Gavage for 3 Months.....</b>	<b>H-7</b>

**TABLE H1**  
**Summary of Reproductive Tissue Evaluations for Male F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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 <sup>6</sup> /testis)	160.4 ± 6.7	168.4 ± 7.8	149.3 ± 6.4	170.9 ± 12.6
Spermatid heads (10 <sup>6</sup> /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 <sup>6</sup> /cauda epididymis)	94.03 ± 2.43	83.90 ± 2.90	89.39 ± 4.30	92.04 ± 4.57
Sperm (10 <sup>6</sup> /g cauda epididymis)	571.6 ± 19.0	505.7 ± 26.3	571.8 ± 35.7	590.9 ± 31.4

\* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test

<sup>a</sup> 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 H2**  
**Estrous Cycle Characterization for Female F344/N Rats in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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 <sup>b</sup>	7/7	8/8	9/10	8/9
Estrous cycle length (days)	5.00 ± 0.11 <sup>c</sup>	5.13 ± 0.13 <sup>d</sup>	5.25 ± 0.13	5.89 ± 0.31 <sup>*e</sup>
Estrous stages <sup>f</sup> (% 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≤0.05) from the vehicle control group by Dunn's test

<sup>a</sup> 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.

<sup>b</sup> Number of females with a regular cycle/number of females cycling

<sup>c</sup> Estrous cycle was longer than 12 days or unclear in three of 10 animals.

<sup>d</sup> Estrous cycle was longer than 12 days or unclear in two of 10 animals.

<sup>e</sup> Estrous cycle was longer than 12 days or unclear in one of 10 animals.

<sup>f</sup> Evidence shows that 150 and 300 mg/kg females differ significantly (Wilkes' Criterion, P≤0.05) from the vehicle control females in the relative length of time spent in the estrous stages.





**TABLE H3**  
**Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female F344/N Rats**  
**Administered Indole-3-carbinol by Gavage for 3 Months**

Stage	Comparison <sup>a</sup>	P Value	Trend <sup>b</sup>
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	
<b>Summary of Significant Groups</b>			
Overall Tests	High vs. Controls	<0.001	
Extended Diestrus	High vs. Controls	<0.001	

<sup>a</sup> Controls = Vehicle Control, Low = 75 mg/kg, Mid = 150 mg/kg, High = 300 mg/kg

<sup>b</sup> N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

**TABLE H4**  
**Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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 <sup>6</sup> /testis)	20.99 ± 1.28	22.56 ± 1.11	22.63 ± 1.60	24.81 ± 0.47
Spermatid heads (10 <sup>6</sup> /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 <sup>6</sup> /cauda epididymis)	18.04 ± 0.58	18.97 ± 1.04	18.52 ± 0.90	18.73 ± 2.35
Sperm (10 <sup>6</sup> /g cauda epididymis)	742.9 ± 28.9	747.1 ± 30.7	820.0 ± 54.9	800.5 ± 94.9

\*\* Significantly different (P≤0.01) from the vehicle control group by Shirley's test

<sup>a</sup> 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 and sperm per cauda epididymis and per gram cauda epididymis measurements).

**TABLE H5**  
**Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Indole-3-carbinol<sup>a</sup>**

	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 <sup>b</sup>	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 <sup>c</sup>
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

<sup>a</sup> 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.

<sup>b</sup> Number of females with a regular cycle/number of females cycling

<sup>c</sup> One of 10 animals was excluded from mean due to cycle length longer than 9 days.



**TABLE H6**  
**Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Mice**  
**Administered Indole-3-carbinol by Gavage for 3 Months**

Stage	Comparison <sup>a</sup>	P Value	Trend <sup>b</sup>
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	
<b>Summary of Significant Groups</b>			
Overall Tests	High vs. Controls	0.004	
Extended Diestrus	High vs. Controls	0.031	

<sup>a</sup> Controls = Vehicle Control, Low = 62.5 mg/kg, Mid = 125 mg/kg, High = 250 mg/kg

<sup>b</sup> N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.



# APPENDIX I

## CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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# CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

## 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. 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 (Hwu *et al.*, 1996; Aldrich, 1997) and the structure of indole-3-carbinol. Representative IR and proton NMR spectra are presented in Figures I1 and I2, 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 I1).

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<sup>®</sup>-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.

### 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.

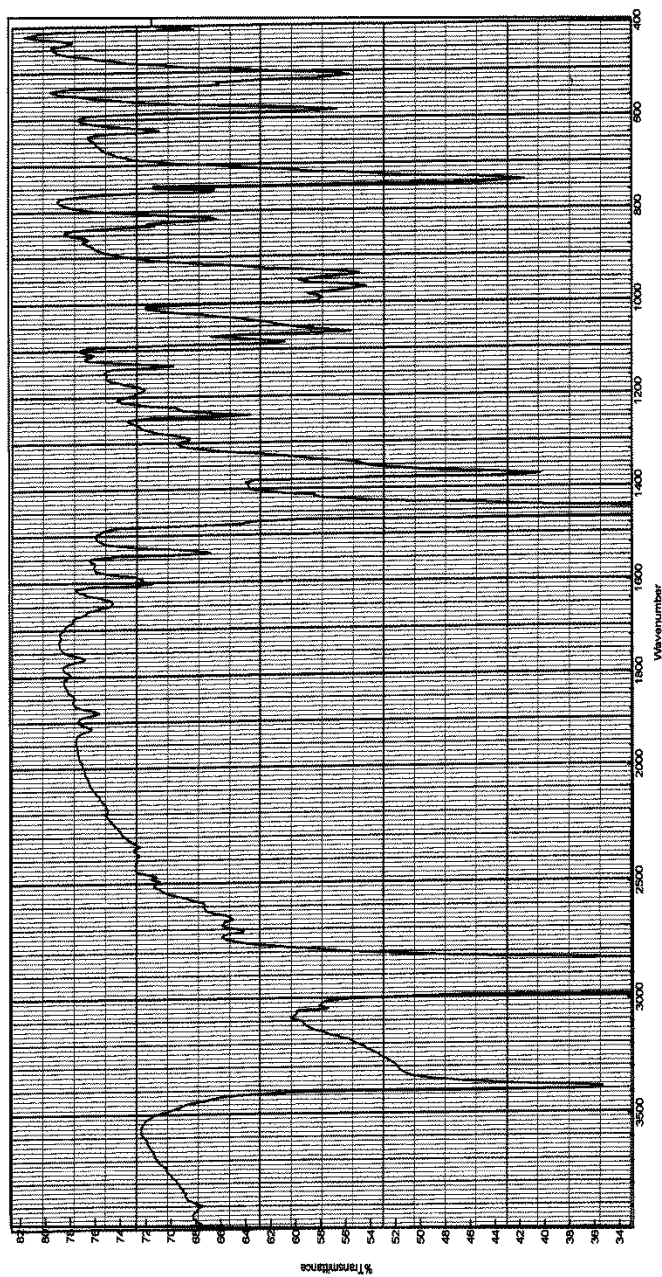


## PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

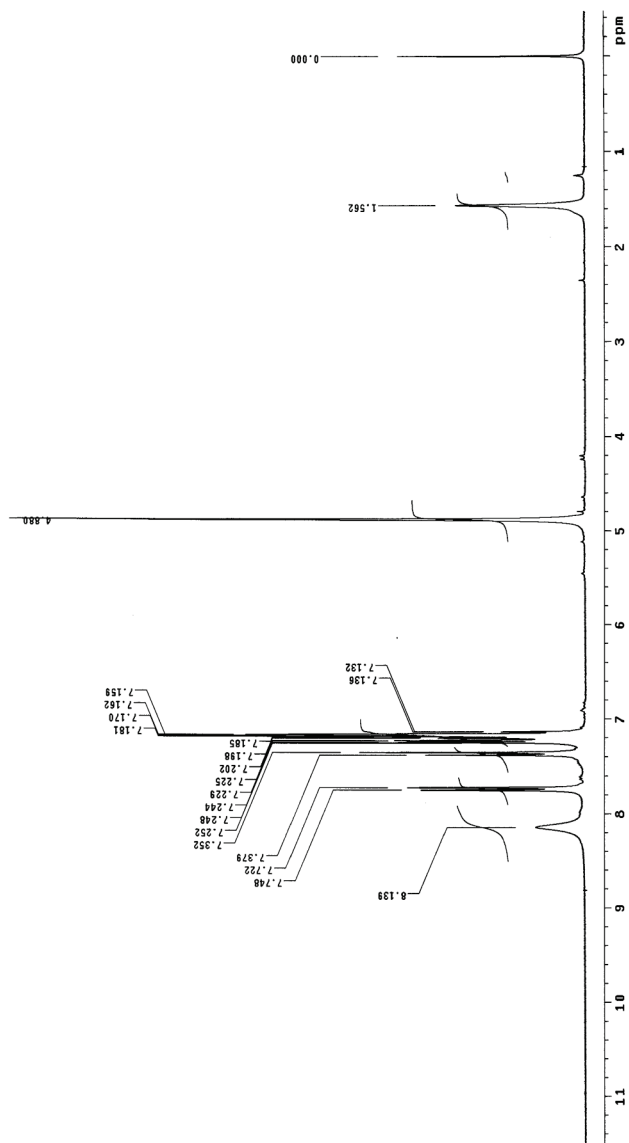
The dose formulations were prepared by mixing the appropriate amount of milled indole-3-carbinol with corn oil to achieve the required concentrations (Table I2). The dose formulations were prepared seven times during the 3-month studies and approximately every 4 weeks during the 2-year studies. Dose formulations were stored in amber glass vials sealed with Teflon<sup>®</sup>-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 I1). 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<sup>®</sup>-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<sup>®</sup>-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 gavageability 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 I3). 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 I4). 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.



**FIGURE II**  
**Infrared Absorption Spectrum of Indole-3-carbinol**



**FIGURE I2**  
**Proton Nuclear Magnetic Resonance Spectrum of Indole-3-carbinol**

**TABLE II**  
**High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Indole-3-carbinol<sup>a</sup>**

Detection System	Column	Solvent System
<b>System A</b> 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
<b>System B</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

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

**TABLE I2**  
**Preparation and Storage of Dose Formulations in the Gavage Studies of Indole-3-carbinol**

3-Month Studies	2-Year Studies
<p><b>Preparation</b>            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.</p> <p>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.</p>	<p>Same as 3-month studies except that dose formulations were prepared approximately every 4 weeks.</p>
<p><b>Chemical Lot Number</b>            CHP801001</p>	<p>CHP801001</p>
<p><b>Maximum Storage Time</b>            41 days</p>	<p>41 days</p>
<p><b>Storage Conditions</b>            The dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5° C.</p>	<p>The dose formulations were stored in amber glass vials sealed with Teflon®-lined lids at 5° C.</p>
<p><b>Study Laboratory</b>            Southern Research Institute (Birmingham, AL)</p>	<p>Battelle Columbus Operations (Columbus, OH)</p>

**TABLE I3**  
**Results of Analyses of Dose Formulations Administered to F344/N Rats and Mice**  
**in the 3-Month Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration <sup>a</sup> (mg/mL)	Difference from Target (%)
<b>Rats</b>				
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 <sup>b</sup>	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 <sup>b</sup>	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 <sup>b</sup>	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
<b>Mice</b>				
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 <sup>b</sup>	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

**TABLE I3**  
**Results of Analyses of Dose Formulations Administered to F344/N Rats and Mice**  
**in the 3-Month Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
<b>Mice (continued)</b>				
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 <sup>b</sup>	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 <sup>b</sup>	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

<sup>a</sup> 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.

<sup>b</sup> Animal room samples

**TABLE I4**  
**Results of Analyses of Dose Formulations Administered to Sprague Dawley Rats and Mice**  
**in the 2-Year Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration <sup>a</sup> (mg/mL)	Difference from Target (%)
<b>Rats</b>				
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 <sup>b</sup>	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 <sup>b</sup>	15	15.4 ± 0.1	+3
		15	15.6 ± 0.1	+4
		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 <sup>c</sup>	+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

**TABLE I4**  
**Results of Analyses of Dose Formulations Administered to Sprague Dawley Rats and Mice**  
**in the 2-Year Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)		
<b>Rats (continued)</b>						
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 <sup>b</sup>	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		
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 <sup>b</sup>	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		



**TABLE I4**  
**Results of Analyses of Dose Formulations Administered to Sprague Dawley Rats and Mice**  
**in the 2-Year Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
<b>Mice</b>				
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 <sup>b</sup>	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 <sup>b</sup>	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 <sup>b</sup>	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
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

**TABLE I4**  
**Results of Analyses of Dose Formulations Administered to Sprague Dawley Rats and Mice**  
**in the 2-Year Gavage Studies of Indole-3-carbinol**

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
<b>Mice (continued)</b>				
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 <sup>b</sup>	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

<sup>a</sup> 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.

<sup>b</sup> Animal room samples

<sup>c</sup> One value only

**APPENDIX J**  
**INGREDIENTS, NUTRIENT COMPOSITION,**  
**AND CONTAMINANT LEVELS**  
**IN NTP-2000 RAT AND MOUSE RATION**

<b>TABLE J1</b>	<b>Ingredients of NTP-2000 Rat and Mouse Ration .....</b>	<b>J-2</b>
<b>TABLE J2</b>	<b>Vitamins and Minerals in NTP-2000 Rat and Mouse Ration .....</b>	<b>J-2</b>
<b>TABLE J3</b>	<b>Nutrient Composition of NTP-2000 Rat and Mouse Ration .....</b>	<b>J-3</b>
<b>TABLE J4</b>	<b>Contaminant Levels in NTP-2000 Rat and Mouse Ration .....</b>	<b>J-4</b>

**TABLE J1**  
**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 <sup>a</sup>	0.5
Mineral premix <sup>b</sup>	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

<sup>a</sup> Wheat middlings as carrier

<sup>b</sup> Calcium carbonate as carrier

**TABLE J2**  
**Vitamins and Minerals in NTP-2000 Rat and Mouse Ration<sup>a</sup>**

	Amount	Source
<b>Vitamins</b>		
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
$\alpha$ -Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
<i>d</i> -Pantothenic acid	10 mg	<i>d</i> -Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B <sub>12</sub>	52 $\mu$ g	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	<i>d</i> -Biotin
<b>Minerals</b>		
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

<sup>a</sup> Per kg of finished product

**TABLE J3**  
**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
<b>Amino Acids (% of total diet)</b>			
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
<b>Essential Fatty Acids (% of total diet)</b>			
Linoleic	3.95 ± 0.254	3.49 – 4.55	23
Linolenic	0.30 ± 0.031	0.21 – 0.35	23
<b>Vitamins</b>			
Vitamin A (IU/kg)	3,601 ± 77	2,350 – 5,720	24
Vitamin D (IU/kg)	1,000 <sup>a</sup>		
α-Tocopherol (ppm)	80.3 ± 21.56	27.0 – 124.0	23
Thiamine (ppm) <sup>b</sup>	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) <sup>b</sup>	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 <sub>12</sub> (ppb)	53.4 ± 38	18.3 – 174.0	23
Choline (ppm) <sup>b</sup>	2,773 ± 590	1,160 – 3,790	23
<b>Minerals</b>			
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

<sup>a</sup> From formulation

<sup>b</sup> As hydrochloride (thiamine and pyridoxine) or chloride (choline)

**TABLE J4**  
**Contaminant Levels in NTP-2000 Rat and Mouse Ration<sup>a</sup>**

	Mean ± Standard Deviation <sup>b</sup>	Range	Number of Samples
<b>Contaminants</b>			
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) <sup>c</sup>	17.8 ± 8.15	10.0 – 42.3	24
Nitrite nitrogen (ppm) <sup>c</sup>	<0.61		24
BHA (ppm) <sup>d</sup>	<1.0		24
BHT (ppm) <sup>d</sup>	<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
<i>Escherichia coli</i> (MPN/g)	<10		24
<i>Salmonella</i> (MPN/g)	Negative		24
Total nitrosoamines (ppb) <sup>e</sup>	8.8 ± 6.32	2.0 – 28.0	24
<i>N</i> -Nitrosodimethylamine (ppb) <sup>e</sup>	2.0 ± 2.27	0.9 – 10.3	24
<i>N</i> -Nitrosopyrrolidine (ppb) <sup>e</sup>	6.8 ± 5.03	1.0 – 17.7	24
<b>Pesticides (ppm)</b>			
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
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

<sup>a</sup> All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

<sup>b</sup> For values less than the limit of detection, the detection limit is given as the mean.

<sup>c</sup> Sources of contamination: alfalfa, grains, and fish meal

<sup>d</sup> Sources of contamination: soy oil and fish meal

<sup>e</sup> All values were corrected for percent recovery.

# APPENDIX K

## SENTINEL ANIMAL PROGRAM

<b>METHODS</b> .....	<b>K-2</b>
<b>RESULTS</b> .....	<b>K-4</b>

## SENTINEL ANIMAL PROGRAM

### 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.

#### Method and Test

#### Time of Collection

### RATS

#### 3-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

#### 2-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

Parvo NS-1

12 and 18 months, study termination

PVM

12 and 18 months, study termination

RCV/SDA

12 and 18 months, study termination



**Method and Test****RATS** (continued)**2-Year Study** (continued)

## Multiplex Fluorescent Immunoassay (continued)

RMV (rat minute virus)  
 RPV (rat parvovirus)  
 RTV (rat theilovirus)  
 Sendai  
 TMEV GDVII  
 (Theiler's murine encephalomyelitis virus –  
 mouse poliovirus, strain GDVII)

**Time of Collection**

12 and 18 months, study termination  
 12 and 18 months, study termination  
 12 and 18 months, study termination  
 12 and 18 months, study termination  
 12 and 18 months, study termination

**MICE****3-Month Study**

## ELISA

Ectromelia virus  
 EDIM (epizootic diarrhea of infant mice)  
 LCM (lymphocytic choriomeningitis virus)  
 Mouse adeno virus-1 (Mad-1)  
 MHV (mouse hepatitis virus)  
 MMV VP2 (mouse minute virus)  
 MPV VP2 (mouse parvovirus)  
 PVM  
 Reovirus  
 Sendai  
 TMEV GDVII

Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination  
 Study termination

## Immunofluorescence Assay

LCM

Study termination

**2-Year Study**

## ELISA

Ectromelia virus  
 EDIM  
 LCM  
 Mad-1  
 MHV  
 MMV VP2  
 MPV VP2  
 PVM  
 Reovirus  
 Sendai  
 TMEV GDVII

End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months  
 End of quarantine, 4 weeks, 6 months

## Immunofluorescence Assay

MHV  
 MPV  
 LCM

4 weeks  
 End of quarantine  
 4 weeks

**Method and Test****MICE** (continued)**2-Year Study** (continued)

## Multiplex Fluorescent Immunoassay

Ectromelia virus

EDIM

LCM

*M. pulmonis*

MHV

MNV (mouse norovirus)

MPV (mouse parvovirus)

MMV

Parvo NS-1

PVM

Reovirus 3

Sendai

TMEV GDVII

**Time of Collection**

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

12 and 18 months, study termination

## Polymerase Chain Reaction

*Helicobacter* species

18 months

**RESULTS**

All test results were negative.

## APPENDIX L

### MICROARRAY ANALYSIS

<b>OBJECTIVE</b> .....	<b>L-2</b>
<b>METHODS</b> .....	<b>L-2</b>
<b>RESULTS</b> .....	<b>L-3</b>
<b>CONCLUSIONS</b> .....	<b>L-3</b>
<b>REFERENCES</b> .....	<b>L-5</b>
<b>TABLE L1</b> <b>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 3 Months</b> .....	<b>L-6</b>
<b>TABLE L2</b> <b>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 3 Months</b> .....	<b>L-6</b>
<b>TABLE L3</b> <b>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 3 Months</b> .....	<b>L-7</b>
<b>TABLE L4</b> <b>Ingenuity Pathway Analysis™ Canonical Pathways Exhibiting Significant (P&lt;0.05) Enrichment in the Liver of Female Sprague Dawley Rats Administered Daily Doses of 300 mg/kg Indole-3-carbinol by Gavage for 3 Months</b> .....	<b>L-7</b>
<b>TABLE L5</b> <b>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 3 Months</b> .....	<b>L-8</b>
<b>TABLE L6</b> <b>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 3 Months</b> .....	<b>L-9</b>

## 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.

## METHODS

### 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.

### Microarray Analysis

The 10 .CEL files representing the 10 liver samples from the study were normalized using the robust multiarray algorithm (RMA) (Irizarry *et al.*, 2003) 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.

### 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](http://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<sup>®</sup> 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.

## RESULTS

### 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 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 L1.

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 Tables L2 and L3.

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 L4.

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 (Nr1h3), Pxr (Nr1h2) and AhR/Arnt (Omicinski *et al.*, 2011). Results of the transcription factor upstream analysis are shown in Table L5.

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) (Dhakshinamoorthy and Jaiswal, 2000; Kensler *et al.*, 2000; Kong *et al.*, 2001; Kwak *et al.*, 2001), AhR/Arnt (e.g., tetrachlorodibenzodioxin, benzo(a)pyrene, 2,3,4,7,8-pentachlorodibenzofuran, and 3-methylcholanthrene) (Denison and Nagy, 2003) and Car/Pxr signaling (e.g., phenobarbital) (Hernandez *et al.*, 2009) exhibit similar patterns of gene expression compared to indole-3-carbinol. Results of the chemical response pattern activation analysis are shown in Table L6.

## CONCLUSIONS

The results strongly suggest that treatment with indole-3-carbinol strongly activates AhR and Nrf2 (Nfe2l2) signaling, but also appears to activate Car (Nr1h3)/Pxr (Nr1h2) signaling. These findings 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 (Ma, 2013). 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 (Bjeldanes *et al.*, 1991). 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 (Refetoff, 1989).
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 (Ameri and Harris, 2008).
3. Activation of *Trp63* 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 (Polyak *et al.*, 1997) that can be elicited by indole-3-carbinol (Brew *et al.*, 2006).

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**TABLE L1**  
**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 3 Months**

Probeset ID	Gene Symbol	Entrez Gene	Fold Change Indole-3-carbinol treated versus vehicle control
<b>Up-regulated</b>			
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
<b>Down-regulated</b>			
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 L2**  
**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 3 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 L3**  
**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 3 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 L4**  
**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 3 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
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

**TABLE L4**  
**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 3 Months** (continued)

Ingenuity Canonical Pathways	–log(P Value)
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 L5**  
**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 3 Months**

Upstream Regulator	Fold Change <sup>a</sup>	Molecule Type	Predicted Activation State	Activation Z-score	P Value of overlap
Nfe2l2	2.075	transcription regulator	Activated	4.399	2.31E-23
Nr1i3	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
Bra1		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

<sup>a</sup> 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 L6**  
**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 3 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
<i>tert</i> -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 $\alpha$	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

