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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Cresols (CAS No. 1319-77-3) in Male F344/N Rats and Female B6C3F1 Mice (Feed Studies)

NTP TR 550

JULY 2008

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF CRESOLS

(CAS NO. 1319-77-3)

IN MALE F344/N RATS AND FEMALE B6C3F1 MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

July 2008

NTP TR 550

NIH Publication No. 08-5891

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.

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

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CONTENTS

SUMMARY

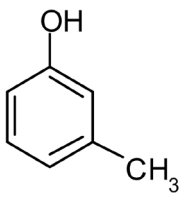
Background
Cresols are petroleum byproducts that are used to make resins, solvents, disinfectants, fragrances, and wood preservatives. We performed tests to determine if cresols caused cancer in rats or mice.

Methods
We fed groups of 50 male rats and female mice cresols mixed into their feed. Male rats were given concentrations of 1,500, 5,000, or 15,000 parts per million (ppm) of cresols in their feed, and female mice were given concentrations of 1,000, 3,000, or 10,000 ppm. Similar groups of 50 animals were given undosed feed as the control groups. Tissues from more than 40 sites were examined for every animal.

Results
The groups fed the highest concentration of cresols weighed less than their control groups. A few rare tumors of the kidney were seen in exposed male rats, and the rate of tumors of the forestomach was increased in exposed female mice. Male rats and female mice given cresols had hyperplasia of the epithelium of the nose. Exposed male rats also had hyperplasia of the kidney, and female mice had hyperplasia of the lung and degeneration of the thyroid gland.

Conclusions
We conclude that the occurrence of forestomach papillomas in female mice was caused by exposure to cresols. The occurrence of a few kidney tumors in male rats may have been related to exposure to cresols.

ABSTRACT



***m*-CRESOL** ***p*-CRESOL**

***m*-/*p*-CRESOL (60:40 mixture)**

CAS No. 1319-77-3

Chemical Formula: C_7H_8O Molecular Weight: 108.14

Synonyms for mixture: Cresol; dicresol; *m*-/*p*-cresol; *m,p*-cresol; methylphenol, mixed; mixed cresols; phenol, methyl-, mixed
Synonyms for *m*-cresol (60%): 3-Cresol; 1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 1-methyl-3-hydroxybenzene; 3-methylphenol; *m*-cresol; *m*-cresylic acid; *m*-hydroxytoluene; *m*-methylphenol; *m*-methylphenylol; *m*-oxytoluene; *m*-toluol; phenol, 3-methyl-
Synonyms for *p*-cresol (40%): 4-Cresol; 1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 1-methyl-4-hydroxybenzene; 4-methylphenol; *p*-cresol; *p*-cresylic acid; *p*-hydroxytoluene; *p*-methylphenol; *p*-methylphenylol; *p*-oxytoluene; *p*-toluol; paramethyl phenol; phenol, 4-methyl

Cresols are high volume production chemicals with a variety of industrial uses. Cresols are used in cleaners, disinfectants, solvents, degreasing compounds, paintbrush cleaners, fumigants, photographic developers, ore flotation processes, explosives, and synthetic food flavors. Cresols are also used as a motor oil additive, textile scouring agent, and surfactant. The chemical acts as an intermediate in the production of phenolic resins and phosphate esters (tricresyl phosphate and cresyl diphenyl phosphate); an intermediate in the manufacture of chemicals, dyes, plastics, and antioxidants; and an organic intermediate in the manufacture of herbicides. Cresols were nominated for study by the National Institute of Environmental Health Sciences because of the potential for occupational and consumer exposure and the lack of chronic toxicity data. Male F344/N rats and female B6C3F1 mice were exposed to a 60:40 mixture of *m*- and *p*-cresol (*m*-/*p*-cresol) (greater than 99.5% pure) in feed for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli,* and mouse peripheral blood erythrocytes.

2-Year Study in Rats

Groups of 50 male rats were fed diets containing 0, 1,500, 5,000 or 15,000 ppm *m*-/*p*-cresol (equivalent to average daily doses of approximately 70, 230, or 720 mg cresols/kg body weight) for 105 weeks. Survival of all exposed groups was similar to that of the control group. Mean body weights of the 15,000 ppm group were less than those of the control group throughout the study. Due to lack of palatability, feed consumption by the 15,000 ppm group was less than that by the control group during the first week of the study but increased to control levels by the second week of the study.

Renal tubule adenomas occurred in three rats in the 15,000 ppm group, and the incidence exceeded the historical control range. One additional renal tubule adenoma was found in a 15,000 ppm rat in an extended examination of kidney step sections. The incidence of hyperplasia of the transitional epithelium of the renal pelvis was significantly increased in the 15,000 ppm group.

The severity of nephropathy was slightly increased in the 15,000 ppm group.

Exposure to cresols resulted in significantly increased incidences of hyperplasia of the goblet cells and respiratory epithelium of the nose in all exposed groups of rats. The incidences of squamous metaplasia of the respiratory epithelium were significantly increased in the 5,000 and 15,000 ppm groups, and the incidence of inflammation was significantly increased in the 15,000 ppm group. The incidence of eosinophilic focus of the liver was significantly increased in the 15,000 ppm group.

2-Year Study in Mice

Groups of 50 female mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm *m-/p*-cresol (equivalent to average daily doses of approximately 100, 300, or 1,040 mg cresols/kg body weight) for 104 to 105 weeks. Survival of all exposed groups was similar to that of the control group. Mean body weights of the 3,000 and 10,000 ppm groups were less than those of the control group after weeks 12 and 9, respectively. Feed consumption by the 10,000 ppm group was decreased compared to that by the control group.

The incidence of squamous cell papilloma of the forestomach was significantly greater in the 10,000 ppm group than in the control group.

Exposure to cresols resulted in significantly increased incidences of bronchiolar hyperplasia of the lung in all exposed groups of mice. The incidences of respiratory epithelial hyperplasia of the nose were significantly increased in the 3,000 and 10,000 ppm groups. The incidences of thyroid gland follicular degeneration were significantly increased in all exposed groups of mice, and the incidence of eosinophilic focus of the liver was significantly increased in the 10,000 ppm group.

Genetic Toxicology

Cresols did not exhibit mutagenicity in tests conducted by the NTP. Each of the individual cresol isomers (*m*-, *o*-, and *p*-) and *m-/p*-cresol was tested for mutagenicity in several strains of *S. typhimurium* and in *E. coli* strain WP2, with and without exogenous metabolic activation; results with all individual compounds and the mixture were negative. *o*-Cresol and *m-/p*-cresol were evaluated for induction of micronuclei (biomarkers of chromosomal damage) in peripheral blood erythrocytes of male and female mice following 13 weeks of exposure in the diet (NTP, 1992); no increases in the frequencies of micronucleated erythrocytes were seen in male or female mice in either study.

Conclusions

Under the conditions of these 2-year studies, there was *equivocal evidence of carcinogenic activity** of 60:40 *m-/p*-cresol in male F344/N rats based on the marginally increased incidence of renal tubule adenoma. There was *some evidence of carcinogenic activity* of 60:40 *m-/p*-cresol in female B6C3F1 mice based on the increased incidence of forestomach squamous cell papilloma.

Exposure to 60:40 *m-/p*-cresol resulted in increased incidences of nonneoplastic lesions in the kidney (hyperplasia), nose (inflammation, hyperplasia, and metaplasia), and liver (eosinophilic focus) of rats. Increased incidences of nonneoplastic lesions were observed in the respiratory tract (hyperplasia in the nose and lung), thyroid gland (follicular degeneration), and liver (eosinophilic focus) of mice exposed to *m-/p*-cresol.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Cresols

	Male F344/N Rats	Female B6C3F1 Mice
Concentrations in feed	0, 1,500, 5,000, or 15,000 ppm	0, 1,000, 3,000, or 10,000 ppm
Body weights	15,000 ppm group less than the control group	3,000 and 10,000 ppm groups less than the control group
Survival rates	33/50, 34/50, 33/50, 31/50	41/50, 43/50, 44/49, 42/50
Nonneoplastic effects	Kidney: pelvis, transitional epithelium, hyperplasia (0/50, 0/50, 2/50, 8/50); severity of nephropathy (1.4, 1.4, 1.7, 2.1) Nose: goblet cell, hyperplasia (23/50, 40/50, 42/50, 47/50); respiratory epithelium, hyperplasia (3/50, 17/50, 31/50, 47/50); respiratory epithelium, metaplasia, squamous (0/50, 1/50, 8/50, 40/50); inflammation (17/50, 19/50, 19/50, 28/50) Liver: eosinophilic focus (14/50, 14/50, 13/50, 23/50)	Lung: bronchiole, hyperplasia (0/50, 42/50, 44/49, 47/50) Nose: respiratory epithelium, hyperplasia (0/50, 0/50, 28/49, 45/49) Thyroid gland: follicular degeneration (7/48, 24/48, 24/49, 21/50) Liver: eosinophilic focus (1/50, 0/50, 2/49, 12/50)
Neoplastic effects	None	Forestomach: squamous cell papilloma (0/50, 1/50, 1/49, 10/50)
Equivocal findings	Kidney: renal tubule adenoma (standard evaluation - 0/50, 0/50, 0/50, 3/50; standard and extended evaluations combined - 0/50, 0/50, 0/50, 4/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	Some evidence
Genetic toxicology		
Salmonella typhimurium gene mutations (*m*-, *o*-, and *p*-cresol and *m*-/*p*-cresol mixture):	Negative in strains TA97, TA98, TA100, TA1535, and TA1537 and in *Escherichia coli* WP2 *uvrA* pKM101 with and without S9	
Micronucleated erythrocytes		
Mouse peripheral blood *in vivo* (*o*-cresol and *m*-/*p*-cresol mixture):	Negative	

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 the 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 in 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 in 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 BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on cresols on May 17, 2007, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On May 17, 2007, the draft Technical Report on the toxicology and carcinogenesis studies of cresols received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.M. Sanders, NIEHS, introduced the toxicology and carcinogenesis studies of cresols by discussing the uses and human exposure for the chemical, the genetic toxicity of the various isomers, the results of short-term studies previously reported in NTP Toxicity Study Report 9, the design of the long-term studies in male rats and female mice, and the neoplasms and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were *equivocal evidence of carcinogenic activity* of 60:40 *m-/p*-cresol in male F344/N rats and *some evidence of carcinogenic activity* of 60:40 *m-/p*-cresol in female B6C3F1 mice.

Dr. Deininger, the first principal reviewer, inquired about the rationale for using only male rats and female mice. Dr. Sanders explained that a 1990 survey of approximately 300 NTP studies showed that in over 90% of the studies where there was a positive response there was a positive response in at least one of those two sex/species groups. He also noted that tumors were seen at the site of chemical contact in the present studies, and he felt that the study results validated the study design. Dr. Deininger also asked for some explanation of the rationale for the *some evidence* conclusion for the forestomach squamous cell papillomas in female mice. Dr. Sanders replied that the conclusion was based on a significant increase in tumors that might be able to progress to malignancy. He added that the weight decrement in that group may be attributable to palatability of the dosed feed rather than to an excessive toxic effect.

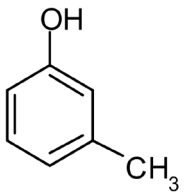
Dr. Soper, the second principal reviewer, said that the kidney adenoma response in male rats was well supported by a highly significant trend test. He agreed with the proposed conclusion of *equivocal evidence* because that tumor type seldom progressed to malignancy.

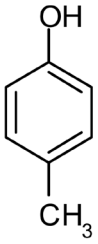
Dr. Novak, the third principal reviewer, inquired if the thyroid gland tumors in male rats were worthy of mention. Dr. Walker noted that those did not rise to any level of statistical significance.

Dr. Kerkvliet said that one written comment was submitted by the American Chemistry Council and that an oral presentation would be given by Dr. J.H. Butala of the Cresols Panel of the American Chemistry Council. Dr. Butala mentioned that a series of reproductive and developmental toxicity studies of cresols had been performed, and he suggested they might be referenced. He also suggested that rodent forestomach tumors may not be relevant to human disease.

Dr. Soper moved and Dr. Deininger seconded that the conclusions be approved as written. The motion was approved unanimously with six votes.

INTRODUCTION





***m*-CRESOL** ***p*-CRESOL**

***m*-/*p*-CRESOL (60:40 mixture)**

CAS No. 1319-77-3

Chemical Formula: C_7H_8O Molecular Weight: 108.14

Synonyms for mixture: Cresol; dicresol; *m*-/*p*-cresol; *m,p*-cresol; methylphenol, mixed; mixed cresols; phenol, methyl-, mixed
Synonyms for *m*-cresol (60%): 3-Cresol; 1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 1-methyl-3-hydroxybenzene; 3-methylphenol; *m*-cresol; *m*-cresylic acid; *m*-hydroxytoluene; *m*-methylphenol; *m*-methylphenylol; *m*-oxytoluene; *m*-toluol; phenol, 3-methyl-
Synonyms for *p*-cresol (40%): 4-Cresol; 1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 1-methyl-4-hydroxybenzene; 4-methylphenol; *p*-cresol; *p*-cresylic acid; *p*-hydroxytoluene; *p*-methylphenol; *p*-methylphenylol; *p*-oxytoluene; *p*-toluol; paramethyl phenol; phenol, 4-methyl

CHEMICAL AND PHYSICAL PROPERTIES

Cresols, monomethyl derivatives of phenol, are obtained by chemical synthesis or by distillation from petroleum or coal tar (*Kirk-Othmer*, 2004). Cresols are colorless, yellowish, brownish-yellow, or pinkish (darkening with age and exposure to light) and have a phenol-like odor with a threshold of 5 ppm (*Merck*, 1996; *Patty's*, 2001). All three isomeric forms of cresol (*ortho*, *meta*, and *para*) have low vapor pressures (0.1 to 0.3 mm Hg at 25° C), are moderately soluble in water (22 to 26 g/L), are soluble in aqueous bases (pK_a of 10 to 11), and are miscible with alcohol, benzene, chloroform, ether, glycerol, and petroleum ether (*Merck*, 1996; ChemID, 2006). Cresols have specific gravities of 1.034 to 1.047 at 25° C and log octanol:water partition coefficients of 1.94 to 1.96. The boiling point is 202° C for *m*- and *p*-cresol and 191° C for *o*-cresol (ChemID, 2006). *m*-, *o*-, and *p*-Cresol have melting points of 11.8, 29.8, and 35.5° C, respectively (ChemID, 2006). *m*-Cresol and cresol mixtures are liquids; *o*-cresol can be a liquid or solid, and *p*-cresol is a solid (*Merck*, 1996). Cresols are flammable, with flash points of 81° to 86° C, and are considered to be fire hazards (*Patty's*, 2001).

PRODUCTION, USE, AND HUMAN EXPOSURE

Cresols are recovered as a by-product in the fractional distillation of crude petroleum and coal tars, which yields a mixture containing the three isomers known as cresylic acid (IPCS, 1995; *Kirk-Othmer*, 2004). Several processes are used to synthesize individual isomers or mixtures of cresols. For example, chlorination of toluene, hydroxylation of the chlorotoluene, and distillation results in recovery of a mixture of *m*- and *p*-cresol (*Kirk-Othmer*, 2004). United States production and imports of individual cresols and cresol mixtures totaled 132.4 million pounds in 1984 (40 CFR, Part 799). By 2003, the capacity for cresol production in the United States had steadily increased to 365 million pounds (ATSDR, 2006). Approximately 2.2 million pounds of cresols and cresol salts were imported into the United States in

2005 (ATSDR, 2006). CAS No. 1319-77-3 is used as a designation for various cresol mixtures including *m*-/*p*-cresol (ACS, 2006).

Cresols are used primarily as intermediates in the production of industrial and consumer products. The majority of *o*-cresol is used to produce novolak resins (*Kirk-Othmer*, 2004). *o*-Cresol is also used directly in solvents and disinfectants (IPCS, 1995). *m*-Cresol is used in the production of antioxidants, fragrances (synthetic musk), herbicides, insecticides (carbamate derivatives and as a pyrethroid precursor), and in the manufacture of the explosive 2,4,6-nitro-*m*-cresol (IPCS, 1995; *Kirk-Othmer*, 2004; ATSDR, 2006). *p*-Cresol is primarily used in the formulation of antioxidants used to stabilize lubricating oil, motor fuels, rubber, polymers, elastomers, and food products (IPCS, 1995). 2,6-Di-*tert*-butyl-4-methylphenol, formed from *p*-cresol, is one of the few phenolic antioxidants approved by the FDA as a food additive (ATSDR, 2006). *p*-Cresol is also used as an intermediate in the fragrance and dye industries (IPCS, 1995). *m*-/*p*-Cresol is frequently used in consumer products as a disinfectant and preservative. Tricresyl and diphenyl cresyl phosphates, produced from *m*-/*p*-cresol, are used as additive flame retardants. Cresol mixtures are used in wood preservatives and in solvents for synthetic resin coatings, degreasing agents, ore flotation, paints, and textile products.

Humans may be exposed to cresols by anthropogenic or natural sources. Cresols occur naturally in oils of some plants and are formed during combustion of cigarettes, petroleum-based fuels, coal, wood, and other natural materials (IPCS, 1995). The present extent of occupational exposure to cresols is uncertain (ATSDR, 2006); however, estimated exposures were 126,000 to 300,000 workers in the United States over 20 years ago (40 CFR, Part 799) and would be expected to be higher today. The general population may be orally exposed to cresols in food and beverages. Cresols have been detected in smoked fish (Guillén and Errecalde, 2002; Guillén *et al.*, 2006; Varlet *et al.*, 2006), fried bacon (Ho *et al.*, 1983), rennet casein (Karagül-Yüceer *et al.*, 2003), buckwheat honey (Zhou *et al.*, 2002), cheese (Suriyaphan *et al.*, 2001), cow milk (Kilic and Lindsay, 2005), and whiskey (Lehtonen, 1982). Humans may inhale cresols in the workplace (Bieniek, 1994, 1997) from cigarette smoke (Nanni *et al.*, 1990; White *et al.*, 1990), vehicle emissions (Morville *et al.*, 2006), emissions from waste incinerators (Jay and Stieglitz, 1995) and coal gasification plants (Jin *et al.*, 1999), and following combustion of wood (Hawthorne *et al.*, 1989; Schauer *et al.*, 2001) and coal (Bezacinsky *et al.*, 1984). Cresols may form as a result of reactivity of toluene with hydroxy radicals in the atmosphere (Dumdei and O'Brien, 1984). Cresols have been detected in sediment and soil (Schwarzbauer *et al.*, 2000; Atagana *et al.*, 2003), surface water (McKnight *et al.*, 1982; Czuczwa *et al.*, 1987; Tortajada-Genaro *et al.*, 2003), and groundwater (Nielsen *et al.*, 1995; Thornton *et al.*, 2001), primarily near point sources. Humans may be exposed to cresol isomers in wood creosote (Ogata and Baba, 1989; Ogata *et al.*, 1995), dermatological creams (Mirza and Tan, 1998), and disinfectant products (Wu *et al.*, 1998). *p*-Cresol is endogenously formed in humans as the result of tyrosine metabolism by gut microflora (Bone *et al.*, 1976). Humans exposed to toluene excrete a portion of the dose in urine consisting of conjugates of *o*-, *m*-, and *p*-cresol (Woiwode and Drysch, 1981; Dills *et al.*, 1997; Pierce *et al.*, 2002).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Detection of cresols and their metabolites in human tissue and urine following occupational, accidental, or intentional exposure to cresol-containing products indicates absorption through the skin, gastrointestinal system, and respiratory tract (Green, 1975; Yashiki *et al.*, 1990; IPCS, 1995). However, cresols are also detected in humans following absorption of other phenolic chemicals such as toluene (Woiwode and Drysch, 1981; Dills *et al.*, 1997; Pierce *et al.*, 2002), and *p*-cresol is endogenously formed from tyrosine (Bone *et al.*, 1976). Absorption of cresols from the gastrointestinal and respiratory tracts, but not skin, has been reported for animals (ATSDR, 2006). Dermal absorption in animals is supported by the observation of movement of *p*-cresol across mouse skin *in vitro* (Hinz *et al.*, 1991). In humans, endogenous *p*-cresol is transported in blood, primarily bound to serum proteins (De Smet *et al.*, 1998); absorbed cresols likely undergo similar transport prior to distribution to other tissues. *m*- and *p*-Cresols administered by gavage to male Wistar rats were detected in blood and distributed to the brain, kidney, liver, lung, muscle, and spleen (Morinaga *et al.*, 2004).

Phenol can be oxidized or conjugated with sulfate or glucuronic acid for excretion in urine of animals (Williams, 1938). Cresols, monosubstituted phenols, are similarly metabolized. However, some qualitative and

quan-titative differences in metabolism of cresol isomers have been observed. Rabbits receiving either *o*-, *m*-, or *p*-cresol by gavage excreted an average of 60% to 72% of the dose as glucuronide conjugates, 10% to 15% as sulfate conjugates, and 1% to 2% as free cresols in urine (Bray *et al.*, 1950). Small amounts of conjugated dihydroxytoluenes were detected in urine from all treated groups. Approximately 10% of the oral *p*-cresol dose was excreted in urine of rabbits as free and conjugated *p*-hydroxybenzoic acid; whereas, no hydroxybenzoic acid was excreted following gavage of *o*- or *m*-cresol. Glucuronidation was also the major metabolic pathway for both *m*- and *p*-cresol administered by gavage to rats (Morinaga *et al.*, 2004). Both isomers underwent sulfate conjugation, *m*-cresol more so than *p*-cresol. No sulfate conjugation of *p*-cresol was detected in rats receiving the chemical by intravenous injection (Lesaffer *et al.*, 2003). Approximately 64% of the dose was excreted as *p*-cresyl glucuronide in that study. Humans exposed orally to wood creosote excreted *p*-cresol in urine primarily as a glucuronide conjugate and as a sulfate conjugate and the free cresol (Ogata *et al.*, 1995). Detection of ᾳ-glutathionyl-*p*-cresol in rat liver microsomes incubated with *p*-cresol indicated formation of a reactive quinone methide (Thompson *et al.*, 1995). The characterization of three additional glutathione conjugates in human microsomes incubated with *p*-cresol indicated the presence of a bioactivation pathway involving formation of the reactive intermediate 4-methyl-1,2-benzoquinone (Yan *et al.*, 2005). Metabolism has been best characterized for *p*-cresol; therefore, a metabolic scheme for the isomer is presented in Figure 1. Based on this scheme and the chemical structure of *m*-cresol and its known metabolism, *in vivo* exposure to *m*-/*p*-cresol might result in the formation of *m*- and *p*-cresol-derived glucuronides, sulfate conjugates, and quinones, as well as *p*-cresol-derived quinone methide and hydroxybenzoic acid.

TOXICITY

Experimental Animals

Median lethal doses (LD_{50}) of cresols administered to animals are presented in Table 1. Cresols are more toxic to mice than to rats when orally administered at similar concentrations. *m*-Cresol is the least toxic of the three isomers within the same species and route of administration.

F344/N rats and B6C3F1 mice of both sexes (five per sex per dose) were fed diets containing *o*-cresol, *m*-cresol, *p*-cresol, or *m*-/*p*-cresol (60:40) at concentrations of 0, 300, 1,000, 3,000, 10,000, or 30,000 ppm for 28 days (NTP, 1992). All rats survived, but some mice exposed to *o*-cresol at 30,000 ppm, *m*-cresol at 10,000 or 30,000 ppm, or *p*-cresol at 10,000 ppm died before the end of the studies. All mice exposed to 30,000 ppm of *p*-cresol died, while all mice exposed to *m*-/*p*-cresol survived. Feed consumption was decreased in all groups of exposed rats during the first week but was not significantly different from that of the controls during the remainder of the study. Mean body weight gains were less than those of the controls in all rats exposed to 30,000 ppm cresols. All surviving mice exposed to 30,000 ppm cresols lost weight except females exposed to *m*-cresol. No clinical signs of toxicity were observed in rats exposed to *o*- or *m*-cresol; however, rats exposed to 30,000 ppm *p*-cresol had hunched posture, rough hair coat, and thin appearance; rats exposed to 30,000 ppm *m*-/*p*-cresol were also thin in appearance. Hunched posture, rough hair coat, lethargy, hypothermia, and thin appearance were observed in some mice exposed to 10,000 or 30,000 ppm cresols. Increased relative liver and kidney weights of both rats and mice were associated with exposure to cresols. Liver weights were affected at concentrations as low as 300 ppm *m*-cresol in female mice and 1,000 ppm *m*-/*p*-cresol in female rats. No lesions were detected in rats exposed to *o*-cresol, but uterine and ovarian atrophy were observed in some female mice exposed to *o*-cresol. *m*-Cresol was associated with uterine atrophy in 30,000 ppm female rats and uterine, ovary, and mammary gland atrophy in 30,000 ppm female mice. Bone marrow hypoplasia was observed in male and female rats and mice exposed to either *p*- or *m*-/*p*-cresol, mostly at 30,000 ppm. Liver atrophy and necrosis were observed in some male and female mice exposed to 30,000 ppm *p*-cresol. Atrophy and regenerative changes in the nasal epithelia of rats and mice were specific to *p*- and *m*-/*p*-cresol exposure. Hyperplasia of the respiratory epithelium was observed in male and female rats exposed to concentrations of 3,000 ppm or greater *p*-cresol and in male and female mice exposed to concentrations as low as 300 ppm. Squamous metaplasia, atrophy, and necrosis of the respiratory and/or olfactory epithelium were observed in some animals exposed to *p*-cresol. Respiratory epithelial hyperplasia of the nasal cavity was observed in male rats exposed to 3,000 ppm or greater *m*-/*p*-cresol, female

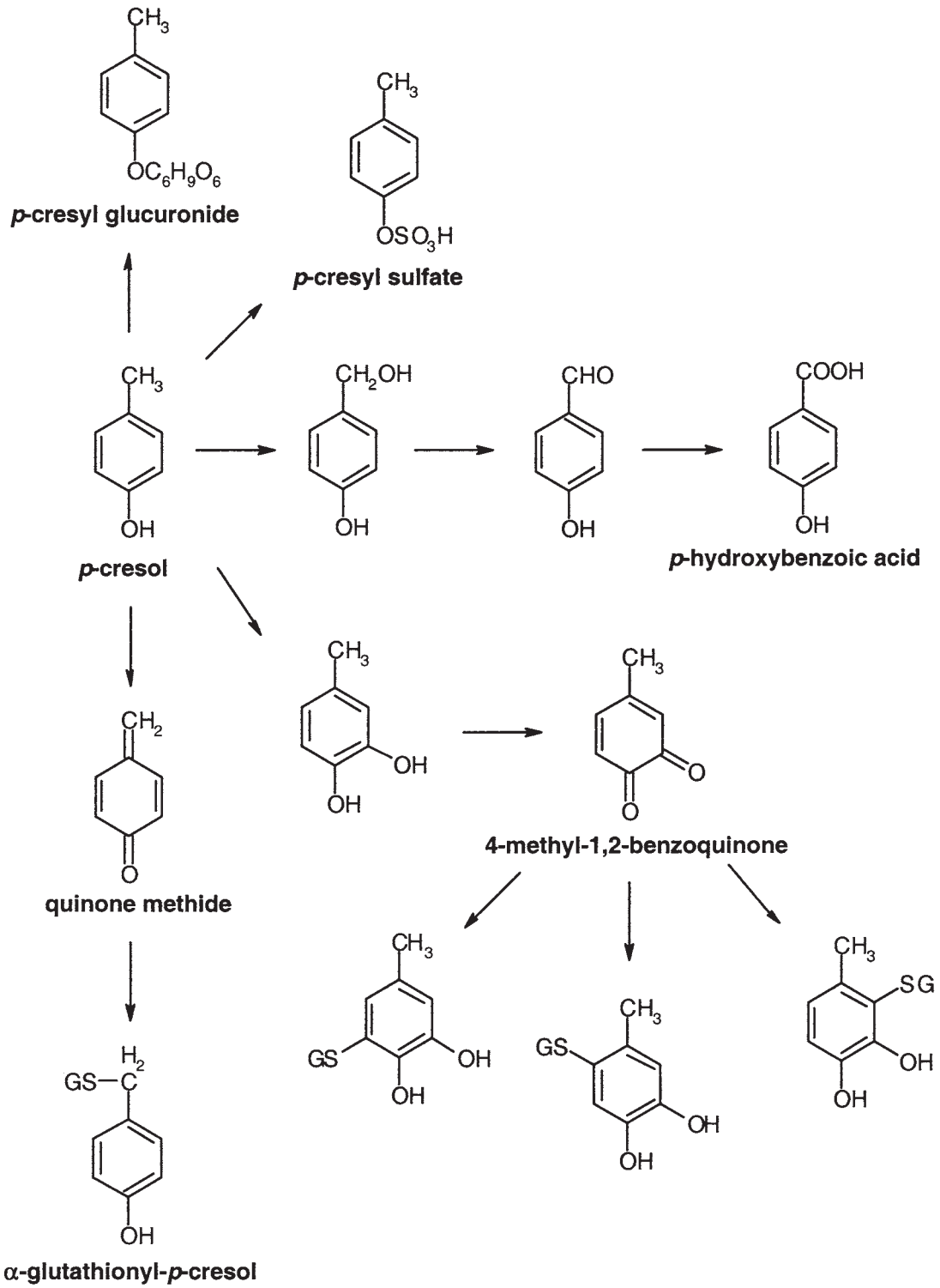


FIGURE 1
Proposed Metabolic Pathways for *p*-Cresol
(Adapted from Yan *et al.*, 2005, and Anderson, 2006)

TABLE 1
Summary of Selected Acute Animal Toxicity Data for Cresols[a]

Cresol	Species	Route of Administration	Vehicle	LD_{50} (mg/kg)
o-	Rat	Oral	10% in oil	1,350, 1,470
m-	Rat	Oral	10% in oil	2,010, 2,020
p-	Rat	Oral	10% in oil	1,430, 1,460, 1,800
m-/*p*-	Rat	Oral	10% in oil	1,625
o-	Rat	Oral	Neat	121
m-	Rat	Oral	Neat	242
p-	Rat	Oral	Neat	207
o-	Rat	Dermal	Unknown	620
m-	Rat	Dermal	Unknown	1,100
p-	Rat	Dermal	Unknown	750
m-/*p*-	Rat	Dermal	Unknown	825
o-	Mouse	Oral	10% in oil	344
m-	Mouse	Oral	10% in oil	600, 828
p-	Mouse	Oral	10% in oil	344, 440
m-/*p*-	Mouse	Oral	10% in oil	651
o-	Rabbit	Oral	10% in oil	940
o-	Rabbit	Dermal	Neat	890
m-	Rabbit	Dermal	Neat	2,830
p-	Rabbit	Dermal	Neat	300
mixed	Rabbit	Dermal	Neat	2,000

[a] Data from Vernot *et al.*, 1977, and from references cited in IPCS, 1995

rats exposed to 1,000 ppm or greater, male mice exposed to 10,000 ppm or greater, and female mice exposed to 3,000 ppm or greater. Metaplasia of the olfactory epithelium and bronchiolar hyperplasia were observed in male and female mice exposed to 30,000 ppm. Exposure to *m*-/*p*-cresol resulted in minimal to mild epithelial hyperkeratosis and/or hyperplasia of the esophagus and forestomach in rats at concentrations as low as 3,000 ppm and in one male mouse exposed to 30,000 ppm. No lesions of the gastrointestinal tract were reported in female mice exposed to *m*-/*p*-cresol or in any animals exposed to *p*-cresol.

In 13-week studies (NTP, 1992), groups of 20 male and 20 female F344/N rats (gross pathology was reported for 10 rats per group) were fed diets containing *o*-cresol or a 60:40 mixture of *m*-/*p*-cresol at concentrations of 0, 1,880, 3,750, 7,500, 15,000, or 30,000 ppm. Groups of 10 male and 10 female B6C3F1 mice were fed diets containing *o*-cresol at concentrations of 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm or a 60:40 mixture of *m*-/*p*-cresol at concentrations of 0, 625, 1,250, 5,000, or 10,000 ppm. There was little evidence of a significant increase in toxic effects of *o*- or *m*-/*p*-cresol in the 13-week studies over those observed in the 28-day studies. All animals lived to the end of the study. Feed consumption was decreased only during the first week for some high-exposure animals. Final mean body weights were less than those of the controls for *o*-cresol-exposed male rats at 30,000 ppm, female rats at 15,000 and 30,000 ppm, male mice at 20,000 ppm, and female mice at 5,000 ppm and higher. Final mean body weight gains were less than those of the controls for *m*-/*p*-cresol-exposed male and female rats at 15,000 and 30,000 ppm and male and female mice at 10,000 ppm. Relative kidney, testis, and thymus weights were increased in some cresol-exposed groups. Relative liver weights were increased in *o*-cresol-exposed male and female rats at 7,500 ppm and higher, male mice at 1,250 ppm and higher, and female mice at 5,000 ppm and higher. Relative liver weights were increased in *m*-/*p*-cresol-exposed male and female rats at 7,500 ppm and higher, male mice at 5,000 ppm and higher, and female mice at 10,000 ppm. Hematology, clinical chemistry, and urinalysis results were generally unremarkable in all studies. However, an accumulation of bile acids was observed in some instances in cresol-exposed rats, indicating decreased hepatocellular function. *o*-Cresol exposure resulted in increased incidences of bone marrow hypocellularity in male rats exposed to 30,000 ppm

and in female rats exposed to 15,000 or 30,000 ppm. The incidence of forestomach epithelial hyperplasia was increased in male and female mice exposed to 20,000 ppm *o*-cresol. Histopathologic changes in *m*-/*p*-cresol-exposed rats and mice included bone marrow hypocellularity, nasal lesions, and atrophy of female reproductive organs (Tables 2 and 3). No kidney or liver lesions were reported. There were few or no indications of adverse effects in male reproductive tissues; however, in females, the estrous cycle was lengthened in mice exposed to 20,000 ppm *o*-cresol and in rats exposed to concentrations of *m*-/*p*-cresol as low as 7,500 ppm.

Atrophy and regenerative changes observed in the nasal epithelia and forestomach of rats and mice following exposure to *p*- and/or *m*-/*p*-cresol in the NTP 28-day studies were presumed to be a result of irritation to the tissues (NTP, 1992). Evidence of nasal irritation (hyperplasia of the epithelium) was also observed in some *m*-/*p*-cresol exposed groups in 13-week studies (Tables 2 and 3); however, no cresol-related effects were observed in the forestomach. All three cresol isomers have been shown to be corrosive to skin following dermal application to rabbits (Vernot *et al.*, 1977). Additionally,

TABLE 2
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Feed Study of *m-/p*-Cresol

	0 ppm		1,880 ppm		3,750 ppm		7,500 ppm		15,000 ppm		30,000 ppm	
Male												
Bone Marrow[a]	10		0		0		0		10		10	
Hypocellularity[b]	0								1	(1.0)[c]	8**	(1.0)
Nose	10		10		10		10		10		10	
Respiratory Epithelium, Glandular Hyperplasia	0		3	(1.0)	8**	(1.5)	10**	(1.6)	9**	(2.6)	9**	(3.8)
Respiratory Epithelium, Hyperplasia	0		3	(1.0)	8**	(1.1)	10**	(1.4)	8**	(2.2)	10**	(3.8)
Thyroid Gland	10		0		0		0		10		10	
Follicle, Increased Colloid	0								7**	(1.1)	9**	(1.6)
Female												
Bone Marrow	10		0		0		0		10		10	
Hypocellularity	0								0		6**	(1.0)
Nose	10		10		10		10		10		10	
Respiratory Epithelium, Glandular Hyperplasia	0		2	(1.0)	6**	(1.3)	10**	(2.1)	8**	(2.5)	6**	(2.8)
Respiratory Epithelium, Hyperplasia	3	(1.0)	1	(1.0)	5	(1.2)	9**	(1.7)	8*	(2.0)	10**	(2.8)
Thyroid Gland	10		0		10		10		10		10	
Follicle, Increased Colloid	0				1	(1.0)	6**	(1.2)	7**	(1.6)	8**	(1.6)
Uterus	10		0		0		0		10		10	
Atrophy	0								3	(1.0)	7**	(1.7)

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

TABLE 3
Incidences of Nonneoplastic Lesions of the Nose in Mice in the 13-Week Feed Study of *m-/p*-Cresol

	0 ppm		625 ppm	1,250 ppm	2,500 ppm		5,000 ppm		10,000 ppm	
Male										
Number Examined Microscopically	10		0	0	0		10		10	
Respiratory Epithelium,[a]										
Glandular Hyperplasia	1	(1.0)[b]					0		2	(1.0)
Respiratory Epithelium,										
Hyperplasia	1	(1.0)					4	(1.0)	8**	(1.0)
Female										
Number Examined Microscopically	10		0	0	10		10		10	
Respiratory Epithelium,										
Glandular Hyperplasia	1	(1.0)			0		0		2	(1.5)
Respiratory Epithelium,										
Hyperplasia	2	(1.5)			3	(1.0)	2	(1.0)	5	(1.4)

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

p-cresol exposure (1.5% in feed for 20 weeks) resulted in an increased incidence of hyperplasia of the forestomach in Syrian golden hamsters (Hirose, *et al.* 1986).

Humans

Toxicity has been observed in humans who have intentionally ingested or been accidentally exposed to cresol-containing products. Exposure to cresols can be fatal; a man died 15 minutes after ingesting a mixture of *m*- and *p*-cresol (Monma-Ohtaki *et al.*, 2002), and a 1-year-old baby dermally exposed (on the scalp) to 20 mL of a 90% cresol solution died within 4 hours (Green, 1975). In the latter incident, clinical signs of toxicity included burns at the site of exposure, hemorrhagic pulmonary edema, liver and kidney necrosis, and swelling of the brain. Generally, cresols are irritating to skin and the membranes of the digestive and respiratory tracts, and exposure-related effects have been observed in the blood, brain, liver, lung, heart, and kidney of humans (ATSDR, 2006).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

The toxicity of *m*-/*p*-cresol has been investigated by the NTP in a two-generation reproductive study in Swiss CD-1 mice receiving 0%, 0.25%, 1.0%, or 1.5% per day in feed (corresponding to 0, 370, 1,500, or 2,100 mg/kg per day, respectively) (Heindel *et al.*, 1997). Some deaths occurred in the F_0 generation but were not considered to be exposure related. The number of litters was not affected; however, the number of pups was reduced; decreased body weight gain was observed in pups, and gestation increased by 4 days at 2,100 mg/kg. Exposed pups gained less weight than the controls by postnatal day 21. At necropsy, F_0 male mice receiving 2,100 mg/kg had decreased body weight gain, increased relative kidney and liver weights, and decreased relative seminal vesicle weights compared to the control groups. F_0 females receiving 2,100 mg/kg also gained less weight than controls and had increased

relative liver weights. F_1 adult males had decreased body weight gain, reduced absolute testis and seminal vesicle weights, and increased relative liver weights at 1,500 and 2,100 mg/kg. F_1 adult females from all cresol-exposed groups had increased relative liver and kidney weights, and mean body weights were less than those of the controls at 1,500 and 2,100 mg/kg. A dose-related increase in hydronephrosis of the kidney was observed in F_1 females. In F_0 and F_1 rats, no histological changes were observed in the reproductive tracts of males or females; sperm endpoints were not affected in males; and no effects on estrous cycle length were observed in females. The only adverse effect on F_2 generation pups was a reduction in body weight at 2,100 mg/kg. However, based on smaller F_1 litters and reduced F_1 and F_2 pup weights, *m*-/*p*-cresol was concluded to be a reproductive toxicant in this mouse strain.

m-Cresol was administered by gavage to newborn male and female Sprague-Dawley rats from postnatal days 4 to 21 at doses up to 300 mg/kg per day (Koizumi *et al.*, 2003). Deep respiration, hypersensitivity upon handling, tremors under contact stimulus, and decreased body weight gain were observed at 300 mg/kg. Clinical effects were observed in some male rats at 100 mg/kg. No abnormalities of physical development, sexual maturity, or functional development of the brain were observed in any animals. The no-observed-adverse-effect level (NOAEL) for neonates (30 mg/kg) was lower than the NOAEL for older rats (300 mg/kg) gavaged for 28 days in the same study. It was speculated that the lower capacity for glucuronidation resulted in less detoxication of the cresol in the neonates.

The embryotoxicity of *p*-cresol was investigated following explantation of embryos from Sprague-Dawley rats on gestation day 10 (Oglesby *et al.*, 1992). The embryos were cultured for 42 hours in concentrations up to 694 µM *p*-cresol with hepatocytes (to model maternal metabolism) or without hepatocytes. Viable embryos (with detectable heartbeats) were evaluated for growth (endpoints were somite number, crown-rump length, and DNA content) and morphological abnormalities (endpoints were tail bud abnormalities, hypoplasia of the first arch, and hindlimb bud absence). Concentration-related effects on all three embryo growth endpoints and on hindlimb bud absence were observed in cultures lacking hepatocytes. In the presence of hepatocytes, the effect on growth parameters was reduced, and no significant increases in structural defects were observed. It was concluded that apparent conjugation of *p*-cresol diminished embryotoxicity.

Unpublished reports of reproductive and developmental studies of cresols administered by gavage were summarized in reviews prepared by the International Programme on Chemical Safety (IPCS, 1995) and the Agency for Toxic Substances and Disease Registry (ATSDR, 2006). In two-generation reproductive studies, no adverse effects on reproductive function or histopathological lesions were reported in any rats exposed to *o*-, *m*-, or *p*-cresol. The high dose (450 mg/kg) resulted in overt toxicity to dams receiving either isomer and body weight effects in their offspring. Slight fetotoxic effects were observed in rats and rabbits in two other studies, but only at maternally toxic doses. The effects were seen in *o*- and *p*-, but not *m*- cresol-exposed rats at the high dose of 450 mg/kg and in *o*-, but not *m*- or *p*-, cresol-exposed rabbits at the high dose of 100 mg/kg. No effects were observed at lower doses.

CARCINOGENICITY

Experimental Animals

The carcinogenic potential of cresols has not been adequately evaluated in animals. Some studies have indicated that cresols are tumor promoters. The tumor initiator, dimethylbenzanthracene, was applied to the skin of mice, followed by application of 20% solutions of *o*-, *m*-, or *p*-cresols in benzene twice weekly for 12 weeks (Boutwell and Bosch, 1959). Mortality was significant in cresol-treated mice; however, increased numbers of skin papillomas were observed in survivors. Benzene had no effect on papilloma incidence. *o*-Cresol (1 mg) administered to mice by gavage twice weekly for 30 weeks along with the known initiator benzo[*a*]pyrene (1 mg) increased the incidence and decreased the latency period for formation of forestomach papillomas and invasive carcinomas (Yanysheva *et al.*, 1993).

Humans

No epidemiology studies or case reports examining the relationship between exposure to cresols and human cancer were found in the literature.

GENETIC TOXICITY

The genotoxicity of cresols has been extensively investigated. Results from a number of *Salmonella typhimurium* mutagenicity assays with either the single

isomers or mixtures of two or more isomers were uniformly negative with and without metabolic activation (Douglas *et al.*, 1980; Florin *et al.*, 1980; Pool and Lin, 1982; Haworth *et al.*, 1983; Zeiger *et al.*, 1992; Kubo *et al.*, 2002).

Results from mutation assays in mammalian test systems are available mainly through industry reports that were summarized in an Agency for Toxic Substances and Disease Registry review (ATSDR, 2006) and a World Health Organization report (IPCS, 1995). The results that were included in these two reports suggest that cresols may be capable of interacting with mammalian DNA *in vitro*, inducing DNA damage and perhaps chromosomal damage in some cell types under certain conditions. However, knowledge of specific assay conditions and protocol details, which are not available, are critical to interpreting these results. The *o*- and *p*-cresol isomers, but not the *m*-cresol isomer, were reported to induce chromosomal aberrations in cultured Chinese hamster cells (Murli, 1988). *m*-Cresol was reported to induce sister chromatid exchanges (indicating DNA damage) in cultured Syrian hamster embryo cells (Miyachi and Tsutsui, 2005), but no induction of sister chromatid exchanges was seen in cultured human fibroblasts following treatment with *o*-, *m*-, or *p*-cresol (Cheng and Kligerman, 1984).

Although the *o*-, *m*-, and *p*-cresol isomers, when tested individually, were reported to be nonmutagenic to cultured mouse lymphoma L5178Y/tk$^{+/-}$ cells (Cifone, 1988), a 1:1:1 mixture of the three isomers was reported to be positive in this assay (PH&S, 1980, 1981). *o*-Cresol was reported to induce DNA damage, assessed by the Comet assay, in mouse spermatids and in human lymphocytes *in vitro* in the absence of S9 activation enzymes (Li *et al.*, 2005). DNA adduct formation was seen in rat hepatocytes and human leukemia HL-60 cells treated with *p*-cresol in the absence of S9 (Gaikwad and Bodell, 2001). Unscheduled DNA synthesis was observed in Syrian hamster embryo cells exposed to *m*-cresol in the presence of S9 activation only (Hamaguchi and Tsutsui, 2000).

Results from *in vivo* genotoxicity assays with cresols produced mainly negative results. Negative results were reported with *o*- (200 mg/kg), *m*- (200 mg/kg), and *p*-cresol (75 mg/kg) administered by intraperitoneal injection in tests for induction of sister chromatid exchanges in mice (Cheng and Kligerman, 1984); for *o*- and *p*-cresol in tests for induction of dominant lethal mutations (chromosomal damage in sperm) in mice (Ivett, 1989a,b); and for *m*-cresol in a mouse bone marrow chromosome aberration assay (Ivett, 1989c). No induction of sex-linked recessive lethal mutations was reported in male *Drosophila melanogaster* treated with *o*- or *p*-cresol (Sernav, 1989a,b). Administration of *o*-cresol (0.5 to 2.0 mg/kg) or *m*-/*p*-cresol (0.1 to 1.0 mg/kg) to male and female B6C3F1 mice in feed for 3 months did not produce increased frequencies of micronucleated erythrocytes in peripheral blood samples (Witt *et al.*, 2000). An increased frequency of micronucleated erythrocytes was reported in a second study in male mice exposed to *o*-cresol (20, 40, or 80 mg/kg) by intraperitoneal injection (Li *et al.*, 2005). Results of this study require replication, as too few cells were scored per treatment group, and the reported increase in micronucleated cells was not correlated with dose.

STUDY RATIONALE

Cresols, monomethyl derivatives of phenol, are present in the environment from natural and anthropogenic sources and are used in a variety of industrial and consumer products. Cresols were nominated for chronic toxicity and carcinogenicity testing by the National Institute of Environmental Health Sciences based on the potential for occupational and consumer exposure and the lack of chronic toxicity data. The results of subchronic toxicity studies of cresols have been published (NTP, 1992). This Technical Report describes the results of 2-year toxicity and carcinogenicity studies conducted in male rats and female mice exposed to a 60:40 *m*-/*p*-cresol mixture. Female rats and male mice were omitted from these studies based on statistical evidence indicating that the carcinogenic potential of approximately 96% of 311 chemicals studied by the National Cancer Institute or the NTP could have been determined using only male rats and female mice (Huff *et al.*, 1991). In those studies, 14 of 161 carcinogens were positive only in female rats and/or male mice. Cresols are structurally similar to phenol and toluene, both previously found to be negative for carcinogenicity in rodents (NTP, 1980, 1990). Therefore, cresols were categorized as lower priority chemicals for testing and were considered to be suitable candidates for evaluation using the modified study protocol. As a result, the number of animals needed to conduct these studies was greatly reduced. The 60:40 *m*-/*p*-cresol mixture was used in the present

studies based on the results of 13-week studies and because the mixture approximated the isomeric ratio distilled from coke-oven tars in the United States (*Kirk-Othmer*, 1997). The cresol studies were originally part of the "Superfund" initiative concerned with identifying hazards of ground water contamination (NTP, 1992); therefore, oral administration was chosen as the representative route of exposure. However, the chemical was administered in feed rather than drinking water based on palatability and solubility of the isomers.

MATERIALS AND METHODS

Procurement and Characterization of Cresols

A mixture of *meta*- and *para*- cresols was obtained from Merichem Company (Houston, TX; lot RC Sample 891) and Merisol USA, LCC (Houston, TX; lot Z000004273). These two lots were combined by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), into one lot (M080901KLH), which was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory, Battelle Columbus Operations (Columbus, OH) (Appendix D). Reports on analyses performed in support of the cresols studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a light yellow liquid, was identified as cresols by the analytical chemistry laboratory using density and boiling point determinations; infrared, ultraviolet/visible, and proton nuclear magnetic resonance spectroscopy; and gas chromatography (GC)/mass spectrometry (MS) and by the study laboratory using infrared spectroscopy. Purity of lot M080901KLH was determined by the analytical chemistry laboratory using thin layer chromatography (TLC), GC, and GC/MS, and by the study laboratory using GC (Table D1). Karl Fischer titration generally indicated a water content of approximately 0.2%. TLC at 254 nm with iodine vapor and reagent detected one major spot with no impurities. TLC at 366 nm and visible light showed no spots, and GC indicated two major peaks and two impurities greater than or equal to 0.05% relative to the major peak areas. GC/MS spectra confirmed that the two major peaks were cresols by comparison with the NIST spectral library; the overall purity was determined to be greater than 99.5%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC. Stability was confirmed for lot M080901KLH when stored in sealed amber glass bottles under a nitrogen headspace and protected from light at temperatures up to 60° C for at least 2 weeks. To ensure stability, the bulk chemical was stored in sealed amber glass bottles at approximately 25° C and protected from light. Periodic analyses were performed by the study laboratory to monitor stability during the 2-year studies using GC. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared monthly by mixing cresols with NTP-2000 feed (Table D2). Homogeneity studies of 1,000 and 15,000 ppm dose formulations of the lot used in the studies and stability studies of a 500 ppm dose formulation of another lot were performed by the analytical chemistry laboratory using GC. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 42 days when stored in the dark in plastic bags at 25° C and for at least 4 days under simulated animal room conditions.

Periodic analyses of the dose formulations of cresols were conducted by the study laboratory using GC. The dose formulations were analyzed approximately every 3 months (Table D3). All of the dose formulations analyzed for rats (54) and mice (27) were within 10% of the target concentrations.

2-Year Studies

Study Design

Groups of 50 male rats were fed diets containing 0, 1,500, 5,000, or 15,000 ppm *m-/p*-cresol for 105 weeks. Groups of 50 female mice were fed diets containing 0, 1,000, 3,000, or 10,000 ppm *m-/p*-cresol for 104 to 105 weeks. The highest exposure concentrations for rats and mice were based on the minimal toxicity observed at these levels in the 13-week studies (NTP, 1992). In the 13-week studies, mean body weight gains were reduced only 7% relative to controls for rats receiving 15,000 ppm and mice receiving 10,000 ppm in feed. The only significant histopathologic changes observed at these exposure concentrations were increased incidences of nasal epithelium hyperplasia in rats and mice and an increased incidence of colloid in the thyroid gland of

rats. The lower exposure concentrations selected for use in the 2-year study represented approximately 10% and 30% of the high exposures in the 13-week studies.

Source and Specification of Animals

Male F344/N rats and female B6C3F1 mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Male mice from the same source were included in the study rooms to ensure normal estrous cycling of the female mice. Rats and mice were quarantined for 11 and 12 days, respectively, before the beginning of the studies. Rats and mice were approximately 6 weeks old at the beginning of the studies. Ten male rats and five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix G).

Animal Maintenance

Rats were housed two or three per cage, and mice were housed five per cage. Feed and water were available *ad libitum*. Feed consumption (by cage) was determined weekly for the first 13 weeks and at 4-week intervals thereafter. Cages were changed at least twice weekly, and cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 4. Information on feed composition and contaminants is provided in Appendix F.

Clinical Examinations and Pathology

All animals were observed twice daily. Body weights were recorded at the beginning of the study, weekly for the first 13 weeks, at 4-week intervals thereafter, and at study termination. Clinical findings were recorded during week 5 of the study, at 4-week intervals thereafter, and at study termination.

Complete necropsies and microscopic examinations were performed on all 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 (eyes were first placed 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 for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. For extended evaluation of renal proliferation lesions, additional sections of both kidneys from the residual formalin-fixed wet tissues were obtained for each male rat, imbedded in separate paraffin blocks, and step sectioned at 1 mm intervals. Three (left kidney) or four (right kidney) sections were examined for each rat. Tissues examined microscopically are listed in Table 4.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. 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 kidney (rats), thyroid gland (mice), and nose (rats and mice).

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, 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 chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the 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).

During the microscopic evaluations of the additional kidney sections for renal tubule proliferative lesions, the step section lesions were compared with lesions observed during the initial standard evaluation to ensure consistency between evaluations and to prevent duplication of diagnoses for lesions already diagnosed in the standard evaluations. Using procedures for the reviews of standard sections, the PWG chairperson reviewed the findings and slides from the step section evaluations. Final diagnoses for the step sections were recorded separately from the standard sections.

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Cresols

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

Strain and Species
F344/N rats
B6C3F1 mice

Animal Source
Taconic Laboratory Animals and Services (Germantown, NY)

Time Held Before Studies
Rats: 11 days
Mice: 12 days

Average Age When Studies Began
6 weeks

Date of First Exposure
Rats: August 5, 2002
Mice: August 20, 2002

Duration of Exposure
Rats: 105 weeks
Mice: 104 to 105 weeks

Date of Last Exposure
Rats: August 4, 2004
Mice: August 19, 2004

Necropsy Dates
Rats: August 2-4, 2004
Mice: August 16-19, 2004

Average Age at Necropsy
Rats: 110 to 111 weeks
Mice: 109 to 111 weeks

Size of Study Groups
Rats: 50 males
Mice: 50 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
Rats: 2 or 3
Mice: 5

Method of Animal Identification
Tail tattoo

Diet
Irradiated NTP-2000 open formula meal (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed twice weekly

Water
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*

TABLE 4
Experimental Design and Materials and Methods in the 2-Year Feed Studies of Cresols

Cages
Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice weekly

Bedding
Irradiated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly

Cage Filters
Spun-bonded polyester (Snow Filtration, Cincinnati, OH), changed every 2 weeks

Racks
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Exposure Concentrations
Rats: 0, 1,500, 5,000, or 15,000 ppm in feed, available *ad libitum*
Mice: 0, 1,000, 3,000, or 10,000 ppm in feed, available *ad libitum*

Type and Frequency of Observation
Observed twice daily. Animals were weighed at the beginning of the study, weekly for the first 13 weeks, at 4-week intervals thereafter, and at study termination. Clinical findings were recorded during week 5 of the study, at 4-week intervals thereafter, and at study termination. Feed consumption was measured weekly for the first 13 weeks and at 4-week intervals thereafter.

Method of Sacrifice
CO_2 asphyxiation

Necropsy
Necropsies were performed on all animals.

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

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 or missing were censored from the survival analyses; 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, A4, B1, and B4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2 and B2) 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., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or 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 and B2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

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 sacrifice; if the animal died prior to terminal sacrifice 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 rats and B6C3F1 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 exposed group with controls and a test for an overall exposure-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).

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997).

The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 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 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance 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 cresols was assessed by testing the ability of the chemical, as individual isomers and as isomer mixtures, to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* strain WP2 *uvrA* and increase 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 C.

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

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

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 (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. 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. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

RATS

Survival

Estimates of 2-year survival probabilities for male rats are shown in Table 5 and in the Kaplan-Meier survival curve (Figure 2). Survival rates of exposed groups of male rats were similar to that of the control group.

TABLE 5
Survival of Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Animals initially in study	50	50	50	50
Moribund	12	10	9	14
Natural deaths	5	6	8	5
Animals surviving to study termination	33	34	33	31
Percent probability of survival at end of study[a]	66	68	66	62
Mean survival (days)[b]	703	696	671	684
Survival analysis[c]	P=0.560	P=1.000N	P=0.950	P=0.710

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A lower mortality in an exposed group is indicated by **N**.

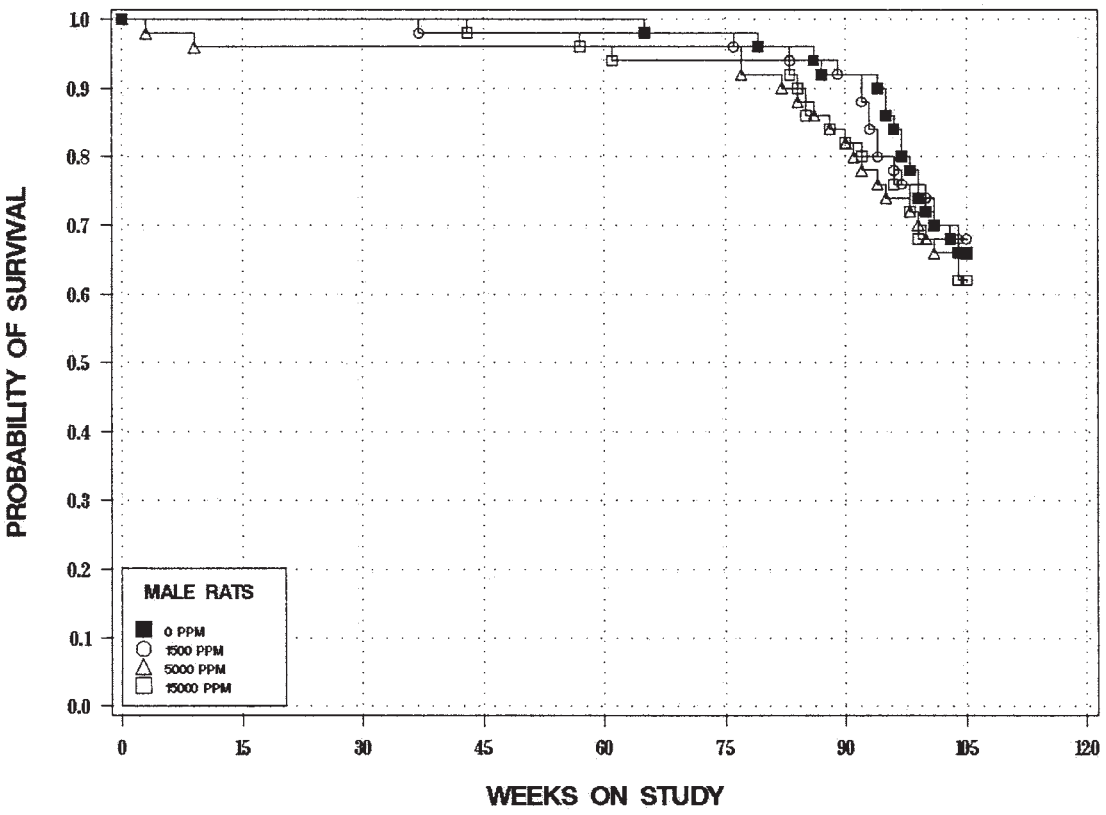


FIGURE 2
Kaplan-Meier Survival Curves for Male Rats Exposed to Cresols in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of the 15,000 ppm group were less than those of the controls throughout the study and decreased to 85% that of the control group by the end of the study (Figure 3; Table 6). Feed consumption by the 15,000 ppm group was less than that by the controls during the first week of the study but increased to within 4% of that by the controls by the second week of the study. Feed consumption by the other exposed groups was similar to that by the controls throughout the study (Table E1). Dietary concentrations of 1,500, 5,000, and 15,000 ppm resulted in average daily doses of approximately 70, 230, and 720 mg cresols/kg body weight for male rats. There were no clinical findings related to exposure to cresols.

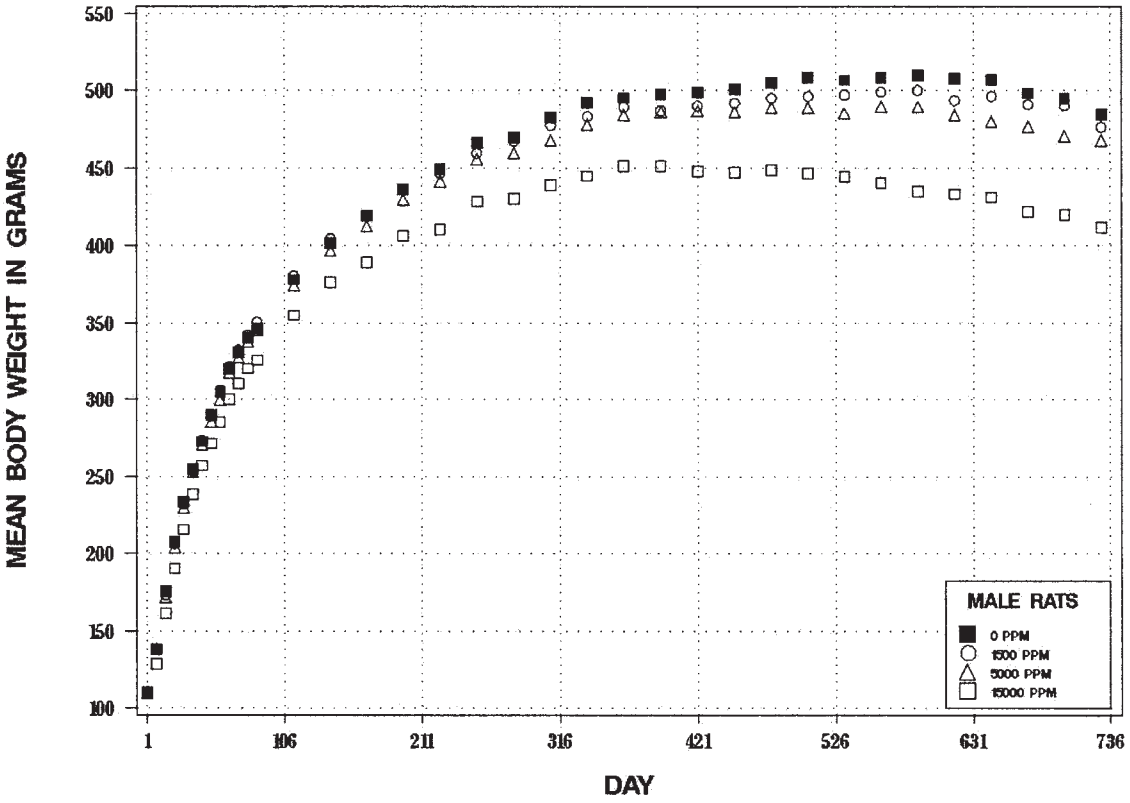


FIGURE 3
Growth Curves for Male Rats Exposed to Cresols in Feed for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Cresols

Days on Study	0 ppm		1,500 ppm			5,000 ppm			15,000 ppm		
	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	110	50	110	100	50	110	100	50	110	99	50
8	139	50	139	100	50	139	100	50	129	93	50
15	176	50	173	99	50	172	98	50	162	92	50
22	208	50	206	99	50	204	98	49	190	92	50
29	234	50	232	99	50	230	98	49	216	92	50
36	255	50	254	100	50	253	99	49	239	94	50
43	273	50	273	100	50	271	99	49	257	94	50
50	290	50	289	100	50	286	99	49	271	94	50
57	305	50	306	100	50	300	98	49	285	94	50
64	320	50	322	101	50	318	99	48	300	94	50
71	331	50	332	100	50	328	99	48	310	94	50
78	340	50	342	101	50	338	99	48	320	94	50
85	347	50	351	101	50	346	100	48	326	94	50
113	378	50	381	101	50	375	99	48	355	94	50
141	402	50	405	101	50	397	99	48	377	94	50
169	419	50	420	100	50	413	98	48	389	93	50
197	436	50	436	100	50	430	99	48	406	93	50
225	449	50	447	100	50	442	98	48	410	91	50
253	467	50	460	99	49	456	98	48	429	92	50
281	470	50	467	99	49	460	98	48	430	92	50
309	483	50	478	99	49	468	97	48	439	91	49
337	492	50	483	98	49	478	97	48	445	90	49
365	495	50	489	99	49	485	98	48	451	91	49
393	498	80	487	98	49	486	98	48	452	91	49
421	499	50	490	98	49	487	98	48	448	90	48
449	503	49	492	98	49	486	97	48	447	89	47
477	505	49	495	98	49	489	97	48	449	89	47
505	509	49	496	98	49	489	96	48	447	88	47
533	507	49	497	98	48	485	96	48	445	88	47
561	509	48	499	98	48	490	96	46	441	87	47
589	510	48	500	98	47	490	96	44	435	85	44
617	508	46	494	97	47	484	95	42	433	85	42
645	507	46	496	98	43	480	95	39	431	85	40
673	499	42	491	99	39	477	96	37	422	85	38
701	495	36	490	99	35	471	95	34	420	85	34
Mean for weeks											
1-13	256		256	100		253	99		240	94	
14-52	444		442	100		435	98		409	92	
53-101	503		494	98		485	96		440	88	

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 kidney, nose, liver, 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.

Kidney: In the standard evaluation of the kidney, the incidence of renal tubule adenoma was increased in the 15,000 ppm group, and the incidence exceeded the historical range for controls in feed studies (Tables 7, A2, and A3). The renal tubule cell adenomas were small lesions, approximately 0.5 mm in diameter. Microscopically, renal tubule cell adenomas were well-circumscribed, discrete, round or oval masses greater than five times the diameter of a normal renal tubule. They were composed of multiple densely packed tubular structures without obvious lumens, having closely packed round to polygonal epithelial cells with varying amounts of lightly eosinophilic foamy cytoplasm and normal appearing nuclei. Bands of homogeneous eosinophilic stromal material were interlaced among some of the tubular structures.

Because the incidence of renal tubule adenoma was increased during the standard evaluation, additional step sections of kidney were prepared from the remaining formalin-fixed tissues. Three (left kidney) or four (right kidney) sections at 1-mm intervals were prepared and examined for each rat. Five additional animals had proliferative renal lesions during the examinations of the step sections. Four lesions were identified as renal tubule hyperplasia, and one was a renal tubule adenoma. The incidences of proliferative lesions in standard and extended evaluations are presented in Table 7.

Two additional benign tumors, both lipomas, were observed in the kidney of rats in the 1,500 and 15,000 ppm groups (Tables 7 and A1). The lipomas were composed of sheets of vacuolated cells with a few residual renal tubules and cystic spaces within the tumors. These lipomas are infrequent tumors in F344/N rats and were considered to be unrelated to exposure. No renal lipomas have been reported in the current historical control data for feed studies, and only two renal lipomas have been reported for all treatment routes combined (Tables 7 and A3).

In the standard evaluation, the incidence of renal tubule hyperplasia in the exposed groups was similar to that in the control group (Tables 7 and A4). Four additional animals had renal tubule hyperplasia during the examinations of the step sections; three of these animals were in the control group. When the single and step sections were combined, the incidence of renal tubule hyperplasia in the control group exceeded incidences in the exposed groups. There was no evidence that exposure to cresols affected the incidence of renal tubule hyperplasia. Microscopically, renal tubule cell hyperplasia consisted of minimal to mild focal segments having single to multiple cortical tubules composed of multiple layers of epithelial cells with pale-staining foamy cytoplasm that partially filled the lumen and enlarged the tubules. The epithelial cells were variably enlarged with distinct cell borders, expanded eosinophilic cytoplasm, variable nuclear size, and multiple enlarged nucleoli. Affected tubules were generally, but not always, larger than normal but less than five times the diameter of a normal renal tubule. Renal tubule hyperplasia was considered to be a preneoplastic lesion and, as defined in the current study, was distinguished from regenerative epithelial changes commonly seen as a part of nephropathy.

The incidence of mild hyperplasia of the transitional epithelium of the renal pelvis was significantly increased in the 15,000 ppm group (Tables 7 and A4). Hyperplasia of the transitional epithelium was characterized by increased numbers of epithelial cells in the lining of the pelvis, often with formation of papillary projections that protruded from the renal pelvic epithelial surface into the pelvic lumen. These findings are common in rats and were considered secondary to the nephropathy in this study.

The average severity of nephropathy was increased slightly in the 15,000 ppm group (Table 7). Minimal nephropathy was characterized by a few scattered foci of tubular regeneration. These regenerative tubules had increased numbers of more intensely stained basophilic cells. Basement membranes, both in glomeruli and around tubules, were slightly thickened. As the nephropathy became more severe, tubular dilatation, proteinaceous casts, and interstitial fibrosis increased. Nephropathy in this study had the typical microscopic appearance of chronic progressive nephropathy, an age-related renal disease in the F344/N rat that is characterized by various degrees of degeneration, regeneration, and atrophy of the tubular epithelium; hyaline tubular casts; glomerulosclerosis; and interstitial fibrosis. Various numbers of scattered tubules were surrounded

TABLE 7
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Number Examined Microscopically	50	50	50	50
Single Sections (Standard Evaluation)				
Renal Tubule, Hyperplasia[a]	2 (1.0)[b]	0	0	1 (2.0)
Pelvis, Transitional Epithelium, Hyperplasia	0	0	2 (2.0)	8** (1.9)
Nephropathy	47 (1.4)	48 (1.4)	46 (1.7)	49 (2.1)
Lipoma[c]	0	1	0	1
Renal Tubule, Adenoma[d]				
Overall Rate[e]	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rate[f]	0.0%	0.0%	0.0%	7.0%
Terminal Rate[g]	0/33 (0%)	0/34 (0%)	0/33 (0%)	3/31 (10%)
First Incidence (days)	—[h]	—	—	729 (T)
Poly-3 Test[i]	P=0.007	—[j]	—	P=0.109
Step Sections (Extended Evaluation)				
Renal Tubule, Hyperplasia	3	0	1	0
Renal Tubule, Adenoma	0	0	0	1
Single and Step Sections (Combined)				
Renal Tubule, Hyperplasia	5	0	1	1
Renal Tubule, Adenoma				
Overall Rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted Rate	0.0%	0.0%	0.0%	9.3%
Terminal Rate	0/33 (0%)	0/34 (0%)	0/33 (0%)	3/31 (10%)
First Incidence (days)	—	—	—	725
Poly-3 Test	P<0.001	—	—	P=0.054

** Significantly different (P≤0.01) from the control group by the the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 0/297; all routes: 2/1,436 (0.1% ± 0.5%), range 0%-2%
[d] Historical incidence for feed studies: 1/297 (0.4% ± 0.9%), range 0%-2%
[e] Number of animals with neoplasm per number of animals with kidney examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Not applicable; no neoplasms in animal group
[i] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[j] Value of the statistic cannot be computed.

by thickened basement membranes and lined with regenerative epithelial cells. Numerous scattered dilated tubules were filled with homogeneous eosinophilic proteinaceous fluid.

Nose: Exposure to cresols resulted in increased incidences of goblet cell hyperplasia, respiratory epithelium hyperplasia and metaplasia, and inflammation (Tables 8 and A4). The nasal lesions were seen primarily in Level I of the nose and occasionally in Level II of the three nasal cavity levels that are routinely examined in NTP toxicity and carcinogenicity studies. Level I is excised immediately posterior to the upper incisor teeth; Level II is excised through the level of the incisive papilla anterior to the first palatial ridge, and Level III is excised through the middle of the second molar teeth. Levels I and II contain the naso- and maxilloturbinates that, along with the nasal passages (meatuses) and septum, are lined by ciliated respiratory type epithelium. Level III encompasses the olfactory region of the nose with ethmoid turbinates and meatuses lined entirely by specialized olfactory neuroepithelium.

The incidences of goblet cell hyperplasia were significantly increased in all exposed groups. Minimal to moderate goblet cell hyperplasia occurred as increases in the numbers of goblet or mucous cells in respiratory epithelium along the nasal septum and was often accompanied by hypertrophy. The hyperplasia with hypertrophy resulted in thickening of the respiratory epithelium and an undulating surface. In more severe lesions, the hyperplastic goblet cells formed acinar structures resembling glands. Affected cells were taller and larger than the normal respiratory cells, and nuclei appeared more numerous. Many goblet cells contained abundant amounts of mucin (Plate 1).

The incidences of hyperplasia of the respiratory epithelium were increased in all exposed groups. Hyperplasia of the respiratory epithelium was a minimal to mild change consistently observed on the tips of the nasoturbinates in Level I; in more severe lesions, the lesion was also seen on the lateral walls and dorsal tips of the maxilloturbinates. A normal respiratory epithelium consists of a single layer of cuboidal to low columnar epithelial cells. In hyperplasia, the respiratory epithelium consisted of three to five layers of cuboidal epithelial cells (Plate 2). In addition, prominent cystic structures, apparently dilated ducts of Bowman's gland, were often scattered within the hyperplastic epithelium.

Squamous metaplasia of the respiratory epithelium occurred with increased incidences in the 5,000 and 15,000 ppm groups. Minimal to mild squamous metaplasia of the respiratory epithelium occurred with hyperplasia and was usually seen within the scrolls of the nasoturbinates; in more severe cases, it was also seen on the lateral wall opposite the nasoturbinates. The metaplasia was characterized by two to four layers of laterally-flattened epithelial cells typical of squamous type epithelium (Plate 3). Close examination of the metaplastic epithelium revealed narrow, clear intercellular spaces bridged by fine eosinophilic filaments that resembled tonofilaments.

Inflammation of the nose occurred with an increased incidence in the 15,000 ppm group. The inflammation was a minimal to mild change that had two distinct

TABLE 8
Incidences of Nonneoplastic Lesions of the Nose in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Number Examined Microscopically	50	50	50	50
Goblet Cell, Hyperplasia[a]	23 (1.1)[b]	40** (1.1)	42** (1.2)	47** (1.6)
Respiratory Epithelium, Hyperplasia	3 (1.0)	17** (1.0)	31** (1.0)	47** (1.2)
Respiratory Epithelium, Metaplasia, Squamous	0	1 (1.0)	8** (1.0)	40** (1.5)
Inflammation	17 (1.5)	19 (1.6)	19 (1.3)	28* (1.4)

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

morphologic appearances. One form of inflammation consisted of one to few focal aggregates of small to moderate numbers of mixed inflammatory cells, mainly macrophages with a few lymphocytes sometimes admixed with a few neutrophils, that were scattered within the lamina propria of the nose beneath the respiratory epithelium. These inflammatory foci were observed in several locations including the nasoturbinates, maxilloturbinates, nasal septum, and the lateral wall. Foci of minimal inflammation in the lamina propria were usually quite small. When inflammatory foci in the lamina propria were more severe, they were larger and more numerous. The other form of inflammation was primarily confined to the nasal lumen and consisted of aggregates of moderate numbers of neutrophils mixed with varying numbers of irregular aggregates of lightly basophilic to eosinophilic material that may have been inspissated mucus. Occasionally, a bit of plant material was also present, suggesting that in some cases, plant material may have been the inciting cause of the inflammation. Secondary hyperplasia of the respiratory epithelium was often adjacent to the areas of inflammation.

Liver: The incidence of eosinophilic focus was significantly increased in the 15,000 ppm group (0 ppm, 14/50; 1,500 ppm, 14/50; 5,000 ppm, 13/50; 15,000 ppm, 23/50; Table A4). Eosinophilic foci consisted of well-demarcated focal areas of enlarged hepatocytes with abundant homogeneous cytoplasm that stained more intensely eosinophilic than normal hepatocytes. These hepatocytes were arranged in normal hepatic plates and had a border that blended smoothly with the surrounding normal parenchyma. There was usually little or no compression of the surrounding normal hepatocytes, although occasionally, limited compression was evident in larger foci. Some foci had areas of cystic degeneration with multiple small cyst-like spaces that contained homogeneous eosinophilic material or free blood. The incidence of angiectasis was significantly increased in the 15,000 ppm group (5/50, 5/50, 7/50, 14/50; Table A4). Angiectasis was characterized by abnormal distention or dilatation of small blood vessels, particularly sinusoids and venules.

Pituitary gland: The incidences of pituitary gland adenoma decreased with a negative trend, and the decreases were significant in the 5,000 and 15,000 ppm groups (0 ppm, 20/50; 1,500 ppm, 14/50; 5,000 ppm, 9/49; 15,000 ppm, 8/50; Table A2). The incidence in controls was at the upper end of the historical range for pituitary gland adenomas in male rats [76/299 (26.3% ± 9.6%), range 18% to 40%]. One pituitary gland carcinoma occurred in the 15,000 ppm group (Table A1).

MICE

Survival

Estimates of 2-year survival probabilities for female mice are shown in Table 9 and in the Kaplan-Meier survival curve (Figure 4). Survival rates of exposed groups of female mice were similar to that of the control group.

TABLE 9
Survival of Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Animals initially in study	50	50	50	50
Missing[a]	0	0	1	0
Moribund	4	4	0	1
Natural deaths	5	3	5	7
Animals surviving to study termination	41	43	44	42
Percent probability of survival at end of study[b]	82	86	90	84
Mean survival (days)[c]	696	717	719	705
Survival analysis[d]	P=1.000N	P=0.716N	P=0.374N	P=0.948N

[a] Censored from survival analysis
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed group columns. A negative trend or a lower mortality rate in an exposed group is indicated by **N**.

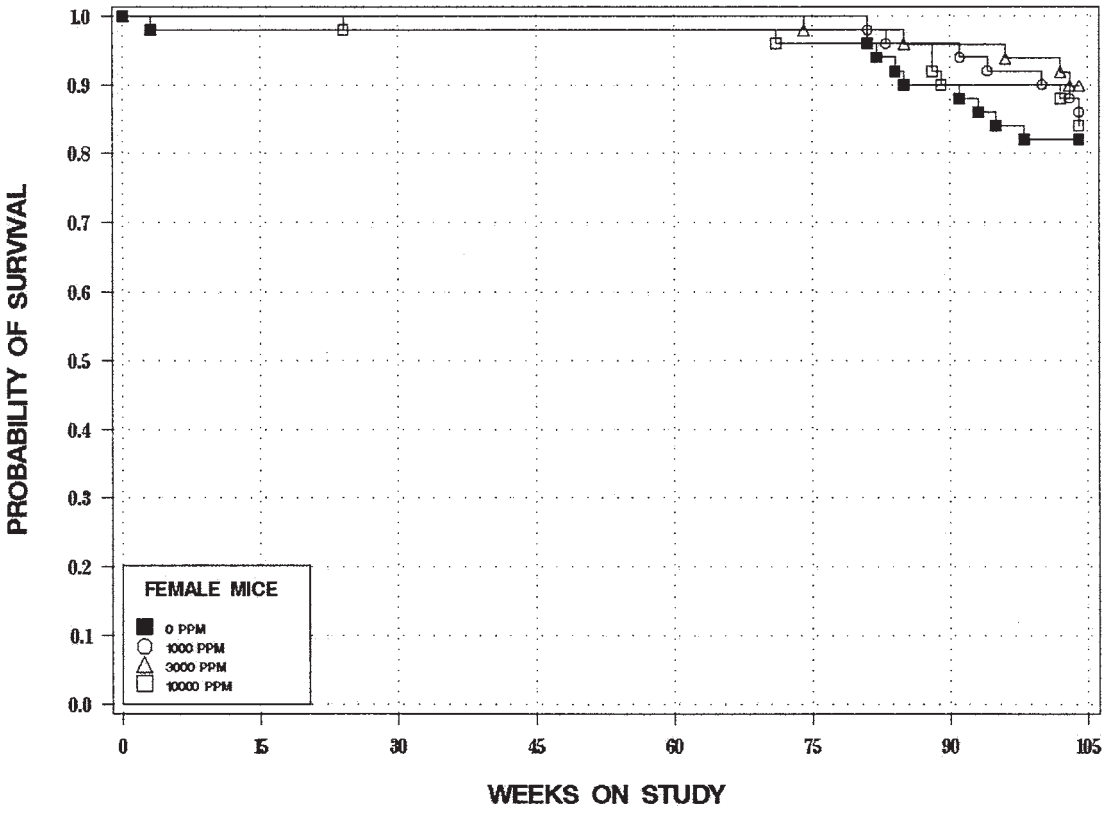


FIGURE 4
Kaplan-Meier Survival Curves for Female Mice Exposed to Cresols in Feed for 2 Years

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of the 3,000 and 10,000 ppm groups were less than those of the control group after weeks 12 and 9, respectively, and decreased to 88% and 75% that of the control group, respectively, by the end of the study (Figure 5; Table 10). The overall feed consumption value for the 10,000 ppm group was decreased 13% relative to the control group (3.3 g per animal per day versus 3.8 g per animal per day) while overall feed consumption by the 1,000 and 3,000 ppm groups was similar to that by the control group (Table E2). Dietary concentrations of 1,000, 3,000, and 10,000 ppm resulted in average daily doses of approximately 100, 300, and 1,040 mg cresols/kg body weight to female mice. There were no clinical findings related to exposure to cresols.

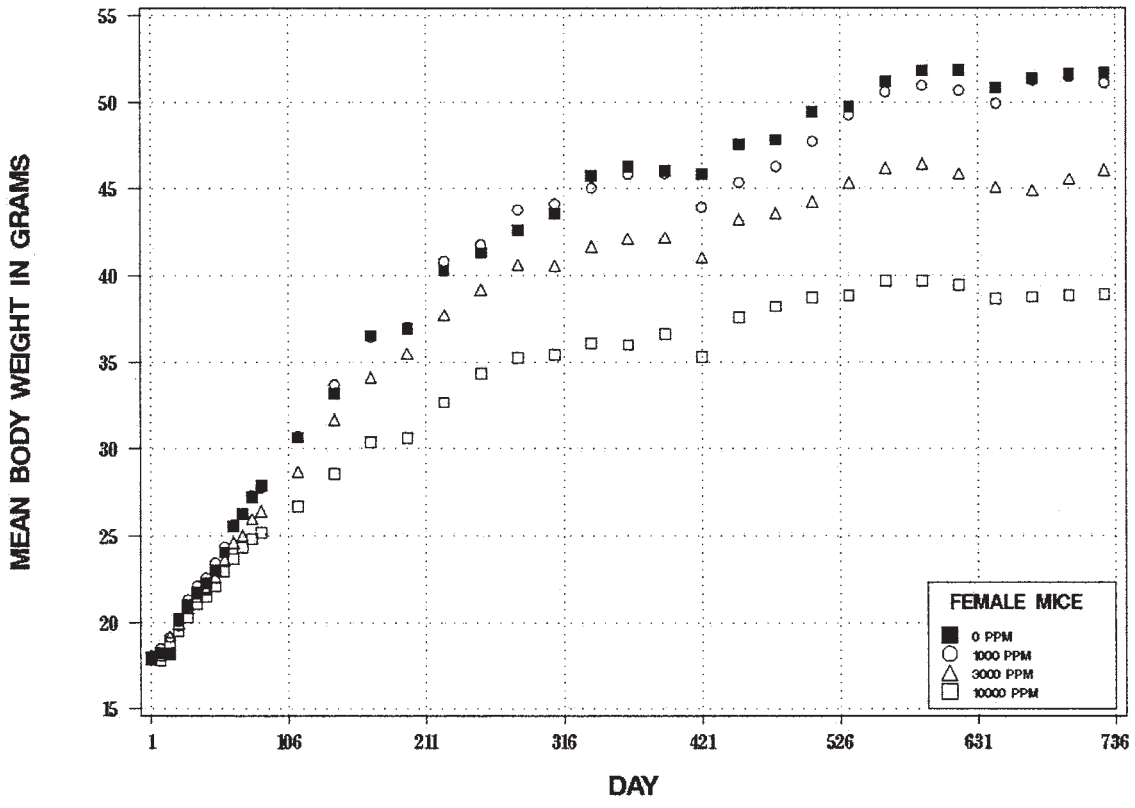


FIGURE 5
Growth Curves for Female Mice Exposed to Cresols in Feed for 2 Years

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of Cresols

Days on Study	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	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	50	18	101	50	18	100	50	18	100	50
8	18	50	19	101	50	18	99	50	18	98	50
15	18	50	19	105	50	19	106	50	19	104	50
22	20	49	20	98	50	20	99	50	20	97	50
29	21	49	21	102	50	21	99	50	20	97	50
36	22	49	22	102	50	22	99	50	21	97	50
43	22	49	23	101	50	22	99	50	22	96	50
50	23	49	23	102	50	23	98	50	22	96	50
57	24	49	24	101	50	24	98	50	23	95	50
64	26	49	26	100	50	25	96	50	24	93	50
71	26	49	26	100	50	25	95	50	24	93	50
78	27	49	27	100	50	26	95	50	25	91	50
85	28	49	28	99	50	26	95	50	25	90	50
113	31	49	31	100	50	29	94	50	27	87	50
141	33	49	34	102	50	32	96	50	29	86	50
169	37	49	36	100	50	34	93	50	30	83	49
197	37	49	37	100	50	36	96	50	31	83	49
225	40	49	41	101	50	38	94	50	33	81	49
253	41	49	42	101	50	39	95	50	34	83	49
281	43	49	44	103	50	41	95	50	35	83	49
309	44	49	44	101	50	41	93	50	35	81	49
337	46	49	45	98	50	42	91	50	36	79	49
365	46	49	46	99	50	42	91	49	36	78	49
393	46	49	46	100	50	42	92	49	37	80	49
421	46	49	44	96	50	41	89	49	35	77	49
449	48	49	45	95	50	43	91	49	38	79	49
477	48	49	46	97	50	44	91	49	38	80	49
505	50	49	48	97	50	44	90	49	39	78	48
533	50	49	49	99	50	45	91	48	39	78	48
561	51	48	51	99	49	46	90	48	40	78	48
589	52	46	51	98	48	46	90	47	40	77	48
617	52	45	51	98	48	46	89	47	40	76	46
645	51	43	50	98	47	45	89	47	39	76	45
673	51	42	51	100	46	45	87	46	39	76	45
701	52	41	51	100	45	46	88	46	39	75	45
Mean for weeks											
1-13	23		23	101		22	98		22	96	
14-52	39		39	101		37	94		32	83	
53-101	49		48	98		44	90		38	78	

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 forestomach, lung, nose, thyroid gland, liver, and bone marrow. 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 B for female mice.

Forestomach: The incidence of squamous cell papilloma was significantly greater in the 10,000 ppm group than in the control group (Tables 11 and B2). One mouse in the 10,000 ppm group had multiple squamous cell papillomas (Table B1). Microscopically, these papillomas were well-differentiated, noninvasive tumors of the squamous epithelium that typically were connected to the unaffected adjacent gastric mucosa by a narrow base or stalk (Plate 4). Squamous epithelium hyperplasia of the forestomach occurred in two mice in the 10,000 ppm group (Tables 11 and B4).

Lung: The incidences of minimal to moderate bronchiolar hyperplasia were significantly increased in all exposed groups, and the severity increased with increasing exposure concentration (Tables 12 and B4). This alteration was more evident in the terminal bronchioles (Plate 5) and was characterized by increased numbers of ciliated bronchiolar cells, which formed fronds of epithelium and extended along alveolar septae in the more severe alterations. Mild to marked alveolar epithelium hyperplasia in a few mice in the 1,000 and 10,000 ppm groups appeared to be extensions of the bronchiolar hyperplasia into the adjacent alveoli.

Nose: The incidences of minimal to marked respiratory epithelial hyperplasia were significantly increased in the 3,000 and 10,000 ppm groups (Tables 12 and B4). Respiratory epithelium hyperplasia was characterized by focal thickening of the respiratory epithelium (Plate 6) caused by an accumulation of nonciliated cuboidal eosinophilic epithelial cells, typically limited to the nasoturbinates in the minimally affected mice but becoming multifocal to diffuse throughout the Level I sections in the most severely affected mice. In some

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Number Necropsied	50	50	49	50
Epithelium, Hyperplasia[a]	0	0	0	2 (1.5)[b]
Squamous Cell Papilloma (includes multiple)[c]				
Overall Rate[d]	0/50 (0%)	1/50 (2%)	1/49 (2%)	10/50 (20%)
Adjusted Rate[e]	0.0%	2.1%	2.1%	21.2%
Terminal Rate[f]	0/41 (0%)	1/43 (2%)	1/44 (2%)	9/42 (21%)
First Incidence (days)	—[g]	728 (T)	728 (T)	713
Poly-3 Test[h]	P<0.001	P=0.510	P=0.507	P<0.001

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year feed studies with controls given NTP-2000 diet (mean ± standard deviation): 6/350 (1.8% ± 1.8%), range 0%-4%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Lung[a]	50	50	49	50
Bronchiole, Hyperplasia[b]	0	42** (1.0)[c]	44** (2.0)	47** (3.0)
Alveolar Epithelium, Hyperplasia	0	1 (2.0)	0	3 (2.7)
Nose	50	50	49	49
Respiratory Epithelium, Hyperplasia	0	0	28** (1.3)	45** (2.2)
Respiratory Epithelium, Metaplasia, Squamous	0	0	0	2 (2.0)
Thyroid Gland	48	48	49	50
Follicle, Degeneration	7 (2.0)	24** (1.8)	24** (1.8)	21** (1.8)
Liver	50	50	49	50
Eosinophilic Focus	1	0	2	12**

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

cases, there was also an infolding of the respiratory epithelium to form pseudoglands. In two 10,000 ppm mice, localized areas of respiratory epithelial hyperplasia were accompanied by squamous metaplasia in which the hyperplastic cells were replaced by flat epithelial cells.

Thyroid gland: The incidences of minimal to moderate follicular degeneration were significantly increased in all exposed groups (Tables 12 and B4). There was no increase in severity with increasing exposure concentration. The follicular degeneration was characterized by the areas of pale-staining colloid, formation of multilocular cysts, and increased interfollicular connective tissue.

Liver: The incidence of eosinophilic foci was significantly increased in the 10,000 ppm group (Tables 12 and B4). The eosinophilic foci varied in size and consisted of well-demarcated collections of enlarged hepatocytes with abundant dark homogeneous eosinophilic cytoplasm. These hepatocytes were arranged in normal hepatic plates that merged with the surrounding normal hepatocytes. There was usually little or no compression of the surrounding normal hepatocytes. Although eosinophilic foci are thought to represent a continuum with hepatocellular adenomas and hepatocellular carcinomas, these neoplasms were not increased in this study, even after adjustment for decreased body weight using the method described by Haseman *et al.* (1997).

GENETIC TOXICOLOGY

Cresols did not exhibit mutagenic activity in tests conducted by the NTP. Each of the individual cresol isomers (*m-, o-*, and *p-*) and a mixture of isomers (*m-/p-*cresol) was tested for mutagenicity in several strains of *Salmonella typhimurium* and in *Escherichia coli* strain WP2, with and without exogenous metabolic activation. Results with all individual compounds (Haworth *et al.*, 1983; Tables C1, C2, and C3) and *m-/p-*cresol (Zeiger *et al.*, 1992; Tables C4 and C5) were negative. *o-*Cresol (Table C6) and the mixture *m-/p-*cresol (Table C7) were evaluated independently for induction of micronuclei (biomarkers of chromosomal damage) in peripheral blood erythrocytes of male and female mice following 13 weeks of exposure in the diet (NTP, 1992); no increases in the frequencies of micronucleated erythrocytes were seen in male or female mice in either study (Witt *et al.*, 2000).

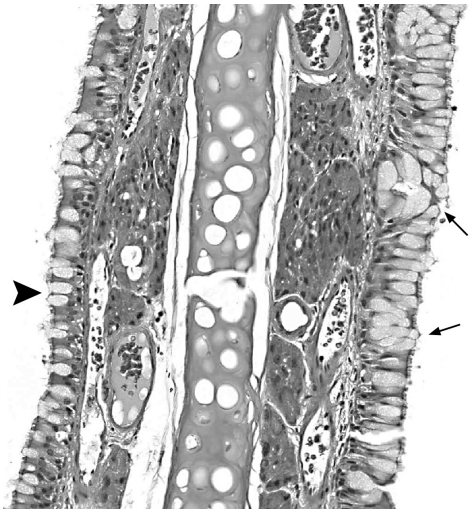


PLATE 1
Hyperplasia of goblet cells in the respiratory epithelium lining the nasal septum in Level I of a male F344/N rat exposed to 15,000 ppm cresols in feed for 2 years. Note increased numbers of goblet cells having large vacuoles (arrows) on one surface of the septum and the normal appearing goblet cells (arrowhead) on the other. H&E

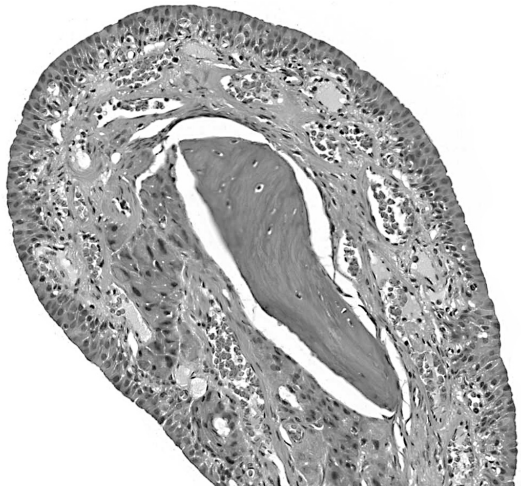


PLATE 2
Hyperplasia of the respiratory epithelium lining the nasoturbinate in Level I of a male F344/N rat exposed to 15,000 ppm cresols in feed for 2 years. Note multiple layers of irregularly shaped epithelial cells have replaced the normal single layer of uniform cuboidal cells on the surface of the turbinate. H&E

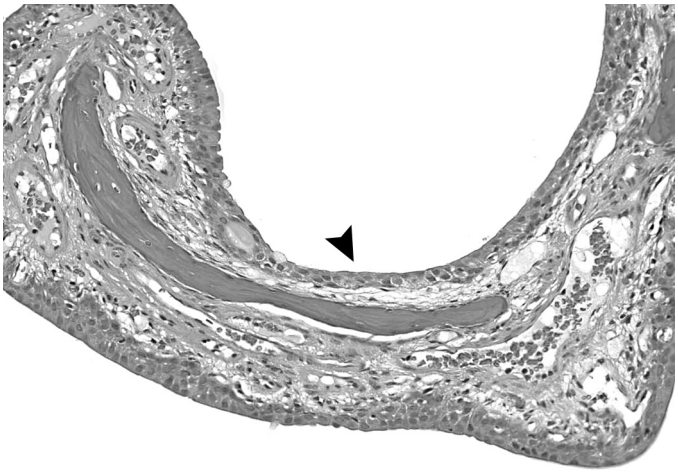


PLATE 3
Squamous metaplasia of the respiratory epithelium lining the nasoturbinate in Level I of a male F344/N rat exposed to 15,000 ppm cresols in feed for 2 years. Note the normal cuboidal respiratory epithelium has been replaced by squamous-like cells having irregular shapes and orientation (arrowhead). H&E

PLATE 4
Squamous cell papilloma arising from the epithelium lining the forestomach of a female B6C3F1 mouse exposed to 10,000 ppm cresols in feed for 2 years. Note the formation of frond-like patterns by neoplastic cells. H&E

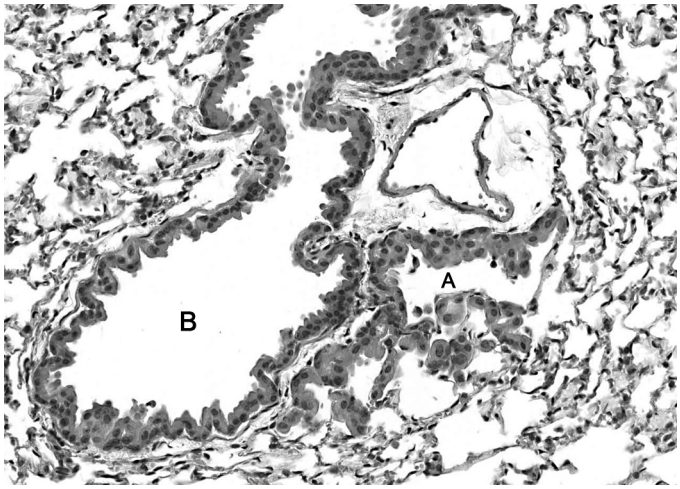


PLATE 5
Bronchiolar hyperplasia in the lung of a female B6C3F1 mouse exposed to 10,000 ppm cresols in feed for 2 years. Note the normal appearing bronchiole epithelium (B) in contrast to the larger cuboidal cells lining alveolar spaces (A). H&E

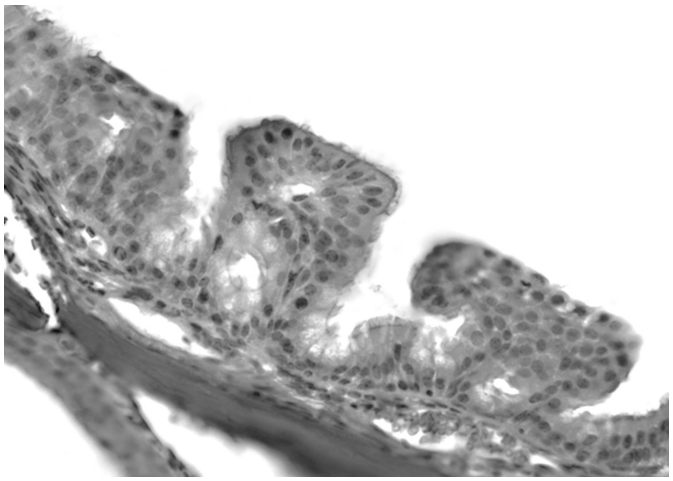


PLATE 6
Hyperplasia of the respiratory epithelium in nasal Level I of a female B6C3F1 mouse exposed to 10,000 ppm cresols in feed for 2 years. Note the increased numbers of epithelial cells along the basement membrane resulting in formation of irregular gland-like projections. H&E

DISCUSSION AND CONCLUSIONS

Cresols occur naturally in some plant oils, are formed during combustion of wood, coal, and petroleum-based fuels, are distilled from petroleum and coal tar, and are synthetically produced (IPCS, 1995; ATSDR, 2006). The three isomeric forms of cresols (*o*-, *m*-, and *p*-) are used individually or in mixtures as solvents, disinfectants, preservatives, and in the production of fragrances, antioxidants, dyes, pesticides, and resins. Cresols have been detected in foods and beverages, air, sediment, soil, and in surface and ground water. The National Toxicology Program (NTP) has performed toxicological and carcinogenicity evaluations on cresols based on their potential for occupational and consumer exposure and the lack of chronic toxicity data. Results from short-term (28-day and 13-week) studies of cresols, including a 60:40 mixture of *m*- and *p*-cresol (*m*-/*p*-cresol) in rats and mice have been reported (NTP, 1992). The 2-year studies, reported here, were designed to evaluate and characterize the potential carcinogenicity of cresols in rats and mice. These studies were carried out using male rats and female mice only. Omission of female rats and male mice was based on evidence indicating that approximately 96% of 311 chemicals tested for carcinogenicity by the National Cancer Institute and the NTP would have been correctly identified using only male rats and female mice (Huff *et al.*, 1991). The NTP decided to evaluate some lower priority chemicals for carcinogenicity testing using this modified protocol. The cresols were categorized as lower priority chemicals based on their structural similarity to phenol and toluene, both previously found to be negative for carcinogenicity in rodents (NTP, 1980, 1990).

In 28-day studies, the pattern of toxicities of the individual cresols was generally similar between rats and mice (NTP, 1992). However, *o*-cresol appeared to be somewhat less toxic than the *m*- and *p*- isomers in the two species. In 13-week studies, few histopathological effects were observed in *o*-cresol-exposed animals, those being increased incidences of bone marrow hypocellularity in rats at higher doses and minimal forestomach hyperplasia in some exposed mice. The changes in the bone marrow were not severe and were considered to be secondary to decreased body weight gains. The forestomach hyperplasia was thought to be the result of direct irritation. A wider range of histopathological effects was observed in *m*-/*p*-cresol-exposed animals. Increased incidences of bone marrow hypocellularity, increased colloid in thyroid gland follicular cells, nasal epithelium hyperplasia, and uterus atrophy were observed in rats; an increased incidence of nasal epithelium hyperplasia was observed in mice. Based on these results, the 60:40 *m*-/*p*-cresol mixture was chosen for the 2-year studies. The mixture was representative of the ratio of the *m*- and *p*- isomers recovered from coke-oven tars in the United States (*Kirk-Othmer*, 1997). The highest exposure concentrations (in feed) selected for the 2-year studies were 15,000 and 10,000 ppm for rats and mice, respectively. These exposure concentrations had no effect on survival, minimal effects on hematology, clinical chemistry, and organ weights, and minimal to mild effects on bone marrow, thyroid gland, nasal cavity, and uterus in 13-week studies.

No differences in survival of cresol-exposed rats or mice compared to controls were observed in the current 2-year studies, nor were any clinical findings related to cresol exposure observed in either species. However, the mean body weights of some cresol-exposed animals were less than controls throughout the studies. The 15,000 ppm rats consumed significantly less feed than the controls did in the first week of cresol exposure, but by week 2, feed consumption had rebounded to within 4% of the controls. As a result, the 15,000 ppm rats gained less weight in the first week, and the mean body weights continued to be less than controls throughout the remainder of the study. Lack of palatability of the feed appeared to be the reason for the initial decreased body weight gain; however, by week 2, the rats seemed to tolerate the taste and/or odor of the chemical in the feed. A severe decrease (relative to the controls) in the final mean weight of mice receiving the highest cresol exposure concentration (10,000 ppm) was observed. These mice weighed 25% less than controls, a result that would not have been predicted from the data obtained from similarly exposed mice in the 13-week study (NTP, 1992). The final mean body weight for the 10,000 ppm mice in the 13-week study and the mean body weights

of the 10,000 ppm mice after 13 weeks in the 2-year study were similar. The decreased body weight gains in the 10,000 ppm mice were associated with an overall decrease of 13% in feed consumption, relative to the controls. Reduced feed consumption in the group seemed to be associated with palatability of the feed, rather than toxicity. Overall, the mice appeared to be healthy, and there was no increased mortality relative to controls. However, it is probable that feed consumption would be further reduced and internal dose difficult to increase at higher exposure concentrations.

Cresols have been shown to be respiratory irritants in humans and animals following inhalation exposure (ATSDR, 2006). This characteristic may account for the lack of palatability of high exposure concentrations in feed. In the present studies, exposure concentration-related effects in the nasal passages of both rats and mice demonstrated the irritating effects of the chemical to biological membranes. Increased incidences of goblet cell hyperplasia, inflammation, squamous cell metaplasia, and respiratory epithelium hyperplasia were observed in rats. The incidences of respiratory epithelium hyperplasia and hyperplasia of the bronchioles were increased in cresol-exposed mice. These nonneoplastic lesions were most likely due to inhalation exposure of the *p*-isomer volatilizing from the feed during consumption and not from systemic exposure following oral absorption. These lesions are considered to be an adaptive response and are among those commonly observed in NTP inhalation studies of chemicals that are known irritants (NTP, 2000). In 13-week studies, evidence of nasal irritation was observed in both rats and mice following exposure to the *m-/p*-cresol mixture. *p*-Cresol, but not *m*-cresol, caused nasal irritation in 28-day studies (NTP, 1992). Furthermore, no increased incidences of respiratory lesions were noted in animals exposed to *o*-cresol for 28 days or 13 weeks. It could be speculated that the increased toxicity of *p*-cresol to the respiratory tract is, at least in part, metabolism-dependent. A minor pathway of *p*-cresol metabolism observed in rabbits was oxidation to *p*-hydroxybenzoic acid through *p*-hydroxy-benzaldehyde (Bray *et al.*, 1950). However, no hydroxybenzoic acids were detected following administration of either *o*- or *m*-cresol in the study. The complement of enzymes necessary for biotransformation of *p*-cresol to an aldehyde is present in the rodent nasal mucosa (Bogdanffy, 1990). *p*-Hydroxybenzaldehyde may be more irritating to tissues than the parent cresol. Aldehydes such as acetaldehyde and formaldehyde are well-known irritants of the respiratory system of humans and animals following inhalation exposure (ATSDR, 1999; USEPA, 1994). Further, goblet cell metaplasia has been observed in the respiratory epithelium of male rats following inhalation exposure to 500 ppm benzaldehyde for 14 days (Laham *et al.*, 1991).

An exposure concentration-related increase in the incidence of hyperplasia in the transitional epithelium of the renal pelvis was observed in rats in the current 2-year studies. The lesion was thought to be associated with nephropathy, which increased slightly in severity but not in incidence over the range of exposures. The only cresol-related neoplastic effect observed in rats was a minimal increase in the incidence of renal tubule adenoma following exposure to 15,000 ppm. Small adenomas were observed in the kidney of four rats in the 15,000 ppm group following standard and extended histopathologic evaluations. The incidence of this neoplasm was not statistically significant but did exceed that of the historical controls. There was no increased incidence of hyperplasia of the renal tubule. In NTP studies, the kidney is the second most commonly affected site for chemically induced neoplasms in the male rat, and these are mostly adenomas (NTP, 2006). In the present study, it could be speculated that the increased incidence of adenoma in the 15,000 ppm group arose by a mechanism of action similar to that proposed for hydroquinone by Lau *et al.* (2001). Hydroquinone markedly increases the number of renal tubular cell adenomas when administered to F344/N male (but not female) rats at nephrotoxic doses. The authors attributed the effect to a minor metabolite, 2,3,5-tris(glutathione-*S*-yl)hydroquinone, a potent toxic and redox-active species. The formation of benzoquinones from *m*- and *p*-cresol and quinone methide from *p*-cresol is inferred from identification of specific glutathione conjugates formed in rat and human liver microsomal incubations (Thompson *et al.*, 1995; Yan *et al.*, 2005). In rats, cresols are detoxicated primarily by conjugation to glucuronic acid or sulfate (Morinaga *et al.*, 2004). Although not reported in the study, it is likely that minor amounts of cresol-derived glutathione conjugates are also formed *in vivo*. However, the potential for formation of quinone-like reactive metabolites from cresols should be much lower than for hydroquinone itself. Therefore, the potential neoplastic response in the kidney should be much weaker in cresol-exposed rats than in rats exposed to similar doses of hydroquinone. No increased incidences of other neoplasms were observed in any other tissues of cresol-exposed rats. However, due to the presence of renal tubule adenomas in the high exposure concentration group, the evidence

of carcinogenicity in rats in the present study was considered to be equivocal.

In the current 2-year studies, an increased incidence of squamous cell papilloma was observed in the forestomach of mice exposed to *m-/p*-cresol (10,000 ppm). The forestomach is used for the storage and triturition of food in rodents; therefore, concentrations of a test article at the site may be high and prolonged (Harrison, 1992; IARC, 2003). Many mutagenic chemicals produce tumors in the forestomach; however, exposure to some nongenotoxic chemicals is also associated with tumorigenesis in the tissue. Evidence presented in the present report indicates that cresols, including *m-/p*-cresol, are nongenotoxic in *Salmonella* and *Escherichia coli* with and without metabolic activation. Therefore, the mode-of-action for tumorigenesis in the forestomach of cresol-exposed animals may be similar to that for other nongenotoxic chemicals. For example, butylated hydroxyanisole (BHA), non-DNA reactive and a skin irritant, produces a low incidence of carcinoma in the forestomach of rodents, presumably as a result of initial cytotoxicity and subsequent cell proliferation (Ito *et al.*, 1983; IARC, 2003). Repeated exposure to nongenotoxic irritants can damage squamous epithelium leading to regeneration involving atypical cellular proliferation capable of progressing to benign and malignant tumors (Harrison, 1992; IARC, 2003). However, in the present studies, there was little evidence of injury to the gastric mucosa. No inflammation was observed in the forestomach of cresol-exposed animals, and hyperplasia (minimal to mild) was evident in only two of the 10,000 ppm mice. Further, no evidence of forestomach irritation was reported in the 13-week studies of *m-/p*-cresol in male or female rats and mice or in the 28-day study in female mice (NTP, 1992). However, in the 28-day studies, hyperplasia was observed in the esophagus and forestomach of some *m-/p*-cresol-exposed male and female rats and in one male mouse exposed to 30,000 ppm. In the current 2-year studies, there was no clear association of irritation to the mucosa with the forestomach papillomas observed in cresol-exposed mice; therefore, the mechanism of their formation is uncertain. It was concluded that the higher incidence of squamous cell papilloma at 10,000 ppm gave some evidence of carcinogenicity in female mice based on the possibility of the progression of these benign tumors to squamous cell carcinomas. No other neoplasms associated with cresol exposure were observed in female mice.

The nontraditional study design used here to investigate the toxicity and carcinogenicity of cresols resulted in a major reduction in the number of animals needed to complete these studies. Further, the modified protocol appeared to be adequate for detecting neoplastic responses in rodents exposed to cresols. In these studies, neoplasms, all benign, were detected at only one target site in either male rats or female mice, and it is unlikely, given the chemical characteristics and biological fate of cresols, that hormonal differences would result in an appreciable difference in neoplastic response in the female rat or male mouse.

CONCLUSIONS

Under the conditions of these 2-year studies, there was *equivocal evidence of carcinogenic activity** of 60:40 *m-/p*-cresol in male F344/N rats based on the marginally increased incidence of renal tubule adenoma. There was *some evidence of carcinogenic activity* of 60:40 *m-/p*-cresol in female B6C3F1 mice based on the increased incidence of forestomach squamous cell papilloma.

Exposure to 60:40 *m-/p*-cresol resulted in increased incidences of nonneoplastic lesions in the kidney (hyperplasia), nose (inflammation, hyperplasia, and metaplasia), and liver (eosinophilic focus) of rats. Increased incidences of nonneoplastic lesions were observed in the respiratory tract (hyperplasia in the nose and lung), thyroid gland (follicular degeneration), and liver (eosinophilic focus) of mice exposed to *m-/p*-cresol.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF CRESOLS

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Cresols[a]

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	10	9	14
Natural deaths	5	6	8	5
Survivors				
Terminal sacrifice	33	34	33	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Intestine large, cecum	(50)	(50)	(50)	(49)
Intestine large, colon	(50)	(50)	(50)	(50)
Sarcoma			1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Polyp adenomatous			1 (2%)	
Intestine small, duodenum	(49)	(50)	(50)	(49)
Intestine small, ileum	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma				1 (2%)
Hepatocellular carcinoma			1 (2%)	
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma		1 (2%)		
Sarcoma, metastatic, mesentery			1 (2%)	
Mesentery	(5)	(7)	(9)	(8)
Sarcoma			1 (11%)	
Oral mucosa	(0)	(1)	(0)	(2)
Squamous cell carcinoma				1 (50%)
Pancreas	(49)	(50)	(50)	(49)
Sarcoma, metastatic, mesentery			1 (2%)	
Acinus, adenoma				1 (2%)
Salivary glands	(49)	(50)	(50)	(49)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(49)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)			
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma			1 (2%)	2 (4%)
Stomach, glandular	(49)	(50)	(50)	(50)
Tongue	(0)	(1)	(1)	(0)
Squamous cell papilloma			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Sarcoma, metastatic, mesentery			1 (2%)	
Schwannoma malignant, metastatic, skin			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	8 (16%)	5 (10%)	6 (12%)	5 (10%)
Islets, pancreatic	(49)	(50)	(50)	(49)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)	1 (2%)		1 (2%)
Parathyroid gland	(46)	(50)	(49)	(46)
Adenoma	1 (2%)			1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	19 (38%)	13 (26%)	9 (18%)	8 (16%)
Pars distalis, adenoma, multiple	1 (2%)	1 (2%)		
Pars distalis, carcinoma				1 (2%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(49)
Bilateral, C-cell, adenoma		1 (2%)		
C-cell, adenoma	6 (12%)	6 (12%)	5 (10%)	8 (16%)
C-cell, carcinoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Follicular cell, adenoma		1 (2%)	1 (2%)	
Follicular cell, carcinoma			1 (2%)	
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Paraganglioma				1 (100%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma	5 (10%)	1 (2%)	2 (4%)	4 (8%)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	42 (84%)	36 (72%)	37 (74%)	43 (86%)
Interstitial cell, adenoma	7 (14%)	12 (24%)	7 (14%)	4 (8%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(5)	(5)	(4)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Spleen	(49)	(50)	(50)	(49)
Leiomyoma				1 (2%)
Sarcoma, metastatic, mesentery			1 (2%)	
Thymus	(47)	(43)	(47)	(48)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Adenoma	1 (2%)			
Fibroadenoma		1 (2%)	2 (4%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	1 (2%)	1 (2%)
Keratoacanthoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Keratoacanthoma, multiple			1 (2%)	
Squamous cell papilloma			1 (2%)	
Trichoepithelioma			1 (2%)	
Pinna, neural crest tumor		1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Subcutaneous tissue, fibrosarcoma	2 (4%)		1 (2%)	2 (4%)
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, lipoma	1 (2%)			
Subcutaneous tissue, osteosarcoma		1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)		1 (2%)	
Subcutaneous tissue, schwannoma benign				1 (2%)
Subcutaneous tissue, schwannoma malignant			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)			
Osteosarcoma				1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Oligodendroglioma benign			1 (2%)	
Oligodendroglioma malignant		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		3 (6%)	1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)	1 (2%)	
Chordoma, metastatic, bone	1 (2%)			
Osteosarcoma, metastatic, skin		1 (2%)		
Sarcoma, metastatic, mesentery			1 (2%)	
Schwannoma malignant, metastatic, skin			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Trachea	(50)	(50)	(50)	(49)
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Sarcoma, metastatic, skin			1 (2%)	
Retrobulbar, schwannoma malignant	1 (2%)			
Harderian gland	(49)	(50)	(50)	(50)
Zymbal's gland	(1)	(2)	(0)	(2)
Carcinoma	1 (100%)	2 (100%)		2 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma		1 (2%)		1 (2%)
Renal tubule, adenoma				3 (6%)
Ureter	(1)	(0)	(1)	(0)
Urinary bladder	(49)	(50)	(50)	(50)
Papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	15 (30%)	21 (42%)	13 (26%)	10 (20%)
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Mesothelioma malignant		3 (6%)		2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	48	50
Total primary neoplasms	122	117	111	120
Total animals with benign neoplasms	50	50	48	48
Total benign neoplasms	97	82	86	93
Total animals with malignant neoplasms	23	29	23	24
Total malignant neoplasms	25	34	25	27
Total animals with metastatic neoplasms	1	3	3	2
Total metastatic neoplasms	1	4	8	2
Total animals with uncertain neoplasms–benign or malignant		1		
Total uncertain neoplasms		1		

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	5/50 (10%)	6/50 (12%)	5/50 (10%)
Adjusted rate[b]	17.4%	11.1%	14.0%	11.6%
Terminal rate[c]	6/33 (18%)	5/34 (15%)	5/33 (15%)	4/31 (13%)
First incidence (days)	665	729 (T)	702	724
Poly-3 test[d]	P=0.370N	P=0.291N	P=0.440N	P=0.317N
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	7.0%
Terminal rate	0/33 (0%)	0/34 (0%)	0/33 (0%)	3/31 (10%)
First incidence (days)	—[e]	—	—	729 (T)
Poly-3 test	P=0.007	—[f]	—	P=0.109
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	0.0%	9.3%
Terminal rate	0/33 (0%)	0/34 (0%)	0/33 (0%)	3/31 (10%)
First incidence (days)	—	—	—	725
Poly-3 test	P<0.001	—	—	P=0.054
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	0.0%	7.0%	2.3%
Terminal rate	1/33 (3%)	0/34 (0%)	3/33 (9%)	1/31 (3%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.532	P=0.503N	P=0.283	P=0.749
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	2.2%	7.0%	2.3%
Terminal rate	1/33 (3%)	1/34 (3%)	3/33 (9%)	1/31 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.632	P=0.757	P=0.283	P=0.749
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	20/50 (40%)	14/50 (28%)	9/49 (18%)	8/50 (16%)
Adjusted rate	42.7%	30.2%	20.2%	18.2%
Terminal rate	14/33 (42%)	11/34 (32%)	4/33 (12%)	6/31 (19%)
First incidence (days)	598	253	534	422
Poly-3 test	P=0.013N	P=0.147N	P=0.016N	P=0.009N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	20/50 (40%)	14/50 (28%)	9/49 (18%)	9/50 (18%)
Adjusted rate	42.7%	30.2%	20.2%	20.1%
Terminal rate	14/33 (42%)	11/34 (32%)	4/33 (12%)	6/31 (19%)
First incidence (days)	598	253	534	396
Poly-3 test	P=0.024N	P=0.147N	P=0.016N	P=0.015N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Preputial Gland: Adenoma				
Overall rate	5/50 (10%)	1/50 (2%)	2/50 (4%)	4/49 (8%)
Adjusted rate	10.8%	2.2%	4.7%	9.3%
Terminal rate	4/33 (12%)	0/34 (0%)	2/33 (6%)	2/31 (7%)
First incidence (days)	449	645	729 (T)	590
Poly-3 test	P=0.429	P=0.107N	P=0.249N	P=0.548N
Skin: Keratoacanthoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	2.2%	7.0%	4.6%
Terminal rate	0/33 (0%)	1/34 (3%)	3/33 (9%)	2/31 (7%)
First incidence (days)	598	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.370	P=0.755	P=0.280	P=0.478
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.2%	2.2%	9.3%	4.6%
Terminal rate	0/33 (0%)	1/34 (3%)	4/33 (12%)	2/31 (7%)
First incidence (days)	598	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.380	P=0.755	P=0.157	P=0.478
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma				
Overall rate	1/50 (2%)	2/50 (4%)	6/50 (12%)	3/50 (6%)
Adjusted rate	2.2%	4.4%	14.0%	7.0%
Terminal rate	0/33 (0%)	1/34 (3%)	6/33 (18%)	3/31 (10%)
First incidence (days)	598	618	729 (T)	729 (T)
Poly-3 test	P=0.290	P=0.494	P=0.045	P=0.283
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.2%	2.2%	7.0%	6.9%
Terminal rate	0/33 (0%)	0/34 (0%)	2/33 (6%)	2/31 (7%)
First incidence (days)	690	648	699	724
Poly-3 test	P=0.182	P=0.758	P=0.283	P=0.285
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	6.5%	0.0%	6.9%	4.6%
Terminal rate	2/33 (6%)	0/34 (0%)	1/33 (3%)	2/31 (7%)
First incidence (days)	653	—	534	729 (T)
Poly-3 test	P=0.550	P=0.122N	P=0.640	P=0.527N
Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	6/50 (12%)	5/50 (10%)
Adjusted rate	8.7%	2.2%	13.7%	11.6%
Terminal rate	2/33 (6%)	0/34 (0%)	3/33 (9%)	4/31 (13%)
First incidence (days)	653	648	534	724
Poly-3 test	P=0.212	P=0.184N	P=0.338	P=0.461

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.3%	6.9%
Terminal rate	0/33 (0%)	0/34 (0%)	1/33 (3%)	2/31 (7%)
First incidence (days)	—	—	729 (T)	625
Poly-3 test	P=0.019	—	P=0.488	P=0.111
Testes: Adenoma				
Overall rate	49/50 (98%)	48/50 (96%)	44/50 (88%)	47/50 (94%)
Adjusted rate	98.0%	98.3%	94.0%	99.1%
Terminal rate	32/33 (97%)	34/34 (100%)	32/33 (97%)	31/31 (100%)
First incidence (days)	449	528	534	576
Poly-3 test	P=0.502	P=0.748	P=0.300N	P=0.672
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/50 (12%)	7/50 (14%)	5/50 (10%)	8/49 (16%)
Adjusted rate	12.9%	15.6%	11.7%	18.7%
Terminal rate	4/33 (12%)	6/34 (18%)	5/33 (15%)	6/31 (19%)
First incidence (days)	449	701	729 (T)	667
Poly-3 test	P=0.293	P=0.476	P=0.557N	P=0.323
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	7/50 (14%)	9/49 (18%)
Adjusted rate	15.1%	17.8%	16.3%	21.0%
Terminal rate	5/33 (15%)	7/34 (21%)	6/33 (18%)	6/31 (19%)
First incidence (days)	449	701	702	667
Poly-3 test	P=0.303	P=0.474	P=0.551	P=0.326
All Organs: Mononuclear Leukemia				
Overall rate	15/50 (30%)	21/50 (42%)	13/50 (26%)	10/50 (20%)
Adjusted rate	31.8%	43.9%	28.8%	22.6%
Terminal rate	6/33 (18%)	11/34 (32%)	7/33 (21%)	5/31 (16%)
First incidence (days)	606	528	534	613
Poly-3 test	P=0.063N	P=0.154	P=0.466N	P=0.229N
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rate	0.0%	6.7%	0.0%	4.6%
Terminal rate	0/33 (0%)	2/34 (6%)	0/33 (0%)	2/31 (7%)
First incidence (days)	—	701	—	729 (T)
Poly-3 test	P=0.400	P=0.116	—	P=0.225
All Organs: Benign Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	48/50 (96%)	48/50 (96%)
Adjusted rate	100%	100%	100%	99.5%
Terminal rate	33/33 (100%)	34/34 (100%)	33/33 (100%)	31/31 (100%)
First incidence (days)	449	253	534	422
Poly-3 test	P=0.996N	—	P=1.000N	P=1.000N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	29/50 (58%)	23/50 (46%)	24/50 (48%)
Adjusted rate	47.2%	59.6%	49.1%	50.0%
Terminal rate	10/33 (30%)	15/34 (44%)	11/33 (33%)	11/31 (36%)
First incidence (days)	548	528	534	299
Poly-3 test	P=0.446N	P=0.153	P=0.510	P=0.471
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	48/50 (96%)	50/50 (100%)
Adjusted rate	100%	100%	100%	100%
Terminal rate	33/33 (100%)	34/34 (100%)	33/33 (100%)	31/31 (100%)
First incidence (days)	449	253	534	299
Poly-3 test	P=1.000	—	P=1.000N	—

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

TABLE A3
Historical Incidence of Kidney Neoplasms in Control Male F344/N Rats[a]

Study	Incidence in Controls	
	Lipoma	Renal Tubule Adenoma
Historical Incidence in Feed Controls Given NTP-2000 Diet		
Benzophenone	0/50	1/50
trans-Cinnamaldehyde	0/100	0/100
Cresols	0/50	0/50
2-Methylimidazole	0/49	0/49
4-Methylimidazole	0/48	0/48
Overall Historical Incidence: Feed Studies		
Total (%)	0/297	1/297 (0.3%)
Mean ± standard deviation		0.4 ± 0.9%
Range		0%-2%
Overall Historical Incidence: All Routes		
Total (%)	2/1,436 (0.1%)	8/1,436 (0.6%)
Mean ± standard deviation	0.1% ± 0.5%	0.6% ± 0.8%
Range	0%-2%	0%-2%

[a] Data as of March 2, 2007

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Cresols[a]

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	10	9	14
Natural deaths	5	6	8	5
Survivors				
Terminal sacrifice	33	34	33	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(49)
Intestine large, cecum	(50)	(50)	(50)	(49)
Parasite metazoan		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Parasite metazoan	4 (8%)	5 (10%)	4 (8%)	2 (4%)
Intestine small, duodenum	(49)	(50)	(50)	(49)
Intestine small, ileum	(50)	(50)	(50)	(49)
Intestine small, jejunum	(50)	(50)	(50)	(49)
Liver	(50)	(50)	(50)	(50)
Angiectasis	5 (10%)	5 (10%)	7 (14%)	14 (28%)
Basophilic focus	24 (48%)	18 (36%)	17 (34%)	7 (14%)
Clear cell focus	25 (50%)	20 (40%)	25 (50%)	27 (54%)
Degeneration, cystic	7 (14%)	9 (18%)	12 (24%)	9 (18%)
Eosinophilic focus	14 (28%)	14 (28%)	13 (26%)	23 (46%)
Fatty change	17 (34%)	20 (40%)	9 (18%)	8 (16%)
Fibrosis	29 (58%)	30 (60%)	29 (58%)	34 (68%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)		
Hepatodiaphragmatic nodule	2 (4%)	5 (10%)	3 (6%)	3 (6%)
Inflammation	35 (70%)	29 (58%)	37 (74%)	38 (76%)
Mixed cell focus	5 (10%)	3 (6%)	3 (6%)	2 (4%)
Necrosis	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Regeneration		2 (4%)		
Tension lipidosis			1 (2%)	
Bile duct, cyst	1 (2%)	2 (4%)	1 (2%)	
Bile duct, hyperplasia	47 (94%)	46 (92%)	46 (92%)	45 (90%)
Centrilobular, degeneration	3 (6%)	5 (10%)	3 (6%)	4 (8%)
Centrilobular, necrosis	2 (4%)		1 (2%)	1 (2%)
Hepatocyte, hypertrophy	1 (2%)			
Mesentery	(5)	(7)	(9)	(8)
Accessory spleen		1 (14%)	1 (11%)	
Fat, necrosis	4 (80%)	6 (86%)	8 (89%)	7 (88%)
Oral mucosa	(0)	(1)	(0)	(2)
Inflammation		1 (100%)		1 (50%)
Pancreas	(49)	(50)	(50)	(49)
Acinus, atrophy	18 (37%)	18 (36%)	16 (32%)	12 (24%)
Acinus, hyperplasia	2 (4%)		3 (6%)	3 (6%)
Artery, inflammation		1 (2%)		
Duct, cyst	10 (20%)	10 (20%)	8 (16%)	7 (14%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Alimentary System (continued)				
Salivary glands	(49)	(50)	(50)	(49)
Submandibular gland, hyperplasia	1 (2%)			
Stomach, forestomach	(49)	(50)	(50)	(50)
Inflammation	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Ulcer	2 (4%)	2 (4%)		
Epithelium, hyperplasia				1 (2%)
Stomach, glandular	(49)	(50)	(50)	(50)
Inflammation	1 (2%)			1 (2%)
Ulcer			1 (2%)	
Epithelium, hyperplasia		1 (2%)	1 (2%)	
Tongue	(0)	(1)	(1)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	42 (84%)	46 (92%)	42 (84%)	42 (84%)
Atrium, thrombosis	2 (4%)	1 (2%)		
Myocardium, fibrosis		1 (2%)		
Myocardium, mineralization	1 (2%)			
Ventricle, thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Hyperplasia	12 (24%)	11 (22%)	10 (20%)	7 (14%)
Necrosis	1 (2%)		4 (8%)	
Vacuolization cytoplasmic	25 (50%)	22 (44%)	21 (42%)	19 (38%)
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)			
Hyperplasia	10 (20%)	12 (24%)	11 (22%)	8 (16%)
Infiltration cellular, lymphoid		1 (2%)		
Necrosis	1 (2%)			
Islets, pancreatic	(49)	(50)	(50)	(49)
Hyperplasia	2 (4%)	2 (4%)	1 (2%)	4 (8%)
Parathyroid gland	(46)	(50)	(49)	(46)
Hyperplasia				1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)
Angiectasis	19 (38%)	9 (18%)	12 (24%)	8 (16%)
Cyst	5 (10%)	5 (10%)	7 (14%)	6 (12%)
Pars distalis, hyperplasia	14 (28%)	13 (26%)	14 (29%)	12 (24%)
Pars intermedia, hyperplasia				1 (2%)
Thyroid gland	(50)	(50)	(50)	(49)
Cyst	2 (4%)	2 (4%)		
C-cell, hyperplasia	25 (50%)	21 (42%)	17 (34%)	6 (12%)
Follicular cell, cyst			4 (8%)	
Follicular cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	4 (8%)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)	1 (2%)	1 (2%)	
Inflammation			2 (4%)	1 (2%)
Preputial gland	(50)	(50)	(50)	(49)
Cyst			1 (2%)	
Hyperplasia		3 (6%)	4 (8%)	
Inflammation	47 (94%)	49 (98%)	45 (90%)	44 (90%)
Prostate	(50)	(50)	(50)	(50)
Atrophy			2 (4%)	
Cyst		1 (2%)		
Hyperplasia	20 (40%)	20 (40%)	16 (32%)	22 (44%)
Inflammation	25 (50%)	19 (38%)	26 (52%)	19 (38%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	
Hyperplasia				1 (2%)
Inflammation				1 (2%)
Testes	(50)	(50)	(50)	(50)
Arteriole, inflammation	1 (2%)			
Germinal epithelium, atrophy	1 (2%)	1 (2%)		
Interstitial cell, hyperplasia				2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			1 (2%)
Hyperplasia	5 (10%)	2 (4%)	6 (12%)	7 (14%)
Necrosis	1 (2%)			
Lymph node	(4)	(5)	(5)	(4)
Mediastinal, ectasia				1 (25%)
Mediastinal, hyperplasia, lymphoid		1 (20%)		
Pancreatic, hyperplasia, lymphoid			1 (20%)	
Pancreatic, pigmentation, hemosiderin				1 (25%)
Lymph node, mesenteric	(49)	(50)	(50)	(50)
Hyperplasia, lymphoid				1 (2%)
Spleen	(49)	(50)	(50)	(49)
Atrophy	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Fibrosis	1 (2%)		1 (2%)	1 (2%)
Hematopoietic cell proliferation	17 (35%)	16 (32%)	23 (46%)	18 (37%)
Infiltration cellular, histiocyte				1 (2%)
Artery, hyperplasia			1 (2%)	
Artery, inflammation			1 (2%)	
Thymus	(47)	(43)	(47)	(48)
Atrophy	44 (94%)	39 (91%)	43 (91%)	47 (98%)
Cyst			1 (2%)	1 (2%)
Ectopic parathyroid gland				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Integumentary System				
Mammary gland	(49)	(50)	(50)	(49)
Hyperplasia	6 (12%)	10 (20%)	6 (12%)	8 (16%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)		1 (2%)	
Hyperkeratosis	1 (2%)	1 (2%)		
Inflammation			2 (4%)	
Epidermis, hyperplasia			1 (2%)	
Hair follicle, atrophy		1 (2%)		
Subcutaneous tissue, metaplasia, osseous			1 (2%)	
Subcutaneous tissue, necrosis		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis				1 (2%)
Joint, inflammation	1 (2%)			3 (6%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hydrocephalus	7 (14%)	4 (8%)	4 (8%)	4 (8%)
Hypothalamus, compression	6 (12%)	4 (8%)	4 (8%)	4 (8%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion			1 (2%)	1 (2%)
Fibrosis		1 (2%)		
Inflammation	26 (52%)	25 (50%)	23 (46%)	24 (48%)
Metaplasia, osseous		2 (4%)	1 (2%)	1 (2%)
Metaplasia, squamous			1 (2%)	
Alveolar epithelium, hyperplasia	7 (14%)	5 (10%)	8 (16%)	4 (8%)
Nose	(50)	(50)	(50)	(50)
Hyperplasia				1 (2%)
Inflammation	17 (34%)	19 (38%)	19 (38%)	28 (56%)
Goblet cell, hyperplasia	23 (46%)	40 (80%)	42 (84%)	47 (94%)
Respiratory epithelium, hyperplasia	3 (6%)	17 (34%)	31 (62%)	47 (94%)
Respiratory epithelium, metaplasia, squamous		1 (2%)	8 (16%)	40 (80%)
Vein, thrombosis			2 (4%)	2 (4%)
Trachea	(50)	(50)	(50)	(49)
Inflammation			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Cresols

	0 ppm	1,500 ppm	5,000 ppm	15,000 ppm
Special Senses System				
Eye	(49)	(50)	(50)	(50)
Cataract	1 (2%)	3 (6%)		1 (2%)
Inflammation	1 (2%)			
Choroid, inflammation		1 (2%)		
Optic nerve, atrophy		2 (4%)		
Retina, atrophy	1 (2%)	4 (8%)		1 (2%)
Sclera, metaplasia, osseous	16 (33%)	29 (58%)	28 (56%)	15 (30%)
Harderian gland	(49)	(50)	(50)	(50)
Atrophy			2 (4%)	2 (4%)
Cyst		1 (2%)		
Hyperplasia	1 (2%)			
Inflammation	1 (2%)	6 (12%)	5 (10%)	3 (6%)
Zymbal's gland	(1)	(2)	(0)	(2)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)			
Hydronephrosis			1 (2%)	
Infarct	1 (2%)		1 (2%)	
Necrosis	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Nephropathy	47 (94%)	48 (96%)	46 (92%)	49 (98%)
Capsule, lymphangiectasis		1 (2%)		
Renal tubule, cyst		2 (4%)		
Renal tubule, hyperplasia	2 (4%)			1 (2%)
Renal tubule, inflammation			1 (2%)	3 (6%)
Renal tubule, mineralization				1 (2%)
Transitional epithelium, hyperplasia			2 (4%)	8 (16%)
Ureter	(1)	(0)	(1)	(0)
Cyst			1 (100%)	
Inflammation	1 (100%)			
Urinary bladder	(49)	(50)	(50)	(50)
Inflammation	1 (2%)		1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF CRESOLS

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Cresols[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	4		1
Natural deaths	5	3	5	7
Survivors				
Terminal sacrifice	41	43	44	42
Missing			1	
Animals examined microscopically	50	50	49	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Periesophageal tissue, sarcoma, metastatic, salivary glands		1 (2%)		1 (2%)
Gallbladder	(50)	(50)	(49)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Intestine large, colon	(49)	(50)	(49)	(50)
Intestine large, rectum	(49)	(50)	(49)	(50)
Intestine small, ileum	(50)	(50)	(49)	(50)
Intestine small, jejunum	(50)	(50)	(49)	(50)
Carcinoma		1 (2%)		
Hemangioma		1 (2%)		
Leiomyosarcoma	1 (2%)			
Liver	(50)	(50)	(49)	(50)
Fibrosarcoma, metastatic, akin				1 (2%)
Hepatocellular adenoma	4 (8%)	2 (4%)	3 (6%)	4 (8%)
Hepatocellular adenoma, multiple		2 (4%)	1 (2%)	1 (2%)
Hepatocellular carcinoma		1 (2%)	2 (4%)	1 (2%)
Hepatocellular carcinoma, multiple	1 (2%)			
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Mesentery	(6)	(3)	(5)	(5)
Carcinoma, metastatic, uncertain primary site		1 (33%)		
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (17%)			
Lipoma			1 (20%)	
Oral mucosa	(0)	(1)	(0)	(0)
Pancreas	(50)	(50)	(49)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Salivary glands	(50)	(50)	(49)	(49)
Sarcoma		1 (2%)		1 (2%)
Stomach, forestomach	(49)	(49)	(49)	(49)
Squamous cell papilloma		1 (2%)	1 (2%)	9 (18%)
Squamous cell papilloma, multiple				1 (2%)
Stomach, glandular	(50)	(50)	(49)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Tooth	(2)	(0)	(2)	(0)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Heart	(50)	(50)	(49)	(50)
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Adrenal medulla	(49)	(50)	(49)	(50)
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(50)	(50)	(49)	(50)
Adenoma	1 (2%)			
Parathyroid gland	(39)	(44)	(40)	(45)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Pars distalis, carcinoma		1 (2%)		
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(48)	(48)	(49)	(50)
Sarcoma, metastatic, salivary glands				1 (2%)
Follicular cell, adenoma			1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(49)	(49)	(49)	(50)
Carcinoma	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (2%)		
Ovary	(50)	(50)	(49)	(50)
Cystadenoma	1 (2%)	5 (10%)	3 (6%)	2 (4%)
Granulosa cell tumor benign		1 (2%)		
Luteoma				1 (2%)
Teratoma benign				1 (2%)
Oviduct	(0)	(1)	(2)	(0)
Uterus	(50)	(50)	(49)	(50)
Leiomyoma	1 (2%)			
Leiomyosarcoma		1 (2%)		
Polyp stromal				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Hemangiosarcoma				1 (2%)
Mast cell tumor malignant			1 (2%)	
Lymph node	(2)	(5)	(7)	(7)
Deep cervical, sarcoma, metastatic, salivary glands				1 (14%)
Mediastinal, fibrosarcoma, metastatic, skin		1 (20%)		
Lymph node, mandibular	(49)	(50)	(49)	(49)
Lymph node, mesenteric	(48)	(50)	(48)	(49)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Hematopoietic System (continued)				
Spleen	(48)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)		1 (2%)
Hemangiosarcoma, metastatic, bone marrow				1 (2%)
Thymus	(50)	(49)	(49)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Integumentary System				
Mammary gland	(49)	(50)	(49)	(50)
Adenoma			1 (2%)	
Skin	(49)	(50)	(49)	(50)
Subcutaneous tissue, fibrosarcoma	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)	1 (2%)		
Subcutaneous tissue, fibrous histiocytoma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma, metastatic, bone marrow				1 (2%)
Subcutaneous tissue, sarcoma	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(49)	(50)
Skeletal muscle	(0)	(2)	(2)	(1)
Fibrosarcoma, metastatic, skin			1 (50%)	
Sarcoma, metastatic, salivary glands		1 (50%)		1 (100%)
Nervous System				
Brain	(50)	(50)	(49)	(50)
Carcinoma, metastatic, harderian gland		1 (2%)		
Carcinoma, metastatic, pituitary gland		1 (2%)		
Respiratory System				
Larynx	(0)	(1)	(0)	(1)
Sarcoma, metastatic, salivary glands		1 (100%)		1 (100%)
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	5 (10%)	3 (6%)	5 (10%)
Alveolar/bronchiolar carcinoma	2 (4%)		1 (2%)	2 (4%)
Carcinoma, metastatic, harderian gland		1 (2%)		
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)			
Nose	(50)	(50)	(49)	(49)
Pleura	(0)	(1)	(0)	(0)
Carcinoma, metastatic, harderian gland		1 (100%)		
Trachea	(50)	(50)	(49)	(50)
Peritracheal tissue, sarcoma, metastatic, salivary glands		1 (2%)		1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Special Senses System				
Eye	(50)	(50)	(49)	(50)
Harderian gland	(50)	(50)	(48)	(50)
Adenoma	6 (12%)	2 (4%)	2 (4%)	5 (10%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Leiomyosarcoma, metastatic, intestine small, jejunum	1 (2%)			
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(49)	(50)
Histiocytic sarcoma	2 (4%)		1 (2%)	2 (4%)
Lymphoma malignant	7 (14%)	6 (12%)	7 (14%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	29	28	26	33
Total primary neoplasms	42	37	36	47
Total animals with benign neoplasms	16	17	14	24
Total benign neoplasms	20	21	19	31
Total animals with malignant neoplasms	20	16	17	14
Total malignant neoplasms	22	16	17	16
Total animals with metastatic neoplasms	2	6	1	3
Total metastatic neoplasms	10	11	1	9
Total animals with malignant neoplasms of uncertain primary site		1		

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

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	2/50 (4%)	2/49 (4%)	5/50 (10%)
Adjusted rate[b]	13.1%	4.2%	4.2%	10.6%
Terminal rate[c]	6/41 (15%)	2/43 (5%)	2/44 (5%)	5/42 (12%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test[d]	P=0.438	P=0.118N	P=0.122N	P=0.483N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	3/50 (6%)	3/49 (6%)	5/50 (10%)
Adjusted rate	15.3%	6.2%	6.3%	10.6%
Terminal rate	7/41 (17%)	2/43 (5%)	3/44 (7%)	5/42 (12%)
First incidence (days)	728 (T)	658	728 (T)	728 (T)
Poly-3 test	P=0.571N	P=0.137N	P=0.144N	P=0.362N
Liver: Hepatocellular Adenoma				
Overall rate	4/50 (8%)	4/50 (8%)	4/49 (8%)	5/50 (10%)
Adjusted rate	8.7%	8.3%	8.4%	10.6%
Terminal rate	4/41 (10%)	4/43 (9%)	4/44 (9%)	5/42 (12%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.425	P=0.617N	P=0.624N	P=0.515
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	6/49 (12%)	6/50 (12%)
Adjusted rate	8.7%	10.4%	12.6%	12.6%
Terminal rate	4/41 (10%)	5/43 (12%)	6/44 (14%)	5/42 (12%)
First incidence (days)	728 (T)	728 (T)	728 (T)	615
Poly-3 test	P=0.373	P=0.530	P=0.392	P=0.391
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	5/50 (10%)	3/49 (6%)	5/50 (10%)
Adjusted rate	10.9%	10.4%	6.3%	10.6%
Terminal rate	5/41 (12%)	4/43 (9%)	3/44 (7%)	4/42 (10%)
First incidence (days)	728 (T)	722	728 (T)	724
Poly-3 test	P=0.564	P=0.599N	P=0.338N	P=0.614N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	5/50 (10%)	4/49 (8%)	6/50 (12%)
Adjusted rate	10.9%	10.4%	8.4%	12.7%
Terminal rate	5/41 (12%)	4/43 (9%)	4/44 (9%)	4/42 (10%)
First incidence (days)	728 (T)	722	728 (T)	713
Poly-3 test	P=0.420	P=0.599N	P=0.478N	P=0.518
Ovary: Cystadenoma				
Overall rate	1/50 (2%)	5/50 (10%)	3/49 (6%)	2/50 (4%)
Adjusted rate	2.2%	10.4%	6.3%	4.3%
Terminal rate	1/41 (2%)	5/43 (12%)	3/44 (7%)	2/42 (5%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.458N	P=0.113	P=0.318	P=0.509
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/49 (4%)	1/50 (2%)
Adjusted rate	4.4%	6.2%	4.2%	2.1%
Terminal rate	2/41 (5%)	2/43 (5%)	2/44 (5%)	1/42 (2%)
First incidence (days)	728 (T)	561	728 (T)	728 (T)
Poly-3 test	P=0.305N	P=0.527	P=0.681N	P=0.491N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/49 (6%)	1/50 (2%)
Adjusted rate	8.6%	6.2%	6.3%	2.1%
Terminal rate	3/41 (7%)	1/43 (2%)	3/44 (7%)	1/42 (2%)
First incidence (days)	570	632	728 (T)	728 (T)
Poly-3 test	P=0.156N	P=0.475N	P=0.487N	P=0.175N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma				
Overall rate	5/50 (10%)	3/50 (6%)	4/49 (8%)	1/50 (2%)
Adjusted rate	10.7%	6.2%	8.4%	2.1%
Terminal rate	3/41 (7%)	1/43 (2%)	3/44 (7%)	1/42 (2%)
First incidence (days)	570	632	719	728 (T)
Poly-3 test	P=0.111N	P=0.337N	P=0.491N	P=0.101N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	1/49 (2%)	10/50 (20%)
Adjusted rate	0.0%	2.1%	2.1%	21.2%
Terminal rate	0/41 (0%)	1/43 (2%)	1/44 (2%)	9/42 (21%)
First incidence (days)	—[e]	728 (T)	728 (T)	713
Poly-3 test	P<0.001	P=0.510	P=0.507	P<0.001
All Organs: Malignant Lymphoma				
Overall rate	7/50 (14%)	6/50 (12%)	7/49 (14%)	6/50 (12%)
Adjusted rate	15.3%	12.5%	14.4%	12.6%
Terminal rate	7/41 (17%)	5/43 (12%)	4/44 (9%)	5/42 (12%)
First incidence (days)	728 (T)	719	515	613
Poly-3 test	P=0.483N	P=0.463N	P=0.570N	P=0.474N
All Organs: Benign Neoplasms				
Overall rate	16/50 (32%)	17/50 (34%)	14/49 (29%)	24/50 (48%)
Adjusted rate	34.5%	35.3%	29.4%	49.9%
Terminal rate	15/41 (37%)	16/43 (37%)	14/44 (32%)	21/42 (50%)
First incidence (days)	590	722	728 (T)	163
Poly-3 test	P=0.042	P=0.554	P=0.381N	P=0.094
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	17/50 (34%)	17/49 (35%)	14/50 (28%)
Adjusted rate	41.8%	34.0%	34.7%	28.8%
Terminal rate	15/41 (37%)	10/43 (23%)	12/44 (27%)	8/42 (19%)
First incidence (days)	561	561	515	493
Poly-3 test	P=0.161N	P=0.281N	P=0.307N	P=0.131N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
All Organs: Benign or Malignant Neoplasms				
Overall rate	29/50 (58%)	29/50 (58%)	26/49 (53%)	33/50 (66%)
Adjusted rate	60.0%	58.0%	53.1%	66.5%
Terminal rate	23/41 (56%)	22/43 (51%)	21/44 (48%)	26/42 (62%)
First incidence (days)	561	561	515	163
Poly-3 test	P=0.209	P=0.502N	P=0.315N	P=0.323

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

TABLE B3
Historical Incidence of Squamous Cell Papilloma of the Forestomach in Control Female B6C3F1 Mice[a]

Study	Incidence in Controls
Historical Incidence in Feed Controls Given NTP-2000 Diet	
Benzophenone	1/50
Chromium picolinate monohydrate	0/50
trans-Cinnamaldehyde	1/100
Cresols	0/50
2-Methylimidazole	2/50
4-Methylimidazole	2/50
Overall Historical Incidence: Feed Studies	
Total (%)	6/350 (1.7%)
Mean ± standard deviation	1.8% ± 1.8%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	28/1,598 (1.8%)
Mean ± standard deviation	1.8% ± 1.3%
Range	0%-6%

[a] Data as of March 2, 2007

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Cresols[a]

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	4		1
Natural deaths	5	3	5	7
Survivors				
Terminal sacrifice	41	43	44	42
Missing			1	
Animals examined microscopically	50	50	49	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Gallbladder	(50)	(50)	(49)	(50)
Cyst		1 (2%)		
Infiltration cellular, lymphoid	6 (12%)	10 (20%)	6 (12%)	9 (18%)
Inflammation, chronic active		1 (2%)		
Intestine large, colon	(49)	(50)	(49)	(50)
Thrombosis	1 (2%)			
Intestine large, rectum	(49)	(50)	(49)	(50)
Intestine small, ileum	(50)	(50)	(49)	(50)
Intestine small, jejunum	(50)	(50)	(49)	(50)
Inflammation, chronic active		1 (2%)	1 (2%)	
Epithelium, ulcer			1 (2%)	
Peyer's patch, hyperplasia, lymphoid		2 (4%)	1 (2%)	
Liver	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)		1 (2%)	1 (2%)
Atrophy	1 (2%)	1 (2%)		
Basophilic focus	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Clear cell focus		1 (2%)		1 (2%)
Eosinophilic focus	1 (2%)		2 (4%)	12 (24%)
Hematopoietic cell proliferation		1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, lymphoid	40 (80%)	43 (86%)	39 (80%)	42 (84%)
Inflammation, chronic active	43 (86%)	44 (88%)	43 (88%)	43 (86%)
Mixed cell focus				1 (2%)
Pigmentation	2 (4%)			
Tension lipidosis	8 (16%)	3 (6%)	6 (12%)	8 (16%)
Bile duct, cyst	1 (2%)			1 (2%)
Bile duct, cyst, multiple				1 (2%)
Centrilobular, hepatocyte, degeneration	1 (2%)			
Hepatocyte, necrosis	1 (2%)	1 (2%)	1 (2%)	
Hepatocyte, vacuolization cytoplasmic	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Mesentery	(6)	(3)	(5)	(5)
Fat, infiltration cellular, lymphoid				1 (20%)
Fat, inflammation, chronic active		1 (33%)		
Fat, necrosis	4 (67%)	2 (67%)	1 (20%)	1 (20%)
Oral mucosa	(0)	(1)	(0)	(0)
Gingival, foreign body		1 (100%)		
Gingival, inflammation, chronic active		1 (100%)		

[a] 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 Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Alimentary System (continued)				
Pancreas	(50)	(50)	(49)	(50)
Basophilic focus			1 (2%)	1 (2%)
Infiltration cellular, lymphoid	21 (42%)	23 (46%)	25 (51%)	24 (48%)
Inflammation, chronic active	1 (2%)	3 (6%)		
Polyarteritis		1 (2%)		
Acinus, atrophy	1 (2%)	2 (4%)	1 (2%)	
Duct, cyst	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Duct, cyst, multiple		1 (2%)		
Duct, inflammation, chronic active		1 (2%)		
Salivary glands	(50)	(50)	(49)	(49)
Infiltration cellular, lymphoid	34 (68%)	36 (72%)	34 (69%)	21 (43%)
Stomach, forestomach	(49)	(49)	(49)	(49)
Epithelium, hyperplasia				2 (4%)
Stomach, glandular	(50)	(50)	(49)	(50)
Mineralization	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Tooth	(2)	(0)	(2)	(0)
Malformation	1 (50%)		1 (50%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(49)	(50)
Aorta, mineralization		1 (2%)		
Heart	(50)	(50)	(49)	(50)
Mineralization	2 (4%)	1 (2%)		1 (2%)
Polyarteritis		1 (2%)		
Valve, inflammation, chronic active		1 (2%)		
Endocrine System				
Adrenal cortex	(49)	(50)	(49)	(50)
Accessory adrenal cortical nodule	1 (2%)			1 (2%)
Hematopoietic cell proliferation		1 (2%)		
Hypertrophy	1 (2%)	1 (2%)		1 (2%)
Infiltration cellular, lymphoid				1 (2%)
Vacuolization cytoplasmic		2 (4%)		1 (2%)
Subcapsular, hyperplasia	48 (98%)	50 (100%)	48 (98%)	50 (100%)
Adrenal medulla	(49)	(50)	(49)	(50)
Hyperplasia	3 (6%)			1 (2%)
Islets, pancreatic	(50)	(50)	(49)	(50)
Parathyroid gland	(39)	(44)	(40)	(45)
Cyst	1 (3%)	1 (2%)		
Cyst, multiple	1 (3%)	1 (2%)		
Bilateral, cyst			1 (3%)	
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, cyst, multiple	1 (2%)			
Pars distalis, hyperplasia	7 (14%)	7 (14%)	3 (6%)	2 (4%)
Thyroid gland	(48)	(48)	(49)	(50)
Ectopic thymus		1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, lymphoid		1 (2%)		
Inflammation, chronic active			2 (4%)	3 (6%)
Polyarteritis		1 (2%)		
Follicle, cyst	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Follicle, degeneration	7 (15%)	24 (50%)	24 (49%)	21 (42%)
Follicular cell, hyperplasia	2 (4%)			2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
General Body System				
None				
Genital System				
Clitoral gland	(49)	(49)	(49)	(50)
Inflammation, chronic active	1 (2%)		1 (2%)	
Ovary	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)			1 (2%)
Atrophy	49 (98%)	49 (98%)	47 (96%)	47 (94%)
Cyst	14 (28%)	9 (18%)	3 (6%)	8 (16%)
Cyst, multiple				1 (2%)
Hemorrhage	1 (2%)			
Thrombosis	1 (2%)			
Bilateral, cyst	1 (2%)	1 (2%)		1 (2%)
Oviduct	(0)	(1)	(2)	(0)
Uterus	(50)	(50)	(49)	(50)
Inflammation, suppurative	1 (2%)		1 (2%)	
Endometrium, hyperplasia, cystic	41 (82%)	46 (92%)	46 (94%)	43 (86%)
Hematopoietic System				
Bone marrow	(50)	(50)	(49)	(50)
Angiectasis	1 (2%)		1 (2%)	1 (2%)
Hyperplasia	1 (2%)	6 (12%)	5 (10%)	2 (4%)
Myelofibrosis	23 (46%)	33 (66%)	18 (37%)	25 (50%)
Lymph node	(2)	(5)	(7)	(7)
Bronchial, hyperplasia, lymphoid	1 (50%)	1 (20%)		
Iliac, pigmentation		1 (20%)		
Lumbar, congestion				1 (14%)
Lumbar, ectasia				1 (14%)
Mediastinal, hyperplasia, lymphoid			1 (14%)	2 (29%)
Lymph node, mandibular	(49)	(50)	(49)	(49)
Hyperplasia, lymphoid	4 (8%)	4 (8%)	5 (10%)	3 (6%)
Hyperplasia, plasma cell			1 (2%)	
Lymph node, mesenteric	(48)	(50)	(48)	(49)
Hyperplasia, lymphoid	4 (8%)		1 (2%)	7 (14%)
Hyperplasia, plasma cell	1 (2%)			2 (4%)
Spleen	(48)	(50)	(49)	(50)
Atrophy	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	7 (15%)	6 (12%)	4 (8%)	6 (12%)
Lymphoid follicle, hyperplasia	14 (29%)	13 (26%)	16 (33%)	12 (24%)
Thymus	(50)	(49)	(49)	(50)
Ectopic parathyroid gland	8 (16%)	10 (20%)	6 (12%)	4 (8%)
Hyperplasia, lymphoid	2 (4%)			5 (10%)
IntegumentarySystem				
Mammary gland	(49)	(50)	(49)	(50)
Hyperplasia			1 (2%)	
Duct, dilatation		1 (2%)	1 (2%)	1 (2%)
Skin	(49)	(50)	(49)	(50)
Cyst epithelial inclusion		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Musculoskeletal System				
Bone	(50)	(50)	(49)	(50)
Osteopetrosis				1 (2%)
Skeletal muscle	(0)	(2)	(2)	(1)
Infiltration cellular, lymphoid		1 (50%)	1 (50%)	
Nervous System				
Brain	(50)	(50)	(49)	(50)
Hydrocephalus		2 (4%)		
Infiltration cellular, lymphoid	1 (2%)	2 (4%)	1 (2%)	
Neuron, necrosis	1 (2%)			
Respiratory System				
Larynx	(0)	(1)	(0)	(1)
Lung	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)			
Infiltration cellular, lymphoid			2 (4%)	1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)		
Alveolar epithelium, hyperplasia		1 (2%)		3 (6%)
Alveolus, infiltration cellular, histiocyte			1 (2%)	1 (2%)
Bronchiole, hyperplasia		42 (84%)	44 (90%)	47 (94%)
Nose	(50)	(50)	(49)	(49)
Edema				1 (2%)
Inflammation, chronic active	1 (2%)	1 (2%)		1 (2%)
Glands, respiratory epithelium, dilatation				1 (2%)
Glands, respiratory epithelium, hyperplasia	6 (12%)	5 (10%)	8 (16%)	10 (20%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)	1 (2%)	2 (4%)	
Olfactory epithelium, degeneration				1 (2%)
Respiratory epithelium, degeneration				1 (2%)
Respiratory epithelium, hyperplasia			28 (57%)	45 (92%)
Respiratory epithelium, metaplasia, squamous				2 (4%)
Pleura	(0)	(1)	(0)	(0)
Trachea	(50)	(50)	(49)	(50)
Special Senses System				
Eye	(50)	(50)	(49)	(50)
Atrophy		1 (2%)		
Harderian gland	(50)	(50)	(48)	(50)
Hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Infiltration cellular, lymphoid	30 (60%)	30 (60%)	35 (73%)	34 (68%)
Inflammation, chronic active		1 (2%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of Cresols

	0 ppm	1,000 ppm	3,000 ppm	10,000 ppm
Urinary System				
Kidney	(50)	(50)	(49)	(50)
Amyloid deposition				1 (2%)
Atrophy		1 (2%)		
Casts protein	29 (58%)	25 (50%)	19 (39%)	25 (50%)
Infarct	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Infiltration cellular, lymphoid	43 (86%)	47 (94%)	43 (88%)	44 (88%)
Metaplasia, osseous	2 (4%)	4 (8%)	4 (8%)	2 (4%)
Mineralization	2 (4%)	8 (16%)	3 (6%)	3 (6%)
Nephropathy	14 (28%)	14 (28%)	11 (22%)	19 (38%)
Cortex, cyst				1 (2%)
Renal tubule, accumulation, hyaline droplet	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Renal tubule, pigmentation				1 (2%)
Urinary bladder	(50)	(50)	(49)	(50)
Infiltration cellular, lymphoid	36 (72%)	35 (70%)	43 (88%)	42 (84%)

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983) and Zeiger *et al.* (1992); in one test with *m-/p*-cresol conducted at SITEK Research Laboratories (Rockville, MD), a slightly modified procedure was used as described below. For the traditional tests, cresol isomers were sent to the laboratory as coded aliquots from Radian Corporation (Austin, TX). Each individual isomer (*m*-, *o*-, and *p*-) and a mixture of isomers (*m-/p*-cresol) was incubated with the *Salmonella typhimurium* tester strains TA97 or TA1537, TA98, TA100, and TA1535 either in buffer or 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 slightly modified protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation and employed *Escherichia coli* strain WP2 *uvrA* pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. The same lot of *m-/p*-cresol that was used in the 2-year bioassay was tested for mutagenicity under this protocol; the compound was sent to the testing laboratory as a coded aliquot. Incubation of bacterial strains with *m-/p*-cresol and subsequent plating were carried out as described above for the traditional protocol.

Each trial consisted of triplicate plates of concurrent positive and negative controls and three to six doses of a cresol isomer or isomer mixture. The high dose was limited by toxicity. All trials were repeated, and those that were conducted with S9 activation enzymes were repeated using the same or a higher concentration of S9.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 13-week toxicity studies with *o*- and *m-/p*-cresol (NTP, 1992), peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.,* 1983) and coded. Slides were scanned at 630× or 1,000× magnification using a semi-automated image analysis system to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) in each of 10 animals per exposure group. Candidate micronuclei were required to exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm UV illumination and orange with 540 nm UV illumination); the minimum size limit for a micronucleus was approximately 1/20 the diameter of the NCE. In addition, the percentage of polychromatic erythrocytes (PCEs) among the total erythrocyte population was scored 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 NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group

(ILS, 1990). 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 exposed group is less than or equal to 0.025 divided by the number of exposed 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.

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 aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots 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 judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Cresols did not exhibit mutagenic activity in tests conducted by the NTP. Each of the individual cresol isomers (*m*-, *o*-, and *p*-) and a mixture of isomers (*m*-/*p*-cresol) was tested for mutagenicity in several strains of *S. typhimurium* and in *E. coli* strain WP2, with and without exogenous metabolic activation; results with all individual compounds (Haworth *et al.*, 1983; Tables C1, C2, and C3) and the *m*-/*p*-cresol mixture (Zeiger *et al.*, 1992; Tables C4 and C5) were negative. *o*-Cresol (Table C6) and the mixture *m*/*p*-cresol (Table C7) were evaluated independently for induction of micronuclei (biomarkers of chromosomal damage) in peripheral blood erythrocytes of male and female mice following 13 weeks of exposure in the diet (NTP, 1992); no increases in the frequencies of micronucleated erythrocytes were seen in male or female mice in either study (Witt *et al.*, 2000).

TABLE C1
Mutagenicity of *m*-Cresol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	82 ± 1.0	92 ± 8.0	120 ± 2.0	139 ± 19.0	112 ± 2.0	133 ± 13.0
	3.3		98 ± 2.0		154 ± 7.0		130 ± 2.0
	10		95 ± 7.0		142 ± 6.0		139 ± 4.0
	33	82 ± 5.0	96 ± 2.0	161 ± 27.0	154 ± 15.0	102 ± 6.0	127 ± 6.0
	100	72 ± 2.0	94 ± 9.0	120 ± 12.0	145 ± 11.0	131 ± 8.0	139 ± 9.0
	333	62 ± 11.0	94 ± 13.0	120 ± 1.0	139 ± 7.0	115 ± 16.0	133 ± 9.0
	1,000	Toxic		Toxic		Toxic	
	3,333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		301 ± 76.0	371 ± 12.0	492 ± 42.0	530 ± 38.0	294 ± 29.0	317 ± 12.0
TA1535	0	12 ± 2.0	12 ± 1.0	15 ± 2.0	17 ± 3.0	11 ± 1.0	14 ± 2.0
	3.3		11 ± 2.0		20 ± 2.0		17 ± 3.0
	10		9 ± 1.0		19 ± 1.0		16 ± 0.0
	33	6 ± 1.0	11 ± 3.0	14 ± 2.0	20 ± 3.0	11 ± 1.0	14 ± 0.0
	100	9 ± 2.0	9 ± 1.0	12 ± 2.0	15 ± 2.0	11 ± 2.0	20 ± 4.0
	333	8 ± 1.0	9 ± 1.0	18 ± 2.0	20 ± 3.0	10 ± 2.0	16 ± 1.0
	1,000	Toxic		Toxic		Toxic	
	3,333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		448 ± 11.0	530 ± 47.0	77 ± 14.0	72 ± 2.0	30 ± 2.0	31 ± 1.0
TA1537	0	4 ± 1.0	5 ± 1.0	5 ± 2.0	7 ± 1.0	4 ± 2.0	9 ± 3.0
	3.3		4 ± 1.0		14 ± 3.0		15 ± 1.0
	10		10 ± 2.0		13 ± 2.0		11 ± 0.0
	33	4 ± 1.0	4 ± 1.0	7 ± 0.0	11 ± 1.0	4 ± 1.0	15 ± 3.0
	100	3 ± 0.0	5 ± 3.0	5 ± 1.0	16 ± 5.0	4 ± 0.0	12 ± 2.0
	333	3 ± 0.0	10 ± 3.0	4 ± 1.0	13 ± 4.0	4 ± 1.0	10 ± 1.0
	1,000	Toxic		Toxic		6 ± 2.0	
	3,333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		81 ± 41.0	122 ± 3.0	28 ± 1.0	71 ± 8.0	20 ± 3.0	30 ± 5.0
TA98	0	10 ± 2.0	16 ± 3.0	21 ± 2.0	34 ± 3.0	25 ± 9.0	26 ± 6.0
	3.3		12 ± 1.0		46 ± 3.0		38 ± 2.0
	10		17 ± 2.0		47 ± 3.0		38 ± 2.0
	33	8 ± 1.0	12 ± 2.0	30 ± 5.0	48 ± 8.0	26 ± 2.0	39 ± 3.0
	100	9 ± 3.0	15 ± 5.0	31 ± 3.0	50 ± 4.0	24 ± 2.0	37 ± 3.0
	333	11 ± 1.0	18 ± 1.0	27 ± 1.0	44 ± 2.0	25 ± 2.0	38 ± 3.0
	1,000	Toxic		Toxic		Toxic	
	3,333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		212 ± 10.0	235 ± 11.0	496 ± 67.0	583 ± 50.0	299 ± 14.0	325 ± 17.0

[a] Study was performed at Case Western Reserve University (Cleveland, OH). The detailed protocol and these data are presented by Haworth *et al.* (1983). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Mutagenicity of *o*-Cresol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	88 ± 3.0	113 ± 10.0	134 ± 12.0	151 ± 11.0	144 ± 13.0	137 ± 3.0
	1		119 ± 5.0		159 ± 9.0		143 ± 7.0
	3.3		101 ± 12.0		143 ± 4.0		149 ± 7.0
	10	112 ± 6.0	115 ± 14.0	149 ± 18.0	163 ± 3.0	131 ± 4.0	146 ± 11.0
	33	102 ± 8.0	148 ± 16.0	138 ± 6.0	Toxic	146 ± 1.0	Toxic
	100	88 ± 6.0	Toxic	133 ± 10.0	Toxic	147 ± 8.0	Toxic
	333	0 ± 0.0		Toxic		93 ± 6.0	
	1,000	0 ± 0.0		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		393 ± 11.0	505 ± 7.0	482 ± 28.0	617 ± 114.0	342 ± 13.0	460 ± 24.0
TA1535	0	10 ± 1.0	12 ± 2.0	17 ± 1.0	17 ± 0.0	14 ± 1.0	14 ± 0.0
	1		17 ± 3.0		16 ± 3.0		15 ± 1.0
	3.3		20 ± 4.0		18 ± 1.0		15 ± 1.0
	10	10 ± 1.0	14 ± 2.0	17 ± 1.0	18 ± 3.0	11 ± 1.0	15 ± 2.0
	33	10 ± 2.0	9 ± 4.0	17 ± 1.0	15 ± 1.0	13 ± 1.0	Toxic
	100	12 ± 3.0	13 ± 1.0	16 ± 2.0	14 ± 2.0	Toxic	Toxic
	333	0 ± 0.0		Toxic		10 ± 6.0	
	1,000	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		450 ± 41.0	475 ± 24.0	51 ± 11.0	76 ± 2.0	25 ± 3.0	43 ± 5.0
TA1537	0	6 ± 1.0	7 ± 1.0	3 ± 1.0	8 ± 3.0	6 ± 1.0	7 ± 1.0
	1		6 ± 0.0		12 ± 3.0		11 ± 1.0
	3.3		7 ± 1.0		12 ± 2.0		7 ± 1.0
	10	6 ± 1.0	8 ± 1.0	3 ± 1.0	8 ± 1.0	9 ± 4.0	9 ± 1.0
	33	6 ± 0.0	Toxic	8 ± 2.0	10 ± 2.0	6 ± 2.0	8 ± 2.0
	100	4 ± 1.0	Toxic	4 ± 1.0	Toxic	3 ± 0.0	Toxic
	333	0 ± 0.0		2 ± 0.0		Toxic	
	1,000	0 ± 0.0		2 ± 1.0		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		326 ± 189.0	841 ± 61.0	42 ± 14.0	64 ± 10.0	27 ± 2.0	59 ± 5.0
TA98	0	21 ± 3.0	17 ± 2.0	44 ± 8.0	33 ± 2.0	36 ± 2.0	30 ± 3.0
	1		15 ± 4.0		30 ± 4.0		31 ± 5.0
	3.3		14 ± 1.0		26 ± 3.0		30 ± 3.0
	10	24 ± 2.0	15 ± 3.0	43 ± 1.0	35 ± 2.0	31 ± 8.0	34 ± 4.0
	33	18 ± 2.0	15 ± 3.0	35 ± 3.0	Toxic	27 ± 1.0	25 ± 2.0
	100	18 ± 2.0	16 ± 2.0	28 ± 2.0	23 ± 1.0	20 ± 6.0	25 ± 4.0
	333	0 ± 0.0		18 ± 7.0		11 ± 0.0	
	1,000	6 ± 1.0		14 ± 4.0		14 ± 1.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		256 ± 18.0	305 ± 6.0	482 ± 116.0	642 ± 34.0	354 ± 15.0	615 ± 29.0

[a] Study was performed at Case Western Reserve University (Cleveland, OH). The detailed protocol and these data are presented by Haworth *et al.* (1983). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C3
Mutagenicity of *p*-Cresol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	131 ± 5.0	113 ± 9.0	175 ± 12.0	158 ± 9.0	158 ± 10.0	149 ± 5.0
	3.3		103 ± 2.0		143 ± 3.0		140 ± 4.0
	10	114 ± 8.0	96 ± 1.0	146 ± 5.0	145 ± 13.0	138 ± 4.0	143 ± 10.0
	33	122 ± 5.0	94 ± 6.0	157 ± 16.0	155 ± 7.0	144 ± 9.0	117 ± 9.0
	100	104 ± 8.0	97 ± 7.0	155 ± 3.0	154 ± 7.0	157 ± 1.0	132 ± 14.0
	333	115 ± 8.0	99 ± 1.0	170 ± 8.0	140 ± 7.0	134 ± 15.0	124 ± 6.0
	1,000	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		455 ± 42.0	492 ± 10.0	618 ± 73.0	631 ± 77.0	326 ± 15.0	501 ± 43.0
TA1535	0	14 ± 2.0	15 ± 2.0	20 ± 2.0	19 ± 2.0	15 ± 0.0	16 ± 1.0
	3.3		22 ± 1.0		19 ± 1.0		17 ± 3.0
	10	18 ± 2.0	19 ± 1.0	20 ± 1.0	19 ± 1.0	19 ± 4.0	20 ± 0.0
	33	18 ± 1.0	24 ± 3.0	22 ± 2.0	20 ± 1.0	23 ± 1.0	19 ± 4.0
	100	21 ± 4.0	17 ± 3.0	18 ± 2.0	18 ± 3.0	19 ± 1.0	20 ± 3.0
	333	Toxic	23 ± 6.0	17 ± 3.0	20 ± 2.0	24 ± 1.0	22 ± 2.0
	1,000	0 ± 0.0		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		511 ± 54.0	466 ± 27.0	94 ± 21.0	87 ± 10.0	41 ± 8.0	44 ± 2.0
TA1537	0	6 ± 0.0	8 ± 3.0	9 ± 1.0		8 ± 2.0	9 ± 1.0
	3.3		8 ± 1.0				10 ± 2.0
	10	5 ± 1.0	10 ± 2.0	10 ± 2.0		10 ± 1.0	11 ± 1.0
	33	5 ± 1.0	9 ± 2.0	9 ± 1.0		13 ± 1.0	11 ± 2.0
	100	4 ± 1.0	9 ± 2.0	8 ± 1.0		11 ± 0.0	9 ± 2.0
	333	4 ± 1.0	6 ± 0.0	11 ± 1.0		11 ± 1.0	10 ± 1.0
	1,000	Toxic		Toxic		7 ± 1.0	
Trial summary		Negative	Negative	Negative		Negative	Negative
Positive control		134 ± 5.0	756 ± 68.0	84 ± 8.0		49 ± 4.0	39 ± 6.0
TA98	0	22 ± 4.0	28 ± 4.0	31 ± 4.0	36 ± 2.0	32 ± 4.0	35 ± 3.0
	3.3		32 ± 4.0		31 ± 4.0		28 ± 1.0
	10	20 ± 1.0	26 ± 5.0	23 ± 1.0	28 ± 2.0	26 ± 4.0	25 ± 2.0
	33	15 ± 1.0	19 ± 1.0	24 ± 1.0	32 ± 3.0	29 ± 3.0	31 ± 1.0
	100	18 ± 1.0	22 ± 1.0	22 ± 1.0	26 ± 4.0	29 ± 2.0	32 ± 2.0
	333	Toxic	20 ± 2.0	9 ± 9.0	26 ± 1.0	Toxic	Toxic
	1,000	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		303 ± 24.0	254 ± 11.0	668 ± 24.0	583 ± 87.0	669 ± 44.0	368 ± 30.0

[a] Study was performed at Case Western Reserve University (Cleveland, OH). The detailed protocol and these data are presented by Haworth *et al.* (1983). 0 µg/plate was the solvent control.

[b] Revertants are presented as mean ± standard error from three plates.

[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C4
Mutagenicity of *m-/p*-Cresol in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
TA100	0	102 ± 8.0	140 ± 20.0	165 ± 3.0	150 ± 10.0	159 ± 6.0	173 ± 6.0
	10		129 ± 8.0				
	33	140 ± 14.0	169 ± 3.0	160 ± 5.0		148 ± 6.0	
	100	132 ± 7.0	178 ± 3.0	171 ± 2.0	157 ± 8.0	141 ± 16.0	176 ± 10.0
	333	120 ± 11.0	160 ± 7.0	162 ± 6.0	188 ± 17.0	152 ± 13.0	179 ± 9.0
	1,000	111 ± 4.0	165 ± 4.0	184 ± 4.0	178 ± 3.0	148 ± 9.0	176 ± 8.0
	1,666	123 ± 10.0					
	3,333			88 ± 8.0[c]	167 ± 5.0	141 ± 4.0	128 ± 7.0
	6,666				95 ± 10.0		54 ± 11.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		1,082 ± 41.0	961 ± 14.0	1,160 ± 25.0	900 ± 13.0	567 ± 6.0	620 ± 25.0
TA1535	0	11 ± 1.0	9 ± 2.0	9 ± 2.0	7 ± 3.0	14 ± 1.0	12 ± 1.0
	10		8 ± 0.0				
	33	8 ± 2.0	11 ± 2.0	8 ± 1.0	10 ± 2.0	12 ± 1.0	14 ± 3.0
	100	8 ± 0.0	11 ± 2.0	10 ± 2.0	10 ± 1.0	13 ± 3.0	7 ± 2.0
	333	5 ± 0.0	10 ± 1.0	9 ± 2.0	9 ± 1.0	8 ± 1.0	9 ± 1.0
	1,000	8 ± 1.0	9 ± 1.0	12 ± 2.0	9 ± 2.0	12 ± 2.0	9 ± 1.0
	1,666	0 ± 0.0[c]					
	3,333			7 ± 1.0	4 ± 1.0	10 ± 2.0	7 ± 3.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		601 ± 67.0	715 ± 68.0	79 ± 1.0	299 ± 50.0	126 ± 8.0	83 ± 11.0
TA97	0	192 ± 14.0	169 ± 7.0	167 ± 8.0	169 ± 10.0	178 ± 8.0	217 ± 3.0
	10		180 ± 10.0				
	33	179 ± 4.0	178 ± 3.0	179 ± 11.0	191 ± 13.0	202 ± 2.0	242 ± 35.0
	100	172 ± 7.0	208 ± 13.0	222 ± 1.0	170 ± 3.0	216 ± 2.0	244 ± 5.0
	333	181 ± 7.0	187 ± 2.0	192 ± 15.0	169 ± 12.0	211 ± 1.0	214 ± 14.0
	1,000	128 ± 14.0	158 ± 9.0	192 ± 6.0	170 ± 10.0	176 ± 7.0	167 ± 11.0
	1,666	0 ± 0.0[c]					
	3,333			143 ± 18.0[c]	100 ± 2.0	149 ± 7.0	131 ± 8.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		335 ± 25.0	546 ± 3.0	818 ± 20.0	549 ± 47.0	529 ± 39.0	360 ± 10.0

TABLE C4
Mutagenicity of *m-/p-*Cresol in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	Trial 2	Trial 3		
TA98	0	17 ± 2.0	36 ± 2.0	44 ± 3.0		
	10		31 ± 1.0	43 ± 3.0		
	33	16 ± 5.0	31 ± 4.0	27 ± 1.0		
	100	14 ± 1.0	32 ± 6.0	36 ± 3.0		
	333	19 ± 3.0	23 ± 2.0	34 ± 5.0		
	666			29 ± 2.0		
	1,000	Toxic	24 ± 4.0			
	1,666	Toxic				
Trial summary		Negative	Negative	Negative		
Positive control		316 ± 14.0	570 ± 51.0	695 ± 43.0		
		+hamster S9		+rat S9		
		10%	30%	10%	30%	
TA98 (continued)	0	33 ± 3.0	30 ± 1.0	38 ± 1.0	29 ± 3.0	
	33	37 ± 2.0		46 ± 5.0		
	100	39 ± 3.0	27 ± 5.0	47 ± 4.0	20 ± 5.0	
	333	33 ± 3.0	24 ± 1.0	39 ± 1.0	16 ± 2.0	
	1,000	36 ± 3.0	20 ± 2.0	46 ± 4.0	17 ± 2.0	
	3,333	28 ± 8.0[c]	12 ± 2.0	30 ± 4.0	19 ± 3.0	
	6,666		0 ± 0.0[e]		17 ± 2.0	
Trial summary		Negative	Negative	Negative	Negative	
Positive control		877 ± 25.0	525 ± 45.0	491 ± 24.0	102 ± 8.0	

[a] Study was performed at SRI International (Palo Alto, CA). The detailed protocol and these data are presented by Zeiger *et al.* (1992).
[b] 0 µg/plate was the solvent control.
Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Slight toxicity and precipitate on plate

TABLE C5
Mutagenicity of *m-/p*-Cresol (Lot No. M080901KLH) in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
TA100	0	54 ± 5.0	70 ± 9.0	76 ± 3.0	77 ± 3.0	44 ± 3.0	63 ± 7.0
	25	53 ± 6.0					
	50	49 ± 2.0					
	62.5					68 ± 3.0	
	100	48 ± 6.0		69 ± 4.0			
	125					57 ± 2.0	
	250	57 ± 4.0	66 ± 6.0	67 ± 3.0	77 ± 3.0	76 ± 3.0	66 ± 6.0
	500	54 ± 5.0	57 ± 4.0	71 ± 4.0	67 ± 3.0	63 ± 6.0	72 ± 2.0
	1,000		45 ± 5.0	58 ± 2.0			84 ± 13.0
	1,500				52 ± 2.0	58 ± 3.0	
	2,000		30 ± 2.0	24 ± 3.0[c]			36 ± 4.0
	2,500				39 ± 5.0		
	3,000		5 ± 1.0[c]				34 ± 4.0
	3,500				16 ± 2.0[c]		
	4,000		Toxic				Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		422 ± 7.0	488 ± 27.0	635 ± 35.0	878 ± 19.0	772 ± 52.0	982 ± 37.0
TA98	0	21 ± 1.0	18 ± 4.0	22 ± 3.0	17 ± 2.0	19 ± 1.0	24 ± 3.0
	25		20 ± 3.0				
	50		19 ± 2.0				
	62.5				23 ± 4.0		
	100		16 ± 2.0	12 ± 1.0			
	125				16 ± 3.0		
	250	19 ± 1.0	17 ± 3.0	14 ± 1.0	23 ± 6.0	27 ± 1.0	17 ± 1.0
	500	19 ± 1.0	21 ± 2.0	14 ± 2.0	23 ± 0.0	24 ± 2.0	15 ± 1.0
	1,000	17 ± 1.0		13 ± 1.0		22 ± 2.0	
	1,500				17 ± 4.0		16 ± 2.0
	2,000	12 ± 1.0		10 ± 1.0		20 ± 2.0	
	2,500						12 ± 1.0[c]
	3,000	5 ± 1.0[c]				18 ± 1.0	
	3,500						Toxic
	4,000	Toxic				Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		693 ± 26.0	654 ± 17.0	445 ± 13.0	1,211 ± 15.0	1,163 ± 77.0	742 ± 37.0

TABLE C5
Mutagenicity of *m-/p-*Cresol (Lot No. M080901KLH) in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
***Escherichia coli* WP2 *uvrA* pKM101 (Analogous to TA102)**							
	0	151 ± 6.0	141 ± 7.0	158 ± 4.0	185 ± 8.0	142 ± 6.0	216 ± 3.0
	25		119 ± 2.0				
	50		141 ± 2.0				
	62.5					214 ± 4.0	
	100	163 ± 15.0	185 ± 8.0				
	125					235 ± 1.0	
	250	151 ± 11.0	181 ± 3.0	157 ± 18.0	170 ± 1.0	223 ± 5.0	236 ± 14.0
	500	146 ± 9.0	192 ± 11.0	152 ± 14.0	179 ± 12.0	198 ± 6.0	240 ± 22.0
	1,000	120 ± 4.0		147 ± 1.0	138 ± 6.0		197 ± 12.0
	1,500					175 ± 6.0	
	2,000	90 ± 5.0[c]		109 ± 5.0			130 ± 6.0
	2,500				120 ± 8.0		
	3,000			53 ± 6.0[c]			17 ± 3.0[c]
	3,500				46 ± 7.0[c]		
	4,000			22 ± 7.0[c]			8 ± 1.0[c]
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Negative
Positive control		1,816 ± 74.0	1,666 ± 163.0	1,955 ± 18.0	993 ± 79.0	823 ± 43.0	1,164 ± 25.0

[a] Study was performed at SITEK Research Laboratories (Rockville, MD). The detailed protocol and these data are presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C6
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *o*-Cresol in Feed for 13 Weeks[a]

PCEs[b]	Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c] %
Male				
NIH-07 Feed[d]	10	1.85 ± 0.17		2.21 ± 0.12
5,000	10	1.76 ± 0.09	0.7012	2.21 ± 0.07
10,000	10	1.69 ± 0.10	0.8284	2.22 ± 0.15
20,000	10	1.93 ± 0.07	0.3320	2.53 ± 0.08
		P=0.300[e]		
Female				
NIH-07 Feed	10	1.34 ± 0.08		1.97 ± 0.08
5,000	10	1.19 ± 0.05	0.8585	2.13 ± 0.08
10,000	10	1.33 ± 0.10	0.5243	2.13 ± 0.15
20,000	10	1.32 ± 0.09	0.5671	2.30 ± 0.11
		P=0.424		

[a] Study was performed at the United States Department of Agriculture, California. The detailed protocol is presented by MacGregor *et al*. (1990), and the data are published in Witt *et al*. (2000). NCE=normochromatic erythrocyte, PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated controls, significant at P≤0.008 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE C7
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *m-/p*-Cresol in Feed for 13 Weeks[a]

PCEs[b]	Dose (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c] %
Male				
NIH-07 Feed[d]	10	0.94 ± 0.14		1.03 ± 0.09
625	10	0.82 ± 0.13	0.7746	1.17 ± 0.09
1,250	10	0.89 ± 0.11	0.6086	1.17 ± 0.06
2,500	10	0.80 ± 0.09	0.8126	1.31 ± 0.11
5,000	10	0.95 ± 0.08	0.4604	1.07 ± 0.10
10,000	10	0.98 ± 0.10	0.3874	1.31 ± 0.08
		P=0.199[e]		
Female				
NIH-07 Feed	10	0.69 ± 0.08		1.27 ± 0.11
625	10	0.60 ± 0.05	0.7908	1.15 ± 0.09
1,250	10	0.53 ± 0.08	0.9406	1.06 ± 0.07
2,500	10	0.55 ± 0.07	0.9060	1.08 ± 0.10
5,000	10	0.50 ± 0.06	0.9636	0.93 ± 0.06
10,000	10	0.55 ± 0.09	0.9087	1.08 ± 0.09
		P=0.873		

[a] Study was performed at the United States Department of Agriculture, California. The detailed protocol is presented by MacGregor *et al*. (1990), and the data are published in Witt *et al*. (2000). NCE=normochromatic erythrocyte, PCE=polychromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated controls, significant at P≤0.005 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX D
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Cresols

A *meta* and *para* mixture of cresols was obtained from Merichem Company (Houston, TX; lot RC Sample 891) and Merisol USA, LLC (Houston, TX; lot Z000004273). These two lots were combined by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), into one lot (M080901KLH), which was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the cresols studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a light yellow liquid, was identified as cresols by density and boiling point determinations; ultraviolet/visible, infrared (IR), and proton nuclear magnetic resonance (NMR) spectroscopy; and gas chromatography/mass spectrometry by the analytical chemistry laboratory. The study laboratory confirmed the identity of the chemical by IR spectroscopy. The density and boiling point were consistent with literature values for cresols (*Merck*, 1996). Absorbances were consistent with literature references (*Sadtler*, 1960a,b), and all spectra were consistent with the structure of the chemical, literature spectra (*Aldrich*, 1983; NIEHS, 1986; NIST), and spectra of frozen reference samples or previously reported spectra of the same lot of the chemical. Representative IR and NMR spectra are presented in Figures D1 and D2.

The moisture content of lot M080901KLH was determined by the analytical chemistry laboratory, Galbraith Laboratories, Inc. (Knoxville, TN), and Prevalere Life Sciences, Inc. (Whitesboro, NY). The purity of lot M080901KLH was determined by the analytical chemistry laboratory using thin-layer chromatography (TLC) and gas chromatography (GC) by system A (Table D1), and by the study laboratory using GC by systems B and C. TLC was performed on 20 cm × 20 cm silica gel 60 F_{254} precoated (250 µm) plates (EM Science, Gibbstown, NJ). The plates were spotted with solutions of the test article and the reference standard (vanillin) and developed in a tank containing toluene:ether (50:50) as the solvent system. The dried plates were examined using UV light (at 254 and 366 nm), visible light, iodine vapor, and a phosphomolybdic acid reagent spray.

For lot M080901KLH, Karl Fischer titration generally indicated a water content of approximately 0.2%. TLC with detection at 254 nm, iodine vapor, and reagent indicated one major spot and no impurities. Detection at 366 nm and visible light showed no spots. GC using system A indicated a purity greater than 99.8%, two impurities at greater than or equal to 0.05% relative area, and an isomer ratio of approximately 60:40 (*meta/para*). GC using system B indicated an average purity of 98.3% relative to a frozen reference sample; reanalysis using system C indicated an average relative purity of 99.5%. The overall purity of lot M080901KLH was determined to be 99.5% or greater.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC by system D. These studies indicated that cresols were stable as a bulk chemical for 2 weeks when stored under a nitrogen headspace, protected from light, at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at approximately 25° C, protected from light in sealed amber glass bottles. Periodic purity reanalyses of the bulk chemical were performed by the study laboratory using GC by system C. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared approximately monthly by mixing cresols with NTP-2000 feed (Table D2). A premix was prepared in a Hobart mixer and then blended with additional feed in a Patterson-Kelly twin-shell blender for 15 minutes using an intensifier bar for the entire time. Formulations were stored for up to 42 days in sealed plastic buckets lined with plastic bags at approximately 25° C or at approximately 5° C for all batches formulated after August 20, 2002.

Homogeneity studies of 500 and 15,000 ppm dose formulations of lot Z000004273 of the test chemical were conducted by the analytical chemistry laboratory using GC by system E and homogeneity studies of 1,000 and 15,000 ppm dose formulations of this lot were conducted by the study laboratory using GC by a system similar to system C (Table D1). Stability studies of a 500 ppm dose formulation of lot Z000004273 were also performed by the analytical chemistry laboratory with GC by system E. Homogeneity was confirmed, and stability was confirmed for at least 42 days for dose formulations stored in the dark in plastic bags at temperatures up to 25° C and for at least 4 days under simulated animal room conditions if losses less than 10% were acceptable; the initial set of dose formulations was accordingly stored by the study laboratory at room temperature. Subsequent analyses by the study laboratory indicated that there was some decrease in test chemical concentration, and all batches formulated after August 20, 2002, were stored under refrigeration at approximately 5° C.

Periodic analyses of the dose formulations of cresols were conducted by the study laboratory using GC by system C. During the 2-year studies, the dose formulations were analyzed approximately every 3 months (Table D3). Of the dose formulations analyzed, all 54 for rats and all 27 for mice were within 10% of the target concentrations. Residual material remaining in the dose formulation storage buckets and animal room samples taken from the animals' feeders were also analyzed. Of the bucket samples analyzed, all nine for rats and all nine for mice were within 10% of the target concentrations. All nine of the animal room feeder samples for rats and five of nine of the animal room feeder samples for mice were within 10% of the target concentrations; all nine mouse feeder samples were within 19% of target. Decreased concentrations in mouse feeder samples were attributed to contamination of the feed with urine and feces.

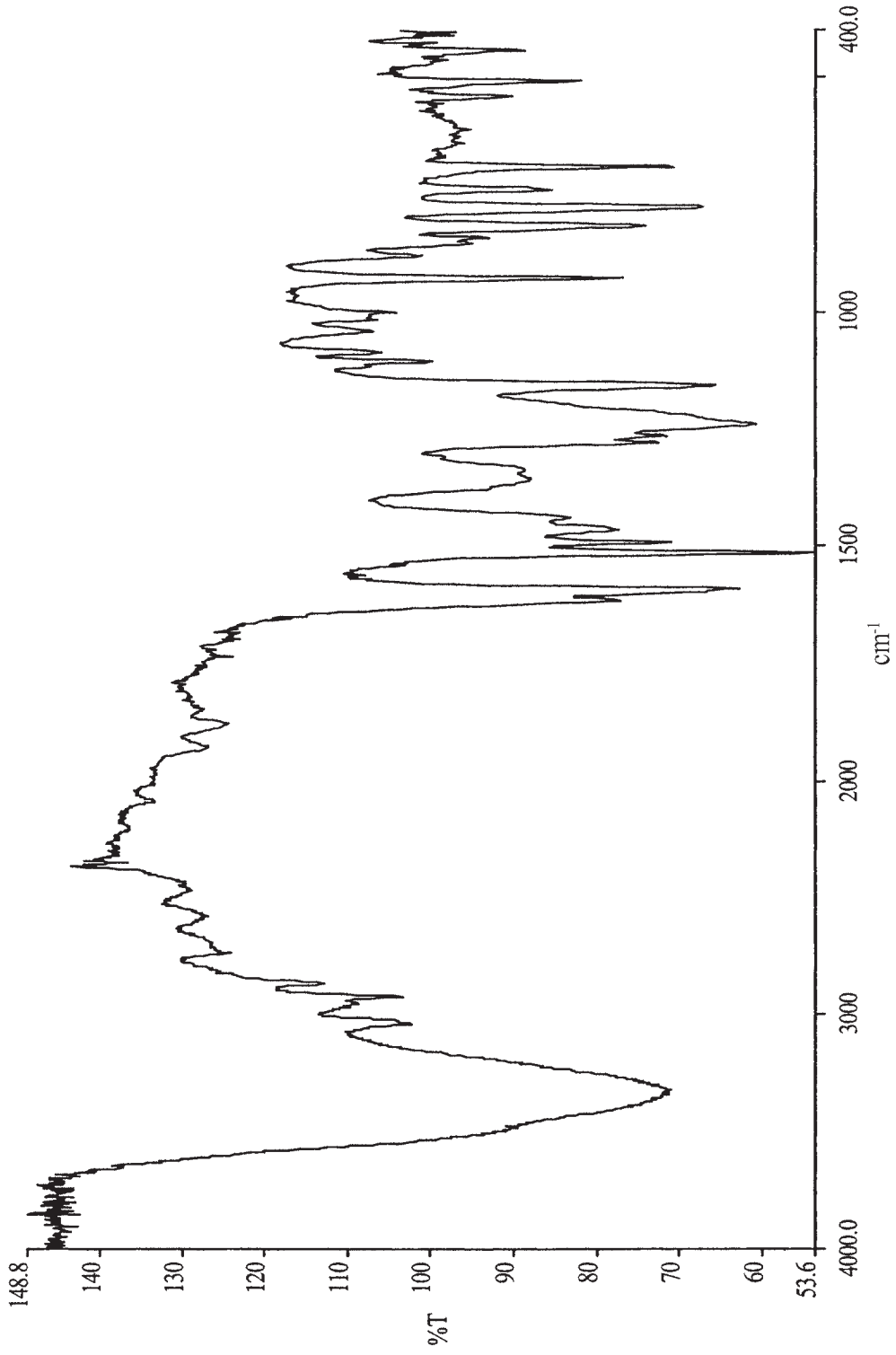

FIGURE D1
Infrared Absorption Spectrum of Cresols

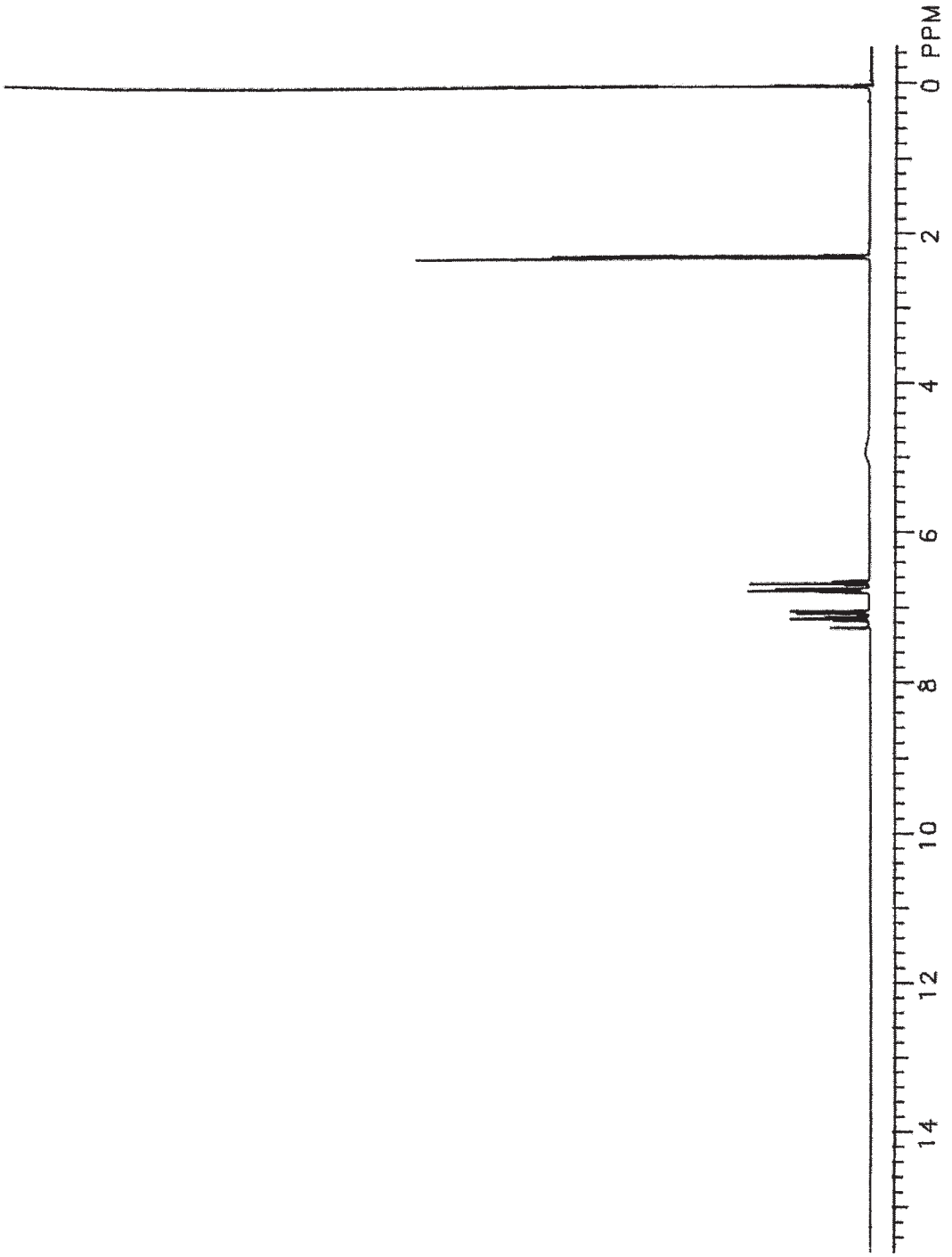

FIGURE D2
Proton Nuclear Magnetic Resonance Spectrum of Cresols

TABLE D1
Gas Chromatography Systems Used in the 2-Year Feed Studies of Cresols[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-FFAP, 30 m × 0.53 mm, 1-µm film (J&W Scientific, Folsom, CA)	Helium at 10 mL/minute	100° C to 200° C at 2° C/minute
System B			
Flame ionization	Stabilwax™ DA, 30 m × 0.53 mm, 1-µm film (Restek, Bellefonte, PA)	Helium at 10 mL/minute	140° C to 170° C at 3° C/minute, held for 5 minutes
System C			
Flame ionization	Stabilwax™ DA, 30 m × 0.53 mm, 1-µm film (Restek)	Helium at 15 mL/minute	140° C to 170° C at 2° C/minute
System D			
Flame ionization	DB-FFAP, 30 m × 0.53 mm, 1-µm film (J&W Scientific)	Helium at 10 mL/minute	Isothermal at 150° C
System E			
Flame ionization	DB-FFAP, 30 m × 0.53 mm, 1-µm film (J&W Scientific)	Helium at 10 mL/minute	140° C to 170° C at 2° C/minute

[a] The gas chromatographs were manufactured by Hewlett-Packard (Palo Alto, CA) (Systems A, D, and E) or Agilent (Palo Alto, CA) (Systems B and C).

TABLE D2
Preparation and Storage of Dose Formulations in the 2-Year Feed Studies of Cresols

Preparation
A premix of NTP-2000 feed and cresols was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender for 15 minutes with the intensifier bar on for the entire time. The dose formulations were prepared approximately monthly.

Chemical Lot Number
M080901KLH

Maximum Storage Time
42 days

Storage Conditions
Stored in sealed 5-gallon plastic buckets lined with plastic bags at room temperature or at approximately 5° C for all batches formulated after August 20, 2002

Study Laboratory
Battelle Columbus Operations
(Columbus, OH)

TABLE D3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Cresols

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
Rats				
July 23, 2002	July 25-26, 2002	1,500	1,474	–2
		1,500	1,522	+1
		5,000	4,976	0
		5,000	5,019	0
		15,000	15,740	+5
		15,000	15,040	0
	September 3-4, 2002[b]	1,500	1,398	–7
		5,000	4,673	–7
		15,000	14,610	–3
	September 3-4, 2002[c]	1,500	1,350	–10
		5,000	4,628	–7
		15,000	14,590	–3
October 10, 2002	October 15-16, 2002	1,500	1,493	0
		1,500	1,496	0
		5,000	5,015	0
		5,000	4,988	0
		15,000	14,880	–1
		15,000	14,840	–1
January 6, 2003	January 8, 2003	1,500	1,443	–4
		1,500	1,459	–3
		5,000	4,975	–1
		5,000	4,966	–1
		15,000	14,780	–1
		15,000	14,770	–2
April 4, 2003	April 4, 2003	1,500	1,498	0
		1,500	1,502	0
		5,000	5,076	+2
		5,000	4,972	–1
		15,000	14,980	0
		15,000	14,860	–1
	May 14-15, 2003[b]	1,500	1,425	–5
		5,000	4,789	–4
		15,000	14,990	0
	May 14-15, 2003[c]	1,500	1,406	–6
		5,000	4,917	–2
		15,000	14,410	–4
June 19, 2003	June 23-24, 2003	1,500	1,427	–5
		1,500	1,431	–5
		5,000	4,718	–6
		5,000	4,793	–4
		15,000	14,500	–3
		15,000	14,380	–4

TABLE D3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Cresols

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Rats (continued)				
September 12, 2003	September 17, 2003	1,500	1,521	+1
		1,500	1,457	–3
		5,000	4,879	–2
		5,000	4,727	–5
		15,000	14,530	–3
		15,000	14,510	–3
December 8, 2003	December 10-11, 2003	1,500	1,481	–1
		1,500	1,504	0
		5,000	5,007	0
		5,000	5,010	0
		15,000	15,030	0
		15,000	14,990	0
	January 22-23, 2004[b]	1,500	1,455	–3
		5,000	4,887	–2
		15,000	14,750	–2
	January 22-23, 2004[c]	1,500	1,431	–5
		5,000	4,771	–5
		15,000	14,710	–2
February 27, 2004	March 3-4, 2004	1,500	1,608	+7
		1,500	1,583	+6
		5,000	5,263	+5
		5,000	5,336	+7
		15,000	16,020	+7
		15,000	16,000	+7
May 20, 2004	May 21-25, 2004	1,500	1,519 ± 62[d]	+1
		1,500	1,567	+4
		5,000	4,999	+0
		5,000	5,284	+6
		15,000	15,250	+2
		15,000	15,350	+2
Mice				
July 23, 2002	July 25-26, 2002	1,000	1,009	+1
		3,000	3,058	+2
		10,000	10,100	+1
	September 3-4, 2002[b]	1,000	916.2	–8
		3,000	2,824	–6
		10,000	9,497	–5
	September 3-4, 2002[c]	1,000	806.5	–19
		3,000	2,543	–15
		10,000	8,926	–11

TABLE D3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of Cresols

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
Mice (continued)				
October 10, 2002	October 15-16, 2002	1,000	1,006	+1
		3,000	2,995	0
		10,000	9,960	0
January 6, 2003	January 8, 2003	1,000	974.0	–3
		3,000	2,964	–1
		10,000	9,852	–1
April 4, 2003	April 4, 2003	1,000	999.8	0
		3,000	2,949	–2
		10,000	9,859	–1
	May 14-15, 2003[b]	1,000	940.2	–6
		3,000	2,880	–4
		10,000	9,828	–2
	May 14-15, 2003[c]	1,000	883.2	–12
		3,000	2,701	–10
		10,000	9,406	–6
June 19, 2003	June 23-24, 2003	1,000	936.7	–6
		3,000	2,932	–2
		10,000	9,620	–4
September 12, 2003	September 17, 2003	1,000	972.9	–3
		3,000	3,012	0
		10,000	9,388	–6
December 8, 2003	December 10-11, 2003	1,000	1,009	+1
		3,000	2,996	0
		10,000	9,891	–1
	January 22-23, 2004[b]	1,000	994.7	–1
		3,000	2,875	–4
		10,000	9,683	–3
	January 22-23, 2004[c]	1,000	936.0	–6
		3,000	2,687	–10
		10,000	9,415	–6
February 27, 2004	March 3-4, 2004	1,000	1,074	+7
		3,000[e]	3,231	+8
		10,000	10,770	+8
May 20, 2004	May 21-25, 2004	1,000	1,061	+6
		3,000	2,992	0
		10,000	10,140 ± 150[d]	+1

[a] Results of duplicate analyses
[b] Samples of residual unused dose formulations stored in plastic buckets
[c] Animal room samples recovered from the feeders
[d] Results of triplicate analyses
[e] This formulation was mixed on March 2, 2004.

APPENDIX E
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES OF CRESOLS

TABLE E1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Cresols

Week	0 ppm		1,500 ppm			5,000 ppm			15,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	15.4	110	16.0	110	219	16.0	110	727	11.3	110	1,547
2	16.0	139	15.8	139	171	15.7	139	566	15.4	129	1,792
3	18.1	176	18.2	173	157	17.7	172	515	16.4	162	1,521
4	19.4	208	19.2	206	140	18.8	204	461	17.7	190	1,395
5	19.9	234	20.1	232	130	20.0	230	436	19.3	216	1,343
6	19.8	255	20.4	254	121	20.1	253	398	19.5	239	1,226
7	20.1	273	20.4	273	112	20.0	271	369	19.6	257	1,144
8	19.5	290	19.9	289	103	19.4	286	340	19.2	271	1,061
9	18.6	305	19.1	306	94	18.5	300	309	18.9	285	993
10	19.7	320	19.9	322	93	19.7	318	310	19.2	300	960
11	20.1	331	20.4	332	92	19.9	327	304	20.1	310	971
12	19.0	340	19.2	342	84	19.3	338	286	19.0	320	890
13	19.9	347	20.3	351	87	20.4	346	295	19.9	326	916
17	20.8	378	20.4	381	80	20.3	375	271	19.4	355	820
21	19.5	402	20.0	405	74	19.2	397	242	19.3	377	769
25	19.6	419	19.6	419	70	19.6	413	238	18.6	389	717
29	21.6	436	21.0	436	72	21.6	430	252	21.6	406	797
33	18.9	449	19.4	447	65	19.0	442	215	17.6	410	643
37	21.0	467	21.2	460	69	21.3	456	234	19.7	429	690
41	19.6	470	20.1	467	65	20.1	460	219	19.6	430	683
45	21.2	483	21.0	478	66	20.7	468	221	19.8	439	677
49	19.3	492	19.4	483	60	19.4	478	203	18.4	445	620
53	18.7	495	18.8	489	58	18.9	485	195	18.7	451	622
57	19.5	498	19.5	487	60	19.2	486	198	19.2	452	638
61	19.3	499	19.5	490	60	19.5	487	200	18.2	448	610
65	19.4	501	19.6	492	60	19.2	486	198	17.8	447	597
69	19.1	505	19.2	495	58	19.0	489	194	18.8	449	629
73	19.0	509	18.7	496	57	18.5	489	189	17.5	447	588
77	18.0	507	18.0	497	54	17.5	485	180	16.7	445	564
81	18.3	509	18.4	499	55	18.8	490	192	17.8	441	606
85	17.8	510	17.5	500	53	16.9	490	173	16.1	435	555
89	18.0	508	17.3	494	53	17.4	484	180	17.2	433	595
93	16.7	507	16.1	496	49	16.9	480	176	16.5	431	574
97	18.0	499	18.5	491	57	18.0	477	189	17.1	422	608
101	16.5	495	17.8	490	55	17.1	471	182	16.8	420	600
Mean for weeks											
1-13	18.9	256	19.1	256	123	18.9	253	409	18.1	240	1,212
14-52	20.2	444	20.2	442	69	20.1	435	233	19.3	409	713
53-101	18.3	503	18.4	494	56	18.2	485	188	17.6	440	599

[a] Grams of feed consumed per animal per day
[b] Milligrams of cresols consumed per kilogram body weight per day

TABLE E2
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of Cresols

Week	0 ppm		1,000 ppm			3,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.3	18.0	3.3	18.1	183	2.9	18.0	485	2.6	17.9	1,455
2	3.5	18.2	3.3	18.5	179	3.3	18.1	547	2.9	17.8	1,630
3	3.5	18.2	3.6	19.1	188	3.5	19.2	547	3.2	18.8	1,700
4	3.5	20.2	3.3	19.8	167	3.7	20.0	556	3.1	19.5	1,589
5	3.3	21.0	3.1	21.3	146	3.5	20.8	504	2.9	20.3	1,429
6	4.0	21.7	4.0	22.1	181	3.9	21.5	545	3.2	21.1	1,520
7	3.6	22.3	3.6	22.6	159	3.2	22.0	437	3.0	21.5	1,398
8	3.6	23.0	3.6	23.4	154	3.2	22.6	424	3.1	22.1	1,405
9	3.6	24.1	3.5	24.3	144	3.3	23.6	419	3.2	22.9	1,395
10	3.8	25.6	3.8	25.6	148	3.4	24.6	414	3.1	23.7	1,309
11	3.7	26.3	3.5	26.2	133	3.3	25.0	396	3.1	24.3	1,275
12	3.7	27.2	3.8	27.3	139	3.4	26.0	393	3.0	24.8	1,209
13	3.6	27.9	3.4	27.8	122	3.2	26.4	363	2.9	25.2	1,152
17	3.6	30.7	3.6	30.7	117	3.4	28.7	356	3.0	26.7	1,123
21	3.7	33.2	3.6	33.7	107	3.6	31.7	341	3.3	28.5	1,156
25	3.7	36.5	4.1	36.4	113	3.9	34.1	343	3.6	30.4	1,185
29	3.7	36.9	4.0	37.0	108	3.4	35.5	288	3.2	30.6	1,044
33	3.8	40.3	3.8	40.8	93	3.9	37.7	310	3.7	32.6	1,133
37	4.0	41.3	4.7	41.8	113	3.8	39.2	291	3.4	34.3	991
41	4.1	42.6	4.3	43.8	98	4.0	40.6	296	3.6	35.2	1,022
45	3.6	43.6	3.7	44.1	84	3.1	40.6	229	3.3	35.4	932
49	4.1	45.7	3.5	45.0	78	3.6	41.7	259	3.5	36.1	970
53	3.9	46.3	4.0	45.8	87	3.9	42.1	278	3.3	36.0	917
57	3.9	46.0	3.9	45.9	85	4.0	42.2	285	3.6	36.6	983
61	3.6	45.9	3.4	43.9	77	3.6	41.0	263	3.3	35.3	936
65	3.6	47.6	3.8	45.4	84	3.7	43.2	257	3.7	37.6	984
69	3.4	47.8	4.0	46.3	86	3.8	43.6	262	3.6	38.2	942
73	3.7	49.5	4.2	47.7	88	3.9	44.3	264	3.6	38.7	930
77	3.5	49.7	4.2	49.3	85	4.1	45.3	271	3.7	38.8	953
81	4.0	51.2	4.2	50.6	83	4.1	46.2	266	3.7	39.7	932
85	3.8	51.8	3.9	51.0	77	3.7	46.4	239	3.5	39.7	881
89	4.2	51.8	4.4	50.7	87	4.4	45.9	288	3.8	39.5	963
93	4.1	50.8	4.0	49.9	80	3.9	45.1	260	3.3	38.7	854
97	4.1	51.4	4.0	51.3	78	3.8	44.9	254	3.3	38.8	851
101	4.3	51.6	4.3	51.4	84	4.3	45.6	283	3.5	38.9	901
Mean for weeks											
1-13	3.6	22.6	3.5	22.8	157	3.4	22.1	464	3.0	21.5	1,420
14-52	3.8	39.0	3.9	39.3	101	3.6	36.6	301	3.4	32.2	1,062
53-101	3.9	49.3	4.0	48.4	83	3.9	44.3	267	3.5	38.2	925

[a] Grams of feed consumed per animal per day
[b] Milligrams of cresols consumed per kilogram body weight per day

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

TABLE F1
Ingredients of NTP-2000 Rat and Mouse Ration

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

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

TABLE F2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

TABLE F3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	15.2 ± 0.46	14.4 – 16.2	23
Crude fat (% by weight)	8.1 ± 0.34	7.5 – 9.0	23
Crude fiber (% by weight)	9.1 ± 0.58	8.1 – 10.6	23
Ash (% by weight)	5.1 ± 0.33	4.5 – 5.9	23
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	6,042 ± 325	3,210 – 1,760	23
Vitamin D (IU/kg)	1,000[a]		
ὰ-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	10.3 ± 5.98	6.4 – 30.6[b]	23
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7[b]	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.993 ± 0.052	0.909 – 1.090	23
Phosphorus (%)	0.604 ± 0.038	0.539 – 0.721	23
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

TABLE F4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.39 ± 0.154	0.14 – 0.50	23
Cadmium (ppm)	0.07 ± 0.022	0.39 – 0.11	23
Lead (ppm)	0.07 ± 0.020	0.05 – 0.11	23
Mercury (ppm)	<0.02		23
Selenium (ppm)	0.20 ± 0.057	0.14 – 0.40	23
Aflatoxins (ppb)	<5.00		23
Nitrate nitrogen (ppm)[c]	15.6 ± 4.31	10.0 – 27.0	23
Nitrite nitrogen (ppm)[c]	<0.89		23
BHA (ppm)[d]	<1.0		23
BHT (ppm)[d]	<1.0		23
Aerobic plate count (CFU/g)	12 ± 8.5	10 – 50	23
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	23
Escherichia coli (MPN/g)	<10		23
Salmonella (MPN/g)	Negative		23
Total nitrosoamines (ppb)[e]	4.7 ± 2.57	2.4 – 12.0	23
N-Nitrosodimethylamine (ppb)[e]	3.1 ± 2.26	1.2 – 9.3	23
N-Nitrosopyrrolidine (ppb)[e]	1.6 ± 0.53	0.9 – 2.7	23
Pesticides (ppm)			
α-BHC	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
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	0.080 ± 0.073	0.020 – 0.280	23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion	0.305 ± 0.502	0.021 – 1.820	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

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

APPENDIX G
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from 10 male sentinel rats and five male and five female sentinel mice at 1 month, five male sentinel rats and male mice at 6 months, six male sentinel rats and five male sentinel mice at 12 months, four male sentinel rats and five male sentinel mice at 18 months, and five 15,000 ppm rats and 10,000 ppm mice at study termination. Blood from each animal was collected and allowed to clot, and the serum was separated; fecal samples were collected from the five male sentinel mice at 18 months for *Helicobacter hepaticus*. Samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	1, 6, 12, and 18 months, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
ELISA	
Ectromelia virus	1, 6, 12, and 18 months, study termination
EDIM (epizootic diarrhea of infant mice)	1, 6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis virus)	1, 6, 12, and 18 months, study termination
LCM (lymphotic choriomeningitis)	1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
MCMV (mouse cytomegalovirus)	Study termination
MHV (mouse hepatitis virus)	1, 6, 12, and 18 months, study termination
Mouse adenoma virus-FL	1, 6, 12, and 18 months, study termination
Parvovirus (MMV VP2 and MPV VP2)	Study termination
PVM	1, 6, 12, and 18 months, study termination
Reovirus 3	1, 6, 12, and 18 months, study termination
Sendai	1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	1, 6, 12, and 18 months
MCMV	Study termination
PVM	Study termination

RESULTS

All test results were negative.

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